So today I want to go through an NMR example and here we have classified it as NMR 1. Now before we continue, it is essential that you've already gone through and have an understanding of the 1D and 2D NMR lectures that are represented on WCAIRs website. So what are we going to be able to understand during this talk? While we want to understand the relationship between 1D and 2D spectra. We're also going to look for is some of the downfalls of some spectra and how other 2D NMR experiments can help you with this understanding. And the ones that we're going to cover today is was deemed to be one of the most essential for understanding structural eludication, which is COSY, HSQC and HMBC. What you going to see in the following slides, is real life examples. Sounds like a movie thing, but you know, real life examples. And there are downfalls to them. I want you to note and watch over them and see how we start to divulge into these downfalls and try to use other spectra to help with this understanding. So the example that has been chosen today is for Fluconazole Fluconazole is a well known drug and I'm not going to go into details about to which its medicinal purposes are who made to etc. For now, we want to be able to just look at its structure and spectra. So here is a 1D NMR of Fluconazole. What do we want to know about the spectra? So first of all, we want to be able to understand how many protons ad up to each one of the signals. And this is known as integration. Where can we find integration? Integration are the numbers that are present below the spectra that you can see here. And what do we do? Well, what we do is, we start to round to the nearest whole number to determine how many protons are making up that signal. So we've taken this one peak here, which has a value of 1.93. So if we were to round this, number up, it is two. So we can therefore say the two protons are equating to the signal. And how should we sought to write this or annotate this on our spectra? Here, we just write H1 and H2. Now you are thinking. What happens if its a CH2? Then technically it's H1 and H1'. Well, we don't know that yet. We want to start to be able to use all of our knowledge, able to fully understand what it is that we have in front of us. So if were to do this integration to all of these peaks on the spectra, we start to see that we have roughly, we not roughly, we have 12 protons in the spectra. What are the next things we want to be able to understand? Well, we now want to be able to understand the regions in the spectra. And what I mean by regions is where do things fall in relation to functionality? We know from previous talks the aliphatic regions regions are about zero to three PPM. Aromatics, for instance, are about six to nine ppm. And what we want to be able to look out here in our spectra is, what is our spectra telling us? Our spectral first of all telling is we have a large set of aromatic peaks, eight of them in total. And we have something that is falling either as an aliphatic or something that is potentially heteroatoms, for instance. What else do we now want to know? Well we now want to be able to understand peak shapes. And what do I mean by peak shapes? I mean by which things start splitting. There telling us how many neighbours something may have. So what we do is. we'll take this first example here. We have two sets of doublets. Using this n plus one formula, we can say that because it equals to two, that n which stands for the number of neighbouring protons, is therefore one. So he can say that each one of these peaks has one neighbouring proton. Now we want to take everything into consideration, everything that we already know. Integration, for instance. We know that each one of these peaks equates to two protons. But we know that each one of these peaks therefore has one neighbour. What region does it fall in the NMR. It falls in either something that is attached on to an electronegative element or something is itself electronegative. So what does this equate to in Fluconazole? Looking at the structure of Fluconazole, we're ideally, first of all, looking CH2. And here we have two sets of them with one of them being highlighted in this diagram. It says here that is one neighbouring proton. So when we actually look at this CH2 would go, okay, let's try and find that neighbour. We look down towards this nitrogen species and realized there's no proton attached onto it. So it can't be this. We then look in the opposite direction and we see a quaternary carbon. Quaternary meaning it's not going to have a hydrogen atom attached onto it. So you say, well, where is it getting this neighbouring from them? Because in theory, it should therefore appears as a singlet. It doesn't have any neighbours. But what we

