

What we're about to go through today is through space correlations. And how the NMR can help us identify these.

Now before you progress is already believed you have undertaken the study of 1D NMR and how to interpret this. And also as well other 2D experiments such as the COSY, HSQC and HMBC experiments, which have been identified in previous talks. So what is it that we are going to be learning throughout this talk? We're going to be learning how the ^1H - ^1H coupling causes a through space interaction that we can actually identify through the NMR.

It's useful because this can help us identify stereochemistry as well as geometry about our molecule. Which is not only important from a functional aspect, but also as well for protein recognition. So what is NOE spectroscopy? We want to be able to understand how to interpret it, where does the information come from and when should we be using it. So an example at the bottom is of two compounds with identical in structure, but they vary in one position only. And this is given by ^3H , which is located here on both diagrams. You can see here that the protons in this diagram are on opposite faces, whereas the protons in this diagram are on the same face. And the NOE, or the NOESY experiment can help identify this. So how does it do it? Well, first of all, we need to be able to understand what is the nuclear overhauser effect and why is it important to me? So the NOE is through space interaction, which is otherwise known as a dipolar coupling. And it's not to be confused with through bond correlation, otherwise known as J coupling or scalar coupling, which you have previously seen when interpreting the COSY, HSQC and HMBC experiments. What the NOE does is what I mentioned previously, it tells us about stereochemical information, about our compound. But in order to understand stereochemical information, we must already have a rough understanding of what is our spectra is already trying to tell us, which means that you actually rely on other NMR techniques in order to be able to understand these NOE are NOESY experiments. What do I mean by these basic experiments? Well, I mean, for instance, like a 1D proton experiment.

For instance, may be even using COSY, HSQC to try and help make that determination of what the molecule is and how it's put together. The NOE is typically optimized to measure about five angstroms through space and to help with understanding what NOE is, I've taken this very simple molecule located at the bottom. Here you can see there are two protons on the same face, and these are separated by four angstroms. We need to be able to learn some terminology here. So the first, we're going to take the proton in black. And this proton it is what is known as the source spin or otherwise given letter "S". It's important that you remember this "S" was all be used in future examples. The proton that's located in blue is known as the interesting spin otherwise given the letter "I". So what do we want to start learning about it? Well we take the source spin and what we do is irradiate it at its Larmor frequency.

And what is a Larmor frequency? Well, in brief, the Larmor frequency is a frequency to which the nuclei will exhibit motion. And for the purposes of this talk, I'm actually not going to go through what the Larmor frequency is further. It will be mentioned in previous talks, as well as the references outlined at the bottom of this slide. So as the radio-frequency has been exhibited to the source spin, what does this look like from an energy diagram? Well, in its happy state, as in before we have given this pulse, you can see that there is a low energy state and a high energy state. And there population differences between the two. You can see that just from this happy state, shall we say, is that there are higher number present in the lower state than there is in the higher state. Something that you should pay particular attention to is these numbers. These numbers are just arbitrary, are not a true reflection of the millions that will be located into each of these different populations. So once this pulse has been applied, what we see is a change or the same happening in these energy systems. And what happens is in the low energy

system that we see is that the nuclei, gets excited and move into this high-energy state. And in doing so, it causes this equilibrium between the low and the high state. Now in previous talks that you have learned NMR is all but measured the difference between something often referred to as relaxation that measures the difference from relaxation. And here what you can see is that the NMR will show this as something that is silent. It can't see what is occurring. But when we switch this pulse off, what happens is, is that this state starts to revert back to its happy state again. And this is what the NMR can measure, i.e. it's all about relaxation. So let's take another step further and let's look at these scary energy diagrams. Again we've taken a molecule similar on the slide previous, but this time what we've done is we've added the interesting spin or the proton of interest onto this CH₃ against the distance is still referred to as four angstroms. So here is an energy diagram. If you've not seen one before. This down at the bottom is a low energy state and is often referred to as alpha alpha. At the top, we have a high-energy state donated by beta beta. And in the middle we have alpha beta, and beta alpha. What do we mean by this? Well, and say that our numbers located at the bottom. So what we learned before by the population of each energy system. But when we mean about high-energy state, what means as we mean with field or against field. Now with field typically refers to an arrow pointing up and against field refers to it pointing down. The down means that there's a higher energy associate with it. Hence why its found in higher energy system. Now what is it that we're wanting to measure or look at? So let's start to look at the spectra because we're not actually going to be drawing this out when we're actually using the NMR, we're going to be interested in something that looks like this. So the first of the transitions that we would like to measure is what is known as the double quantum transition. And what do we mean by this? Well, it refers to the movement between the beta beta, or the high-energy system, all the way to the low energy system, otherwise known as alpha alpha,.

It is called double quantum because with the spin, the source spin, sorry, in black and interesting spin in blue, it takes with it both of the nuclei to, to make it go up field? And how does the NOE look? So what happens is because I said to you, anything that's in close proximity, you are irradiating the source spin, which is in black, and you're effectively measuring the interesting spin. Now what we see here is that with this double quantum transition, we are actually seeing a positive NOE, whereby the signal starts to increase. And we'll explain more as to why this is positive in the next slide. The second of the transitions that are important for us to remember is the 0 order Quantum Transition, otherwise known as W₀. And this occurs between the intermediate energy levels, the alpha beta, beta alpha. And remember, what we are actually thinking about here is we're always wanting to get the source spin back to it's happy state. So you can see here it was against the fields on, it's now withfield. And here It was against field and now it's with field. And what happens is, is when you are measuring a 0 order quantum transition, you actually see a negative NOE. Now something to bear in mind is that small molecules tumble quickly and they are the ones that produce these positive NOEs and large molecules tumble slowly, so give these negative NOEs. Now, let's start to look at this in more detail. Let's take that same energy diagram again. Now remember, we're only interested in measuring the interesting spin, given by the blue arrows. You see the population differences between them with the lowest population present in this high-energy state here. Now when we produce this pulse, gives the source spin its pulse. What we see is a difference now, as in the nuclei started to move into these different energy systems. And what can we see here? Well, we see that the alpha, alpha state has lost. The intermediate of this site here has gained. This intermediate side has lost, and the high-energy system has gained. Now how do we know that's what was going to occur? Remember I said to you in previous slides to remember what S And I for? Well, here is the S transition, the source spin, and here is the I, the interest spin of what we are monitoring. So bearing that in mind, this is what is happening to the source spin. But now we want to see how did these transitions affect what we see in the NMR. So let's start with

