So today I want to speak about 2D NMR analysis. What will not be talked about today is how to interpret, say, and when to run a 1D NMR. WCAIR have listed a variety of topics covering 1D NMR analysis and it it is assumed that you have already read and understood that material before moving on to 2D NMR. So what is going to be covered in these learning objectives? So today we are going to be looking at how to understand and interpret some 2D NMR and have some things such as the simple 1D NMR experiment can be blown up to something that is not only on one axis, but two. And how these dotted lines can interpret something as complex as a structure. What will we be focusing on? We'll be focusing on three of what is believed to be the most critical of 2D NMR experiments. And these are the COSY, the HSQC, and the HSBC. What's going to be covered is a small amount of its methodology, how to run it and went to run but also as well, what are some of the downfalls to running these sorts of experiments? So what is 2D NMR? So to be able to understand what 2D NMR, it is critical to already have an understanding of what 1D NMR is. Here is a cartoon representation of what an 1D plot looks like. We have a frequency axis which is donated by the x-axis, and we have intensity measured here on the y-axis. So higher is the 2D plot different? The 2D plot has a frequency both on the x as well as the y-axis was a third axis being donated by intensity, which is represented here by this contour plot. So what is 2D NMR doing? 2D NMR is typically measuring the transfer of magnetization through a series of radio frequency pulses, now being called RF pulses, using scalar coupling or otherwise known as J coupling. So let's divulge more into detail. Here we have a typical pulse sequence. It is listed into four different sections. We have preparation, we have evolution, the mixing, as well as detection. We also have two time intervals donated by T1 and T2. What occurs during T1 is the first of two radio-frequency pulses. And what is on the second is the second of these two radio-frequency pulses donated by T2. But what does an actual spectrum look like? So let's take this example here on the left-hand side. We have frequency given on both the x as well as the y-axis. F1 is present on the Y and f2 is present on the X. So what are we looking at here? In this first example we have F1 is equal to 35 hertz, and F2 is equal to 80 hertz. This is referring to the signal that was generated during the first T1 produced a frequency at 35 hertz The same signal then transferred another signal to something later on, which was present at 80 hertz found at T2. And here is represented by this intensity signal dotted here. Let's then move on to another example. The middle example shows that there's a peak at F1, 35 hertz, and F2 at 35 Hertz. During T1, the initial signal produced at F1 remain unchanged during mixing peanut and therefore continue to produce the signal at F2 of 35 Hertz. Seen here. Now there are two differences between these two spectra. The first is there is no transfer of magnetization and the frequency for both the F1 and F2 remain the same. This is given by what is called on the diagonal or the line of correlation. The first example is where we see evidence of a transfer of magnetization, and in this case, complete transfer. The signal that was generated 35 Hertz as being completely transferred to the F2 frequency, which is that 80 hertz. And this is what can be donated as a cross peak. So how does this actually look in a 2D NMR experiment? And this is where this third plot comes into the hand. Here we have F1 is equal to 35 hertz. And this signal here is producing an F2 of 80 hertz, seemed to us, there's a transfer of magnetization. The peak that appears now on the diagonal is saying that F1, there was 35 hertz and F2, but there's also 35 hertz so there was no transfer, however, because these are both present on the same spectrum. What does this mean? So the signal that was present during T1, which was 35 hertz, transferred some, but not all of its signal during the mixing period to something pleasant at 80 hertz. The other part of the signal remained on unaffected, unchanged, and therefore continued to produce a signal at 35 hertz as seen here. So overall, what does this mean? In a nutshell, something that remained unchanged, stays on the diagonal and something that transferred by magnetization is present on a cross peak. And this is how a COSY spectra, amongst some other 2D experiments also work. So the first of which we're going to start to talk about today is a COSY experiment. What is a COSY experiment? COSY stands for Homonuclear Correlation Spectroscopy. So let's look at the first word, homonuclear. Homonuclear means that it is between the same nuclei

and in COSY spectra, it measures the high abundant nuclei. Now there are a few of them donated here, but the one that we're going to focus in on is what's believed to be one of the most commonly used is the proton experiment. Correlation spectroscopy is referring to the transfer of magnetization, and a COSY typically does it between roughly three bonds. So how does this look pictorially? Here we have magnetization being given to this nuclei signal here. And these other signals here are representing this transfer of this magnetization to a neighboring nuclei, in this case, another proton. How does this look? Well, it's referring to what are its neighbours? This proton can distinguish what are its neighboring nuclei are neighboring protons. And it does this through using scalar coupling, or otherwise known as J coupling. COSY I said toyou, you can measure roughly between three bonds. And what it does is, it has the ability to measure both germinal as well as vicinal coupling. So here, this no coupling is represented by two different, two of the same nuclei on two different carbon atoms neighboring protons. Geminal is referring to where two protons are present on the same carbon. You can see there's already a downfall to this. How do I know which one is neighbouring? How do I know which one is present on which carbon? We'll get into that later. COSY he is also able to do some form of long-range