[SQUEAKING] [RUSTLING]

[CLICKING]

JOHN GRIMES: OK, well, I guess I'll get started, and let people trickle in, if anybody else is coming in. So my name is John Grimes. And I work in the chemistry department's Instrumentation Facility. And down there, we've got a number of different instruments.

We have five mass spec instruments. That is not my specialty. So other than being able to point them out, I can't really tell you that much about them.

I help run NMRs. And so what I do is I teach students how to use the NMRs. I will help them select what experiments they possibly need to use in order to give them the answer that they're looking for. And I'll also help them interpret the data, or at least get them started off on interpreting the data so that they can do that on their own when they're doing their own research.

So what I hope to talk to you about today is what an NMR instrument is and what it actually consists of as far as the parts, what the analytical technique of NMR is, and how we measure the signal, and then go into some examples of how to interpret the data. So here's a picture of one of our instruments there. And here's an example spectrum of adenosine.

So nuclear magnetic resonance is what NMR stands for. And it's the study of molecular structure by measuring the interaction of radio frequency energy with a collection of nuclei that you've taken and you've put into a strong magnetic field. So it's an analytical technique that is based on a nucleus's-- or nuclei-- intrinsic angular momentum. It is a nondestructive analytical technique.

And that's important if you're a graduate student in the chemistry department, wherever, even an undergraduate, and you have worked long and hard to synthesize some natural product that's 15 steps into a synthesis, you've only got a half milligram of that, and it's a year's worth of your life's work, you don't want to destroy that sample analyzing it. So you can take that sample, and you can put it in a small, little, cylindrical glass tube. You can analyze it. And then you can take that sample back out and use it for something else.

So the technique allows you to determine connectivity within a molecule. So I've drawn-- this is something I'll bring up a spectrum of later. It's just three heptanone. But it will allow you to see that protons on this terminal carbon are connected to that and next to a carbon here that has two protons on it.

So it looks through bond connectivity. And it won't necessarily give you connectivity all the way through the molecule, but you can build up, say, this chunk of the molecule and this chunk. And then you can link it together-- picture linking together a chain that allows you to put together the whole molecule.

It will also show you interactions through space. I'm not going to embarrass myself and try and draw a protein. But there can be two parts of a molecule that are hundreds of atoms away from each other if you were to try and go through the chemical bonds. Yet they're held near each other in space. And you can monitor how close they are. And that helps determine the three-dimensional structure of molecules. And so all the time protein structures are solved by NMR.

And you can use it to monitor other processes too, such as whether a protein has bound a small molecule, whether there's hindered rotation about a bond, so something like-- where did my chalk go? If you take something like dimethylformamide, this bond here has partial double bond character. And you can see separate peaks for those methyl groups. And you can rationalize it by the hindered rotation around that bond.

And there even other techniques. And so everybody is going to be familiar with what NMR is. And hopefully none of you have had to have one, but it's the same physical technique as an MRI.

So here's an MRI of, unfortunately, my daughter's head after she swam into the pool end in a swim match. Nothing happened to her, but you can take pictures with NMR. In the past, I've used it to take pictures of insects.

You can also do analytical techniques of in vitro diagnostics. So there's a test out there called the NMR LipoProfile. And it will analyze your cholesterol, i.e. the density-- or the concentration of lipoproteins that's circulating around in your blood.

So what is an NMR instrument? NMR instruments come in two flavors. Or at least-- maybe they come in more flavors, but, here at MIT, we have two types of NMR instruments. There are ones that are referred to as high-resolution instruments, which usually have a stronger magnet, and they're bigger. There are also desk-- they're not desktop, but benchtop instruments, which is what you're going to use in your lab.

So each of these has the exact same constituent parts. I didn't go over and try and take your benchtop instrument apart to get a picture of those parts because I wouldn't have gotten it back together. And it would have never worked. So I'm going to go through one of our instruments and show you the individual parts, but keep in mind it's the exact same thing that's in the instrument that you'll be using.

So you've got to have a strong magnetic field to immerse your sample in. And obviously that's supplied by a strong magnet. So magnets will come in two flavors.

In the benchtop instruments, they are a permanent magnet. Has anybody ever taken apart old computers, and you can get the hard drives, and there are strong magnets in there that are sort of silver colored? Those are made from a neodymium-iron-boron alloy. And that is what they use for the permanent magnets that are in the benchtop systems.