said before about some NMR is that the ability to detect geminal and vicinal coupling. Vicinal meaning neighboring, geminal meaning attached on to the same carbon. And perhaps, maybe what we're seeing here is general coupling, but we want to use further NMR experiments to help us make that conclusion. So let's now look at another peak. Here we have sets of singlets and by meaningful singlets. It contains no neighbouring protons. What do we know about these singlets. Well, they fall within the aromatic region. So we are looking for hydrogens or rotons that are connected onto an aromatic species that do contain no neighbours. So we look at our spectra, our structure sorry, and we see that there are two sets of triazoles and one substituted aromatic ring. Taking the triazoles into account, which we've highlighted here in green, you can see that these green protons do not contain any neighbours. And looking at 1D NMR spectra, they should appear a singlets. So perhaps these are them. However, I said to you to take everything into account. Now, an aromatic proton can only be one, as in the sense of it integrates to one. And why is that? An aromatic carbon can only contain one hydrogen species on it. So if we look at integration here in the aromatic region, why do we see two protons? We're looking at it, we're thinking, OK, there's two protons here. By can only technically have one proton attached on to an aromatic carbon, contains no neighbours. Do I have some form of symmetry in my molecule then that these two protons are identical. There in the same chemical environments to one another and the NMR can't distinguish the difference, but they still also contain no neighbours. Looking at the structure of fluconazole, we'd have two identical triazole rings. So perhaps what we are seeing here is two protons, one for each of the rings. But again, we want to use some further experiments to help us determine that conclusion. Something as well that is too great use as well is this other aromatic set of protons, three of them in total. What are they? Well, we have one remaining ring that we're not really too sure about or we haven't actually started to look at yet. What do we know about these? These are splitting into multiplets. There are three protons like I said to you equating for them, but with one of the multiplets appearing to have two protons associated with it, protons 5, and proton 6. We're then saying, okay, what do, what can this tell us about it? Well, for now, we want to leave as it is. We're just saying that there's three aromatic protons, we're not too sure what and how they see, because perhaps some of these see these, perhaps these both see each other? Right now, we don't know. What we could do is, is that we could divulge into the J coupling and blow up these peaks and measure that the coupling between them. And perhaps, maybe find the distance between these two protons. And been able to say which peaks are able to see you on another. However, in this example, I want to also incorporate 2D NMR. So for this purposes, I'm not going to divulge into that. However, is a technique that you should use when understanding your 1D NMR spectra. So let's start looking at some of these 2D spectra. Let's first start looking at the COSY spectra. Again, not going to go into detail about it, but the COSY spectra is a correlation experiment between neighbouring protons. How did we first start to look a COSY spectra? We first of all start to look at the diagonal. And what we are most interested in now in COSY, is the cross peaks. The cross peaks are telling us where potential coupling is happening. And what do we first of all want to do what? We want to start studying what protons can see each other. So taking the first example, here, that we can now see that protons 5 and or 6 can see number 7. So this is now telling us that these two sets of peaks can see one another. Which proton? Because remember there's two underneath here. Can see number 7, again, you still don't know this. What else do we know about the spectra? Okay, so we look at the next set of coupling and we can see here that protons 9 and or 10 can see 11 and or 12. Okay, that's something that we speculated before. We knew that perhaps they could see each other. Now this is not confirmed in the COSY experiment. What now can the COSY tell us? The COSY now tells us as well that protons 1, 2, 3, 4 and 8 are all COSY silent. Which means that there is no coupling taking place. So it's good to see a good correlation between the 1D as well as the COSY experiment. We already knew that was coupling between these two or these sets of peaks here. And we knew that because of the singlets that there was no coupling. But it's always nice this has

been confirmed in the COSY experiment. So where do we now want to take our 2D experiment? Well, we now want to start to say, okay, I want to know direct coupling. And what I mean by this is. I want to start to understand which proton is connected onto which carbon species. And to do that we need to use a HSQC. In very brief.

