NMR spectroscopy for Structural Biology NS
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Lecture: 15
General Concept of Multidimensional NMR - 1

So, far we considered the conventional Fourier transform from NMR and there what we had was one frequency axis and when the plotted we get the intensities on the other side on the second axis. So, now we enter into a new revolution which is called as multi-dimensional NMR the beginning of which is of course the 2-dimensional NMR this came into existence in the 1970s and this actually was the main revolution for applications in structural biology.

In earlier situations it was all limited to chemistry and small molecules of few atoms and tens of atoms and things like that of simple molecules. Now it was unthinkable to go into things like proteins and nucleic acids and things like that which have hundreds of protons and so many other nuclei. It was impossible to do it to analyze this spectrum I will show you this by an illustration here.

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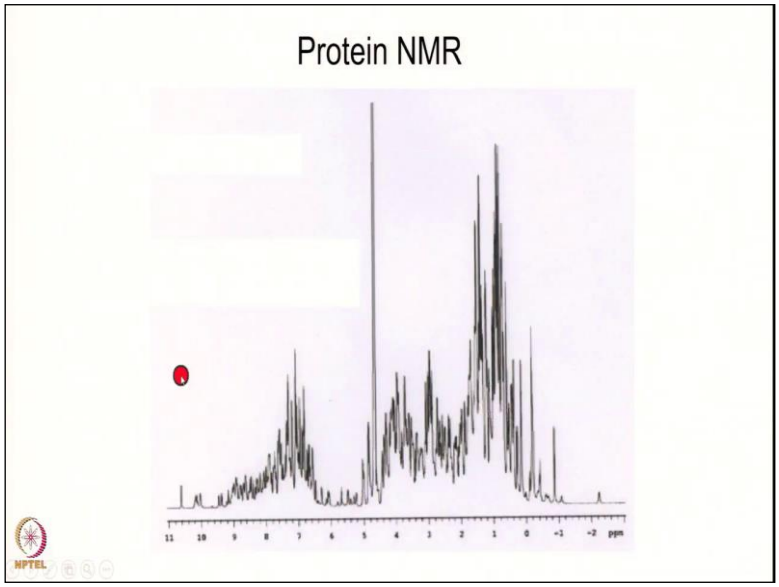

Here is a spectrum of a protein the spectrum of a protein this has about 400 protons and the proton spectrum the proton spectrum spans as it is here from 0 to 11 ppm of course you have some shifted ones here -1 this is the spectrum of lysozyme recorded on a 900 megahertz

spectrometer and you can see how much is the overlap of signals you cannot count the number of lines here.

Forget about measuring the coupling constants which I forget about individual assignments to 400 different protons how will you assign them and how will you get to the structure of them this is a quite an ambitious project to get to the structure with such a large protein such a large molecule with so, many lines without the assignments you can do nothing in NMR. You need to have to have the assignments first.

And this is the water line here we know from the chemical shift difference all of that what we have talked about is now coming into practical use. This is a water line quite substantially reduced and this sample is one millimolar in protein concentration recorded in water, water is completely suppressed here in the one dimensional spectrum and these are all the aliphatic protons the methyls here and these are the aromatics here and then you have the NH protons here in this area.

But you certainly cannot count them you cannot count to them and therefore you cannot assign them then what do we do. So, this is where the revolution occurred that. Now you try to spread this information in 2 different axes instead of one frequency dimension here this is one frequency dimension why not we generate 2 frequency dimensions and establish a correlations between the various signals in the one dimensional spectrum in a 2 dimensional way.

And that is the motivation that was a fantastic idea but how to implement it. You have to generate 2 frequency axis that is the first principle. How do we generate 2 frequency axis?

