JOANNE STUBBE: So we're talking about cholesterol homeostasis. And I said at the very beginning, the first two lectures are going to be focused on the terpenome and how you make cholesterol. And in the beginning in Monday's lecture, we had gotten through the first few steps in cholesterol biosynthesis, starting with acetyl CoA.

And we'd gotten up to the position where we had condensed three molecules of acetyl CoA using Claisen and aldol reactions to form this molecule, hydroxymethyl glutaryl CoA. And at the end of the last lecture, we were talking about HMG CoA reductase, which is abbreviated in the Brown and Goldstein papers like that, which requires two molecules.

This should be "NADPH." I announced that before all of the notes. "NADH" should be replaced with "NADPH."

We're doing biosynthesis. We talked about the mechanism of how you go from this CoA analog to a double reduction to form an alcohol. And the question is, why is that interesting and important? And it's interesting and important because it's the major target of $30 billion drugs, the statins, which specifically target HMG CoA reductase.

And so what I want to do-- I'm not going to spend a lot of time on this, but I want to say a little bit about this, given its central role in many people's health nowadays. So how do these analogs end up working?

And so we have an intermediate in this process, which I drew out in detail last time. I'm not going to draw it out again. So we're going to go through two reductions.

The first reduction forms a thiohemiacetal, which then kicks out CoA to form the aldehyde, which then gets reduced again. I'm not going to write that out. This is the first intermediate you see. And what we'll see is the inhibitors all look like this intermediate.

So when they look like the normal substrate or an intermediate along the pathway, those are called "competitive inhibitors." So these are competitive inhibitors. If you don't remember what

a competitive inhibitor is or how it's described, you might want to go back and look it up in Voet or whatever your basic biochemistry textbook is.

And so what we'll see is all of the compounds that are actually used clinically look like this. And so if you look at this, they're not exactly the same. But it's proposed to be a model of this particular intermediate in the reaction. So this is the thiohemiacetal.

But here, I've drawn a lactone rather than an acid. And in general-- whoops. In general, lactones are actually given as the drug, rather than the acid.

And does anybody have any clue as to why that might be true? Why would you want to use this molecule, as opposed to the ring-opened species, which would look like this?

AUDIENCE: Uptake.

JOANNE STUBBE: Pardon me?

AUDIENCE: I said, uptake issues.

JOANNE STUBBE: Yeah. So it's uptake issues. So what happens is you give this-- and you'll see this is true also in the last section in purines and pyrimidines. How you deliver the drug, it needs to be able to get across the membrane in an efficient fashion. And so they give them lactone, but the lactone rapidly ring-opens inside the cell.

So that's the analog. I don't know if you can see it, but it's the same analog I've drawn up there and I drew last time on the board. And then these are the drugs.

And so the drug-- I've written this part looks like this part. So this is where the competitive part comes from. And what's down here? Down here is, remember, the CoA. And down here in the drugs is stuff.

And the key to this stuff is hydrophobicity. And so the key to almost all drugs is hydrophobicity. So it can't be so hydrophobic that it's insoluble. But lots of times, you have pockets that you can't see in protein structures. It inserts itself in, and you get a lot of binding energy.

So if you look at these, these, again, are on the PowerPoint presentation. What you see here, these are all the ring-opened here and ring-closed. And then if you look down here, what you see is all the stuff. And you see the stuff is dramatically different. You can look at it, but each

company has tried to get its cut of the $30 billion market.

And what I'm going to show you on the next slide is this molecule. So again, the key thing is what's common to all of these inhibitors is this moiety. And when you look at the structures, you can see this guy. And then this guy will have slightly different orientations within the structures.

So if you look at the structures of HMG CoA reductase, what you can see-- and if you pull this up and stare at it, it looks like a mess. But it really isn't a mess.

If you look at this, you're going to see in all the structures, here's the carboxylate. Here's a carboxylate. And this is the hydroxyl group. So that's the key part over here, the carboxylate in the hydroxyl group.

