Welcome to an NMR tutorial on the effects of dynamics on the NMR time scale and the conformation of molecules. For all the amazing information NMR gives us. It is both insensitive and slow. The sensitivity we previously addressed, it results from the small population difference we observe in our spin states using the magnetic field. But what do I mean when I say slow? Well, the above diagram, marks out oughly the timescales over which physical processes occur, and the timescale over which various techniques can report at the top and the Bottom respectively. So we can see the very fast atto- to femtosecond time scale here. That are not very many options for monitoring, but there are important phenomena occurring. So there's vibrations, excited states. As we get down to the picosecond time scale, there are more options available, and some of those include NMR based techniques, which are shown in these three-dimensional shapes. However, the NMR information we're most interested in is the J coupling and chemical shifts of the molecules. You will see that they are at the slow end. They are slower than many processes, including structural changes of our molecules, which are significantly faster. As we mentioned in the last tutorial, This means that the structures we see in NMR are normally averages. And this is where we touched on this before: Our rotating alkyl chain, here, has various potential coupling constants depending on the angle. But in reality, it rotates too quickly. What we see is an average with a J value somewhere around seven. This is only true because it's free to rotate, however, if we locked it, the story would be quite different. Now I don't think it would be difficult to persuade you this proton beside the fluorine is quite different from this proton, which is beside another proton. But these two protons here are also inequivalent, as are these two here. And this is due to different coupling because of their spatial arrangement. So the fact they're locked in a ring means that these are no longer interchanging. And in fact, it's easier to see in this type of conformation. Even if the molecule was symmetrical, You would find the protons on the faces are in fact different to one another. And indeed these are differentto one another as well. However, these two protons and those two protons are going to be the same. And that's because their spatial relationship Two other protons to which they can couple is the same. We will talk about diastereotopic protons, rotamers and atropisomers. And I will even send a couple of personal examples Now for an the example to demonstrate the values of j couplings and shape in structural determination. I have no idea if this molecule actually exists. There is one reference for it on scifinder and a 2010 patent on electrochemical energy storage. That doesn't actually matter because this specta is simulated. What can the spectra tell us about the arrangement of our atoms in space? Well, we're going to approximate that this cyclic ether system behaves like a cyclohexane chair conformation. So here any of our groups could be in an axial or equatorial position. Any of these, as you can draw two conformations of your cyclohexyl chair, the up or down, you can observe on this flat structure, it does not tell you anything about whether these are axial or equatorial and how they relate to one another. So the most downfield protons in this structure are going to be the ones that are attached to the carbon bearing two oxygens, attached to two electronegative groups. I'll go a bit further, information not easily proven, but this sitting here, this downfield one, is that proton. We'll come back to that later because I actually wanted to start here at this other downfield proton. Now, the reason we start there is there is a large J coupling. And in the context of this ring system, we know then that the only way we get that J coupling is to have an axial, axial relationship between two protons. And this allows us to define the non-hydrogen substituents that are attached. So if we say this is axial, this scene. Then we know that this hydroxyl is equatorial and this methyl is equatorial. So let's look for the next signal. We are looking for large J coupling doublet. And then also a quartet for this methyl group. Now, this bond can rotate freely So we should expect J coupling of around about 7 Hz. It's easy enough to identify its this triplet of quartets here. Now, you might be a bit confused by the fact that it's apparently a triplet. Well, this is telling us is that we have two very similar couplings and we can't separate them, so we're only seeing one J coupling, which looks like a triplet. That's telling us then that we have an additional axial

coupling, which means this position must also be axial.

This is equatorial. Now when it comes to identifying this proton, we know that we have a large axial coupling, And we know that being attached to a carbon with an oxygen, that it should be further downfield. Then the signal we've just looked at before. So I think it's quite obvious in the system that we're looking at C.

So here we have the large J coupling we spoke about. And we also have smaller J coupling, 3.5 Hz. And this means that we have either an axial/equatorial or equatorial/equatorial. coupling. We've already said we know our proton, is axial. So it must be an axial-equatorial coupling. And that means this proton is equatorial, and this substituent is axial. And actually this fits what we know about how these Chair confirmations must work because if this hydroxyl position is pointing up, then the next equatorial substituent must be pointing down. So this position must be axial.

