# Principles and Applications of NMR spectroscopy
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**Module 5**
**Lecture No 21**

From this class onwards now we will move on to 2D NMR spectroscopy. Till the last few part of last part of the course we have looked at 1D NMR.

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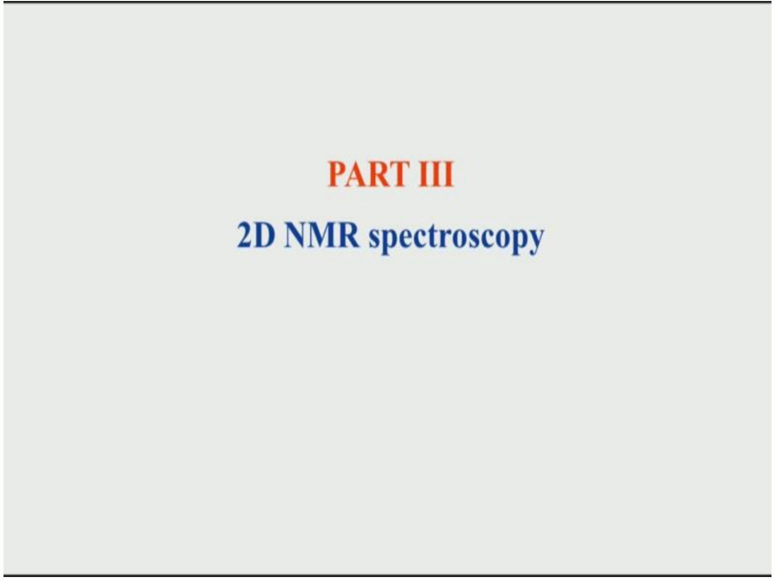

PART III

2D NMR spectroscopy

So now let us start with the important aspect of the NMR spectroscopy which is Two Dimensional NMR. Here we are going to see, how 2D NMR spectrum is recorded? what are the basic principles involved in 2D NMR? and how it can be interpreted and also in the last part of the course, we will be now looking we will be looking at how we can use 2D NMR for different applications.

Typically 2D NMR applications has is basically in the case of organic molecules, are very useful and also it is very useful in the case of peptides which are amino made up of amino acids. So we will see that in this the last part. Right we will now start with the 2D NMR basics.

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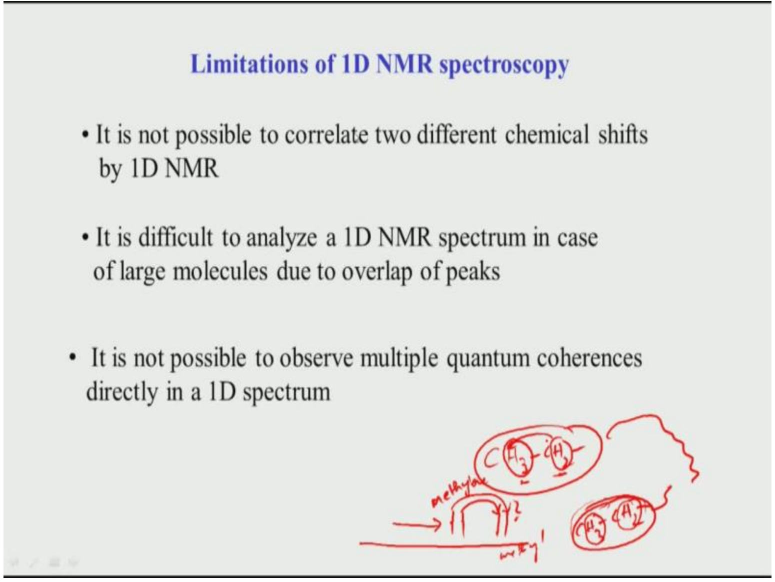

## Limitations of 1D NMR spectroscopy

- It is not possible to correlate two different chemical shifts by 1D NMR
- It is difficult to analyze a 1D NMR spectrum in case of large molecules due to overlap of peaks
- It is not possible to observe multiple quantum coherences directly in a 1D spectrum

So you can see as we saw here in the case of 1D NMR, there are few limitations of 1D NMR which you cannot the information which some information you cannot obtain from 1D NMR. So let us see what are those drawbacks of 1D NMR. Number 1, it is not possible to correlate two different chemical shifts by one by 1D NMR. So what do we mean by this? What it means is for this you have to imagine the 1D spectrum, let us say of ethanol molecule where we have CH2, CH3 for hydrogen.

