JOANNE STUBBE: What I want to do is sort of introduce you to the second half of the course, where we're going, and what topics we're going to be covering. And then I'll start in module 5. So as with the first half of the course, we have four modules. The first half the courses was pretty well organized-- that is, you went from here to here to here. It all sort of made sense.

This half of the course doesn't do that. We're talking about-- there are hundreds of topics in biochemistry, and any one of them is exciting and important. And these are the ones we're talking about this semester. And again, the focus will be sort of trying to get you to think about, what is the evidence that supports the model I'm going to present. So in module 5, we're going to be talking about the terpenome. And I'm going to be talking about, most of you have seen in the last module, with polyketide synthases, you have aldol reactions and claison reactions to form carbon carbon bonds.

In this module, you're going to be introduced to another way to form carbon carbon bonds. And that's through C5 units. And C5 units are the basis for forming cholesterol, which is really the focus of module 5. And what we're going to be talking about is initially cholesterol biosynthesis, that will be this lecture and probably most of the next lecture. And then you all know from eating McDonald's hamburgers, you get a lot of cholesterol in your diet. And the question is, how do you control cholesterol levels?

And so this semester, the second part of the semester is really focus on the question of homeostasis. Cholesterol is essential. If you have too much you've got problems. Doesn't matter what pathway you're talking about, if you have too much or you have too little, you have problems. So how do you control everything? And we're going to be talking about cholesterol sensing and regulation. And we're going to come back to a topic you covered in the first half of the course with ClpX and ClpP protein mediated degradation, because it plays a central role in controlling cholesterol homeostasis.

So that will be the first module, module 5. Module 6 is also going to be a module on

homeostasis. It's my feeling that in introductory courses, people don't get introduced enough to metals. And 50% of all the proteins inside of us bind metals and do something with them. And so in this module, I'm going to talk about metal homeostasis. And I'm really going to focus on iron. And you'll see why I'm going to focus on iron when we get there. But we're going to talk about iron sensing and regulation, initially in humans, and then we're going to focus on the war between pathogenic organisms for iron and humans for iron, since iron is essential for almost all organisms to survive.

The third module is sort of-- module 7-- sort of follows from module 6. And it's a topic that I've been following for 30 years, and it irritates the hell out of me. So everybody talks about reactive oxygen species, and how bad they are. You can't-- you can listen to NPR, you can read it in *The New York Times.* What are reactive oxygen species? As a chemist, what are they?

So we'll define what reactive oxygen species are. Many of you know that they're bad. That's why you eat vitamin C and vitamin E. And they're involved, in fact, in defense against some microorganisms. But also they're good. They're now known to be involved in signaling. So again, it's a question of homeostasis. And so you need to understand the chemistry of what these species do, and where they can go astray, or where you can harness the reactivity to do something important.

And the last section-- hopefully we'll get there this year, I'm trying my best-- this is the area I know most about, we're going to talk about nucleotide metabolism. In 5.07, they don't talk about nucleotide metabolism at all. And I would say in the next decade, you're going to see a lot about nucleotide metabolism. Where have you seen ATP and GTP in the first part of this course? Everywhere. How do we control all of that? Pretty important. What are the questions we're going to be asking? Where does it come from? And how do we control the levels, which is central to all of metabolism? Hopefully we'll get there and discuss that.

So the required reading has now been posted on Stellar. And there are-- there is really sort of three things you need to read. One is about a short review on the terpenome, which is what I'm going to start talking about. The second is lipid metabolism that's been taken from Voet & Voet, you can go back in any basic textbook and read the section, because it's central to thinking about what's going on with cholesterol.

And then you'll see that these are two of my favorite guys, Brown and Goldstein. They won the

Nobel Prize for their work, but in reality they should have won at least two Nobel prizes for their work. I mean, you can never not listen to them talk and not get excited. I mean, they always have something important and exciting to say. So they-- last year we used this review. There's a new review. They're different, they're both pretty short. Pick which one you want, they cover the material. And then this one doesn't cover the most recent material as well. So here's another short review that covers that material. So these guys here will give you an overview of what I'm going to be talking about. And they've all been posted on the website already.

