Principles and Applications of NMR spectroscopy

Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 5
Lecture No 22

In the last class, we started with 2D NMR spectroscopy. We saw how basically how it helps to resolve overlaps? How it helps to correlate to chemical shifts in for example carbon and proton? How it helps to correlate two protons which are coupled to each other and these are the various advantages of 2D NMR and much more which we will see down the line. what, let us have a look again to what we actually how we record a 2D experiment. So for that let us go back for some time to 1D NMR.

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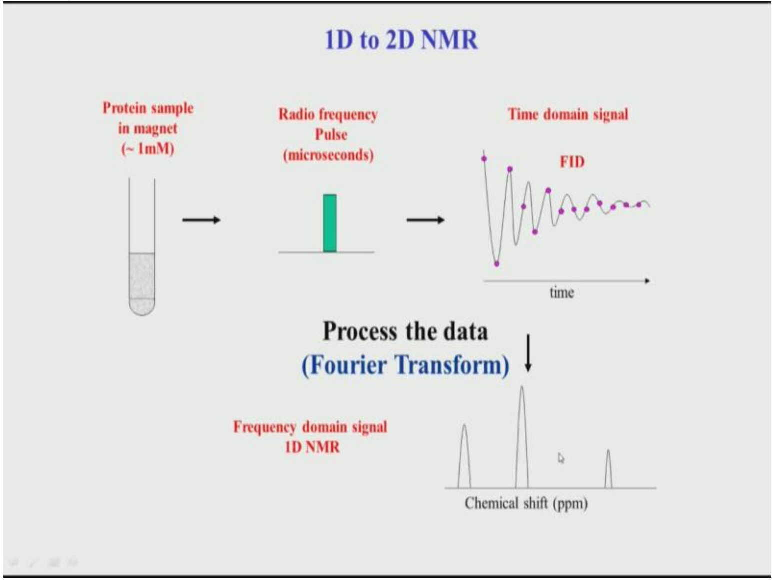

1D to 2D NMR

Protein sample in magnet (~ 1mM)

Radio frequency Pulse (microseconds)

Time domain signal

Process the data (Fourier Transform)

Frequency domain signal 1D NMR

So this is shown here, so we in 1D NMR, we saw that first you have a sample which is a typically in NMR tube which is of this 6 5 mm NMR tube and typically the concentration we use is about a millimolar and this sample we is put in a magnet and then we record 1D NMR experiment applying by applying a pulse. It is a RF pulse which is about a few microseconds long. Once the pulse is applied and the magnetisation starts relaxing back to z-axis and in the xy-plane it starts oscillating.

So this is what is captured as an FID and this oscillation is nothing but the chemical shift of the atoms and this decay is because of the relaxation. So as molecule is relaxing atoms are relaxing their intensity in the xy-plane decrease because they start dephasing and also their magnetisation along z-axis starts building up. So this decrease is basically a damped exponential and that is what we see here and the oscillation is the chemical shift that is the frequency in the xy-plane.

And then this FID is then subjected to Fourier transform which is a mathematical technique which separates now resolves this FID into the frequency components. So the FID may not be a simple sinusoidal, it may have a mixture of sinusoids different frequencies and different chemical shifts from different atoms in the same molecule. So that those frequencies are all together present in the FID but by doing a Fourier transform we can separate them into peaks. So this is basically what we saw as far as 1D NMR is concerned. So 2D NMR now we extend this concept little bit more.

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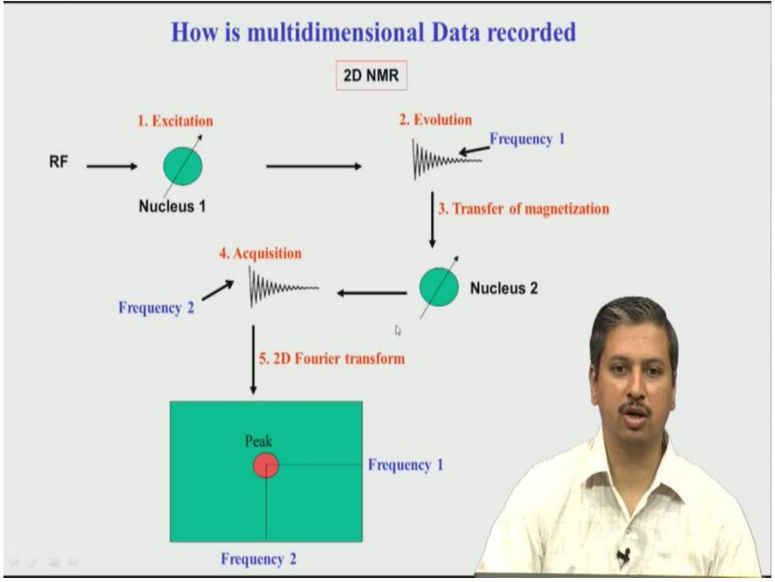

How is multidimensional Data recorded

2D NMR

RF

1. Excitation

Nucleus 1

2. Evolution

Frequency 1

3. Transfer of magnetization

Nucleus 2

4. Acquisition

Frequency 2

5. 2D Fourier transform

Peak

Frequency 1

Frequency 2

So when you go to 2D NMR, you start now from again a RF pulse just like the 1D and you excite means you apply to a nucleus. Let us say we have a nucleus 1, some nucleus or some atom in the molecule, hydrogen atom. So you apply excitation pulse on that nucleus, then as soon as the pulse is applied and after the pulse is removed, like we saw in the 1D, the system now starts to relax back to equilibrium.