There's been a great improvement in those in the past, I guess, 15 years or so. There used not to be any benchtop instruments. It was difficult to engineer and machine permanent magnets that would give a uniform magnetic field. But they've been able to do that. And so there are a lot of benchtop instruments that are out there now.

The standard NMR-- well, they're termed high-resolution NMRs because they have stronger magnetic fields that then can be generated from permanent magnets-- use what are called superconducting magnets. So the magnetic field is generated by the circulation of electric charge in a superconductor. And, if you're familiar with superconductors, usually, they have to be below some specific temperature in order to maintain their conductivity.

While the temperature, the critical temperature, has come up in recent years for superconductors, as far as producing something that's easily machinable into wire that you can wrap into a coil, the higher temperature-- higher critical temperature superconductors aren't easily malleable. So you still have to use something that you've got to get really cold, i.e. down to liquid helium temperature.

So, in a superconducting magnet, you've got-- think of it as a giant thermos, which is what this can is. You've got a hole through the center, which is called the room temperature bore. It's room temperature because it is not cold.

Your sample, which is in this little tube that I showed you generally, will be held in what's called a spinner, so this little blue thing. You will put it in the top of the magnet. And it will just ride down on a cushion of air somewhere to about there in the center of magnet-- in the center of the magnet.

On the benchtop instrument, what's nice-- let's see if I can back up a slide. All you do-- you can see it, and it'll be obvious when you run it in the lab for yourself. You just put the tube right down in there. You don't have to put it in any specific holder.

So your sample tube goes down that bore. From underneath the instrument comes the NMR probe, which I'll talk about in a second. So that superconducting wire is wound in a coil around that room temperature bore. This chamber here, which, if I could see, is number 6, that is a chamber that is full of liquid helium.

So, when you set up one of these magnets, you cool it off. You fill that with liquid helium. And then you put a charge on this superconducting wire. And the charge is about 100 to 200 amps.

And 200 amps is the amount of charge that goes through a medium-sized house. So it's got a good amount of electricity on there that's circulating around. As long as you keep it cold, meaning as long as you top it off with liquid helium every few months, it's going to remain a magnet and generate a powerful field.

So, if you just had liquid helium touching metal, the outside of that metal would be a big chunk of ice. So this has an evacuated-- not layer, but I guess a portion of it that's evacuated with a high vacuum to provide insulation. Outside of that is a layer of liquid nitrogen. And we fill that weekly. And that just cuts down on any thermal transmission, even though you've got a high vacuum there.

And then outside of that is another high vacuum layer and then the room. And so you can walk up to the can, and you can touch it. And it won't feel cold at all. So that's what the magnet consists of.

The NMR measurement is based on the strength of the magnet-- so B0 is what I'm calling that-- and the gyromagnetic ratio of the nuclei that you're looking at. And we're going to talk about hydrogen today. And so you've got to be able to synthesize precise frequencies with precise durations and power levels in order to send those to your sample.

So you've got this console. It's got amplifiers in there. 100-watt amplifiers is pretty much the standard. Actually, I shouldn't even say that's the standard. I think ours have 500-watt amplifiers in there.

You've got different boards. It used to be that these all were plugged in to these long things with connections. And it talked through what was called a backplane, but, now that these are modern digital consoles, it all talks via ethernet. So everything has an address, and it talks to each other. It routes the signal to where it needs to go. You've also got some preamplifiers here.

So all this material or everything that's in the console will generate the signal that is sent to your sample in order to excite it the way that you need to in order to get the information out that you want to. This also will take the signal that your sample gives off. And it will amplify it and digitize it and send it to the computer so that it can be processed into something you can use.

To send the sample-- well, so the console generates all that signal, but it's got to be broadcast to your sample somehow. And so we think of the probe as the NMR antenna. So probes can come in a number of different formats. There can be probes for looking at solids. So you wouldn't even dissolve your sample in a liquid.

There can be what are called flow probes where you've got just-- we call it a cell, but it's just a container that's a certain volume. And you pump your sample up through a tube into that cell, analyze it, and then you pump it out. There's what we call a micro coil probe, which just has a small cell. So I've used ones or had them in the past in other labs where it had a 5 microliter cell. So you could look at just a very small amount of things.