So cholesterol homeostasis-- I never end a lecture on time. You'll find out that I'm trying my best, anyhow. We have about five lectures we're going to be covering. And this is where we're going. And the first one, today's lecture, we're going to be talking about a new way to form carbon carbon bonds through C5 units. And we'll be talking about the terpenome, where there are a huge number of natural products, distinct from polyketide synthases and non-ribosomal polypeptide synthases that you just finished talking about.

We want to get to-- we'll see in the biosynthetic pathway to get to these C5 building blocks, we need to get to a metabolite called mevalonic acid, that's front and central in cholesterol biosynthesis. So we need to get that far. And then we need to get from mevalonic acid into cholesterol. And so we'll be talking about this the first couple of lectures. In addition to making cholesterol, you get a lot of cholesterol from your diet. And so the question is, how does it get from your diet, transferred through the plasma, and taken up into cells?

And this section, we'll describe the discovery of receptor mediated endocytosis, by Brown and Goldstein, that's now known to be prevalent everywhere. So the module-- module 6, you take up iron by receptor mediated endocytosis. In module 7, growth factors are involved in receptor mediated endocytosis. So it represents a general paradigm that happens all the time over and over again in biology.

And then we're going to ask the question, how do you sense the cholesterol? And we're going to be doing two recitations on this. And then we'll have a few lectures on the machinery that sense cholesterol sterile responsive binding proteins, in a molecule called SCAP, and another molecule-- both of these are proteins-- called INSIGs. Everything is located in a membrane-- that's something you haven't been exposed to. How do you control everything when you have stuff stuck in a membrane.

And then we'll come back at the end to look at the rate limiting step in formation of mevalonic acid, HMGCoA reductase, and how that plays a key role, since it's involved in making cholesterol. How do we control and regulate that protein, also in the ER membrane. And we'll see it requires ubiquitin mediated protein degradation. And so that's why I'm going to come back, and we're going to spend a little bit of time talking about this process in eukaryotic systems.

And actually, in all the other modules, you're going to see ubiquitin mediated protein degradation. And finally, this week in recitation, there's a new-- not new, it was discovered in 2003-- a new target for drug therapy in controlling cholesterol levels. And there was a paper published this year to show that this is, in fact, a good target. And this paper used CRISPR-Cas9 technology. So I'm going to-- even though I'm not an expert in that, my lab hasn't used it-- I'm going to introduce you to this technology, and then focus on why we think this is a good new target, and what the targeting is.

So that's where we're going. So the terpenome-- let's see if I can remember what I'm going to say. The first thing I want to say is-- let me just get all this-- OK, the first thing I want to tell you something about is the nomenclature-- and all terpenes are either called isoprenoids or terpenoids, and they're all made from C5-- a C5 hydrocarbon skeleton. And this C5 hydrocarbon skeleton is an isoprene.

So this is the key building block that you're going to see over and over again over the course of the first couple of lectures. So an isoprenoid is, in general, linear. And it's made of n of these C5 units. So n can be 2 to thousands. And I'll give you examples of these. And again-- and then the terpenoids-- so let's just use terpenoids over here-- in general are also made of C5-- C5 units. But often, they're oxidized, cyclized, and sometimes rearranged.

So my goal today really sort of is introduce you to this huge class of natural products. And give you some examples of this, and then start focusing on how we get the building blocks. Do you think iso-- this isoprene can be a building block? It's chemically not very reactive. So no, we have to convert-- we have to convert this into the chemically reactive building block. So while isoprene gives you the C5 unit, our focus today is going to be on creating the building blocks.