There in the hemiacetal, the carboxylate's not there yet. So you can't put in the active species, or they turn over. So what you're doing is throwing in components to prevent turnover but to give you a feeling for where the different substrates bind.

CoA is not attached to this moiety, but CoA is attached here. And I think what's unusual about this-- I don't know if you looked at any structures in the polyketide synthases. But you would think at the end of CoA, if you look at the structure, it has an adenine moiety on it.

And you would think you would get a lot of binding energy onto this hydrophobic adenine moiety, which can also hydrogen-bond. And almost all structures to that part of the molecule never bind to the protein. It's stuck out in solution. So this is sort of typical.

Why nature designed things this way, I don't know. She's got this huge-- she could have used a thiomethyl group, and the chemistry would have been exactly the same. But she uses this huge CoA moiety. And in most cases, you see the chain extended and the adenine on the outside.

So if you look at that, the chemistry is going to come over here. And if you look at this yellow, yellow is sulfur. So that's where the sulfur would be connected to the hydroxymethyl glutarate.

And then what do you see over here? Hopefully, you can see this. But this is another adenine ring. And then over here is the pyridine ring. And we went through the mechanism last time that transfers the hydride. So this green part is the redundant, and this part is a mimic of the hydroxymethyl glutaryl CoA.

And in all cases-- we have hundreds of structures now-- what you will see if you look at the inhibitor binding is you have changes in conformation in this region. And the Km for binding of hydroxymethyl glutaryl CoA is 4 micromolar. But the KD for binding of the inhibitors is about a nanomolar. So you're gaining a lot by sticking this hydrophobic mess on.

And what happens-- so here's, again, this hydrophobic mess. And what you can see, this is one of the analogs I had before. Again, hydroxymethyl glutarate-- the glutarate and the two carboxylates are there. And then they stick on something hydrophobic in this part of the molecule.

And if you look at the conformation of these helices in this region-- and you really have to look at the three-dimensional structure to see something-- that's the region where you see changes in binding. So it's an induced fit mechanism of binding. And in fact, this induced fit occurs in all of the analogs that are looked at.

And so if you look here-- and you can look at this. I would say take it home and spend some time looking at it. Again, this part of the molecule in all of these analogs is exactly the same. And what you had different is this hydrophobic mess in this part of the molecule and changes in this region of binding.

And people are still working on it, trying to make-- usually, it's the first couple of drugs that make all the money. And if you're third or fourth, you don't make enough money. But there are lots of problems that keep coming up. And so people are still really heavily focused on trying to lower cholesterol levels.

So HMG CoA reductase-- I told you last time, it's a huge protein, 880 amino acids. Half of it's stuck in the ER. You can cut off the-- the other half that is soluble is in the cytosol.

And we're going to come back to this because this rate-limiting step plays a key role in sensing of cholesterol levels. So the end of lecture three and into lecture four, we're going to come back to HMG CoA reductase because of its central role in cholesterol homeostasis.

So we're not here yet. Remember, the goal of the terpenome was to get to the building blocks. We still haven't gotten to the building blocks yet.

What were the building blocks? Isopentenyl and dimethylallyl pyrophosphate. Remember, the common building block is an isoprene, but the isoprene is not the reactive species we needed

to get it into some form where you can actually do chemistry on it. So our goal has been to get to IPP, and we are here.

So the rest of the biosynthetic pathway to get to IPP is pretty straightforward. I'm not going to draw out the details at all. But we go from mevalonic acid. And so we use ATP and a kinase. And we use a second ATP and a kinase. And then we use a third ATP.

And so if you look at the pathway here, what happens is you're phosphorylating the alcohol that we just created with HMG CoA reductase. So we're sticking a phosphate on. Another ATP sticks a second phosphate on. And in the end, we need to get to isopentenyl pyrophosphate.

And so we have a third enzyme that is going to phosphorylate to facilitate, finally, conversion of the C6, three acetyl CoAs, into the C5 isopentenyl pyrophosphate. So if you look at what's going on in that reaction, we have a third ATP. And the ATP is used to phosphorylate the alcohol. So what we're doing basically is making it into a good leaving group.