So now there in case of ethanol is very straightforward, there is a CH2 system H2 and there is a CH3H3 and they are J-coupled to each other and one gives you a triplet and other gave us a quartet, right? So CH2 gave us a quartet and CH3 gave us a triplet. So that was simple. But now if I go to some other molecule, we will see down the line some examples. But if I go to some other molecule, where there are more than these pairs of protons so CH2CH3, I call them as a pair.

So if I see such multiple pairs in a proton spectrum, I do not know which pairs are correlated with each other, which CH2 and CH3 are correlated with each other? So that information is not obtainable from 1D spectrum. So one has to therefore, use a 2D NMR which will help us to know which pairs are possible. So let me illustrate this with a picture we can look at a schematic drawing for example, let us say I have a hypothetical molecule where I have a CH3 and a CH2 somewhere in the molecule and somewhere down the molecule, so this is the same molecule, I have another CH2 and another CH3.  Ok?

So there is some hypothetical molecule like this. Now what will happen is this CH2 this proton is coupled to this proton so you expect a triplet for this, a quartet for this but the same time, similar things is happening here. Here also I get a quartet for this proton and I will get a triplet for this proton. So if I record a spectrum, I will get two triplets in the methyl region and I will get two quartet in the methylene region. So now, I have do not have information that which two are connected to each other, I do not have that information.

So that information of connection between which methylene which methylene is connected to which of its respective methyl, that information is not present in the simple 1D NMR. All the 1D NMR gives me is a simply a set of lines. It tells me there is a this is a methylene group this methylene group and so on it (does) for example it tells me that this is a methylene molecule, methylene moiety, this is a methylene moiety, this is a methyl and this is a methyl. But it does not give me the information of the connectivity that is which methyl is connected to which methylene.

That information, connected in the sense like this connection coupling and that information is not provided in this 1D spectrum. So if you want to know which methyl peak here is matching with which methylene peak here, which are the coupling partners, you will not get that information because this will also be a quartet, this will also be a quartet, this will be a triplet, this will be a triplet because of the J-coupling but that connection is not there information. So that is for that we need to go to 2D NMR. So this is one of the limitations 1D proton NMR and secondly, you get can resolve overlaps again we will see some examples, how we can resolve overlaps.

So what happens is when you have a large number of peaks in a proton spectrum, use the peaks will start overlapping, they will start crossing each other, they will lie on each other so there is a lot of degeneracy. Degeneracy is the word used for saying that there is an overlap of peaks. So to resolve those degeneracies, or to resolve those overlaps, we need to go to 2D NMR. And a third aspect is more technical point, a point is that in NMR which we did not see because of that will be not possible in this course to deal with we will look at that in an advanced version of the course later.

The thing is that what is called coherences in NMR. Whenever you apply NMR pulses you generate coherences. So if you have two protons coupled to each other by J-coupling, there is a possibility of generating multiple quantum coherence. So I will not go into detail of this terminology because this is not part of this course but as information is useful to know that multiple quantum coherences cannot be detected by an NMR.

They can be generated, they cannot be detected directly. So to detect multiple quantum coherences, we have to do it by 2D NMR only. In 2D NMR what we do is, we generate some multiple quantum coherences and when we want to detect them, we convert it into one into a single quantum coherence.

So for the time being you if it is okay if you do not fully understand these concepts of the multiple quantum coherences but remember in mind, keep in mind that 2D, one of the reasons 2D NMR is very useful is that you can indirectly detect multiple quantum coherences because in 1D you cannot directly detect them. So now let us see, look at these three of these at least two of these two cases how 2D NMR helps to resolve.

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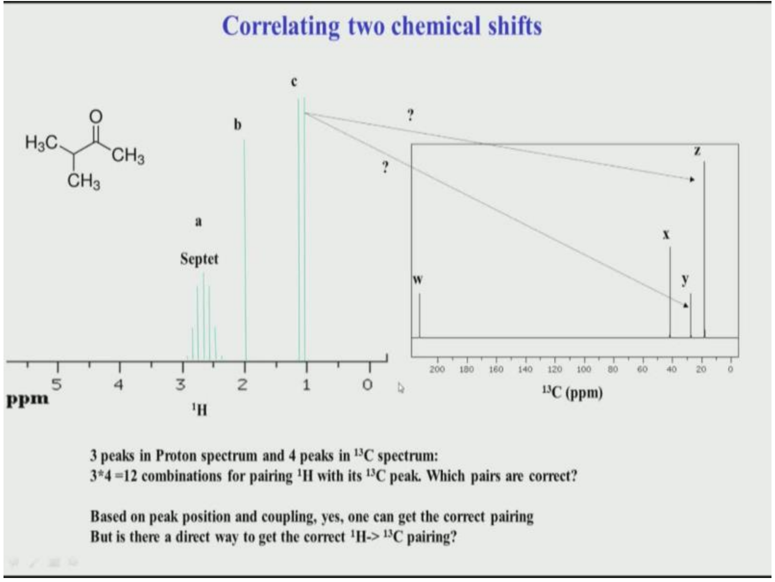