If we identify now this signal, And it must have, again a J of roundabout seven quartet, and a small coupling, for its axial-equatorial coupling and have the same things we previously observed. There's a similar coupling that gives us a quartet and that coupling is to this proton. This is an equatorial-equatorial coupling.

And that means This is equatorial and that is axial. And so we have defined the positions in space of all our substituents of this chair conformation. Now you might have wondered. In the previous slide, why it was, we never saw any coupling to the OH protons. And that's because we exchange these using hydrogen-deuterium shake. And I'm going to now talk about what is as an exchangable proton. What are we talking about when I say that, and when should you expect such protons? So the colour is exchangeable protons. And, some reference to this has been made previously in interpreting signals. And I hope to give you a little bit more detail about what's happening here. So this doesn't mean anything sophisticated. It is literally swapping protons. And in practice, it usually refers to hydrogen to hydrogen or hydrogen to deuterium exchange. The important point for hydrogen to deuterium exchange is the D is a spin equals one nuclei. And that means that within the range that we're looking at for proton NMR, we are not able to observe a signal. Okay.

And these are calculated or literature pKa values. So I want to start with this proton here as iy has a reasonably low PKa and is possible, because of that For this proton to come off that, and go on to water, and a proton from water to be exchanged onto this oxygen. And that's is literally all we are talking about with exchangeable protons. Protons that can exchange with other protons in the same molecule, with impurities or with the solvent on a timescale quicker than our NMR experiment. I said within the same molecule, and you can see here, this is an example of what that would look like So these proofs have been flipped. And there are some rules of thumb for identifying these: So you're looking for low pKas, the ability to hydrogen bond and attachment to a heteroatom. These are not necessarily independent of one another. And they are normally broad. This is due to averaging the various exchange states. And they don't display coupling in many cases because the coupled protons are exchanged over the course of the experiment. I should say that we normally treat exchange as a binary proposition. Back to why in a moment. But it's just worth bearing in mind that in fact different protons exchange at different rates. The rates vary differently over the pH of your solvent. It can also be concentration and solvent purity dependent. And this means the some features like the appearance of coupling are actually quite variable sometimes even for the same molecule. And the reason we treat it as binary is normally our way of confirming the existence of exchangeable protons is simply to add a large quantity of deuterium compared to the small amount of hydrogen we have present And shake. And then the vast majority of the protons that are exchangeable on our molecule will be changed to deuterium, which we will not see in the NMR experiment. And the

signals will disappear. To give you an idea of what that looks like. Here is an antibiotic molecule. And you can see there are few broad signals. This one you would suspect, due to its breadth and absence of coupling, to be an exchangeable signal. It makes sense for that to be our NH2 here. And this is a bump in our baseline which may or may not be signal at all. But we can see after exchanging with D2O that both of these vanish and we are able to see those signals with exchangeable protons and in fact we have our amine and our acid. So I'm going to move on now to the concept of diasteretopic protons. So this is a bit of an extension of the previous discussion. But this time, protons can rotate. So although they are not locked in place, they are still inequivalent. And this might seem odd. Especially when you look at this flat molecule here and I'm telling you that these two protons are not equivalent. But look at the three-dimensional model and I think this becomes clear. You can see there is a group off the stereo centre, here, that is orientated onto one face of our molecule. If we look at these two protons. And I asked you, rotate this in your head, I think, what you will realize is that it is impossible to have these two protons become exchangeable, to become equivalent to one another. And this is because the two faces of the molecule are different. This means they are inequivalent and they couple, and this coupling is going to be 14 to 17. hertz - its a big one. It is the presence of the chiral center which causes this. So you can see if I was to remove that chiral center, you would then have two equivalent faces. And if you then rotate this, these bonds, the protons become equivalent they invert in the environment. Now, you don't have to make three-dimensional models to test this. There's actually a very simple test for diastereotopicity. So take your center, identified this one here, and replace an atom on it. One of the atoms. On your diastereotopic centre That are equivalent to one another, with another atom. So I've replaced this red hydrogen with a fluorine, and that gives us one state. We then take that backoff, so we're back to our original molecule, and instead make this substitution on the other hydrogen. We end up with this molecule. If our protons are diastereotopic, These should be diastereomers of one another. No diasteretopicity requires there are two or more two or more symmetry elements after replacing the proton, Not that there was one previously. So I'd like to direct you to the example BOX. This molecule has no chiral centers. However, if you replace either of these protons, both the exchange site and the central atom will become chiral. As a result, these protons are actually diastereotopic. The same actually applies to enantiotopic protons. Although in most cases this is less important. So this is where a single substitution would make enantiomers.