And again, the building blocks are going to be-- these are the guys you're going to see over and over again. 1, 2, 3, 4, 5-- so does everybody know what PPI means, so I don't have to write it out? pyrophosphate-- we're going to see this over and over again. You've seen this in

the first part of the semester. And this isopentenyl pyrophosphate, or IPP. And we're going to see this-- if you look at this this hydrogen, what's interesting about this hydrogen, chemically?

What's the pKa of that hydrogen, compared to if it was this hydrogen? It's much lower. Yeah, so it can form allylic cations or anions. And so this can readily isomerize to form this species, which also plays, which is dimethylalyl pyrophosphate, which is the other key building block that we're going to be looking at. So currently, it's estimated from the latest paper that I've read that these are the building blocks for what we call the terpenome.

And it's estimated that there are greater than 70,000 natural products. Now in contrast to what you've learned about with non-ribosomal polypeptides synthases and polyketide synthases, where you sort of can find everything in an operon, and you can sort of understand how your molecules could be put together. It's not so trivial with terpenes. There is no such logic in these systems. So let's just look at what some of these molecules actually are so you know why they're important.

And, OK, so so in the center of everything are these two guys, our two building blocks. And these building blocks can go to the fat soluble vitamins-- so for example A and K. So if you look over here, you have vitamin A-- I knew this was going to happen. My late-- my pointers are not working very well. I need-- OK, so anyhow you have vitamin A and you have vitamin K. And where do you see-- you can see here readily that you have these C5 units somehow stuck together. And you have to ask the question, where does the rest of this come from?

So fat soluble vitamins, which we're not going to talk about, what you also have is prenylated proteins. And prenylated proteins are shown here. So quite frequently, you have small little g proteins, GTPases, you've seen these before. EFTU, EFGG, they're all over the place. There are hundreds of g proteins-- we talked about them in the recitation section on Rodnina that I gave you. Anyhow, a lot of those little g proteins go to the membrane and come away from the membrane.

They do that by sticking on a tag. This tag can be geranylated or gerynalgeranylated-- it just the hydrophobic tag that allows things to interact with the membrane, increasing the effective molarity. Nature uses this trick over and over and over again. Another thing you can generate is natural products of medicinal interest. And I just show here taxol and artemisinin. So taxol is used in the treatment of breast cancer. Artemisinin, anybody heard of that?

Yeah, so this has been the major target-- in fact, this pathway we're talking about today has

been a major focus of many synthetic biologies, trying to make this mevalonic acid pathway so they can make potential drugs, but also jet fuels-- which, again, you want some kind of hydrocarbon. So this pathway has been studied a lot from a point of view of metabolic engineering. It's also involved in-- this is one of my favorite-- the perfume.

You can tell from the way I smell the perfume industry. Any of you ever break a pine cone? A pine needle? Yeah, doesn't it smell great? No, you don't think so? I think it's wonderful. It's called pinene. Anyhow, it has-- I think terpenes wonderful smells, and it's the hallmark of the fragrance industry. Is that on here? Yeah, so menthol. Limonene is orange-- in fact, if you were here when Barry Sharpless used to teach-- I can't digress, because that's why I never get to the end of the course. But anyhow, Barry used to bring to organic class-- he had boxes of smells. And he used to pass around the smells, and they were all wonderful. And they were almost all terpenes.

And we're going to be looking at things like dolichol-- we aren't going to be looking at it. We will see it a little bit. But what you can see here, Suzanne Walker is giving a talk here April 4th. And she works on-- one of the things she works on is peptidoglycan biosynthesis. And so sugars are carried around on these lipids. Some of them are C19 to C55. If you look at these, you can see these little units stuck together.

Barbara Imperiali, in our department, the biology department, works on a asparagine-linked glycosylation. Again, the sugars are carried around by these kinds of terpenes. So plays a central role in putting sugars onto systems. And then what we're going to be focused on today-- and this is the focus in general-- is on cholesterol. Do I have that up there? I think so. So what we're going to-- do I have cholesterol up there? Yeah. OK, here it is. Cholesterol.