There are also probes that are known as cryogenic probes. Those, the electronics that are in the probe are held at liquid helium or liquid nitrogen temperature. And, by doing that, it cuts down on the inherent electric noise that's present in the circuits. And it makes them more sensitive.

So I've brought a probe here, and you're not going to be able to see this from where you're sitting back there, but you can come up and look at it later if you want. Your benchtop instrument will have the same stuff in it. It's just not going to look like this exactly. So this is what gets inserted up from the bottom of the magnet. And it's just-- it's screwed in, and it stays in the bottom of the magnet.

There's only a couple of connections where the wires from the console are hooked up to this. And so this one, I can take the cover off of. And you can look at this when you come up.

Up in the very top of the probe, there's a little glass insert. And there's some flat ribbons of wire that are wound around here. And so I've got that pointed out. In fact, I think that's the same probe that I took a picture of.

And so your sample will go down, and it will go right into where those coils of wire are. And so the coils of wire will send the signal to your sample that's being generated in the console. And then, when that signal gets turned off, your sample will relax back to its equilibrium state. And it will induce a small voltage in these coils, which gets picked up, sent back through that console and off to the computer to generate your spectrum.

So I will leave this right here. If you come up later to look at it, feel free to pick it up. Just be very careful. This is glass. And you don't want to bang on it or bend it because it will-- you can break it, not that it's working anyways anymore, but we like to have it for demonstrations.

OK, so the NMR signal itself, it's generated when a collection of nuclei, meaning your sample, is placed in a strong magnetic field and irradiated with radio frequency energy of the appropriate frequency. And we'll see what that is in a second. The signal is a very small amount of energy that's given off, as the nuclei in your sample transition back to their equilibrium state. And it's really-- it's a time-dependent current that's induced in the coil in the probe on the order of microvolts.

So it possesses four different-- four properties and only four properties that we make use of. So pictured right here is an actual NMR signal. It's what we call a free induction decay. It's just a damped sinusoid. And, out of that, you can get these four properties that you can make use of.

The one that we usually make the most use of is the frequency. And so I'll tell you how you can convert this to this in a few slides, but where lines will appear on this graph tells us something about the molecular environment that whatever gave rise to that signal is in. So that's when-- most of the time, that's what you use.

The next most common piece of information you use out of it is the intensity. So the intensity of a resonance in the NMR spectrum is going to be directly proportional to the number of nuclei that give rise to that signal. And that doesn't mean just-- well, it also means just every nuclei that's in there, but specifically, for instance, if you've got one signal from this group of protons and one signal from this group of protons, their intensity is going to be equal because three protons gave rise to this signal, and three protons gave rise to that signal.

So you can use that as an internal check in molecules to make sure that you're identifying peaks correctly. You can also use it to quantify molecules, different molecules that are present in a sample. And you can even make a sample where you've spiked a known amount of something in there to serve as a standard and then quantify the amount of an unknown that you've put in there.

Another property is the phase of this signal. So what I'm showing here is not something you're going to acquire. It's called a two-dimensional spectrum, but what I'm trying to highlight is that each of these colors, be it-- I called it red-- I think it might be some blend of that or blue-- are a different phase.

Specifically, think of this. Does anybody know what a two-dimensional map is? Ever look at a topographic map where you've got mountains that are outlined by contours?

So you can think-- what did I call it? So I said blue is negative. So think of the negative-- think of the blue cross peaks as being holes or going down into the plane. And think of the red ones coming out of the plane.

So the way that this experiment is acquired, it makes methylene groups have a negative phase. And it makes methyl and methine groups have a positive phase. So that's really useful when you're looking at an unknown because, right off the bat, you can just say, OK, I know that this, this, this, and this are from a methyl or methine. And I know that these blue peaks are from methylenes. And then, specifically, I can look at it and say, OK, since this one and this one are in a line next to each other, I know that these are protons on the same carbon.

The last piece of information that comes out of an NMR signal is the duration of the decay. So it can be longer. It can be shorter. You can use that like for things like I hinted at before where you have-- small molecules usually have a long decay. Large molecules usually have a very short decay.

So, if you have a protein that's binding a small molecule substrate, its decay is going to transition from something long to something short. And you can use that to tell that your molecule has been bound. Any questions?

All right, so unfortunately not all nuclei can be measured by NMR. Any nucleus that possesses angular momentum will exhibit a magnetic moment that will interact with a magnetic field. Just like electrons, if you've learned about in general chemistry where you learned the quantum numbers and you learned about spin, it's the same for nuclei.