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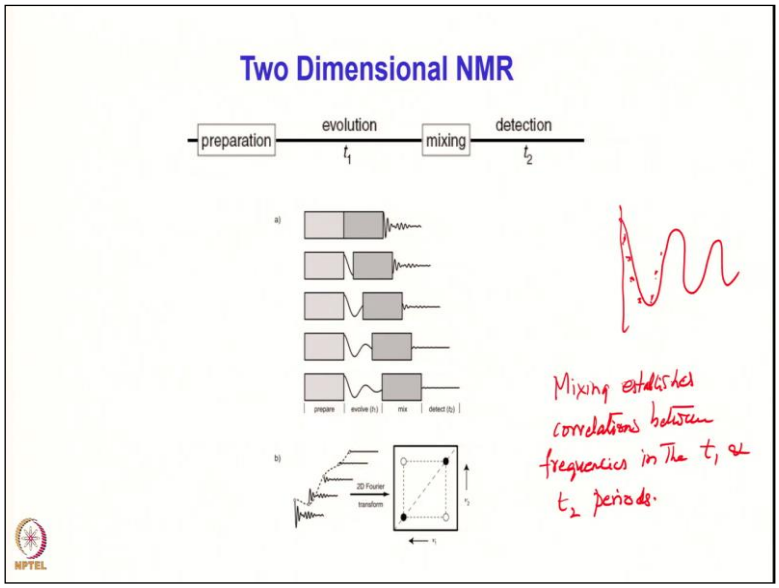

And this is the idea which was put forward and this was initially given by Jane Jenner in from Belgium and it was picked up later by Richard Ernst from Zurich and now it really caused a huge explosion in the applications of NMR in chemistry and biology. Now the idea is following. In 2 dimensional NMR you have to generate 2 frequency axis. In the normal FT-NMR you had one time domain time domain signal.

And the time domain signal after Fourier transformation you get the frequency domain spectrum which will give you the spectrum. Now we want to generate 2 frequency axis. So, what we will do here is let us divide this time axis like this let us divide the time axis like this. So, let us say we have so, called preparation period the preparation period means we can prepare the spin system in any manner we want.

It can be an equilibrium state or it can be non equilibrium state whatever you want here this is called the preparation period then you have a so called evolution time which we call it as t 1 then you have what is called as a mixing time we have we have come across this mixing time and also such concepts earlier when you talked about the NOE we talked about the mixing and then we have the detection time which is called t_2.

This is where the actual FID is collected this evolution time is an indirect detection period as I will show you later this is the detection time actually FID is collected during this period only. Now how is the experiment done you do a series of experiments you do a series of experiments that is this preparation block remains the same and you do once a with $t_1 = 0$.

Then you have the mixing you collect the FID this is FID number one then you increment this t_1 value give a small value here increment that means you separate this preparation and the mixing by certain value that is the increment and after that you collect the FID again this is FID number 2 then you continue to increment it in similar way if the increment here is δt_1 here it will be 2 δt_1 here it will be $3\delta t_1$ your $4\delta t_1$. So, you systematically increment this period and then you collect the FID every time.

So, third second FID third FID fourth FID; so this is like doing the digitization when you are collecting the FID you're not collecting the FID. So, we have this the FID when it is collected we are collecting in a digital manner right. So, we have various points here is what this is what we are doing we are collecting the FID also. So, here we are actually generating this is another time domain here and this time is incremented.

So, just as this time is increment 1 point to another point to third point four, five, six seven like that. So, you keep doing the first point and then the second point the third point 4th point fifth point and so on. So, forth you generate a various set of experiments you collect. So, many FIDs therefore what you get? So, that is what is indicated here FID number 1, 2, 3, 4, 5 etc. So, therefore you have.

So, many FID so now each one of them is dependent on the value of this t_1 whatever is the t_1, value here that will modulate the signal that is detected in this FID. So, this FID what you are collecting will be dependent on this time because during this period the magnetization will be precessing in the transverse plane it will be going around in the transverse plane. So, therefore this these will have frequency labels.

This will have frequency levels during this period and the mixing is a crucial thing which allows you to establish correlations between the spins here and the spins there. Mixing establishes correlations between the evolution and the detection. Let me write that here mixing establishes correlations between frequencies in the t_1 and t_2 periods. In other words magnetization may get transferred from one spin to another spin what if a particular spin was precessing with a certain frequency here.