Correlating two chemical shifts

3 peaks in Proton spectrum and 4 peaks in $^{13}C$ spectrum:
3*4 =12 combinations for pairing $^1H$ with its $^{13}C$ peak. Which pairs are correct?

Based on peak position and coupling, yes, one can get the correct pairing
But is there a direct way to get the correct $^1H \rightarrow {}^{13}C$ pairing?

So let us take this example, again this is a molecule which we saw in the previous class, when we were looking at carbon 1D NMR analysis , there we saw that we look we had a 2D spectrum 1D spectrum of hydrogen and we also had a 1D spectrum of carbon. But the one question I raised last time was that there is no connection information in from these two. What is happening is we have recorded these two spectra separately in isolation. Means this was recorded initially then you record this but I do not know which proton peak here corresponds to which carbon peak here because all of them are CH peaks CH peaks but of course, you can say by logic again if you use a logical idea you can say that look this will correspond to this and this will correspond to that and so on but that is based on some kind of a logical arguments.

But if I want to directly see that which proton peak here, this is let us say on proton peak because this together is one and this end in doublet, this is one and this is another one. This is a bunch of one peak corresponding to a J-multiplet. So this particular peak, one peak corresponds to which carbon here. Of course, remember this is coming from ketone so we are not going to worry about the ketone because there is no proton attached for ketone.

So I am not looking at the ketone, I am only looking at this carbons and again this quaternary carbon remember is not, sorry this is a proton here, single proton. So there is one carbon, these two are equivalent, so two carbons and this is a third carbon. So we have three carbons here. Similarly there are three protons here because there are this methyls, these two methyls correspond to one methyl.

This correspond to this methine is one hydrogen, one type and this is the third type of hydrogen, so three hydrogen. So we have three hydrogens on this side, three carbons on this side, so can we match from 1D which is which, that is the question which is shown in this diagram. For example, can I say that, this about particular doublet, one hydrogen corresponds to which carbon here, that information is not present in this 1D and this is one of the drawbacks of doing 1D separately but if I do a 2D I should be able to find out. So that is the question.

So suppose I have I label this, let us see how a 2D will help us in this information. So right now again keep in mind we are only going through idea of how 2D can be 2D can help us to resolve as we go along, we will see more details of 2D. So in the next slide we will see a simple 2D but I am not going to right now we will not see how 2D is recorded. We will come back, come to that later. So let us now look at how a 2D can help for this problem.

So what is the problem in hand? The problem in hand is as follows : we have three hydrogens which is labelled A, B, C and we have three carbons which is labelled X, Y, Z. Now my job is to match and my problem is to match  which proton here is paired with which carbon here. So there are three pairs possible means three pairs are there. Which pair is the correct pair? So for that, that is what is shown here. If I try all possible combinations, I have twelve combinations possible because this can go with any of this three, this can go with any of these three, this can go with any of these three.

So there is basically they are nine possible but there are actually four peaks in the thirteen here, so if you see there are four. So of course, as I said by logically you can say that this is not attached to a proton so you can ignore that combination but if you take brute force, a mathematical approach. A mathematical approach tells me that given four peaks here and three peaks here, I expect twelve combinations or twelve pairs. Okay?

But we do not know which pair is correct, but only one three pairs are correct because this has to be paired with one, this has to be paired with another carbon, this has to be paired with another. So only three pairs are the actual pairs but based on this spectrum separate alone I am not getting that information.