And that, during that period again there is an oscillation. So in the 1D NMR, we captured this as a FID but in 2D , we do not do that. You do not take the FID at this stage, you let it evolve, you let it oscillate like this in the xy-plane and let it decay. So we allow it to do that but what happens is during that period when this is happening, we use the word evolution. So we say that the chemical shifts, which is oscillation is evolving means it is happening in time.

Now during that period, this nucleus 1 maybe couple to another nucleus by J-coupling. So remember we saw the J-coupling can be two bond in case of proton or it can be three bond the also in case of proton whereas in the case of carbon and hydrogen, it is one bond. So right now, let us focus only on hydrogen to hydrogen coupling because for carbon you cannot do with the scheme which I am showing now. For that you need a slightly different scheme.

So we will now look at only the proton to proton correlation. So in this case, when you excite one proton, it starts evolving because of this chemical shift and during that period, it starts also transferring its magnetisation to a second nucleus because of the J-coupling. So this happens spontaneously, there is no need for any anything to be done from the spectrometer side, no pulse to be applied. It just during the period when it is in the xy-plane, it starts transferring the magnetisation to the neighbouring spin, neighbouring to which it is coupled.

Now once it has transferred the magnetisation, it will now, we have sitting at the nucleus 2 is now ready, so if I excite second nucleus by a second another RF pulse then the energy or the magnetisation which has come from here to here, now the nucleus 2 starts evolving so basically you can see there are like a two 1Ds. I apply 1D here and another 1D here.

So the second pulse which I apply, now brings the nucleus 2 in the xy-plane because all the time this was z-axis as we considered and this we considered along x-axis. So during the transfer, it remains along z-axis, that is nucleus 2 but when we apply a pulse on nucleus 2 now, this will become this will come on the xy-plane and that now starts evolving. So this is now captured in the hard hard way and this is the FID which is recorded or detected physically by the hardware console electronics. Ok?

So this portion was not captured but this is captured. Now what has happened is that when this is evolving, this intensity, so if I, the intensity of this evolution at what time it transfers to this is that is captured in this FID. So this FID, although it has only the oscillations of the nucleus 2, it also has the information of nucleus 1 because when the nucleus 1 was going evolving, during that period, it had transferred the nucleus to magnetisation to nucleus 2.

So that evolution of nucleus 1 is indirectly indirectly present in whatever transfer which happened and that when you further excite and create a second FID, that information of this FID is actually present in this. So this mathematically, we will see a little bit later but the idea here is that we are not directly detecting the FID here, we are indirectly transferring that to this to this one and then detecting this FID and doing a Fourier transform.

So when we do a Fourier transform now, we will not do a simple 1D Fourier transform like we did in 1D NMR, we will do Two dimensional Fourier transform. Why? Because this is also one time domain, so when it was evolving, it was also time and then here also it is time so we actually had two time evolutions. One evolution for nucleus 1 and the second evolution for nucleus 2.

So therefore, the data which you get is not a one dimensional data now, it is basically a two dimensional data because for every point here, you have a corresponding point here. So we can we will see more in a in a short while, how mathematically this happens but the idea here is we have two time domain data, two FIDs, one is indirect, means not detected, one is direct which is detected.

So this matrix is like a matrix of points, if you subject it to Fourier transform, you will now get frequencies on both axis. So now in 1D NMR if you recollect, we had one time domain one time domain which was converted to one frequency domain and 2D NMR we have another time domain, now which is plotted along y-axis and that has a frequency also because every FID is basically oscillation which is again a frequency component, that frequency is along this axis.

So now if the nucleus 1 has the frequency 1 and nucleus 2 has frequency 2, now what has happened is because of this joint FID kind of detection, you will get now if you do a Fourier transform the frequency to which comes here like a 1D. Frequency 1 is also 1D but now these together, they will give a cross peak at this position which is corresponds to the frequency 1 here and frequency 2 here.

So this is very similar to the analog analogy we saw in the last class, that if I plot name on one axis and surname on another axis, I am actually correlating all the name with the corresponding surname in a 2D. Similarly here, if I have one atom with 1 frequency in molecule, second atom with another frequency and if these two are (())(8.58) in this case by J-coupling then there is a J-interaction between them through J-coupling and that interaction results in a transfer of magnetisation between the two and that is therefore they are now becomes one unit they are coupled to each other, so their together they are captured as this tube cross is a cross peak.

Now this cross peak has 2 frequency, each corresponding to the one nucleus. So this is the basically the general idea of a 2D NMR and there are many many variations of this as we go go along, we will see that. Now let us see how physically, I mean this is a schematic, this is all was done as a qualitative explanation. Now actually in NMR instrument, how do we record or how do we carry out a 2D?