coupling, which can't be donated by this W geometry. W geometry or coupling tends to have a smaller coupling than normal. Roughly about two hertz. It's still small, but it can still be detected. And this coupling tends to happen in some cyclic systems which have this co-planar arrangement that can't be adopted. And in this example here we have cyclic butane represented in the cis configuration, otherwise known as the W geometry. So 1,2,3 and 4. So I wanted to talk about more about, well, how does a COSY spectra become a COSY? So this is just going into slightly little bit more detail from what we previously talked about on the previous slides. So COSY has two frequency axis, F1 and F2. Because it's homonuclear, It means that F1 and F2 at some point will be the same. And this is represented by this diagonal line or line of correlation, meaning any dot that is present on this line is the same in both the F1 and F2 axis. But what they're most interesed in? We're intrested about when there's a transfer of magnetization to find out what is something coupling to. And this is where we get these cross peaks. So here we can take the example in the middle. We can draw a line through the middle to show this diagonal or line of correlation. And we can start to read, what are we reading? We take a line or an intensity signal on this diagonal line and we look up and along. We look to see where there's a cross peak and what is it correlating to. In this example here, we have two doublets. and in here, this COSY experiment its telling you that these two doublets are actually neighbouring. They are potentially, potentially next to each other. Cause remember, a COSY experiment cannot distinguish the difference between geminal and vicinal protons. So overall, what is it that we're trying to actually say here? Well, we are trying to say that anything that is on this diagonal line is present in both the 1D experiments. And anything above and below is the thing that is of interest. Where does the coupling happen? So, what are the downfalls? The downfalls, like I said to you before, is this inability to distinguish the difference between germinal and vicinal protons. And this can be very guilty, is that some of the saturated systems or the aliphatic systems that we can see. Because of this sometimes in a COSY experiment, these aliphatic regions in the NMR can be very heavy, or very heavy populated, busy, and there's quite an awful lot of overlap. Meaning being able to interpret this COSY experiment can sometimes be incredibly difficult. Now these next two points refers to almost all of the 2D NMR experiments. Because you're asking more of the NMR instrument is only natural that you are using longer acquisition times and because there is more information being generated, more sample is often required for these experiments. Something else has to pay some particular attention to is that if in some cases where the coupling is measured to be 0 Hertz, although they could be neighbouring, for instance, they are still COSY silent so you're unable to detect them. So a COSY experiment is looking at the neighboring protons. Now we want to know more about our spectra. We now want to know as well how is this in relation to another nuclei. So this is where we start to look into these heteronuclear experiments. And the first that's going to

be talking about today is the HSQC experiment, standing for heteronuclear single quantum coherence, heteronuclear, meaning between two different nuclei. And it's often between proton highly abundant and another nuclei. In this particular case and throughout this talk, I'll be talking about carbon, but it's not just limited to carbon. You can also use nitrogen, phosphorous. And in some instances fluorine. Ss what does the HSQC measuring? It is measuring one bond distance away. So the direct coupling of. Here we can see in this example, carbon one contains two protons. Proton one and proton one prime. Carbon two contains just proton number two. And the HSQC is measuring which proton is connected to which carbon atom. And how does it do it? Because it's now heteronuclear. This cartoon representation of this HSQC is not giving any indication that there's a line of correlation, but is still giving these intensity signals. These cross peaks are where these two nuclei are actually attached onto. So let's take our first example. Let's look at carbon one and proton number one. How does the spectra read this? Proton one by reading down on a long and along to it's carbon spectra. You can say that whatever is making or accounting for this proton signal here is directly coupled onto this carbon signal here. Now, in this particular example, because carbon one has two protons, 1 and 1 prime, this experiment, can distinguish the difference between geminal and vicinal. Here we can see here that proton one prime, is also read by that same carbon atom, telling you that carbon number one actually contains two proton signals responsible for it and is therefore a CH2. So moving on from the HSQC signal, by being able to identify the direct coupling of something. We want to also start building a picture in our heads. The COSY experiment has already taken us to neighbouring protons, the HSQC has taken us to what are each of those protons directly attached onto? Now we want to start by looking at longer-range couplings. And this can be done through what is known as the HMBC, the heteronuclear multiple bond coherrance. And this example here, I will be talking about the proton and carbon coupling. What can the HMBC do? The HMBC is typically set up to measure roughly ten hertz, which is typically two to three bonds lengths. So what does it look like? Again, because it's heteronuclear by this cartoon representation, there is no diagonal line, just as what was seen on the HSQC signal. And the cross peaks are where these nuclei are linking towards one another. So let's take a look at how to interpret this spectrum. Let's take a look at carbon number one. And in this case we'll look at to what the HMBC is going to measure. So between 2 and 3 bonds, carbon number one, if we start counting one bond, two bond three bond, carbon number one in theory should see