During this period during this mixing it may get transferred to another spin and that will go with a different frequency. It can go with the different frequency but whether this transfer will

be complete or not complete one does not know it may be partially transferred. Now therefore what we have got here we have got a time domain data which has 2 dimensions that is what is indicated here.

So now let me go back here. So, you have a time domain data which has 2 dimension that is a t 1 and t 2 therefore if I do a 2 dimensional Fourier transformation now I do a 2 dimensional Fourier transformation this is 2 dimensional Fourier transformation I generate 2 frequency axis which we call it as ν_1 and ν_2 whatever is the frequency present during the t_1 period will appear along this axis whatever is the frequency present during this period t_2 period will appear on the on the ν_2 axis.

Therefore have generated 2 frequency axis by doing this trick I have given an additional t_1 period additional evolution period during which the various spins process with their characteristic frequencies. Now the mixing may cause transfer of magnetization from one spin to another spin we have seen this how it happens in the various examples. We saw that in the case of INEPT we saw in the case of NOE how magnetization transfer can happen depending upon what you do.

It can go from one spin to another spin all this is boxed up in this so called mixing. So, therefore we can establish correlations when you transfer the magnetization meaning we establish correlations and the transfer may go 100% or it may go 50% or whatever. So, depending upon the way the mixing is done. So, you have a partial transfer or full transfer this is what happened in the case of NOE for example.

NOE entire magnetization is not going there part of the magnetization part of the perturbation is getting transferred to this period to another spin and therefore then that spin will carry the information of the past. So, this is the idea. Now since we increment this t_1 I generate another time variable I generate another time variable and the evolution carries this information of the precessions during the t_1 period. Therefore various frequencies are present here and I have the labelling can be done during this period.

Therefore this evolution time can be called as indirect detection period evolution time is also called as indirect detection. Although there is no directly a FID collected in the digitizer in the digitizer of the FT-NMR spectrometer there is no direct data collection there but we collect the

data in the form of many FIDs whose evolution is modulated by the evolution during the t_1 period.

Therefore that modulation is dependent on this t_1 period therefore if a Fourier transform along the t_1 axis then I get that information of modulation by the evolution here and this is explicitly shown in this particular example here.

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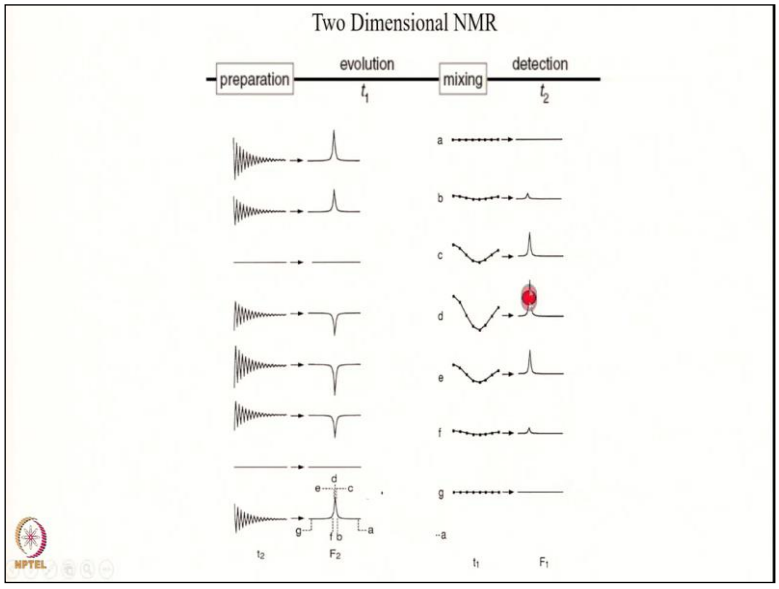

So, I have here various frequencies this is the first FID first FID after I do a Fourier transformation I get a line like this. Now I have the second FID I Fourier transform I can the second spectrum but now you see its intensity is not the same as this it is slightly reduced this is because of the attenuation here because of the evolution here. Because of the evolution in the t_1 the FID is looking different the third one is zero nothing there.