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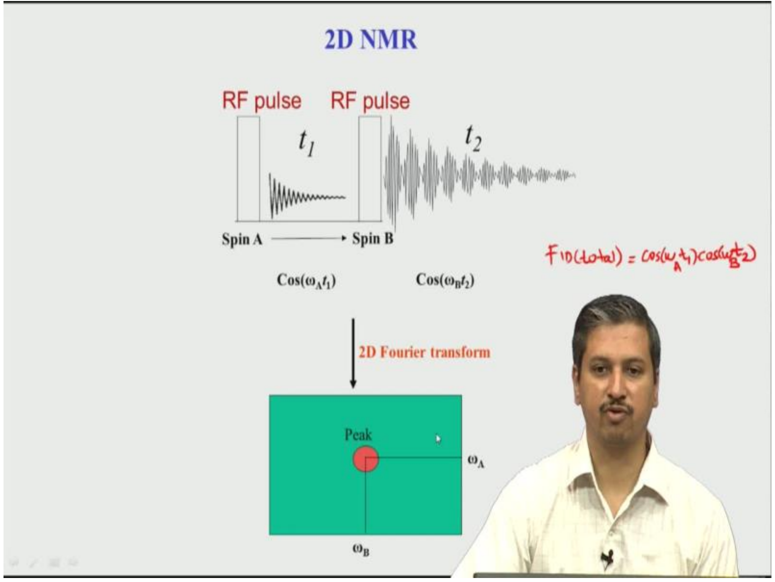

2D NMR

RF pulse RF pulse

t_1 t_2

Spin A → Spin B

$\mathrm{Cos}(\omega_A t_1)$ $\mathrm{Cos}(\omega_B t_2)$

$FID(total) = \cos(\omega_A t_1)\cos(\omega_B t_2)$

2D Fourier transform

Peak

ω_A

ω_B

So we will see here. So this is what we start, again the same if you go if you remember the last the previous slide. We saw that we apply first an RF pulse. Now here we are not showing to which nucleus you are applying. So let us make it a very general scheme right now. So we are applying generally a pulse which will affect all the protons. Ok? So it is not only applicable to one particular proton, it will be applied to all the protons in the molecule. So all of them now get excited, means they start they come to the xy-plane.

When they do that, they start evolving. So this is what we saw and there is a chemical shift evolution and that we call it as a time domain t1, so this is time, so x-axis. So when is evolving during that period it also transfers the energy of magnetisation to the second nucleus and now the second nucleus at this point you apply another pulse which will now excite the second nucleus and it will come now to xy-plane and that will start now evolving. Ok?

So this is similar to what we saw in the previous slide but this is now coming more to the actual way we record NMR experiments in the spectrometer. So we can call this as spin A then it goes. So now let us see at the mathematics of it. So we have spin A which is basically excited let us say, so in first case we talked about generality, now we will go to a specific spin. So let us imagine that you are applying this RF pulse, the first RF pulse to a spin A, one molecule one atom in the molecule.

Now that will start evolving by chemical shift, so that evolution remember if we recall we saw in in the 1D NMR part that this is actually written as cosine omega t1 or t , cosine omega t where omega is the chemical shift or frequency and t is the time; and there is also another factor which is this decay, exponential decay but for time being we can we can ignore it in the for the sake of the actual description, it does not matter so but remember also there is one more factor here which is exponential decay because of the relaxation.

But now coming to this part here, so A is now the spin A. So we are talking about the chemical shift of one spin A and t1 because we call this part as t1, so this is just a mathematical notation to label this variable. So this is a variable means it is varying and therefore we call it as t1 and (())(12.24) t , the second t is t2. Now after this transfer because of the J-coupling interaction and the magnetisation has gone from here to here.

Now I apply the pulse on spin B and now that will start evolving oscillating in the xy-plane with its chemical shift Omega B. Ok. So this is now captured as a FID physically. So this is what is detected physically but this is not detected physically. So now if you see here, my final FID now will be actually a multiplication of this multiplied by this. Ok? So let me write it mathematically here. So my final, I will say FID total is basically Cosine Omega-A t1 into Cosine Omega-B t2.

So this is the main point here, that what has happened that if you see mathematically, we have basically multiplied the two FIDs, ok? So now the total FID, so if you look at now look, focus your attention on this part here, Omega t2, if you see the Omega t2, the intensity of this frequency which is the amplitude. We are now talking about this amplitude is multiplied with another factor. Ok?

So therefore, this intensity is now having the chemical shift information of A because this factor which is Omega A, cosine of Omega A is now modulating the second FID. So we use the word modulation. Modulation means modifying or changing, so it is this the the second the FID which is detected that is during t2 is now being modulated by the FID during t1. So you see now here is where the interlocking in the sense interconnection comes.

Now these two have got interconnected to each other. Ok? So now if I do a Fourier transform of this FID mathematically; what I will do is I will not do a single fourier transform, I will do Fourier transform two times. First I will fourier transform along one that is t1, so if you look at t1, it is also modulated by t2. So you see this is called intermodulation, both of them are modulating each other, that means any FID I detect for B is now affected by A and any FID along A is affected by B. Ok?