a HSQC does not have a line of correlation because on one axis we have a 1D proton experiment and on the other axis we have a carbon. So HSQC is a heteronuclear experiment. What did we want to start to take from this piece of information? We want to start to look to see, well, what carbons are there in my spectra. So the first thing that we noticed is that for proton number 8, if we are to look down on the spectra here, we don't actually see any peak that presented at all for this proton. And because of that, we can make the suggestion that perhaps proton number 8 isn't associated with any carbon. And perhaps it's actually a proton that's connected onto something that the spectra can't see, such as a heteroatom. Now again, because we're fortunate we have the structural of fluconazole. Perhaps it could be this OH species here. Moving on from this, when we start to actually look at the HSQC, remember we read down and across to the carbon species here, we can see for all of these peaks that we just highlighted here are all associated with aromatic carbons. And how do we know that? Because they all fall within the aromatic region of the carbon spectra. What it should be noted is that when you are understanding or interpretating an HSQC, what is key is is that you write down the ppm shifts of those carbons or you give those carbons numbers. So for instance, the green one that is highlighted here, that we can see here. We can say that this is carbon number 1, carbon 2, because this is proton 1 and proton 2. In the blue example, we can then say this is carbon number 3 and carbon number 4 and so on. What else do we want to be able to interpret from this HQSC? Looking at this HSQC, we have on the purple here that 9, 10. 11 and 12 are associated with the same carbon. And I, because remember we looked to at the 1D experiment and it said that these integrated for two each. So it's meaning there's four protons and total we can't have a CH4, but we can, but that is methane. But then that would be the structure over. So it's not that it's two sets of CH2 because they are coming off in the same shift. Because of that then, we then say, okay, perhaps they are identical CH2. And again, this is where it comes into do we have some form of symmetry. in our molecule. Now just looking at the structure of fluconazole, again, we have these two identical CH2. So perhaps one set is from one and the other set is from the other. So, this is as much as we can take from the HSQC. The HSQC is confirming what we've already speculated, but it's helping us now, for instance, understand the, we have some sort of geminal system happening here. So this is telling us, there is a CH2. Going a step further, we want to start to look at well, understood, how a COSY spectra works. So the coupling between neighboring protons. We looked to see which carbon is associated with which proton. And that we want to understand what start to build a map in our heads. How do we start to link all of these things together? And we can use what is known as the HMBC experiment, which is a heteronuclear multiple bonds coherence experiment. And it's read exactly the same as an HSQC. You work your way from your proton and you figured out to which carbon signal it is being able to see. So in this spectra here, black is indicated by the HSQC and red is indicated by the HMBC. I would always advocate that reading a HSBC is always good practice to overlay your HSQC because it offers an awful lot of understanding to the spectra. So what's the first thing we want to start looking at? Well, let's maybe take a look at these peaks down here. What can we say? Well, protons 1 and 2 and 3 and 4 look to be potentially three bonds away because the HMBC can see roughly three bonds away, two to three. Roughly three bonds away, which is good. So we've worked our way from the black and we've worked away till we see HMBC signal, which is located here. How do we know what this one is? Well, there's the black line there. It's telling us that these see each other. So let's start to work again. Now we're going to look at the peaks 9 10 and 11 12. And we've said here that they can both see each other. So looking here, you can see the black for the HSQC, but underneath, particularly this one for example, as well, you can actually see a hint of red indicating that these can both see each other. Now

remember a HMBC can measure typically three bonds away. So what does this look like? We said that these looked to be CH2, the COSY and the HSQC confirmed this. But because the HMBC saying they can see each other, it suggests that they're not next to each other, but they are separated by some sort of atom. Now what this atom is, it could be a quaternary, it could be a heteroatom. Right now we don't know what we know that there's something potentially separating these two. What else can we say? Well, that these CH2s can see peak number H8, so proton number 8. We said before that proton number 8 looked to be some sort of heteroatom. So is this heteroatom in the middle? Again, we're having to speculate. and see. We can also say that these CH2 can also see sets of quaternary carbons. And how do we know they're quaternary carbons? Well, the ones that we've highlighted here is the reason being third black dot on this line to signify that there's any coupling taking place between something already contains a proton on it. Suggesting to us that these are therefore quaternary carbons. But nonetheless, proton number 8 is also shading this coupling between this quaternary