So we say a nucleus possesses spin in reference to its spin quantum number, which is labeled I. It's easy to think of nuclei as cute little balls that are rotating around, but remember they're not spinning. It's just an inherent physical property that seems like they're little spinning balls when they're not.

So there are rules for determining if a nucleus has spin or not. It's present in either half integer or integer values. You don't really have to worry about that for this class.

We're going to be looking at spin 1/2 nuclei protons, which are the easiest to interpret. And luckily protons have a 99.9885% natural abundance. So it's the most sensitive and strongest NMR signal that you can get out of any nucleus.

There are, like I said, the rules for determining whether something has spin. Nuclei with even protons-- an even number of protons and an even number of neutrons do not have nuclear spin. And so they're NMR inactive.

Unfortunately, the next most common thing that we would love to look at as organic chemists is carbon. And carbon has six protons and six neutrons for carbon-12. And so we can't. It's NMR inactive.

Luckily for us, though, carbon has an isotope that's 1.1% naturally abundant, which is C-13. And we can look at that. We take a hit on sensitivity, but we can still get an NMR spectrum of that. So that's good.

Some nuclei have multiple NMR-active isotopes. That doesn't mean they always appear in the same spectrum. They will have different gyromagnetic ratios. So they will appear at different overall frequencies, but you can look at-- sometimes, you can use that to your advantage. You could take a spectrum of 10 boron and a separate spectrum of 11 boron. Or a proton, we use the signal from deuterium as a lock signal for the magnet to focus on and counteract its inherent drift.

So what's the physical basis of the NMR signal? In a magnetic field, all those little magnetic moments are going to adopt an orientation relative to that field. The allowed orientation of these moments is going to be explained by quantum mechanics. Luckily, we don't have to really get into that.

And the process that nuclei undergo during an experiment can be rationalized in two ways. You can think of it in terms of vectors, so which way those little magnetic fields are pointing, or you can think of it in terms of the energy levels that the nuclei adopt when they're put in that magnetic field.

So I tried to make a little cartoon here. With no magnetic field, these are supposed to just be all randomly oriented, but, when I immerse my collection of molecules that's in my sample in a magnetic field, they will line up and be either with the field or against the field.

And so, if we look at one nucleus when we place it in a magnetic field, quantum mechanics tells us that it's not just going to point straight up. There's got to be some uncertainty in where that nucleus is pointing. And so it is going to precess, meaning it's going to rotate around, the direction of the magnetic field with a characteristic frequency, which we call the Larmor frequency. It's named for-- what's his name? Joseph Larmor who was a physicist back in the late 1800s, early 1900s.

That frequency is going to be directly proportional to the strength of the magnet that you put your sample in. I've unfortunately-- so gyromagnetic ratio can be specified in either radians per second per tesla or in hertz per tesla. I apologize for not being too careful in that I will jump back and forth between the two.

And so, for a proton, if we take a magnet, that's 11.75 tesla and we put our sample in there, it's going to precess around the field at 500 megahertz. And so, when we talk about NMR instruments, just as far as what size they are, they are not specified by the strength of their magnet. They're specified by the frequency that a proton precesses in a field with a magnet of that strength.

So, if you come down to the DCIF, I'll say I have a 500, or I have a 600, or I have a 400. In the undergraduate teaching labs, you have a 300. And I think the benchtop is a 60 megahertz. And I'd have to go look up in a table or do the calculation how strong in tesla a 60 megahertz NMR magnet is, but that's the way it's specified.

So that's for one nuclei when you put it in the magnetic field, but our sample is actually a bunch of different nuclei. Or not-- well, yes, it's a bunch of different nuclei. It's a collection of nuclei.

We don't have to keep track of every individual nucleus and deal with the quantum mechanics when we look at an NMR experiment. We can treat the whole sample as a collection of the nuclei, so summing up all the individual magnetic moments, and just look at the bulk magnetization. And so we can use statistical mechanics in order to figure out what's going on.

So we put our nucleus-- here's our sample. We've got, say, 10 milligrams of material. We've got it dissolved in about 600 microliters of a deuterated solvent. We drop it down so it goes in our probe in the magnet. And our collection of nuclei will start precessing either aligned with the field or aligned opposite the field.