So, therefore here also there is nothing the fourth one it has changed sign why does this happen this is because of the evolution during the t_1 period what is the situation it has reached here the spin has reached here that shows up in this here that shows up in this initial point of the FID and then you see again this will now it has become negative this is more negative again it decreases in the negative because it starts going modulation right.

Positive decrease zero negative increase negative decrease again go positive and so on and so forth. So, therefore I get a series of spectra like this after they do a Fourier transformation along the F_2 all the FIDs will collect them like this. Now we look at this look at this various points this is look at this various points find out the condition of that in each of this spectra in the

spectra I have this points a b c d e f g these are all the points on the on the line here in the f 2 dimension.

This is my F_2 dimension this is the t_2 period therefore I have the F_2 dimension here after the Fourier transformation. I am collect looking at these points here at a b c d e in this and I see through this at this particular point what is the value here what is the value here and I plot that here I plot that here. So, therefore you see at the point a there is nothing it is all 0 all over and it is all 0 here.

Now we come to the point b there is a small dip there is a small dip in the middle therefore if I were to Fourier terms on this I get a small signal here. Now we go to point c there is a big dip as I go take point c and go along like this and plot the value of the signal here it will look like this. Now it has a time dependence right this is the what is along this axis this axis is t_1 here this is t_1 right.

So, it is indicated here this is the t_1 axis therefore if a Fourier transform this this is like an FID also right this is also like an FID. So, if a Fourier transform this I get a signal. So, go to point d greater dip greater intensity go to e dip has decrease smaller intensity go to f again it has decreased further small intensity go to g here then no signal zero intensity therefore you see here as a result of this kind of an experiment.

I have got the frequencies along both the F_1 and the F_2 axis. So, this is what is the principle of two dimensional NMR which is summarily described here.

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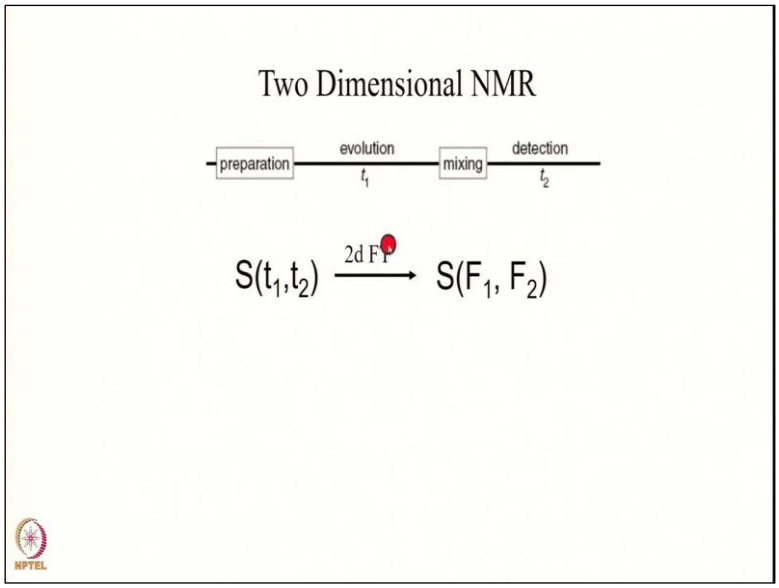

So, you have the 2 dimensional NMR you have $S(t_1,t_2)$ time domain signal has 2 time components t_1 and t_2 you have to do a 2 dimensional Fourier transformation and generate a frequent 2 frequency axis F_1 and F_2 . So, these are independent Fourier transformations you can do either one whichever order you want to do it does not matter okay all right.