That's what we're going to be focusing on. And that's not a C5, But a C30. So how do we get from these C5 units into the C30 units? So that's an introduction to the terpenome. They're everywhere. And so you can't become a biochemist without seeing carbon carbon bond formation by these C5 units, in many, many kinds of reactions-- in both primary and secondary metabolism. They're very important.

So what I want to do before we get into looking at one of the pathways that you can make the building blocks, IPP and DMAPP-- this is abbreviated DMAPP. One of the ways is through the mevalonic acid pathway, and here's a picture of a cell that I took from something off the web. But I want to introduce you to where we're going to be going, cholesterol biosynthesis. So

where do we break down fatty acids? Does anybody know? You remember from your introductory course?

I want to try to put this into the big picture on metabolism, so you're not-- we're just not pulling it out of the air. Anybody know where you break down fatty acids from the diet?

AUDIENCE: You're asking in the cell, specifically?

JOANNE STUBBE: There in the cell. Yeah, where in the cell? These are-- we're talking about eukaryotes now. Bacteria don't make cholesterol. Yeah. What? OK, you don't even know that. OK, so you should go back and read the chapter on fatty acid biosynthesis and degradation. That would be a good thing for you to do. Anyhow, fatty acids are broken down in the mitochondria. We'll see this in a second. So the mitochondria play a role.

We're going to see today-- so here's the nucleus, here's the endoplasmic reticulum. The endoplasmic reticulum is the key sensor in cholesterol homeostasis. And so we're going to-- and we're going to see that it controls transcription factors. Transcription factors are stuck in a membrane in the ER. That's-- how weird is that? Because where do transcription factors need to go? They need to go to the nucleus.

So how can you do that? How can you take something stuck in a membrane and get it to the nucleus? So they need to go through a golgi stack. They do some stuff we're going to learn about to eventually get into the nucleus, where they control not only the levels of cholesterol proteins, but also of metabolism of phospholipids, triacylglycerols. so this takes us into the big realm of all lipid metabolism, which most of the time people don't spend a lot of time talking about in an introductory course.

And I mean, one of the interesting questions-- we're going to see the key rate limiting step in cholesterol homeostasis. The protein is bound to the ER membrane, and a lot of the proteins involved in cholesterol biosynthesis are in the ER membrane. 50% of all the cholesterol ends up in the plasma membrane. how does it get there? Does it just go through solution? You need to think about the properties of cholesterol.

So when you get confused about where we're going, come back to the picture. And I'm going to show you one other big picture, which we use when I teach-- when I've taught 5.07 with Essigmann-- again, this is the picture we can back to over and over and over again. Because everything is interconnected. So we're going to be talking about cholesterol biosynthesis.

We're going to see a key player is acetyl-CoA. Where have you seen that?

You've learned a lot about acetyl-CoA in biosynthesis, and use in biosynthesis and polyketide. Synthases, you talked about biosynthesis of fatty acids. So fatty acids are biosynthesized in the cytosol. But I just told you fatty acids are degraded in the mitochondria. What are they degraded to? Degraded to acetyl-CoA. Can acetyl-CoA get from the mitochondria to the cytosol? Nobody knows?

Let's get some energy, you guys. What do we know about acetyl-CoA?

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: It's what?

AUDIENCE: A transport system [INAUDIBLE]--

JOANNE STUBBE: So you think is the transport system that takes it from the mitochondria to the cytosol. So that's wrong. And in fact, this is again another thing that I think maybe isn't emphasized enough in an introductory course. A lot of these things cannot transfer across these membranes. So-- and this may or may not be logical to you, when you take this and you're saying, oh my god, this is so complicated. It's really not that complicated when you put all primary metabolism into the big picture.