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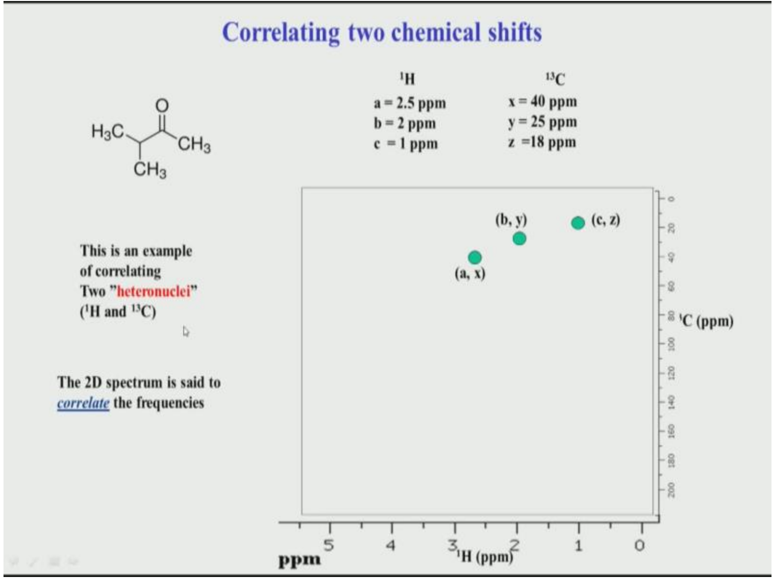

## Correlating two chemical shifts

$^{1}H$
a = 2.5 ppm
b = 2 ppm
c = 1 ppm

$^{13}C$
x = 40 ppm
y = 25 ppm
z =18 ppm

This is an example of correlating Two "heteronuclei" ($^{1}H$ and $^{13}C$)

The 2D spectrum is said to *correlate* the frequencies

So this is what can be seen, suppose I do now a 2D NMR, Ok? So if I do a 2D spectrum, that means on this axis, I have put the proton and in this axis, I have the carbon. Ok?  Now this is the three pairs. Ok? So this is already known to us but I am just trying to show here that if I do a 2D spectrum, I will get the correct spot, because the correct peak which is in the carbon side here which was coming at twenty in our previous slide will show up now it is correct partner from the proton side. So you see, this is simple 2D coordinates.

This C comma Z is nothing but is telling me that the proton number C which we saw in the last slide which we assigned as C that has this partner in the carbon slide corresponding to Z which was in this slide. So a 2D spectrum if I record, immediately out of those twelve combinations, gives me the right combination because it connects the right carbon with the right proton. How it does it? We will see that later. But the advantage here of 2D is that of the twelve combination which we saw, I am able to get the right combination simply by recording a 2D spectrum and the right partners, the combinations are showing up here.

So similarly if you look at the second combination, B to Y, it comes in the 2D spectrum because that is the only one which matches with this peak and corresponding to its partner, if you draw a line, a straight line here that is that peak which was at 2 ppm value and that now corresponds to this carbon. And the last combination was a septet. So if you recall in the last slide, we saw a septet and the septet was basically at around 3 ppm, 2.5 to 3, and that septet is what is coming here and its corresponding carbon is basically here at 40 ppm. That is what is shown here.

So this is basically what we saw that if you see X Y Z, the A matches with, the C matches with Z, Ok? And B matches with Y and A matches with X. so this is basically what is how the 2D information helps us to resolve the ambiguity. So what was the ambiguity? The ambiguity from the two separate 1D was that we are do not know, we do not know which proton peak matches with which carbon? Which two carbon proton are paired with each other?

But from the 2D that pairing directly comes because that is how the 2D is done where the correct pairing shows up by a simple this axis, on the y-axis is your chemical shifts of carbon and x-axis is the chemical shifts of the hydrogens. So this is what we say correlation. So the word correlation is used in NMR in 2D NMR very often. the idea here is that we are correlating the two frequencies means we are connecting or matching the two chemical shifts.

One is carbon chemical shift is hydrogen and by connecting those two I am able to figure out which proton is correlated with which carbon chemical shift. Ok? So this is an example of what is called as a heteronuclear NMR experiment. Why hetero? Because we are connecting a carbon with proton. If I have proton on both sides y and x, we will use the word homonuclear. Homonuclear means same nucleus, same type that is hydrogen and hydrogen. If I use heteronuclear means it is hydrogen and carbon. It can be carbon-carbon also, so carbon carbon on both axis can also mean is a homonuclear.

So remember again, I have not generated this 2D spectrum from my 1D spectra. 1D spectra was done separately and 2D spectrum is recorded separately. So please do not confuse that this 2D spectrum which I gave an example is coming from the combination of 1D which we saw in the previous slide. That is a separate thing, we recorded separately 1D and we recorded 2D separately. This is not generated by combining the two 1Ds, it is generated by different method which we will see. By doing a 2D, I am able to get that ambiguity resolved.