So mathematically now they are connected to each other. So when I do a independent I do a fourier transform along t1 which is for this FID, I will get Omega-A. Ok. but if it is also affected by Omega-B, so if do another fourier transform along Omega-B, I get the respective chemical shift Omega-B. But because these two are affecting are intermodulated, the fourier transform will give me two frequencies. One frequency for a this, one frequency for this because t1 is different from t2.

So in a mathematical sense, you will now get, end up with getting a frequency now on one side, on one dimension that is around t1 corresponds to the A because it is coming from this factor; and along B, along second frequency dimension, it is coming from the second frequency. So basically this is the whi thech the central point which you have to keep in mind, that when you have in a 2D, the two FIDs now are modulated by each other.

So although in in real time in actually when we do when we first get this FID then we go for this FID but in a mathematical sense, they are now all intermodulating each other. So therefore there is a connection between the two. So that means if I have a unique frequency A in the molecule and have a unique frequency B in the molecule and if those two are connected to by J-coupling because of that only we are getting this.

So when I do a fourier transform, this peak will be unique now to that particular two atoms, because those two atoms have a very distinct chemical shift. Now in in a molecule like this we may have several pairs. Ok? So I have just given an example of one pair and and we looked at one pair but like that I may have C and D for example, so I may have Omega-C Omega-D.

Again those two hydrogens coupled to each other so you will get a cross peak from some anywhere here, some other frequencies where that corresponds here to C and this may correspond to D. Ok? So that is basically how we generate at a cross peak, this remember although we call it a peak, we use specifically the word cross peak. We will see that how it looks in the overall 2D spectrum but the cross peaks are coming because of this point in that two frequencies are now connected to each other because the atoms, respective atoms are J-coupled to each other.

So in this way, every pair of atoms which are connected to each other by J-coupling will come in the spectrum. Ok? So that is basically how we get the correlation in terms of chemical shift between two atoms.

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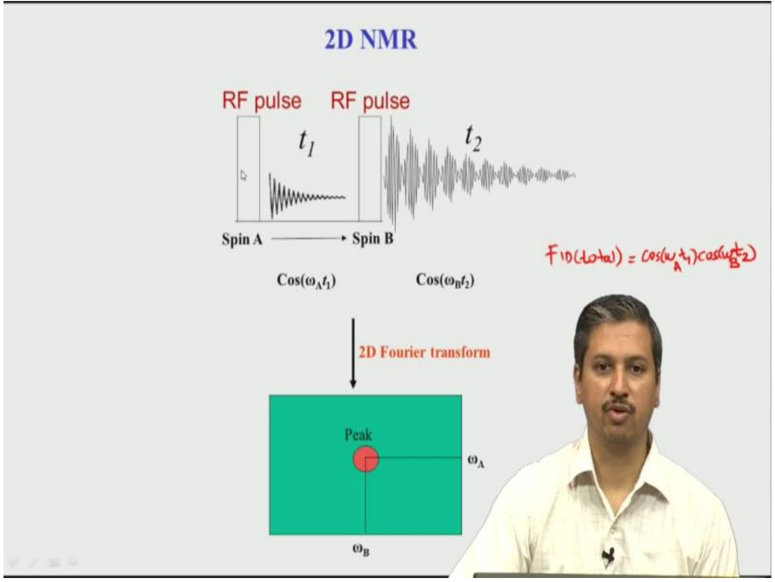

2D NMR

$\text{Cos}(\omega_A t_1)$ $\text{Cos}(\omega_B t_2)$

$FID(total) = cos(\omega_A t_1) cos(\omega_B t_2)$

So let us go further and see now what happens. So one thing you have to remember is that in the previous slide, here we looked this as spin A and we said this is applied to spin B but in reality, this is not the case. In reality, this pulse affects both A and B at the same time and this pulse again affects both A and B at the same time.

So you see there are both A and B get excited here, both A and B evolve here, both A and B transfer to each other and both A and B now are excited finally and both A and B will evolve here.

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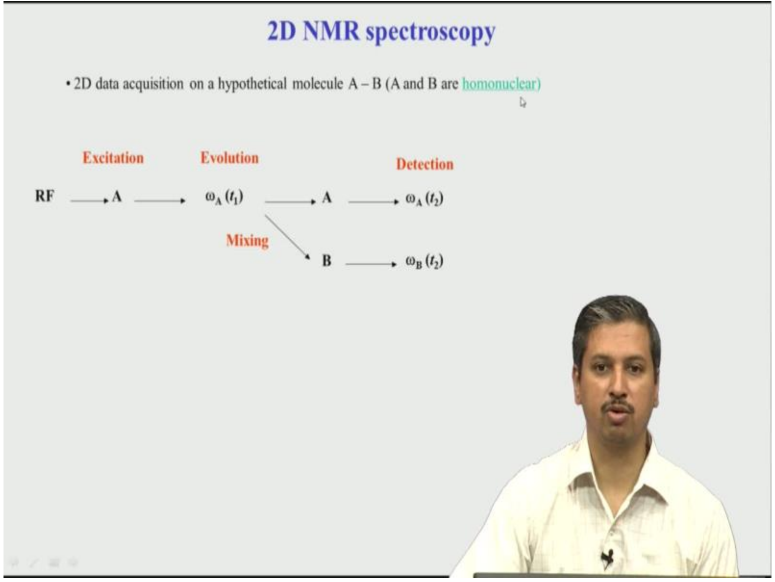