Acetyl-CoA goes into the TCA cycle, and it condenses with oxaloacetic acid to form citric acid. We're going to see citric acid again with iron homeostasis. Anyhow, it's citrate that is able to go across the mitochondrial membrane, as is malate, as is pyruvate. Acetyl-CoA is not able to do that. And so to get acetyl-CoA, then you have to enzymatically break down citrate. So if you don't know what citrate is, it's a central metabolite. You're going to see it again and again. Pull it up, Google it. Put it in your brain. You form acetyl-CoA.

An acetyl-CoA, as you learned in the first part of course, can form fatty acids. Where do fatty acids go? They can attach to glycerol. And where does glycerol come from? It comes from breakdown of sugars through the glycolosis pathway. And they come together to form phospholipids, which make up all our membranes. Pretty important. What else can fatty acids do?

They can interact with glycerol without a phosphate, to triacylglycerols. Triacyl-- esterified triacylgycerols. Does everybody know what glycerol is? Everybody know? OK, so-- and this is

another thing we're going to find. We have huge amounts of phospholipids and triacylglycerols in our diet. So we have to deal with those things. But acetyl-CoA, we'll also see, is the building block to form mevalonic acid through a pathway we're going to describe now.

So mevalonic acid is a key player, and its formation is rate limiting in cholesterol biosynthesis. The enzyme that makes mevaloinc acid is located in this little messy thing here, and that's the ER. So it's bound to the ER. It makes cholesterol. And then ultimately, how does cholesterol move-- and a lot of the precursors to cholesterol stay solubilized, and then get distributed to all the membranes. 50% of the cholesterol, for example, is found in the plasma membrane.

So that's the big picture. And so when you get confused about where we're going, you need to go back and see how central a player acetyl-CoA is to everything. And so its regulation, you're going to see, is governed by the same transcription factors that regulate cholesterol homeostasis. Because they were all linked. So where are we going? Let's see if I can remember where we're going. So where are we going?

So I'm giving you an overview of where we're going. And I'm only-- this is a long pathway to get to lanosterol. I'm not going to look at all the steps in the pathway. I'm just going to tell you how we use these C5 units to generate terpenes. So we've got to get to the C5 units over here. We've got to get to these two intermediates. And then we're going to use them to get eventually to a C30. And we'll see the same chemistry, once we know a few rules, just like with aldol reactions and claison reactions, are used over and over and over again.

There are a few basic rules. Every protein is different, but I'm going to make sweeping generalizations, which is a good place to start. So we have to use three molecules of acetyl-CoA. So you're all-- you all should be very familiar with acetyl-CoA. And we're after trying to form C5. So three of these gives us C6, so we have to get rid of a carbon. So from this we need to lose whatever the pathway is. It turns out we lose one carbon as CO_2.

And this whole process-- so this can be multiple steps-- is called initiation. And it forms IPP, which can isomerize to form DMAPP. And then, again, this is our C5 unit that we're after. So this is C5. And we've lost one carbon as CO_2. So then we're going to have what I'm going to call elongation. And what we're going to see is that to get to lanosterol, all we need to have a C30. So we need six of these guys.

So we have six C5 to form a C30. Which is lanosterol. This is a precursor to steroids. [INAUDIBLE] talk about cholesterol-- uh oh. I knew that that was going to happen. I jump

around. Usually this falls off, so you'll have to get used to this. It's a good thing-- I spent all morning trying to figure out where the-- where this cord came, because I knew my cord wasn't going to fit, and the cord was going to be shot, and then I was thinking, how am I going to run from one door to the next? I need to get this back on me. Can you hear me? OK.

Let me get back on gear. So C30-- but then what's going to happen-- so we get to lanosterol, and then from lanosterol, and going to lanosterol here, we're going to have to do, like, I think, the most amazing chemistry in the whole world. We're going to have to do an oxidation and a cyclization. So this is going to be a terpene. So we're going to put these things together to form a C30, a linear C30, and then they have to come together to form this guy.