So, now that was the principle of 2-dimensional I will give you certain examples here what all things whereas various kinds of 2D experiments we will not go into the theory of these calculations we are going to use these ideas and describe some experiments how these experiments what are the example sequences here and what is the information that comes in these experiments. One of the very early experiments that was done was 2D J-resolved spectroscopy.

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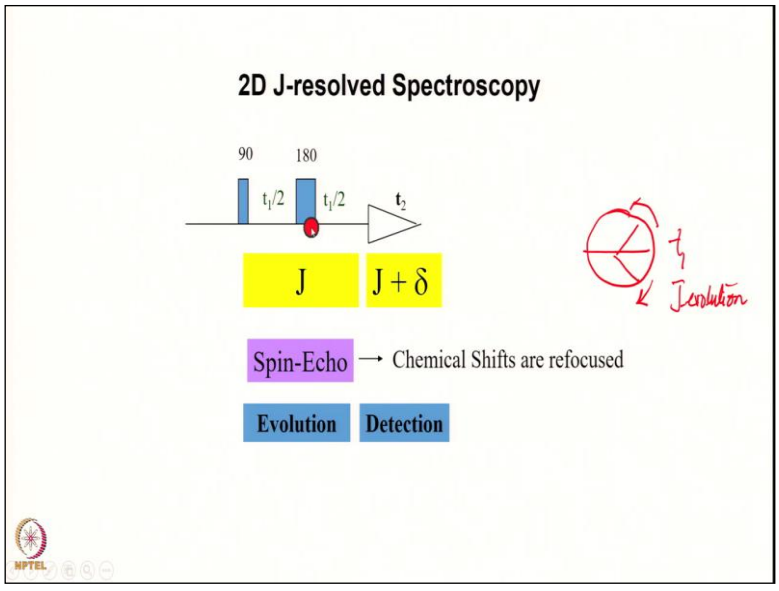

The J-result spectroscopy is done in this following manner. So, you have a first a 90° pulse 90° pulse. Now the evolution period is from here to here evolution period is from here to here but we have put the 180° pulse in the middle itself that does not matter we can do that but the time is incremented here. Now what is the consequence of this the consequence is from here to here this is an incremental time you are collect going to collect various FIDs with incremented t values right.

So, as a function of t_2, now from here to here it is the same as the spin echo this point is the spin echo. So, this sequence 90 180 τ 90 τ 180 τ that is a spin echo. So, during the spin echo what happens that is indicated here during the spin echo chemical shifts are refocused right. In the spin echo chemical shifts are refocused but the J remains the J information remains the coupling constants are not refocused in the spin.

This is what we saw earlier and also in the INEPT experiment we explained we explained that how the J information is not refocused in the spin echo period and therefore the J information is present here and this is my evolution. Therefore what is the information present during the evolution period the t_1 period that is a J information. So, frequencies correspond which describe the J are present.

So, it is like sitting in the center of the line and looking at the 2 transitions A_1 and A_2 transitions how they are precessing how they are processing you sit at the center of one line will have and A_2 are the 2 transitions of the ace pin you are sitting in the center because your chemical shifts are refocused the chemical shift information is not present here anymore. So, during this t_1 increment what is changing the 2 transitions A_1 and A_2 they keep moving let me write that here.

So, they keep moving during the t_1 period they keep evolving okay they are in the center this happens for every spin. For every spin because the chemical shift information is removed one it is at zero frequency chemical shift does not have information at all. So, only the J values are present during this period that is the effect of the spin echo. I repeat here again the chemical shifts are refocused no matter what the chemical shifts are.

That is completely eliminated at the time of the echo until this period which means it is like sitting at the center of the multiplet for every nucleus because the chemical shift information is not present it is like sitting at the center of every multiplet for in your spectrum. Therefore we

consider only this kind of I take a 2 single doublet the 2 transitions keep evolving as a function of t_1 therefore what is getting modulated as a result here.