And we've got the two phosphates on there by the first two kinases. And so now what we want to do-- I forgot a methyl group here. And so now what we've done by phosphorylating this is we've activated this for a decarboxylative elimination reaction.

And so now, where are we? We're now finally at isopentenyl pyrophosphate. So we've gotten to our C5. And the key thing, remember, is we started with acetyl CoA. During this reaction, we lose CO2. And that's why we've gone from a C6 to a C5 during this reaction. We lose that.

And again, we see ATP used over and over again. And GTP, you see the same thing. It's used to make things into better leaving groups and facilitate the overall chemistry. So I'm not going to talk again about the details of any of these steps. The steps are all straightforward.

You've seen these steps in primary metabolism with the role of ATP over and over again. But the key thing we want to talk about is the terpenome. And to get to the terpenome, we needed to get to isopentenyl pyrophosphate and dimethylallyl pyrophosphate so that we can look at the new way of forming carbon-carbon bonds with C5 units.

So we've gotten through the first few steps. That's what I call the "initiation process." We started with acetyl CoA and got to IPP, dimethylallyl pyrophosphate. If you look at these hydrogens, hopefully, you know allylic hydrogens are moderately acidic. And there's an isomerase that can convert this molecule into this molecule.

And so this is dimethylallyl pyrophosphate. And we're into the second part of the biosynthetic pathway for cholesterol. So we're through the initiation. We've got our building blocks.

Now, what we want to do is do the elongation step. And so now we're going to use this. So IPP-- we're now going to look at the elongation reactions. And I guess I'll use a second board over here. And so let me do it over here, and then I'll do the next one.

And so what we want to do then is take C5 plus C5. And we're going to look at this reaction in detail. And the enzyme that's going to do this is called FPP synthase, farnesyl pyrophosphate synthase. I'll write that down in a minute. And you form C10.

And C10-- then this is the same enzyme. So it's also FPP synthase. FPP is the product, farnesyl pyrophosphate. And IPP gives us C15. So this is a major elongation reaction.

And what I want you to see is that this C15, three C5s stuck together, is linear. And if you go back and you look at your notes from last time, we talked about isopernoids and terpenoids. And you can make linear molecules that can go from a couple of units, like geranyl, geranyl, C10-- sorry-- geranyl pyrophosphate.

That's C10. That's called a "monoterpene." And you can add another C5, isopentenyl pyrophosphate. That's a C15. That's called a "sesquiterpene." "Sesqui" comes from 1 and 1/2.

So what you'll see in the next thing, if you add another five, you have a C20. That's a diterpene. And if you go to a C30, that's a triterpene. You can google it, but the nomenclature's complicated. But that's where they come from is the different C5 units.

So really, this chemistry is the basis for all the reactions in the terpenome. So what we're going to do is go through that chemistry. How do you form a new carbon-carbon bond using these building blocks? What are the general principles?

And I showed you the hundreds of different kinds of natural products that you can find in humans and plants and bacteria all over the place. They play an incredibly important role in primary and secondary metabolism. And what we're going to look at is the general way that these carbon-carbon bonds are made.

All right. So again, let me stress that this is linear. And you'll see that when we actually look at this. So FPP-- let me write this down. So it's farnesyl PP, pyrophosphate. We talked about this last time. It's a central player in many, many, many reactions, and it's a C15.

So the farnesyl pyrophosphate synthase was the first enzyme characterized for parental transfer reactions, for these C5-forming reactions. It's been studied extremely extensively by Dale Poulter's lab at Utah, and it's served as a paradigm, really, for thinking about all of the biochemistry.

Did any of you guys ever hear of Saul Winstein? No. It shows how old I am. Anyhow, Saul Winstein was a faculty member at UCLA many years ago, probably in the 1970s.

But if you've taken 5.43, hopefully, they still talk about-- or what's the advanced physical organic chemistry course you guys take? Any of you'd had that? Anyhow, you've had-- have you heard about Saul? You've never heard of Saul-- bad, bad.