So there will be a slight energetic preference for those nuclei to have their magnetic moment aligned with the field. And that population difference will result in this net magnetization that we call the bulk magnetization. Let me put that down.

We can jump from thinking of vectors to thinking of energy levels. So everything that is aligned with the field is going to be at a lower energy level. And we call that just the plus 1/2 nuclei. And everything that is aligned against the field is going to be at a higher energy level at negative 1/2.

And so the difference between these two levels is going to increase as the strength of the magnetic field increases. And so maybe over here, on the 60 megahertz, the difference is only that much, but, when you get to our 600, it's a lot more.

Here's where the measurement-- or the principle behind the NMR measurement comes in. You're making nuclei transition from one energy level to the other energy level. So you're perturbing their equilibrium distribution, and then you're letting them relax back. And that's what gives off the signal that we make use of in NMR.

So I guess I jumped too far ahead in my verbiage. So we have our sample in there. It's lined up. And we perturb it with an RF pulse.

So now I've jump back to vectors. If you think of it in terms of vectors, when you generate that radio frequency pulse that has the frequency equal to the difference in those energy levels, you are, in essence, generating a small magnetic field that is aligned. In this case, it's arbitrary, but we'll say it's aligned along the x-axis.

That acts to topple-- or not topple, tip this bulk magnetization vector off of being pointed with the main field over into the xy-plane. It's still precessing around at that characteristic frequency. And, when it has a component in the xy-plane, that will generate a current in these little coils in the probe, which is then received back at the console and sent to the computer.

So we pulse our sample. And depending on how long we turn on that pulse for-- and it's usually the order of microseconds. I think our pulses are set up to be about 10 microseconds. We can tip this over to some varying degree.

And so usually we try and tip it over by a specific amount, which is a 90-degree pulse, because that will generate the maximum amount of signal that we can get out. So we tip it over into the xy-plane. We turn off that pulse. And then we let it relax, and we collect our signal.

Our signal-- I think I said this before-- is called the free induction decay. And so here's another diagram of it. We've turned off that pulse. The signal is precessing here, but it relaxes back so that the component here gets shorter and shorter while we grow back the component in the z-direction. That gives us this voltage-- and my laser died-- that gets picked up in the coil.

We can do that multiple times. So, if you've got a strong sample, you can just take one scan, but usually we'll take multiple scans because you can do what's called signal averaging, which is add them together. And that can help you remove artifacts, increase your signal to noise, and other things. So that's what the free induction decay is in the NMR signal.

I put this back in my slide pack after I gave the slides to Professor Dolhun to print. So you don't have this in your handouts unfortunately. This is just-- I was thinking this is still a good way to show about the NMR signal, but in terms of the number of spins and the energy levels.

So here's your equilibrium. You've got more spins pointing. I'm saying they're pointing down in this lower energy level. You pulse it, and that equalizes the energy level. So I forget how many are in each, but these are supposed to be an equal number of arrows.

Then, after you turn off that pulse, the spins that got transitioned to the upper energy level will relax back down. So there's a predominantly-- they're more predominantly in the lower energy than the upper energy. As they do that, they give off the free induction decay, which is your NMR signal. Are these any questions?

OK, so what do we do with that FID? Now, I've been doing this-- I don't know-- since 1999. I can look at an FID. I can say, well, that's a long one. I can look at an FID and say, yeah, that's got several different frequencies in there. But, as far as looking at an FID and saying, oh, that comes from DMF or, oh, that comes from ethyl acetate, no, I can't do that. And I doubt that anybody else could.

So we have to transform that and somehow make sense of it. And so that is done using a Fourier transform. And so a Fourier transform takes something that is in the time domain. And it transforms it to the frequency domain. And, a long time ago, before the advent of computers, NMR wasn't done using Fourier transforms because it was computationally too difficult.

And so someone back in the '60s-- actually, someone famous at IBM developed a way to do this to make it a little bit easier, but they still had to print out little cards. And you'd punch holes in them. And it would take days and days to feed it into a machine. And nowadays your phone can do it. It's got more computational power than something that had a whole room.

So everything is done by Fourier transform now. That gives us our spectrum, which consists of lines, which we can look at, and we can interpret and figure out. And, no, I couldn't do a Fourier transform or solve one in my head or even on paper. So it's just something the computer does.