2D NMR spectroscopy

- 2D data acquisition on a hypothetical molecule A – B (A and B are homonuclear)

So you see that is what is shown in the next slide here. So that is what happens, we start, let us say we consider spin A, the A side. So we have excited A, it evolves by its chemical shift during t1. Now the second pulse is called a mixing pulse.

Why is it mixing? Because it has now excited B, but remember it has during the evolution it has transferred the magnetisation to B. So therefore it has, when we excite the second pulse we saw that B gets excited but that process of actually starting from A transferring it to B and then exciting B, we use the word mixing. So this is how so this is mixing is different from evolution.

During evolution the only thing happening is the chemical shift is evolving and there is a transfer of magnetisation but actually during mixing, we transfer the magnetisation from here to here or the coherence is basically interchanged. Now, what happens is when it is evolving, it may not transfer complete magnetisation to B. It may keep some magnetisation with its own and this is typically what happens in homonuclear cases.

So we are looking at proton proton now, so in a homonuclear NMR 2D NMR what the magnetisation which is supposed to transfer to B does not completely transfer. It keeps something with itself. Ok?

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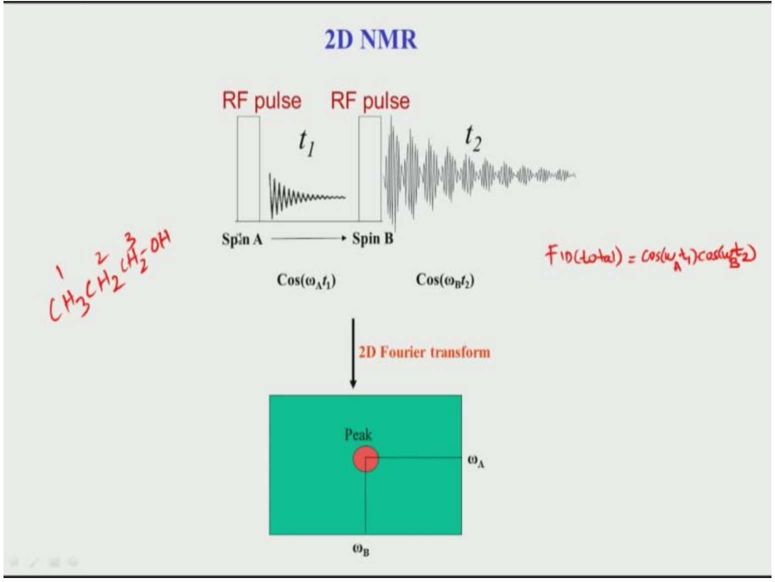

So that is what happens is, therefore when after if you go back in this picture here, in this particular time here where the arrow is pointing at this time now the magnetisation is not completely gone to B, it is still some of it is remaining in A.

So this portion, the portion which goes to B can be as little as 5 percent means it can happen that 95 percent remains only on A and only 5 percent has gone to B or it can go as much as 50-50, 50 percent has remained on A and 50 percent has gone to B. So what it reminds this kind of efficiency, so we use the word transfer efficiency. So this is a very standard nomenclature jargon in NMR. this, what is the transfer efficiency?

See the transfer efficiency is basically how much percentage of magnetisation A during this period evolution has been transferred to B. Now that depends on variety of factors. Number 1: It is basically the strength of interaction. So if spin A and spin B are nicely well coupled to each other then in that case the efficiency is high but if they are very weakly coupled, then the efficiency is low. So basically one of the main factors is it depends on the J-coupling of the spin.

Second factor which matters, is the relaxation. So it may happen that this molecule relaxes very fast. So, by the time I give a time delay here time gap here, by the time this time is over, the spin has A has completely died down because of relaxation. So in that scenario it will not be able to transfer any magnetisation to B. So that is another actor determining the transfer efficiency. So basically it is J-coupling and the evolution.

And third parameter is which is more little affects in many cases is called passive coupling meaning when spin A is interacting with spin B, there is another spin C which also interacts with A. So what is happening is, A's magnetisation now is getting divided into two into two other protons. Ok? So it is basically one another proton is quietly drawing the energy from A or by coupling whereas our main focus was to go to this side. Ok? So that is called passive coupling.

That is because A has multiple couplings, it may not be just that it is coupled to one proton. It may be coupled to another proton. For example, consider this molecule propenol. CH3 CH2 CH2 OH. So let me draw this to give you an idea, so this is this. We looked at ethanol all the while, let me now consider molecule propenol. So here, we have three protons. One, two and three.