proton number 3. So how does this look on the spectra? Here you can see that proton number one, if we read down to the spectra until we find an intensity peak and then read along, we can see here that proton number three can see carbon number one. So let's look at different peak. For example, let's now look at proton number one. Proton number one, counting three bonds away, can see carbon number three. And how does this look on the spectra? So here we can see, proton number one going down onto long, can be seen by a single carbon atom. However, because we already know that carbon number one is that germinal system. It contains a CH2, so two protons. That if we were to look at the spectra again, we can actually see there are two intensity signals for each one of these protons given off. So again, highlighting ability to detect geminal and vicinal coupling. Something that isn't shown in this example, is the ability for the HMBC to detect quaternary carbons. So notice with the COSY experiment, it was limited to just looking at a proton signal, proton to proton coupling. The HSQC was limited by only being able to look at a proton that is connected to a carbon. And what happends to these quaternary carbons? These to are enriched in your sample. How can you measure them? The HMBC has the ability to detect these quaternary carbons. So for instance, if carbon number one did not contain any Protons, Proton number three would still be able to see carbon number one. So just reiterating, what does HMBC measure? It measures optimally between two to three bonds away. However, when should you run it? So by the COSY experiment it needs to be run with another proton experiment because it's using two proton experiments to be able to create its picture. The HSQC needs to be run with a proton experiment, but then you can go straight into measuring the HSQC experiment, so the proton carbon coupling.

The HMBC however, should be run in conjunction with a HSQC. I'm for the reasons of this diagram is highlighting why. So let's take a look. HSQC is given in red and HMBC is given black. Now in order to interpret the HMBC, it's essential that we know where all the carbon atoms are on who they are connected onto in order to start building this picture in our head. So let's take a look. Here again is the same example that we've been using previous. The HSQC, the detects proton one directly coupled to carbon number one. Now, because we know where carbon number one is in our spectra, we know that if we were to count three bonds away, we would eventually reach proton number three, which is annotated here in black. We knew this by simply looking at the structure. However, sometimes you may not always be given the structure and you may have to solve an unknown, for example. So here is where it becomes difficult. The HSQC, like I said, is limited between the detection of ten hertz is its optimal range for detecting two to three bonds away. How can we start to overcome some of these issues? Well, this is where we can refer to some of our other 2D NMR experiments, even once we have learned today, for example, the COSY. So let's take a look at the spectra here. What happens if we were to say, well, what happens if this peak that we are seeing, between carbon number one and proton number three is actually two bonds away. So what I'm saying here is what happens if proton number three is actually in this position here, the middle? How would we be able to detect whether proton number three was two or three bonds away? This is where we refer to our COSY spectra. Our COSY spectra remember, tells us the direct, well not direct but measuring our neighboring protons. So here, if proton number three was actually in the middle, this would be reflected in the spectra. The spectra that is drawn here is showing you the structure that is drawn here. So let's take a look. Shall we? Here is proton number one. So let's look on our diagonal peak here, proton number one. So this is where both F1 and F2 are the same. We talked about measuring a COSY experiment, where we look up and along or down and along until we see some of these cross peaks. So let's see where our first cross peak is. If we go up and along from here, we come to another line on the diagonal telling us that this is responsible for H one prime. What's this measuring? It's measuring the germinal coupling. Now let's look to see if there's any other coupling that is evident. So let's look here. Proton number one can be seen also by this signal given off here. And if we scroll along back to the diagonal, we can see that the proton signal is responsible for this is actually proton number two. So in this example, what have we determined? The HMBC is actually reading three bonds away because of the signal that is given off here. How is this actually measured? We can see in the COSY experiment that this is highlighting that this is true. That proton number one can see proton number two. So in summary, we covered today, we covered some of the most essential, are believed to be the most essential 2D NMR experiments to at least start you off on helping to undertake some structural eludication. We went through the COSY experiment, which was typically homonuclear. And in this example we talked about the proton-proton coupling. We also talked about two heteronuclear experiments such as the HSQC, and HMBC. And here is just a summary of what has been covered. The HSQC the direct coupling between a hydrogen and carbon. Whereas the COSY is the coupling between homonuclear neighbouring protons, and the HMBC is a longer range coupling between proton and carbon. However, these 2D experiments, these are not the only ones you can run and what hasn't been covered today is through some space correlations such as the NOESY and the ROESY experiments, which will be discussed in later talks. So its hoped that when you're undertaking just structural indication that not only running just a simple proton and possibly carbon experiment, but you also consider the use of some of these 2D NMR experiments. Their run times maybe longer, but it's well worth the wait. This talk was sponsored to you by WCAIR at the University of Dundee, Scotland, UK.