So, what is the modulation that is happening J evolution is contributing to the modulation. So, therefore J evolution. So, therefore if I were to Fourier transform along the t1 period what I am supposed to get I should get the J information J is the frequency I should get that frequency right the angle modulation creates the different phases and the FIDs will be modulated by that evolution.

Therefore if a free transform along this I should be getting the J information what happens here in the t_2 period t_2 period I have done nothing I am collecting the data as if it is in the normal one-dimensional spectrum. I collect the data as if it is the normal one-dimensional spectrum. One-dimensional spectrum has both chemical shift and the coupling constant therefore I have the J + δ both informations are present here during the detection period.

So, during the spin echo during the t_1 period I only have J and during the detection period I have plot J + δ therefore this experiment is called as J resolved spectroscopy.

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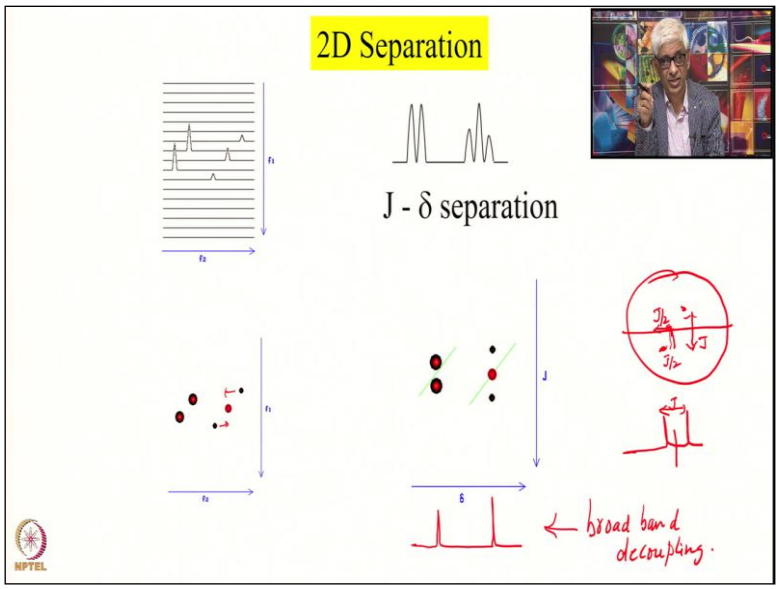

How does this look. So, you see this is explained here is called as 2D separation here. Now this is consider this sort of a system here you have a one doublet and a triplet there. Now along the F_2 period I have both the J and the δ. Now this axis is delta plus j this axis is δ + J and this axis is only j. So, δ + J and J what it will give me along this frequency axis I will have the J information this axis I have the chemical shift information.

Therefore for a doublet I will have these 2 lines oriented at a certain degree at a 45° here they will be oriented at 45° with the center line because there is a J/ 2 and J/ 2 both places right. So, this is the center and this is a doublet right. So, this is J/ 2 here and this is also J/ 2 the doublet. So, the doublet this is J this total is J from the center to the end it is J/ 2.

Therefore one of them goes up here and this is and then the total from here to here is J that is what is called the J separation along one axis I have the J information along the other axis I have both J + δ. So, therefore when they consider in the 2 dimensional spectrum I will have these peaks appearing at an angle of 45° because you have J/2 and J/ 2 which is an isothermal triangle.

As well as triangle means it is a 45° angle therefore I will have here the doublet appearing like this and the triplet will also appear like this and we have the in the triplet of course we will have the one line at the center therefore triplet will be right. Now what we do we do not want this angle we do not want this angle what we do is during the precessing process we will move this fellow to this center.

We will move this fellow here we will move this fellow to the left move this fellow to the right and similarly we move this fellow to the left move this fellow to the right. So, that we bring all of these on the same line and we bring these 2 in the same line that is what is done here this is done by moving one of them this way other one this way and here this also again move this way and move this way.

So, let me indicate that here when you move this. So, they will all come on the same line. So, as a result I will have this spectrum. Now you see on the J on this is the F_1 axis or is also called as the J axis. Now I have simply separate looking at the separations here I can measure the coupling constant. And if I take the projection of this I will have only one line if I take the projection here I will have only one line correct which means I have decoupled them which have decoupled the 2 spins.