So we're going to talk about this reaction, because it's such a cool reaction. Anyhow, we're going to talk about how this linear molecule gets to this. That's-- that is the coolest reaction, in my opinion, in biology. I remember when I first heard about this when I was in graduate school in 1968. A long time ago. That's what made me decide I didn't want to be an organic chemist. I said, how amazing is this? That you can do one step and you can put in all of these asymmetric centers and 100% yield.

So that was it. That was a turning point in my life. Anyhow, hopefully it'll be a turning point in your life too. So we have C30. And then we're not there yet. So this is going to be, for us, the-- after the elongation, these cyclizations and oxidation is going to be the termination to get to this ring structure. But then to get to cholesterol, we have 19 more steps. So this is really complicated pathway. And I'll tell you what had the chemistry actually is not so hard to understand, but the details are really still not understood. Because all of the proteins are membrane bound.

So what I want to do now is come back over here. And we're going to talk about initiation, elongation, and the termination steps. And I'm going to focus on a few of the reactions that I think are important, and a lot of the details are written down-- are written down on the PowerPoint. So let's look at the first few steps. And so let's start the pathway.

And the first molecule we're dealing with is acetyl-CoA. And what is special about acetyl-CoA. Why is nature-- you've just had a whole bunch of units on acetyl-CoA-- why does nature use thioesters? What are the two key things you need to think about in terms of its reactivity? You guys should be experts on this now. Yeah?

AUDIENCE: There's a low pKa [INAUDIBLE].

JOANNE STUBBE: Right, so you have a reduced pKa, is reduced from, say, 22 to 18. So this is the alpha hydrogen acidity. And what else does a thioester do? What is the other reactive part of CoA? This should be like the back of your hand. I mean, this is part-- this is central to everything in biochemistry. Yeah.

AUDIENCE: [INAUDIBLE]

JOANNE STUBBE: Pardon me?

AUDIENCE: The leaving group.

JOANNE STUBBE: The leaving group. You are going to have a leaving group. But that's-- and that is important, but that's not the key important thing. That is a part of the game. It can drive the reaction to the right, if you look at the free energy of hydrolysis. What else is activated when you have a sulfur ester as opposed to an oxygen ester?

AUDIENCE: Carbonyl.

JOANNE STUBBE: The carbonyl, because of the decreased resonance stabilization. So what you've done then is you have activation for nucleophilic attack. And you see this-- nature uses this in cholesterol homeostasis as well, over and over and over again. So in the first step, and I'm not going to draw out the details, what you can see here is that you're taking two molecules of acetyl-CoA and you're forming acetoacetyl-CoA-- that should be good practice for you for thinking about the exam on Wednesday.

And this is an example of a claison reaction, one of the three types of mechanisms, to form carbon carbon bonds. The next step, we need three acetyl-CoA's to get eventually to isopentenyl pyrophosphate and dimethylallyl pyrophosphate. So we're going to use another molecule on acetyl-CoA to form hydroxymethylglutaryl-CoA. So we need to add another one of these guys. And this is HMG-CoA synthase.

So before I go there, let's go through what we know. So this is-- so we're starting here. So here-- acetyl-CoA plays a central role. Why thioesters? It's important in claison reactions. Here's the example of a claison reaction involving a carbanion intermediate that you guys should all be experts at at this stage. What about this step? The next step? Formation of hydroxymethylglutaryl-CoA?

So here it turns out that this enzyme uses covalent catalysis. Frequently enzymes-- you've already seen this as well, we're going to see this again and again over the course of the rest of the semester. One of the major mechanisms of rate acceleration is covalent catalysis. Here the thioester-- the CoA ester has been removed and it's attached to a cysteine in the active site of the enzyme. And then this can react with, in this case, a ketone like molecule in an aldol reaction.

So here's the second example. We take acetoacetyl-CoA. We add another CoA. And what we form, then, is hydroxymethylglutaryl-CoA. And what we're going to see is, during this reaction, we also have to do a hydrolosis reaction. Because we start out with acetyl-CoA and we only add-- end up with a single thioester. And this reaction forms hydroxymethylglutaryl-CoA.