So what happens is when I when this proton number two, it may while it is when you excite this proton, let us say this spin A is this proton number two. After exciting this proton its FID during the evolution, it starts transferring its magnetisation, not only this side but it is also coupled to this side. Ok? So you see this is the concept of passive coupling.

When I am considering this as an active, a pair this pair I have to now see that this pair is now draining away the magnetisation. Similarly, if I consider these two as an active pair, this is getting drawn away from this. So basically this magnetisation now is getting divided, so its efficiency of transfer to this or this is reduced but if you consider these two hydrogens, 1 and 3, they have only they are only coupled to 2. So for them, you can have a very good efficiency because they are only unidirectional.

Unidirectional means they are only going to this side, there is no other spin which is taking away the magnetisation. So this is how we understand, how the efficiency matters and this is a very very important concept because one of the main area of research in NMR is to maximize this transfer efficiency; because the sensitivity depends very much on how much you get the transfer to be.

So for example, if I keep 99 percent on A and I can transfer only 1 percent to B, my system is very weak in intensity. My B spin energy when I finally get this FID, will not be very good signal to noise. So optimizing, increasing, maximizing this transfer is a important area of research.

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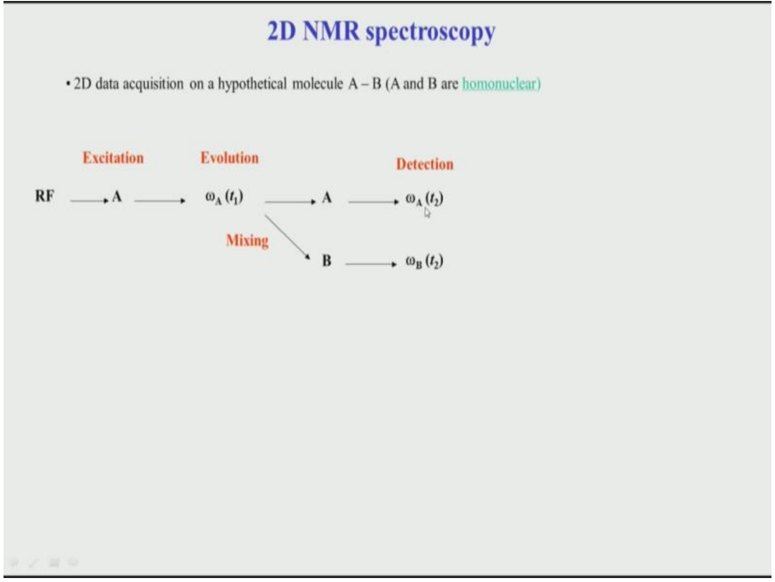

2D NMR spectroscopy

- 2D data acquisition on a hypothetical molecule A – B (A and B are homonuclear)

So this is basically what is shown here, so you have the A has kept something with its own, it could not transfer completely to B and now when I apply the second pulse.

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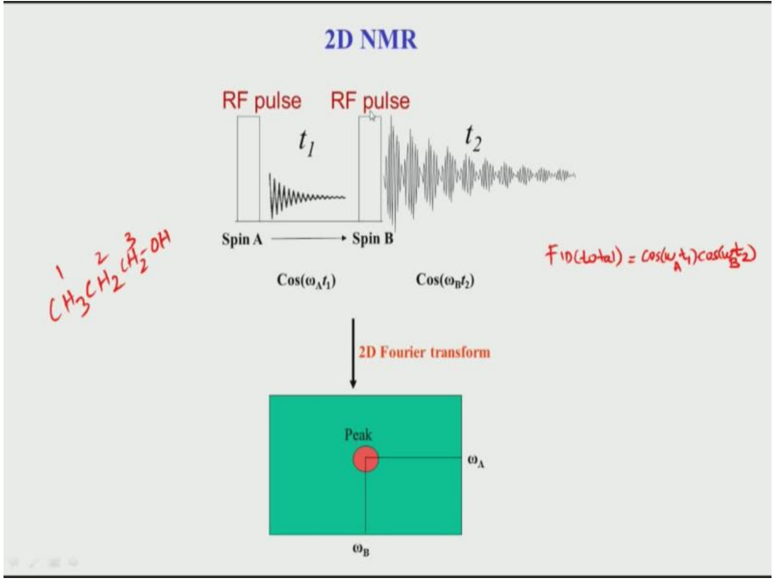
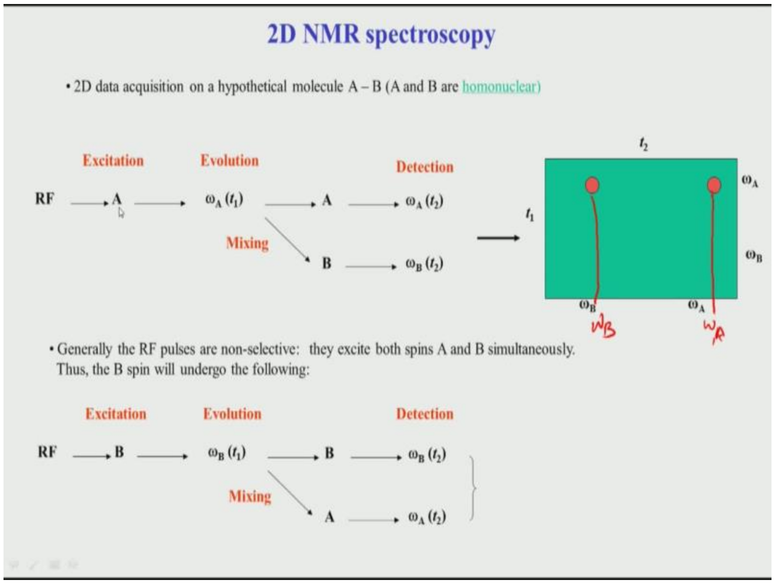