So let's back up. I've given away a lot of this because I've already showed you several spectra, and you see that there are multiple lines. But here's the free curve for the equation for the frequency being proportional to the magnetic field strength.

And you might think, OK, I have protons. Why don't I just get one peak because I have protons? And I would have one peak for protons and one peak for other nuclei.

If it did that, I wouldn't be up here talking to you about this because, while it might be a fine and dandy measurement for a physicist to use, it'd be useless for us in chemistry because we'd only get one peak for all the different protons that are in our sample. Luckily, when you put your sample in a magnet, whatever the local magnetic environment is that the nuclei reside in, that is going to modify the [INAUDIBLE] or the external field that those nuclei feel. And so that's what spreads out our single signal from a proton into the different regions of the spectrum.

So this is supposed to be ethyl acetate. I've got a doubly bound oxygen here. I've got a single bound oxygen here, a methylene, methyl, and methyl.

So oxygen, I think everybody might know is electronegative. It's really greedy. It doesn't like to share its electrons. So it pulls electron density away from things that are nearby it. And that's why I've got these little deltas up here, indicating the partial negative charge.

The things that are bound closest to it and even further out, they're going to be more positively charged or partial positively charged than they would have been because the electron density is being pulled away from it. So that's what helps spread out those signals.

We call this chemical shift. It's denoted on a spectrum as ppm. And, oftentimes, it's denoted with delta, not to be confused with the delta I've used for the small charges, but you might see that on the axis of it.

So anything that can perturb electron density will affect we call it the shielding of the nucleus, not only electronegative things, where nuclei are oriented in relation to double bonds. In a benzene ring, you've got the clouds of electron density in those pi orbitals. And things that get oriented directed into that will be more shielded. Things that are sticking off the ring equatorially-- or not equatorially, but in the plane will be deshielded. So that has an effect. So all these things will combine together to spread out what's in your spectrum.

So here is just a spectrum of my ethyl acetate. And so notice I've spread it out. Well, I haven't spread it out. It is spread out into three separate signals.

You can use NMR solely as a fingerprint and tabulate. I know that only such and such resonances come at 2 point-- I don't know. We'll call that 2.01. And so you can go look in a table and say, oh, well, this must be from one of these small subset of molecules because they only have something at 2.01.

It's more important to be able to rationalize where things appear by-- or yeah, where things appear on this spectrum by where they are in the molecule because that will help you analyze the spectrum better. So, if we look at this, we've got three peaks.

Now, I talked about intensity being one of the properties of the signal earlier. I don't know if you can see this, but there's a 3.10 under there, a 3.10 under here, and a 2 under here. So that's the relative intensity of those signals.

Now, one thing that is common when you're first learning NMR is to think that the integrals are dead on exact so that it should be 3.00. No, it's going to be close, but it's not going to be exact. You've got to take great care when you're acquiring the spectrum to get your integrals to be as close to perfect.

So we'd call 3.1 3. So we know-- we can look at our molecule. We've got two methyl groups. So we can guess that these are both from the methyl groups because they integrate the 3 and that this is from the methylene because it integrates to 2.

So why is this methylene all the way down at 4 and these are up here? Well, what's the methylene next to? It's bound straight to the oxygen. So the oxygen is withdrawing the electron density away from that methylene. And it is shifting the peak what we call downfield, which means to the left on an NMR spectrum.

That's a leftover term from in the old days when they would sweep. You would actually adjust the frequency-- or not frequency. You'd adjust the strength of the magnet. So anything that was down in this direction used a weaker magnetic field strength. Anything that was up in this direction used a stronger magnetic field strength.

Nowadays, we don't do that. The magnet is always just what the magnet is, but those terms are still around. So anything to the left is downfield. Anything to the right is upfield.

So this one is deshielded the most. It is at 4.1. The next peak is this one. Now, we've got to choose. Is it from this methyl, or is it from this methyl?

And so you would look, and you'd say, OK, both of these are bound to carbon, but the carbon that this one is bound to is a carbon that is doubly bound to an oxygen. So electron density is being drawn away from that carbon by the oxygen. So that's still going to also have electron density being pulled away from this methyl. And so that's going to shift it down here.

This carbon is just bound to a plain carbon without a double bound oxygen on it. And so it's not deshielded as much. Although, if I took a spectrum that had-- so, if I just had an alkane like pentane and I looked at the terminal methyl on pentane, it would be over here because it would not be deshielded at all compared to this.