So we've lost-- in this reaction over here, you can see where this is lost. So you have an acetyl-CoA to form the thioester in the active site. You you've lost a CoA. Is everybody with me? So you're using the third molecule of acetyl-CoA. You've already lost the CoA. And then what you end up with in the end is you have to hydrolize this off. So if you didn't realize it went through a covalent intermediate, it would be like you just lost a CoA, which you did. But you lost it in this step, because you went through a covalent intermediate.

And you're not responsible for the details. Many, many enzymes that use acetyl-CoA go through covalent intermediates just like this. But you have to study each one to figure out why they do that. Why do they do that? Because covalent catalysis gives us rate accelerations of 10 to the 4th, 10 to the 5th. So nature has used that as a repertoire of defining how to catalyze reactions at amazing rates.

So now we're at this stage. And the next step in this pathway-- so we're still trying to get to the C5 over here. And to get to this now, we're going to have to do a reduction. And we're trying to get to-- so we did this reaction here. I will fix my thing for next-- OK. So now what we're doing is we're going from hydroxymethylglutaryl-CoA to mevalonic acid. And this is wrong. They should all be NADPHs. When you're doing biosynthesis, what do you use? You don't use any NADH. You use NADPH in almost all biosynthetic pathways.

So what happens? You're reducing, basically, the thioester down to-- a thioester down to an alcohol. Everybody should know at this stage how this kind of reaction goes. Everybody, this is one of the two major redox co-factors and all of biology. Can somebody tell me how this redox reaction goes? This is one of the vitamins on your bottle, niacin, which gets metabolized into

NAD, NADP. How does that do a reduction? Can somebody tell me? Yeah.

AUDIENCE: Form an aromatic ring by eliminating the hydride-- so the hydride attacks the--

JOANNE STUBBE: Right, so that's it. And where does the hydride attack?

AUDIENCE: The carbonyl.

JOANNE STUBBE: What part of the carbonyl?

AUDIENCE: The carbon.

JOANNE STUBBE: Yeah, OK. So this is something that I fight with kids all the time in 5.07. It doesn't attack the oxygen. It attacks the carbonyl because it's polarized delta plus delta minus. So you have-- this, again, of all the vitamins on your vitamin bottle, this is the simplest one. So hydrogen moves with a pair of electrons that's called the hydride, to do this reduction. And over here, I think I have the details written out.

So I'm not going to write this out in more detail. But you generate this intermediate-- this intermediate-- this intermediate may-- or the oxygen may or may not be protonated. You need to look in the active site of the enzyme. But then what happens to this intermediate? This intermediate-- so tetrahedral intermediate's not very stable. It can break down to liberate CoA. And what are you left with? You're left with an aldehyde.

So that's one reduction. And where do we want to go? Where we want to go-- and so I'm not going to draw the whole thing out, but I'll draw a part of this out-- so this gives us, then, through a tetrahedral intermediate, an aldehyde. And then what can happen to the aldehyde? We use another molecule of NADPH? And what happens with that? The same thing.

You now do a hydride transfer. And so we need another molecule of NADPH to form the alcohol. So this is a mevalonic acid. So this is written out in more detail here, for those of you who have trouble trouble thinking about this. But of all the factors that nature has evolved to help us expand the repertoire of reactions in biology, NADPH-- NADH is the simplest. Hydride-- it's always hydride. Flavins, much more complicated. We'll see some of those-- the chemistry is much more complicated. This is really straightforward.

So what do we know about this? Why are people interested in this? And this enzyme is called HMG-CoA reductase. And in your handouts, it's abbreviated-- I think these things are terrible. I

will give you a list with all the acronyms on them. I can't remember the acronyms. And people change them. And people name things-- enzyme names are extremely difficult. The older they are, the worse the issue is. Because do you know what NAD used to be called? Any of you have a memory of that? Any of you read the old literature?