2D NMR

$\overset{1}{CH_3}\overset{2}{CH_2}\overset{3}{CH_2}-OH$

$FID(total) = \cos(\omega_A t_1)\cos(\omega_B t_2)$


2D NMR spectroscopy

- 2D data acquisition on a hypothetical molecule A – B (A and B are homonuclear)

- Generally the RF pulses are non-selective: they excite both spins A and B simultaneously. Thus, the B spin will undergo the following:

So now let us go back to this picture. So when I apply this pulse, the second pulse, it is going to be applied on both A and B because as I said, we do not consider A and B as separate, we call all of them are hydrogens so the pulse is applied to all of them at the same time. So when that happens, now if I do a Fourier transform, I will get two peaks instead of one. Why is that? the reason is the following, that this combination Omega-A Omega-t1 that is cosine, remember?

We saw here that cosine modulation in the previous slide. So this is cosine Omega-A t1 gets multiplied with Cosine Omega-A t2. So you see in t2 and t1 in both the dimensions. Consider t2 as one dimensions and t1 as another dimension. In both the dimensions now, we have A and A. So that means, that will correspond to this. So ok? So here basically what has happened is this frequencies A and this so will let me correct this because of slight shift here. So basically this, is Omega-A and this is Omega-B. Ok?

So now in both the axis, the corresponding frequencies are Omega-A here and Omega-A here. Ok? Because see if you look here, both the dimension t1 and t2, you just cosine Omega-A t1 and cosine Omega-A t2. But now if you consider the second transfer, I mean the other transfer which has happened to B. If you look at that FID, that has got now cosine Omega t1 during evolution. But during t2 it is cosine Omega-B t2 that is it is evolving with chemical shift of B.

So when I do a Fourier transform of that FID which cosine of this multiply by cosine of that, you will get now on the t1 axis that is this dimension Omega-A that is because it is Omega-A during t1. But around B, around second dimension, it is Omega-B because it is Omega-B t2 which is this axis as shown here, our horizontal axis is t2. So and this is t1.

So this is what is scenario if you consider A. But remember we started from A here, but as I said this pulse generally the pulses are non-selective which means they (())(26.58) A and B simultaneously. Thus the spin B will also undergo the following which we will show here. So now this excitation is for both A and B.

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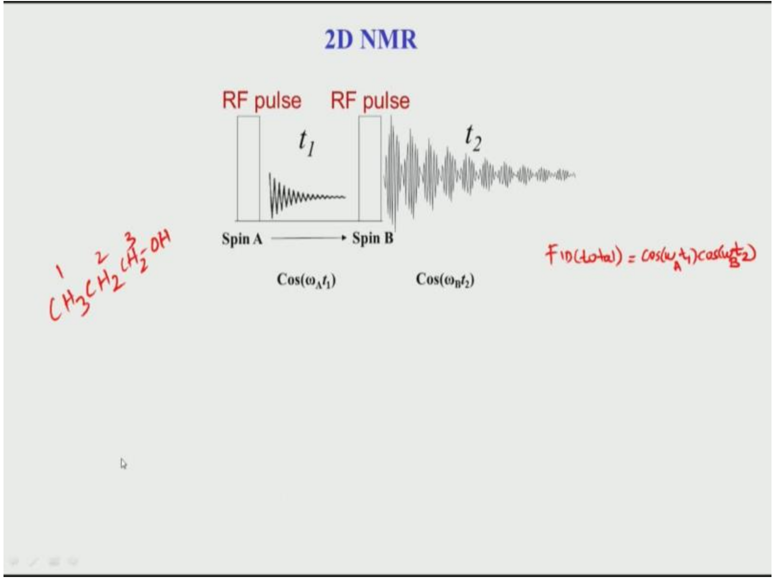
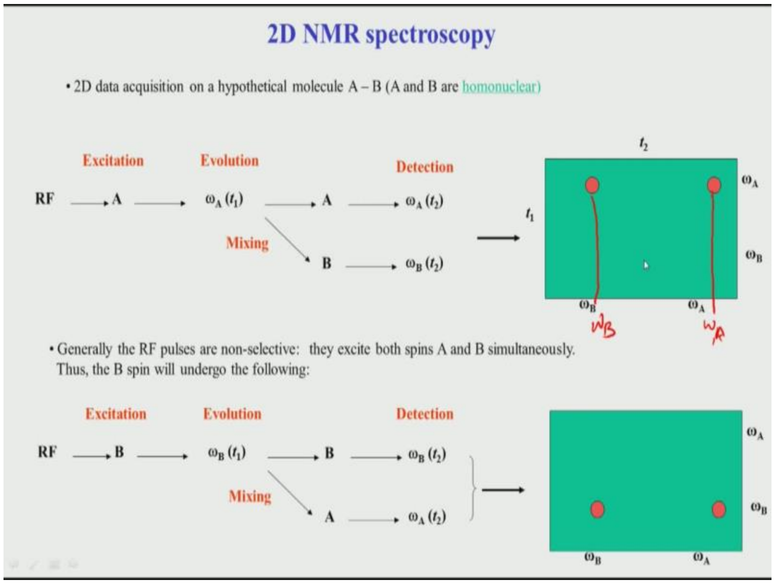