Enantiomers are normally equivalent in our NMR spectra. And so these protons are also normally equivalent. However, in principle, in the presence of chiral molecules, be it a solvent, Lewis acid, whatever, the two sides of my molecule here. Could again be made inequivalent. And, if that happened, I would again essentially have diastereotopic protons and I will be able to see these enantiotopic protons separately. They would become inequivalent. So there is a difference between protons which are enantiotopic and therefore can be made inequivalent, and groups which can never be made inequivalent, and thats whats I'm demonstrating here: If you can draw an axis of symmetry through the central atom, then your group is homotopic. And these will always be equivalent.

Enantiotopic Protons, on the other hand, are mirror images of one another. Again, there is no need for these protons to actually be attached to the same center. These protons here are enantiotopic. So from the exchange of protons, let's move on to situations where the only exchange is of the position of one atom in space. This conformational interchange involves the interconversion of two or more conformations. I mentioned earlier, that conformational changes is fast compared to NMR. This is not always the case. So in this context, we're talking about changes that are close to the NMR timescale. We have an example here. These two methyl groups interconvert. And you can see that if there's rotation around this bond, these should be equivalent. So at room temperature, you do indeed see a single

peak, showing these can interconvert. However, they're not as fast as they might be in a molecule. Less prone, to rotational problems. And so, and they're quite broad, so they're still relatively slow. They're approaching the NMR timescale. As you cool this even a little bit, you see the signal broaden as the range of states that are being seen by our NMR experiment increase until this starts to form two distinct populations. And at this point, we are seeing the protons of the methyl groups on each site as inequivalent, slowly interchanging. As the interchange becomes yet slower. So it isn't affecting what we see on NMR timescale at all, these peaks become progressively sharper until at minus 50. They are essentially completely different signals and they're not broad. And these methyls are now inequivalent. Should say that if you get stuck somewhere in here, it is possible to see pretty much nothing, so the peaks vanish. So that example involves rotation around a bond. But you can also have an inversion around an atom. So if we take an amine with three identical groups, and here we have an arrangement, it goes 1-2-3. Then we can rotate that easily around this axis here. And we can have 3-1-2 and 2-3-1, however, can't get us to 2-1-3. So if we try and rotate this group, one further to this place here, we end up 2-3-1. So the only way to interconvert these two states is to push through a pyramidal inversion. So you end up at this more linear transition state, which is higher in energy and therefore slower. Nevertheless, in the case of amines it happens pretty fast. So we're trisubstituted a means in principle could be chiral actually they interconvert too quickly. However, if we go down a row in the periodic table, phosphenes are much harder to interconvert. In fact, this example is specifically synthesized. To do so well. Now I want you to look at look at Ha and b on this diagram. The system is too hindered to rotate around here. So these should be inequivalent. The reason they're inequivalent is that we have this phosphorus popping up out of the plane. So this is all one plain, however, the two planes are inequivalent because of this phosphorus. However, the interconversion that actually happens here is the phosphorous interconverting between being above the plane and below the plane. So we can see at minus 50, we can see Ha and Hb and indeed a number of other signals as being distinct. And as we warm up and the interconversion of this phosphorous center increases, these signals vanish. Ha and Hb disappear. They would eventually coalesce and we'd see signals reappear if we warmed it up enough and the molecule was stable enough to allow that. Now, I've shown you that kind of inversion of around a centre. For organic chemists it is actually more common to observe rotational interconversion. So we're back where we started with our simple alkyl chains. Now, where we have different conformations that we can reach by rotation, We have rotamers. So these two energy minima with energy and energy high between them. So these are rotamers of one another. An energy gap is overcome to go from one to another. Now, the timescale for interconversion of these rotamers is proportional to the energy of the molecule, or the Temperature and the energy of the barrier to rotation. For most molecules like our alkyl chain, It's faster than NMR measurement. As we see before.