What was a doublet here. Now appears as a single line what was a triplet here will also appear as a single line. So, this is a homonuclear broadband decoupling. So, this amounts to broadband decoupling. The coupling information is removed therefore your lines will get simplified right.

So, your spectrum will get simplified you will have multiplet information multiplicity is removed.

So, you will have one line per spin therefore you will have you have very higher resolution in this kind of a spectrum but you are not lost to the coupling information though the coupling information is retained along the along the other dimension. So, therefore this experiment is called as 2D separation or J - δ separation. In a 2 dimensional experiment we have achieved this sort of a result.

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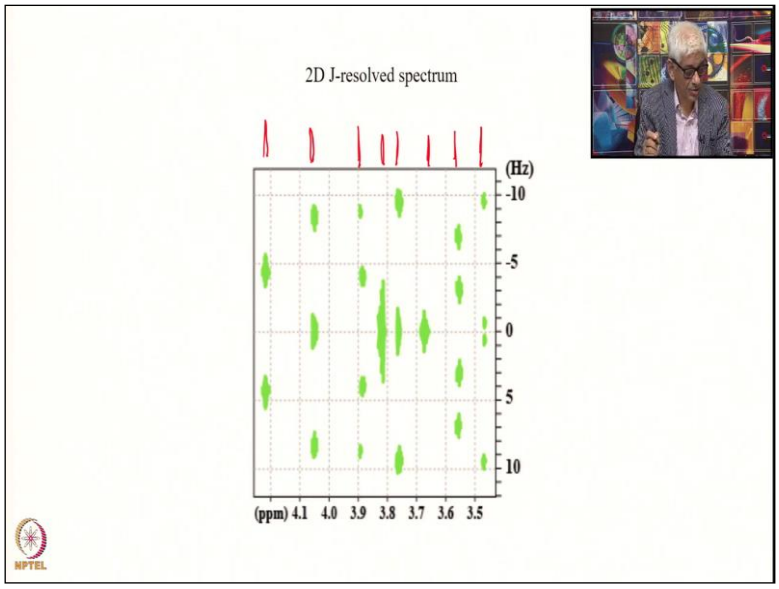

So, now we see here it is a more complex system there we have many spins and I have just shown the spectrum here for your for information and I do not even know what molecule it is but nevertheless it does not matter you have here a doublet you have a triplet then you have a doublet of a doublet here then you have again possibly a triplet here but with a smaller coupling constant. So, here you have a triplet again a single line and then you have a doublet of a doublet again doublet of a doublet.

So, you can see all the coupling information can be obtained and look at the range here the range is -10 hertz to +10 hertz. So, 20 hertz this is about I mean you do not have any fine splitting which is more than that. So, you will have here the separation which is like about 10 hertz you are able to measure this 10 hertz separation and this will be 20 hertz here and so on so forth. The coupling information is present and if you take the projection down here each one of them will give a single line all of them will give you a single line.

So, this will be a single line this will be a single line a single line here a single line there a single line there again a single line there a single line there and a single line there. If you took this projection onto this axis you will have only one line. So, there is a completely chemical shift information is on one axis and the coupling information is on the other axis. So, therefore this is an extremely useful technique for handling large molecules.

Because you achieved a good resolution enhancement along the F_2 dimension by decoupling and but you retain the coupling information along the F_1 axis therefore you can extend the coupling constant also you have not lost it. So, it is like sitting on the line the spectrum and rotating it by 90° like this. So, you have the spectrum like this sit in the center and rotate it by 90° therefore you have a much better resolution in your final spectrum well.