that W_2 double quantum transition. Now remember the I, the interest spin, is what we're measuring. When we talked about the W_2 what it is the drop in population between the high energy to the low energy. Therefore, the high-energy will lose and the low energy will gain Now because we're measuring the interesting spin, we want to measure the difference between these populations. But first, we need to be able to look at, well what was at the beginning? So taking the difference between these two here, you can see that the difference of 8 separates the interesting spin in the ground state. This W_2 transition here, now the change or the difference between these populations is now 10. Therefore, there has been an increase, which is why we see a positive NOE. Let's look at it in a different transition, which was the W_0 or W naught. And here, what we saw previous was, was the change across the intermediates. Which means in this state here will lose and in here, shall gain. And this is evident by this diagram here. Remember when we're talking about the difference in populations, we're still measuring the same transition for the interesting spin. And if we are to measure this difference, we will see the difference now is 6 less than what was present in this ground state here. And therefore, we can say that W_0 transition has a negative NOE. So how does this actually look in spectral form? So let's take, this molecule again, a slightly different reiteration from previous slides, hence why the carbonyl. Now, I'd like to outline as well that this is a very simplistic diagram. Everything has been donated as a singlet, which is in theory not true. These are all CH_2 . But for simplicity of the diagram, apart from this one actually is really just a CH , I have given it just as a single proton that's being measured. And what are we interested in? Well, we would like to know the configuration of which CH_3 is which. So what we will start to do is we irradiate each CH_3 at a time to tell us what's happening. If we irradiate the first, what we see is a disappearance in the green and blue signals, but the maintenance of the red and the amber. Now because the NOE is going to be showing us anything that's in close proximity to the thing that we have irradiated, that this CH_3 must be close to, and can see, the red and the amber. If we do the same to the next CH_3 , we can see that there's a disappearance of the reds and the emergence of the green and blue. What does this tell us? Well, this tells us that this peak highlighted in blue is actually the CH_3 here. Why? Because we can still see this red peak in the NOE difference spectra. And the peak that is indicated in red is actually this CH_3 here.

And why? Because we were able to see the green and the blue because it is within close proximity to the CH_3 . So this is the NOE difference spectra but I said to you that there was some 2D experiments that we could also learn. And this is what referred to as the NOESY experiment. Whereby the difference being is that the NOESY records all the NOEs for a single experiment. What it does is that F_1 and F_2 are equating to a 1D proton spectra.

Very very similar to a COSY. This therefore means that there's a line of correlation across the diagonal which is indicated by a positive NOE, and the cross peaks are negative NOEs. Now the cross peaks are referring to the interesting spin. These are the ones that we are interested in. So let's look at this with a nice diagram here so that let's take this molecule here. And the key thing that we're wanting to know is, what is the stereochemistry of H_5 and H_{10} by using the spectra. So let's take a little look. Again, what we see is, is that we've got these cross peaks which appear above and below the line of correlation of our simplistic reasons, i have not or are not going to take into consideration until it falls below the line as it's matched above. So let's take for instance, H_4 , and we already know its stereochemistry, but we're interested in the H_5 , which is the one next to it. Now we look our spectra, we say, okay, can we see any cross peaks? Anything is telling us that there's an interesting spin occurring and therefore it's in close proximity to this H_4 and indeed there is. We can see here that its H_5 . The facts that H_4 can see H_5 tells us that they're in close proximity. So represented on the diagram, you can therefore add in the stereochemistry for this proton as it is present on the same face as H_4 . Let's look a step further unless

take H10. The fact that H10 can also see H5 also tells us that H10 is on the same face as H5. And in doing so, it can get this assignment of stereochemistry. So by using this 2D experiment, even though we already knew the assignments of the 1D. Now remember I said you that NOESY experiments can't be used on their own, used in conjunction with other experiments. But because we already had this assignment, we were able to very quickly start to measure our NOESY experiment and tell us what the stereochemistry was of this molecule, here. This was a very quick introduction to through space Correlation Spectroscopy. There are other experiments available to you, such as the ROESY and TOCSY experiments, but these have not been mentioned here. What should be noted is that the ROESY experiment, for instance, how it differs between the NOESY, is that it can go and read further into the molecule. So it can extend that a bit further than the NOESY. The NOE difference spectra shows how individually irradiating a peak shows only peaks are in close proximity to that source spin. So the interesting spins if it where.

it where. The 2D NOESY is read fairly similar to the COSY and this is why it was advised that you also read and understand the 2D NMR COSY sections before you are to move on to the likes of the NOESY experiments. There is a line of correlation, very similar again to the COSY and there's a donated by the difference in the NOEs. I said previously that small molecules tumble quickly and therefore produce a positive NOE, whereas large molecules tumble slowly and therefore produce this negative NOE. And remember the thing that we are always interested in reading is the interesting spin. Otherwise donated by I. The talk was brought to you by WCAIR, University of Dundee, Scotland, UK.