Anyhow, he's the one that figured out how to think about classical and non-classical carbocations. And Dale Poulter worked from him. Dale Poulter moved into enzymatic reaction systems and really sort of unravelled how these things work. And the paradigm I'm going to give you-- every enzyme's different. But the paradigm I'm going to give you I really think came partially founded on the physical organic chemistry and from Dale's lab.

So these are pretty important contributions. And what we'll see is this is called a "type I synthase." And if you read the assigned reading, you'll see there are type II synthases. So there's more than one structure of the enzymes involved in these systems. We're going to specifically focus in class on the type I synthase. And it's basically an alpha helical bundle.

And I think on the next-- if I could remember what I have. Yeah. So this was taken out of the article you are supposed to read. And so this is FPP synthase.

This is a monomer, but it's a dimer. And all I want you to see if you take the 30,000-foot view, there are five helices here. They're in red. If you look at this long helix, it's everywhere. Everything's a little bit juggled around.

You can see you have a couple blues here and a couple of blues here. So they're structurally homologous to each other. And I think what's most remarkable about this-- so FPP synthase takes two C5s, makes a C10-- this is a C15. So it's a linear.

Squalene synthase, which we'll look at in a minute, takes two C15s and makes a C30, the precursor to making the ring structure for cholesterol. But these two guys in the middle, which look-- and this is linear, as well. These two guys in the middle, which look remarkably similar--

actually, structurally, if you superimpose the structures, they're really similar-- form cyclic terpenes.

So they form cyclic sesquiterpenes. I'll show you this in a minute. And they use FPP. So here, all of these enzymes use FPP. And they all look alike sort of from the 30,000-foot point of view.

And the question is then, how do you control what the chemistry is in the active site? So they have homologous structure. So these are all structurally homologous.

Another thing that you need to remember about these systems is that they have similar metal binding motifs. Now, if you look at the reaction of IPP, if you think about this, this isn't what PP looks like.

Does everybody know what PP looks like? Hopefully, you've seen this over and over again. What would the metal be or metals?

AUDIENCE: Magnesium.

JOANNE STUBBE: Yeah. So whenever you have pyrophosphates or ATPs or GTPs, you always have magnesium. Magnesium plays a central role in everything in biology. And we can never look at magnesium because the ligands are fast-changing. It moves around all over the place.

So it's hard to freeze out and understand the function of magnesium. But it turns out most of these proteins require three magnesiums. And as with many metal-based reactions, if you line things up, you really don't find that much sequence homology. But if you know where to look, you find sequence homology around where the metals bind.

So what you see in the case of the linear farnesyl pyrophosphate, you see a DDXXD motif. And you find that in almost all of these enzymes. And if you go to the terpenoids-- so the non-linear ones-- you see a D.

Again, I don't expect you to remember something like this. But I do expect you to remember that these metal motifs, once you know how to think about something, are actually very helpful in trying to define the function of an unknown open reading frame, if you know how to look.

And more than 50% of all annotated genes code for proteins we have no idea what they do. So looking at these kinds of motifs can actually be quite informative. So then what you need to do to really understand what they're doing is dive in and look at where the metals bind, if

you're lucky enough to be able to get a structure with the metals bound.

So we have alpha helical motifs and metal binding motifs. And then the other kind of motif that I think is really interesting for the linear system-- so that's farnesyl pyrophosphate-- is how you control chain length.

So FPP synthase-- that's what we're talking about-- is a dimer. And the metal binding motifs sit up there. So the metal binding motifs sit in the top, the way I've drawn this here. So you have metals. There are thought to be three metals.

And then we're building C5, C5, C5. Where does the chain go? And what you'll see is there is a cone shape which migrates towards the bottom of the structure. I'll show you a picture of this in a minute.

And so then the question is, what controls chain length? So if you end up looking at the structure, what you see is a phenylalanine. And the phenylalanine is a molecular doorstop. So the chain is extending, because we're going from C5 to C10 to C15.

Why don't we go to C50? And I showed you in the first lecture dolichol and lipid II have C20s, C55s. How do you control the chain length?