2D NMR

$F_{ID}(\text{total}) = \cos(\omega_A t_1)\cos(\omega_B t_2)$



2D NMR spectroscopy

- 2D data acquisition on a hypothetical molecule A – B (A and B are homonuclear)

- Generally the RF pulses are non-selective: they excite both spins A and B simultaneously. Thus, the B spin will undergo the following:

So let me just go back to this previous slide here that we are talking about this pulse now, the first pulse. It is not just only applicable to A it is applicable to B also, all the spins, so that is what is meant here. Now again during evolution, in t1, the B is evolving now because we have excited B and B is transferring now again partially to A but it may keep something on its own because efficiency is never going to be 100 percent.

So now, during the second pulse, after the second pulse, when we actually detect the signal that, during that time the B which is remaining on B which did not get transferred to A, that will get excited and that will now start evolving with the frequency B because it is remaining on B and that will be during t2 and what happens to the second transfer, or the transfer which (())(27.59) to A, that now gets excited as A and that will evolve or move oscillate with a chemical shift of A.

So now let us see how the fourier transform happens. So if you see here, this frequency, this is now again as shown in this picture, it is t2 on this axis horizontal and t1 on the vertical axis. So now if you see during t1, it is B here and t2, it is B here, so both of them are same, B and B but so therefore that will be this peak here, cross peak which will basically correspond to B this side and B on this side.

Ok now look at the other transfer, it has become A during t2 and it has B during t1 so that will be the cross peak between A and B. so again this is basically this peak here, this line here. So the Omega-A is here and Omega-B is here so you get a cross peak between A and B because of this transfer. So now do you see bot of this picture now are happening at the same time in the molecule, because the first pulse will excite both A and B and the second pulse again excites both A and B.

The second pulse, remember? We use the word mixing pulse. It is called a mixing pulse. So the mixing pulse is again applicable to both A and B. So now if you look at it, you have four FIDs, one this combination, one is this combination, third is this combination and the fourth is this combination. So we have four FIDs, each FID remember gives one cross peak. So four FIDs will give you four peaks.

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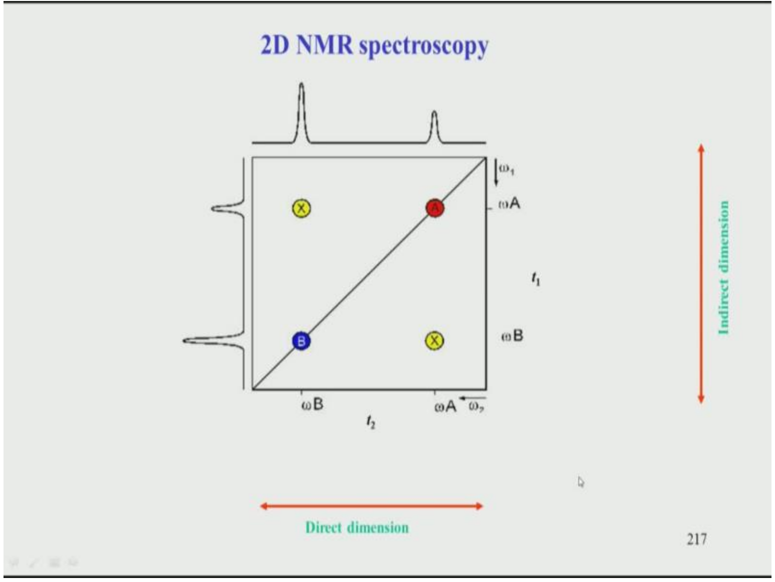

2D NMR spectroscopy

So these two now you have to combine, because we analysed them separately, we will combine together and this is what you will get. You will get basically a 2D spectrum looking like this and at this is called the diagonal peak. Ok? And the diagonal peak basically contains the same frequency in both sides and this we saw comes because 100 percent of the magnetization is not transferred to each other to the second.

Whereas this is called a cross peak and that comes because A has been transferred to B or B has gone to A. so you see all the four will come because we are not discriminating or distinguishing between A and B. So this is what is concept of 2D NMR.

It the next class, we will continue like this and see some more details of how 2D NMR can be analysed.