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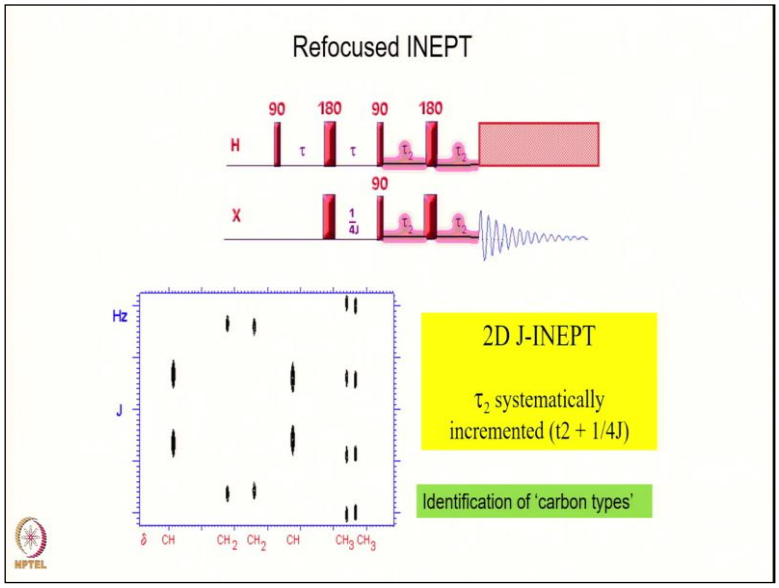

So, this is a little bit more modified technique of refocused INEPT and as a cons we already discussed the refocused INEPT earlier and this experiment is now converted into a 2 dimensional J INEPT experiment in this case what we have seen this earlier this experiment we discussed earlier refocused INEPT. So, basically and we do a decoupling of course we can get this. Now what we do is instead of this period being a constant time period what we do is we make it as an evolution time.

We make as an evolution time when we do that you generate the second frequency axis here when you do the second frequency axis you are able to produce the frequency axis along the F_1 dimension Fourier transformation you get such a beautiful clean spectrum and 2D J INEPT.

Identification of carbon types by looking at this you can identify what kind of a carbon it is along this axis you have the carbon frequencies and along this axis you have the J's.

So, the single doublet therefore this is the CH group and this is the CH_2 CH_2 you have a much larger separation here in this then another CH_2 then you have the CH here then you have the CH_3 which you saw earlier we saw in the case of one dimensional spectrum how these ones will appear. So, you can identify the carbon types by looking by doing such a kind of an experiment.

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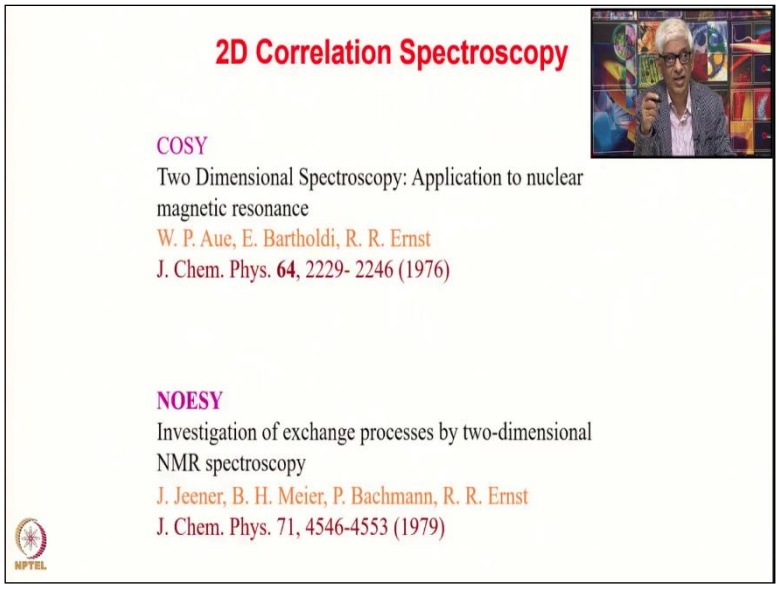

So, I think we can we can stop here we will go into this next kind of 2 dimensional experiments in the next class.