Another thing you might be questioning is, why are these ones split into multiple peaks, but this one is only a single peak? Let me make sure I kept my slides in order. OK, I did. That is due to what is called coupling.

So protons that are bound to a carbon will, in essence, talk to protons on neighboring carbons through the bonds. And it's called spin-spin coupling or scalar coupling. And so you will have protons split apart into characteristic patterns.

What else? So the splitting on those peaks is not dependent on the main field so that, if I took that ethyl acetate spectrum-- and I forget what strength. I maybe will say I acquired that at 500 megahertz.

And I measured the difference between those peaks. And we'll say it's 8 hertz. If I take that molecule, I put it in a gigahertz machine, which is twice as strong, and I measure the splitting, it'll still be that same value.

So, for simple spectra, the splitting follows what's called the n+1 rule. And so you look at the number of protons on neighboring carbons, and you add that up. So I guess I should have drawn another molecule. Where did my chalk go?

So, for instance, we'll take this one here. So CH3, I've got two protons over here. So it's going to give me a simple spectrum. So it's going to be split by 2 plus 1 equals 3.

And let's see. I'm going to close this and bring up my real spectrum. So this spectrum is of 3-heptanone. And the methyl here and the methyl here are these two resonances over there. And so you can, in fact, see that those are split into three peaks because each of them has two protons next to it.

Now, not everything behaves that way. When you get into more complicated molecules, splitting can be completely non-rationalizable. So there are several ways that can happen. The most common way is, when the difference in chemical shifts between two nuclei is not much bigger than their coupling constant, you'll just get a whole bunch of different peaks.

I can drag up an example of that in a second. So let's see. Oops, OK, so that crashed. Oh well, so I won't do that. OK, so I've given you a table in there that gives some of the common splitting patterns that you will observe. Yes?

AUDIENCE: So what do you mean when you say simple spectrum?

JOHN GRIMES: A simple spectrum is where the splitting obeys this n+1 rule. And so all the multiplets will be analyzable that way. And you'll know it. If you look at something and you don't see these common patterns, you can automatically say, OK, there's more complex splitting that's going on in or non-first-order.

The intensities of those peaks in a first-order multiplet will obey or agree with the coefficients of a binomial expansion, i.e. Pascal's triangle. So, for n equals 2, you will get 1 to 2 to 1 and, for 3, 1 to 3 to 3 to 1. So they will obey that. If it was non-first-order, you might see four peaks, but you wouldn't have that same intensity pattern.

So here's some other examples of NMR spectra. Here's a simple one, ethanol. We can look at this, and we can rationalize where the peaks appear in the same fashion. We've got a methyl group and a methine.

So, if we look at the methyl group, we see that there's one, two protons next to each other. So we would expect the resonance for that to be split into three separate peaks. And there, in fact, we see a triplet. And then, for the methylene, not that it's default now because there's nothing-- or that's the only other one, you would expect one, two, three, four peaks. And you see that.

And now this is tricky. Why do you not get three peaks for the OH, even though it's got two protons next to it? OH and NH have exchangeable protons. And so the proton is coming on and off that molecule in a faster time period than the NMR measurement. So you just see an average of the chemical shifts, meaning it gives you a single peak, rather than splitting into a triplet.

These didn't come out very big, but it's just showing you, with bigger molecules, you get more peaks. If you zoom in on these, you'll see that a lot of these do not give simple triplets or quartets or doublets, that you've got a lot of more complex splitting going on in the molecule.

And so here's an example of an ester. I think that you all are going to be synthesizing esters. So, if we look at these two compounds-- and something I didn't point out, well, I'll show you this in another slide-- anything that appears in an aromatic ring is going to be deshielded. So it's going to be far downfield.

And I think I said that before. You've got the electron density of the pi orbitals above and below the ring, which would shield something. But those protons are held not there, but straight out from the ring. So they're in a deshielded region.

So, right off the bat, we can look, and we can say, OK, we know that this must be-- or these peaks must be from the protons on the phenyl rings. Unfortunately, there's no integration on here, but you also, if I had the integration values, you would see that those both integrate to 5. And that would tell you something about it too.