So that's an interesting question in polymer biochemistry. Here, we control it by a molecular doorstop. So if I replace the phenylalanine with an alanine, what might happen? Go.

AUDIENCE: You'd have longer.

JOANNE STUBBE: Yeah. So they made up to-- I can't remember. I haven't read the paper in a long time. But they can make C50-mers. So they can actually see, and they're not uniform.

So that's a key thing. You want them to be uniform. So if you look-- I think in the next has a picture of this. Again, this is graphics from really quite some time ago now, 1996. So the picture's not very good.

But you can see sort of the tunnel, and the metal binding sites are actually up there. And if you look at the structure, you can see the phenylalanine. So that's what we know sort of about the type I synthases. They're involved in making the C15 farnesyl pyrophosphate.

So now we want to look at the chemistry, what's going on in the chemistry. And can we make a generalization about how this chemistry is used to put all C5 units together? So that's what I

want to focus on next. Whoops.

So what I'm going to now look at are the proposed mechanisms. And I'm not really going to go into much detail. I'm going to give you a generic overview of the things you need to remember if you encounter something like this. The first guess would be a mechanism similar to the one I'm proposing now, but then you have to look at it in more detail to figure out what's really going on.

So what do we have? We have dimethylallyl pyrophosphate. And I have a cartoon for you to look at there, but I'm going to draw it differently than this cartoon. But you can just watch me because, again, the key thing is thinking about how you form the carbon-carbon bond and what's going on in these reactions.

So we just looked at the pyrophosphate. And if you look over there, what do you need? You need to have a bunch of metals bound.

And recently-- this is a fairly old paper. They have better papers. I think I took all the pictures out, because it's hard to see things without looking at it in detail. But in fact, the magnesiums are interacting with the pyrophosphate and adjacent to the pyrophosphate.

And it's clear they play a key role in catalysis. But whether they move during the transformation, again, I think we just don't know that much at this stage. It's hard to trap it in an informative state, like it is with all crystallography.

So here's dimethylallyl pyrophosphate. Here's isopentenyl pyrophosphate, the two guys we were after. And the first step in all of these reactions is ionization.

So this is an unusual reaction in biochemistry. There are almost no examples of carbocation in biological transformations. This is one of the few places where you see this. So this is the ionization step. And all of the reactions we are going to be looking at involve ionization, but other kinds of chemistry can also happen that we're not going to discuss.

So what have we generated? We generated an allylic cation. And what we also have is we lost pyrophosphate. And I'm being sloppy. I'm not drawing out how these are interacting with metals, but the charges are pretty much neutralized in some form that we don't know the details of.

So you can't forget about the charges. And so we can just put down magnesium 3+. And then

what we want to do, we want to make a carbon-carbon bond.

Whoops. Let me get this right. If I make a mistake on the board-- like sometimes, I always get mixed up with four or five carbons-- raise your hand and say, you've got the wrong number of carbons. You tell me. You be the cops.

So what we're going to do now is we're ready to form a carbon-carbon bond. And we're going to be forming a new carbocation. Hopefully, you remember from introductory chemistry that carbocations that are tertiary are more stable. And when you look at terpene types of chemistry, you see tertiary carbocations used over and over and over again.

That being said, I'm putting brackets around this because despite the fact that I draw this intermediate, no one's ever seen it in the enzymatic reaction using the normal substrates. So you have to play games to study mechanism, just like you have to do in organic chemistry.

So what happens now is you're set up to form the carbon-carbon bond, which has been the goal of what we've been trying to do in the first couple of lectures. And so what do you generate? You generate the new carbon-carbon bond, which is the skeleton for geranyl pyrophosphate.

You generated a new carbocation, and it's a tertiary carbocation. And our pyrophosphate is still sitting in the active site.

And so now what we're ready to do is we're going to form our C10, geranyl pyrophosphate. And we'll see one of the types of reactions that you see over and over again when you make carbon-carbon bonds is loss of a proton. And that gives you the C10, which is these two things stuck together, which is a monoterpene, which is called "geranyl pyrophosphate."