carbon. What else can we say about this spectra? Well, H 1 and 2, 3 and 4, we already determined, could see each other by this coupling happening here. But what else can we say about the spectra? Will actually, the CH2 is close to it because the CH2 can actually see this aromatic peak here. So what does that actually look like when we're looking at the structure of Fluconazole. So here we determined from our 1D experiment that we have some aromatic peaks. And these aromatic peaks look to be 1, 2, 3 and 4. We said already these aromatic peaks contained two neighbours. That they were on their own. And this is here represented by this diagram we can see here that 1,3 and 2, 4. Because right now we can't distinguish the differences between these. What I've done is 1 and 3, because one set and one set, because 1 and 2 they are they are the same carbon but on different rings. Looking again, 9, 10,11,12, we said was a potential CH2. And again, we can't determine the differences between them. So perhaps maybe 9 and 11 are part of the same and 10 and 12 are part of the same. Or perhaps 9 and 10 are one of these. An 11 and 12 or one of these. Right now it's making it difficult to make that conclusion. So let's take a look again. So this is just looking at it from Fluconazole's point of view. As in structure. Here, I've labeled the structure, I've only taken part of the molecule we're interested in explaining. The black line is indicated by one coupling length. The red is indicated by two, and the green line is represented by three. And this is present on all of these. The red boxes around it is referred to the red box present on the spectra, and the blue box is referring to a blue box around the structure here. So what do we want to now say? Ok, we have two sets of quaternary carbons here, and it is shared by the CH2 as well as this potential heteroatoms. So how does that actually look? Here we have an aromatic carbon because it falls within the aromatic region. So looking at the structure of Fluconazole and counting for, let's say the CH2 at least, let's count the bonds. 1, 2 and 3 to be able to reach this quaternary carbon. And I've indicated it as being in green when counting for the heteroatom. So the OH, for instance in Fluconazole, we count one bond to the oxygen, two bonds is to the quaternary and three bonds is to this aromatic peak here. So we can say that this chemical shift that is present here, is accounting purely for this quaternary carbon that is present here. So the substituted automatic ring. When looking here at this one. We can then say, okay, well, what's this quaternary carbon? Then we start counting up to. We can say that just by looking that the carbon spectra, its some form of aliphatic peak because of where it falls in the carbon spectra. So again, we can start to do our counting and then I realise actually, that this is accounting for two bonds away. 1 and 2. Because here's the quaternary carbon that's aliphatic, going the opposite direction for hitting aromatic peaks here. So this is the quaternary carbon. So remember I said to you before that the HMBC can measure three bonds away, but it is optimal and it's set to read about ten hertz, which is approximately two to three bonds. So let's now look at the blue structure here. What can we determine from this? The CH2 can only see one set of aromatic peaks. And aromatic peak that's associated with this triazole ring. But which one, which one can it see? What is this carbon signal here? So looking at the bonds again, black, red, and green. Going in this green

direction here for three bonds away, you end up on this nitrogen atom that doesn't contain a hydrogen on it at all. Now that's okay because HMBC can measure quaternary elements, but because nitrogen in the HMBC that we measured was a proton carbon correlation means that this cannot be seen in HMBC. Going in the opposite direction, here now we've reached a carbon attached on to hydrogen species, evident here. So we can therefore say this carbon, this here, is actually this carbon on the triazole ring. Which means that this one, because it is not seen by the CH2, is the one that's furthest away. Which is the one that is present here. So what are the conclusions of what we've learned? Well, we did manage to be able to determine the
structure of Fluconazole. And we're able to assign each of the carbon peaks and the proton peaks to that structure. But how did the experiments help us? The HSQC was key to being able to understand that Fluconazole had perhaps geminal coupling and that the COSY, identified this coupling. The HSMC was very, very powerful experiment. Why? Because it was able to identify the quaternary carbons. And not only that, but make the distinction between which of those aromatic proton, 1 2 or 3 4, where it was on that triazole ring. You also notice as well that the carbon spectra was unbelievably weak. There was high background noise that you saw on it. However, the HSQC and HSMC made up for this downfall. The HMBC was able to see those quaternary peaks, which are often very weak and a carbon spectrum. Anyway, the HMBC though had another one of its downfalls because it was measuring a proton carbon coupling. It could not detect the coupling to nitrogen, but also knows as well, HMBC can be run with a proton to nitrogen coupling. So you can see here that all of the information, the 1D COSY the HSQC and HMBC all enhanced the power to be able to understand the spectra and the structure of Fluconazole. This talk was brought to you by WCAIR, Scotland UK.