So now the question becomes, which splitting patterns in this region down here agree with what we see up here? So, if we look at our ester, we see that, on this ester over here, we've got-- and we saw this ethyl acetate. We have the methyl and the methylene bound to the oxygen. So we know that the methyl and the methylene are going to split into a triplet and a quartet. And we know that that quartet is going to be shifted downfield because it's bound to that oxygen.

Now, that's not to say that also, in this one, they're not going to be split into a triplet and a quartet. But this carbonyl carbon is not going to drag the peak as far downfield as it does over here. So we can say that this compound here is from this one.

And this peak right here is my single methylene that doesn't have any protons next to it, whereas this compound over here is this one here. Did I just do that? No, I just said that backwards.

This compound here is this one. This compound here is this one. So here's the single methylene, and here it is. Here's the methylene that's split into a quartet. And it's right there, whereas, on this one, this methylene is shifted a little farther downfield because now it's bound to the oxygen. Does that make sense to everybody? OK.

I'm not going to go over this completely. You can look at this. You can also look up-- you can find stuff like this online everywhere. This is a fellow over in England who puts out these graphics called Compound Chem. I don't know if anybody follows him on Twitter, but he's got all sorts of great chemistry education graphics.

This is one that just gives you an idea of the different chemical shift values and where things appear. And so you can see an OH on a carboxylic acid is going to be very deshielded. Amide protons are going to be in this region and et cetera. So that I can give you some idea of where to look for resonances.

I said that we can take spectra of carbon. Carbon is a lot less sensitive than proton. In fact, sensitivity-wise, if you just compare the gyromagnetic ratio of carbon to proton, it's a fourth of proton. So, like I said, we specify our magnets by the precession frequency of protons. So, on a 500-megahertz instrument, it would be a 125-megahertz carbon instrument.

So you take that one fourth of a hit because of the gyromagnetic ratio, but then you also take a big hit because only 1.1% of the carbon present in your sample is made of carbon-13, which is the NMR-active carbon. The rest of it is carbon-12, which is NMR inactive.

But you can still acquire carbon spectrum. A carbon spectra has a much bigger frequency spread. So you don't have overlap as much. That can be very useful.

And this combined with a proton can tell you a lot about your molecule. You're not going to acquire these on the benchtop instrument. Although, I guess it probably will if you have a very, very concentrated sample.

This is just to show an example. You can do very complex samples or experiments. So this is a three-dimensional spectrum of a protein. So this is something you would use in determining a protein structure. I've never acquired one of these. So all I can tell you is that you're looking both at carbon resonance-- what do we have up here? Oh OK, sorry.

So it's the protein was expressed by bacteria that were growing in food that was labeled with N-15 and C-13 so that there's incorporation of those nuclei in there. And it will give you a signal, and you can use that. Let's see. I'm almost finished, but I think we're about out of time.

Sample preparation, when you're preparing your samples, it is important to prepare clean samples. There should not be particulate matter in your samples. You should filter them if you do have particulate matter from your reaction because that will-- having particles in there will lead to poorer spectra that will be difficult to interpret.

NMR are the very thin, glass-walled tubes. So be careful with them. If you snap this, you can easily stick it in your hand. So be very careful with that.

Use a deuterated solvent when you're preparing your samples. I think you will be given that by your TAs anyways. And never put a dirty tube in the instrument. You should always clean the tube off on the outside.

And then I think the last slide that I have is about shimming. Shimming is adjusting the homogeneity of the magnetic field. So, if you remember from that equation-- and you don't even have to know how to solve this-- so your frequency is directly proportional to the magnetic field strength.

Shimming means making your magnetic field homogeneous so that the top of your sample feels the same strength field as the bottom of your sample. The machine will do it automatically, but, if the top of your sample feels a different magnetic field strength than the bottom, then it's going to have two separate frequencies for where those protons precess, which means the line broadens out. And, if that line broadens out, it makes it harder to interpret.

And then I'll skip over this one. The last thing, I guess, questions, I put in some references in here. This is a great thing to do with old magnets. If you're artistic with cutting tools, I would love to have my own pizza oven with an old magnet. So, if anybody has any questions, please let me know.

AUDIENCE: Pretty cool lesson.

JOHN GRIMES: And no, that's not a friend of mine's either. I just snagged that off the web.

AUDIENCE: That's hilarious.

JOHN GRIMES: Anything else? Thank you.

[APPLAUSE]

And, if you want to look at this if you have time, you can come up.