So what's interesting about this-- and I think this is sort of something that's pretty general-- is the pyrophosphate in the active site. If you look in the active sites, they're amazingly hydrophobic. And the pyrophosphate in some way stereospecifically-- I haven't drawn the stereochemistry here-- removes the HR proton to generate the olefin.

So what you've now generated is geranyl pyrophosphate.

So here's C10, and this is geranyl pyrophosphate. Let me also put brackets around this intermediate.

Again, we haven't seen this intermediate. And how do we know this is true? Because we know a lot from Winstein and Brown about carbocation chemistry. And people have been really creative in figuring out how to show that this model is in fact correct.

Hopefully, I have C10 there. And so this is an intermediate because we're still going to go on. The enzyme doesn't stop at C10. It adds another isopentenyl pyrophosphate.

So if you want to think about how nature might design that, if you look at this molecule and you look at this part of the molecule and replace it with an R group-- so we have an R here. What does this look like? It looks just like dimethylallyl pyrophosphate.

But we need to put the R group somewhere. So in the case of FPP synthase, we're going down the tunnel. So we're getting it out of the way. But we're going to do the same chemistry that we just did over again, and we just replaced a methyl with an R group.

So that's the basic chemistry. It's pretty straightforward, the only chemistry that I'm aware of in biological systems that involves carbocations. These are special carbocations. That is, they're, in general, stabilized. They're allylic. Or in many cases, they can be tertiary.

So let's emphasize that. Again, if you don't remember your organic chemistry, you should go back and look up the sections on carbocations. So I told you the farnesyl pyrophosphate is sort of central to many things. And farnesyl, in this case-- I'm not going to draw out farnesyl pyrophosphate.

The chemistry's the same. You can repeat it yourself. But here is our farnesyl pyrophosphate, but look what it can form. Remember, you saw all those smells. If you break a pine needle, you have pinene.

What you see is this one intermediate can form all of these compounds. So the question is-- with an enzyme that looks just like farnesyl pyrophosphate in three-dimensional structure. So that's sort of amazing.

And what you're doing here is taking a linear molecule. And in this particular case-- and I'm not going to talk about this slide in detail, but I will talk about one case in detail-- what you're now doing is getting it to do alternative chemistry. So how would you design the active site of your enzyme to end up doing that, to use the same chemistry, ionization?

And then you have to do cyclization and loss of a proton or whatever. How does nature design

all of this? So once we get through this set of lectures, I would suggest this would be something you could go back and practice on. How do we get to all these guys?

I'm going to show you one example of that. I'm not going to go through this slide. It's way too complicated, but I think it shows you sort of the amazing diversity of the terpenome, using farnesyl pyrophosphate. So what I want to do is give you an overview of the rules. And then I'll go through one specific example.

So let's make general mechanistic comments. And in the original, version of the PowerPoint, this slide wasn't in there. Anyhow, the first thing is you've already seen up here, and this is going to be common, is you lose a proton.

So the first step is ionization. So ionization happens in almost all these reactions. There are exceptions to this, but most first steps are ionization.

The second step can involve proton loss. And I'm going to write down what the steps are, and then we'll come back and look at a specific example. And we're going to see this in cholesterol. One can have with carbocations-- if you go back and you think about what you learned if you've had the second semester of organic.

With carbocations, you can do hydride transfers. So that's a hydrogen with a pair of electrons. We can have hydride transfers.

We're also going to see-- and both of these are key in cholesterol biosynthesis. We can have methyl anion transfers. And the other thing is these reactions all go stereospecifically.

And that's one thing. If you become an enzymologist, you realize that's what's cool. That's why you have such big huge enzymes, so they can control the stereochemistry of everything.

So they do everything with 100% EE. And they don't have to worry about it like chemists worry about it, but they pay a price. They have a big huge protein.

The third thing-- and this is going to become important. It was just important in the slide I showed you previously-- we're going to see cyclizations. And cyclizations require, in general, protonation of an olefin-- I'll give you an example of that-- or protonation of an epoxide.

So in some way, you're going to have to do some more chemistry to get your olefin. Everybody know what an epoxide is? So we're converting an olefin into an epoxide. We're

going to protonate it, and then we're going to do cyclizations.