So what what we get as an average. However, if it's really hindered, interconversion might be very, very slow. This is a chiral ligands or catalyst known as BINAP. And it can be bought as either pure R or pure S. With the chiral centre actually being axial chirality, around this axis. And the calculated interconversion temperature for these two is about 500 degrees Celsius. And they come as powders.. What we often end up with is something in between. And a classic example of that is when the the interchange is slow enough to see in the NMR, but too fast to give two distinct compounds you can separate and handle. The reason this is particularly worth knowing about is that at some point you will have a spectra that looks impure but actually just has NMR timescale atropisomers. And the way to confirm is by making the peaks change proportions. that can be done changing the solvent or the temperature. So here is a literature example. This is a tertiary amide. Such structures often have slow rotation. And rotation is hindered around this axis. And the proton we are observing is this one. So you can see you have a slightly broad signal from this. However, it is still coupling To this proton here.

Now they have tried a range of different solvents. And think of these inter conversions, conformational changes as being like chemical reaction in the sense that some solvents will allow it to happened more easily, it will be favorable, and other will make it more difficult depending on the interactions between the molecule and the solvent. And here in the less polar media, chlorobenzene and chloroform. We can see the interconversion is quite slow and we see separate signals for two different conformational forms. However, for polar, aprotic solvents, DMF and DMSO, this interchange is faster and we see only a single signal. So the way they then check that these signals, they use chlorobenzene as it happens, are in fact rotamers of this form is that the hea it up from 25 degrees C, we can see the signals start to disappear as they come to the NMR time scale. And then the cool it down again to make sure that they are not in fact just observing decomposition. And the change in the proportion of these, and the shape tells us that this was a conformational effect. I just want to finish off with a personal example. Here there are two sets of geometric isomers on the NMR time scale.

the NMR time scale. there are those generated by the through ring plane of rotation, that is along here, along this carbon-nitrogen bond. And the readout for that is these diastereotopic protons here. And there is also a plane of rotation through this nitrogen in the other direction. So through the Boc group, and that is is the tbutyl group spinning around here. So you can see the two inequivalent diastereotopic protons, this is the room temperature NMR. quite nicely defined. They are completely inequivalent to another. Rotation around this axis is slow. However, these signals are broad, and these signals are the result of the rotamer The Boc group.. And I want to draw your attention to two things in particular. Firstly, the easiest way of seeing this is the Boc group here because it should be a single peak and it's not. So you get two peaks here. The second is that these are not proportional to one another. So where our diastereotopic Protons must give us one proton for each position. They are one structure that is not interconverting, rotamers can represent two different populations so they are the whole molecule in two different conformations. And these do not have to be equally favorable. And as a result, these peaks can be different sizes. So this is the room temperature NMR, here. And you can see that these are broad. As we heat up to 55 degrees, we actually start to lose some definition on our rotameric peaks. And this is where we're starting to spin around here. Just a little bit quicker. If we spin around that axis as fast enough then we will no longer have axial chirality. around that axis, and so these protons would no longer be diastereotopic. However, the tbutyl group, the Boc group which was not fast enough to be interconverting at room temperature, at 55. it now rotates quickly enough that you don't see two conformations. Instead you see an average. And this is particularly clear in this Boc group here. And I'll just draw your attention to the fact that the peak is not actually situated on either of the peaks above - it is in between. So it's an average states.