It used to be called DPN-- dipyridine nucleotide. So this is pyridine, and that's where they got the name from. And I used to teach with somebody, [INAUDIBLE] a long time ago, and everything was DPN. So anyhow, if you've read the literature, nothing will be called in NAD, NADH. And in fact, a lot of the seminal experiments that elucidate the pathways came out of the old literature.

So why is this protein interesting? So we're going to spend a little bit of time on this protein. People have spent a huge amount of time looking at this protein in detail. Does anybody know why?

AUDIENCE: [INAUDIBLE] people target it-- like statins target it, or cholesterol.

JOANNE STUBBE: So the key thing in this system is it's the rate limiting step in cholesterol biosynthesis. And it's the target of, I would say, a wonder drug-- the wonder drugs of the statins. So people really care about the detailed mechanism. We don't care about the detailed mechanism. We do care that hydride attacks the carbonyl, and it attacks the carbon and not the oxygen.

But the details, if you're interested in that, you can go read about this in the reference. A lot of people have focused a lot of energy on this, trying to make better statin inhibitors. So what do we know about this? And there's a few things I want to say about this. And so if we look at the protein, we're going to come back to this in lecture 3. So this is important to remember.

So this is the protein. I'm going to use this cartoon. And Liz used these cartoons as well. But what we're going to see is the protein has eight of these things-- eight. Each one of these things-- OK, [INAUDIBLE], and we need three more. I'm not going to fit this. So it has a transmembrane helices. And the protein itself is, again, 888 amino acids. And what's interesting about this, if you have this many transmembrane helices, where's the protein going to be located? It's going to be located in a membrane.

So these five are called the sterile sensor domain. HMG-CoA reductase is going to be a key player in regulation of cholesterol levels. And it exists-- it's found, this protein is found in the ER membrane. And SSD is the sterile sensor domain. And we're to come back to this when we

start talking about homeostasis. We're going to see that there are other proteins that also have transmembrane helices that somehow bind and sense cholesterol that are going to help us control cholesterol levels.

Now, what's really interesting about this-- so the protein is huge. It's stuck in membrane. What's really interesting is that you can cut the protein in half, about in half. That's what you're looking at there. You have a soluble protein, they're much easier to crystallize than membrane proteins. And it turns out, if you cut the protein in half, this guy, if you cut, is active. And it's soluble. And the activity is the same as the protein bound to the membrane. So it has very high activity. So it's like you have two separate domains.

Furthermore, if you cut this in half, you can still target this to the ER membrane, and you can still sense cholesterol. So somehow these two things have come together. They have two really independent activities. But we're going to see, they work together to control cholesterol levels. So what I want to do-- how am I doing? Oh, see, time goes by too fast. Isn't time going by too fast for you?

Anyhow, I want to show you-- and we'll come back to it next time-- is that the statins are the target of HMG-CoA reductase. I mean, this is like an amazing thing. Cholesterol biosynthesis was only elucidated in 1955. And it turns out this guy, Endo, was the first one to discover a natural product that somehow could lower cholesterol in 1976. And actually, when I was a young person, Al Alberts used to work at Merck. I used to consult for Merck back in those days.

It was incredibly exciting times, because he discovered really sort of the first real statin that worked, that wasn't toxic-- lovastatin. And really, within a period of only seven years, this was approved by the FDA. So that's an amazing observation. People are still gobbling down statins everywhere. There are issues with them, but it makes $30 billion for the companies that own this.

So you now might have heard of Lipitor or Crestor-- anyhow, they are there. And it really is a wonder drug. And it works, we're going to see next time. Because it looks like the substrate hydroxymethylglutaryl-CoA-- So that it acts as a competitive inhibitor for binding to the active site of HMG-CoA reductase, and prevents the reduction process. And we'll come back next time and look at a little bit at the details. We're not going to spend a lot of time looking at the details, but then finish on to get to IPP and dimethyl APP, the building blocks we're after to

make all terpenes. OK.