And the third general type of reactions is water addition. So if you have a carbocation sitting around. You add water, bang, you have a reaction and form an alcohol.

So the other generalizations I want to make-- so that's the chemistry. We're going to see this chemistry play out over and over again because I've selected examples of this for you to look at. But it's quite common.

The second thing besides these mechanistic issues is, how do you distinguish between linear versus cyclic? And you've already seen the strategy with farnesyl pyrophosphate. You really sort of have a tiny little cavity where the IPP and the dimethylallyl pyrophosphate bind, and then you have a long tunnel.

What do you have in the case of cyclic terpenes, which you saw in the previous slide to this one? And the key thing is the shape of the active site. And what you will see if you look at a lot of these active sites is, in general, they're very hydrophobic.

Why is that true? So somehow, you've got to take care of the pyrophosphates. But they're very hydrophobic because we're dealing with these hydrocarbons, which are hydrophobic. So the question then is, can you take this farnesyl pyrophosphate and fold it?

And folding it in different ways-- if we go back to the last-- whoops. If we go back to the last slide, if you look at it here, for example, and you ionize here to form a carbocation, you can have a cis or a trans carbocation. And that then can lead to further types of chemistry, where you form different kinds of ring structures.

So it really is all about folding in the active site of the enzyme. So the active site is the key to determine which of these many kinds of things that can happen that if you did this in solution, you might actually get a mixture of all of these kinds of things. So the key then is hydrophobic and the shape of the active site.

And then another key thing is I'm going to show you that in many of these reactions, you go through-- like we saw up there-- these carbocation intermediates. Well, there might be three different carbocation intermediates you could go through. How do you decide?

How do you decide-- how did enzymes evolve to give you specific carbocation intermediates? How might you stabilize a carbocation intermediate? Anybody got any ideas?

What would you expect to find in the active site then? I'm going to show you on the next slide, which is sort of a generic active site of a terpene that can cyclize. Any guesses? How would you stabilize a carbocation?

AUDIENCE: Negative mixtures.

JOANNE STUBBE: Yeah. So one way-- you might have an aspartate. Nature doesn't do that. So that might-- well, the problem is if you do that and you form a covalent bond, that's the end of your reaction. So

So how you do this is I don't think we really totally understand it. But how else could you stabilize it? Anybody else? What did you learn about weak non-covalent interactions in biochemistry that could help us?

Everybody hates waiting on covalent interactions, the key to everything-- key to everything in how enzymes function.

AUDIENCE: You could just have something [INAUDIBLE] in general.

JOANNE STUBBE: But electron-rich-- but that would be doing-- that's what she was suggesting. You have a carboxylate, an aspartate or a glutamate. Then you would form a bond, and then you would be stuck.

So the way nature actually does this is she uses aromatics. And it was discovered maybe about 15 years ago that you can have an aromatic whatever. And you have some kind of a cation. So this is called a "pi cation interaction."

Usually, the pi cation interactions are with metals. But here, we have a carbocation. So the model is that you might find in the active site tryptophans, tyrosines, phenylalanines. And so these become really key.

And in fact, if you look at an active site-- so I don't even remember which enzyme this is. And somebody was trying to study something, and they have a small inhibitor in the active site. But you notice you don't have a long site where this chain can extend.

What you've done is constrained the active site much more, and that shape is going to be key to the many different reactions you could have. And then if you look carefully, you can't really think about this. But you have phenylalanine, tyrosine, tryptophan, and another tyrosine in the active site. And that's what you see in many of these protein structures all over.

Again, we have FPP synthase, which has this thing. And then we have these terpene cyclases, which have this thing. And each one of them is different. And so the difference is related to the shape.

And it's proposed that this stabilizes this interaction. It's been challenging to show this chemically, but these interactions are worth quite a bit. These are also hard to measure, but it's something that was discovered and now has been actually widely observed.

And the other thing I want to mention about these enzymes, which I think is interesting and distinct from other enzymes that you've encountered, is that, in general, they're really not very specific. So if you start looking at these-- look at this. How could you make one cation here versus the three others?

If you start looking at how to get to these cyclized products, you say, how the heck did nature ever do that? There's no way you could guess at what the product would be, in my opinion. So what happens is these enzymes actually when you start looking-- we have good analytical methods-- are really promiscuous.

So they might produce a predominant product, but they always produce a bunch-- 1%, 5%, sometimes even more-- of other products. And I think if you look at the chemistry that we've been talking about, basically, all of this sort of makes sense.

So what I want to do now is give you an example of all of these reactions in one case. And this case, I guess I didn't write down the references. But I took it out of the literature. It's from David Christianson's lab.

So here, we have farnesyl pyrophosphate, and here's the product we want to get to. So you'll have something like this on a problem set that I'm going to ask you, and it will be simple. I won't give you something that's so hard to see.

But for me, lots of times, when you look at these rearrangements, it's easier if you make models. I don't know if anybody ever uses models anymore. I still use models, because you have to bend things in the right way to see what's possible and if the orbital's overlapping in the right way. You've got to really think about the stereochemistry.

So what do we have here? So the first step is ionization. So we would form an allylic cation here.

That's what we just did over here, which I hid. So that's what we just did over here. Oh, we didn't do it over here. Here-- over here, we formed this allylic cation.

And once you do this, then they didn't show you that intermediate. They went on to the next step. So once you generate a cation there, they drew the conformation such that this thing could cyclize.

But when you cyclize, you have electron deficiency at this carbon. So you have a second carbocation. This is not allylic, but it's a tertiary carbocation.

So now the question is, what can happen? And again, you've got to keep your eye on what your goal is way down at the end. And you could probably draw more than one mechanism to get from A to B. And then you have to figure out experiments of how you would test it if you really care about that.

So what happens here? You're losing a proton. And again, the pyrophosphate is acting as a general base catalyst. So that's exactly what happens in the case or what's proposed to happen in the case of farnesyl pyrophosphate. So you generate this species.

Well, this might be stable. You might actually be able to isolate that as an intermediate along the reaction pathway. But we know in the end, we end up with two six-membered rings with this stereochemistry and with methyl groups in certain places. And so then you have to think about how can we get there.

So remember that I told you that terpenoids do cyclizations. And one way they can do it is to protonate the olefin. So here, there might be a group in the active site. Maybe it's the phosphate that would help facilitate. You've just used it as a general base catalyst.

Now, it's got a proton. It could now function as a general acid catalyst. You could protonate this position and now form two six-membered rings and a new carbocation.

So in general, the nomenclature is when you draw these things, if you have a stick like that, that means you've got a methyl group. If you want to put a hydrogen there, you put a hydrogen on it. So if there's nothing there because CH3 takes up more room and they become very complicated to draw, the methyl group has methane.

And the hydrogen, you put on. So you can distinguish one from the other. So now what happens is remember, one of the mechanisms I told you is hydride transfer.

And again, I think looking at the stereochemistry of these systems helps see how this could happen. But these are all stereospecific. So you have hydride transfer from this position to this position.

And when you have hydride, a hydrogen with a pair of electrons, what you have left is a new tertiary carbocation. And this new tertiary carbocation-- let me see what's going on-- is now-- in the end, we get a methyl group here. We have no methyl group there.

Now, we have a CH3- group migrating. And that's the third method that I described. So the CH3- group migrates, giving you a new carbocation. And then the last step is, again, loss of a proton.

So here's an example. This is a complex example, but there are 70,000 of these guys. So these are sort of the general rules. Nature has figured out how to make all these different kinds of natural products.

So what I want to do now-- so those are the general overview of how these systems work. What am I doing? Oh, I'm sorry. I get so lost.

Anyhow, I wanted to get through cholesterol. But next time, we'll come back. And in the very beginning, we're going to see how we take C15s to go to C30s and then how you cyclize this in the most, in my opinion, amazing reaction in biology-- other than ribonucleotide reductase, anyhow.

See you next-- see you Friday.