So let us see at the second so this is what is shown here, that is what I said that you are actually correlating two different types of nuclei, so we use the word hetero-nuclei.

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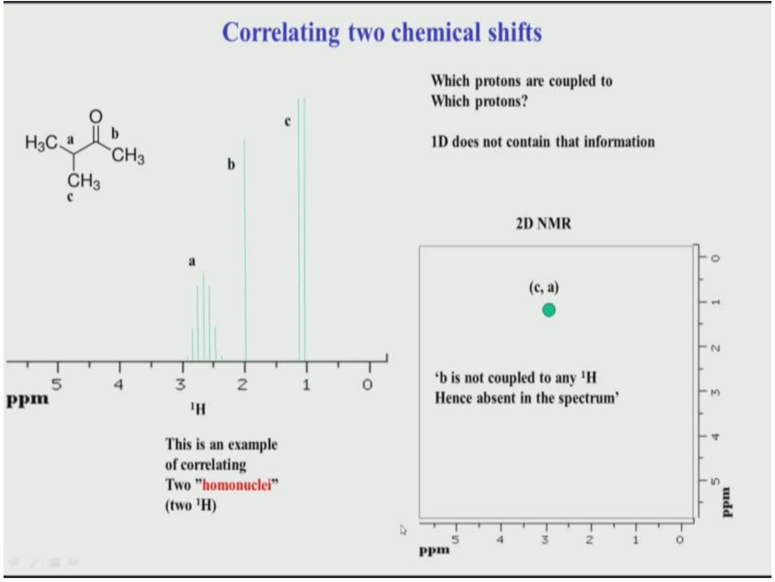

Correlating two chemical shifts

Which protons are coupled to
Which protons?

1D does not contain that information

2D NMR

(c, a)

'b is not coupled to any ¹H
Hence absent in the spectrum'

This is an example
of correlating
Two "homonuclei"
(two ¹H)

Now let us look at again this example, in a different way, so there is one more case, the question is which protons are coupled to which protons. So this is a separate problem. In the first case we saw, which proton is coupled to which carbon. That is what we saw in the previous slide for the same molecule now now we will not look at the carbon, we are looking at only the hydrogens. Now can we say that A is coupled to B? Because of this it is getting this multiplet or is it that A is coupled to C.

Now which is A, which is B, which is C, we do not know, we just label these three protons, three peaks as A, B and C. So the question we are trying to ask here is that, given a peak doublet here, means it is coupled to some proton. It cannot generate a (ss) doublet if this is not coupled to anything. This for example, is not coupled to any proton. So therefore there is no coupling of B to any of the protons but C should be coupled to A only because of that it should be able to get a doublet.

Similarly A should be coupled to C only then it can give a multiplet so this information of which two hydrogens, these are all hydrogens now; which two hydrogens are coupled to each other? That information is not coming from 1D. in this case , yes, In this case it is easy to say because this B is not coupled to anybody therefore it is a singlet. So I can say, conclude that yes, A should be coupled to C. But remember, in a more complicated system, this information is not going to be there and therefore it is required to do a 2D NMR to give you this information.

So let us see how 2D NMR gives that information which is shown here. So you see if I record a proton to proton spectrum, means both axis is proton now or hydrogen now here, compared to the last example where we saw carbon was shown here. But now we are looking at hydrogens on both axis so this is a 2D homonuclear experiment and that type of experiment, what it is doing? It is correlating, again remember the word correlation, it is correlating the proton with another proton and how is protons correlated, it is taking a proton which are coupled to each other, are correlated.

So if you see here, this this proton which is at one ppm which is a C is it is showing that that is correlated to the proton which is A in the x-axis. What is A? A is this proton. So that is what we expected based on our logical arguments that B is not coupled to anybody because it is an isolated CH3 as shown here. But A and C, we expect to be coupled to each other. Of course, so that is why it was easy to see from 1D but the example here is to show that a 2D gives you that information.

So in this particular case, information was obtained not directly, but by our logical arguments. But here the information is directly obtained and you can actually see and this particular case that you can see that there is a coupling which means there is a correlation between the two protons. So for example, if I have many CHCA pairs. Ok? So in this case there was only one A one C but remember, I showed you in the beginning of the class today that you can have many CH3CH2 (())(19.20) in a big molecule.

So you will have many such AC pairs, you may have many isopropyl groups. Right? You may not have only just one isopropyl in a molecule, you may have multiple isopropyls. So in all those isopropyl groups how do you know the CA pairs? Because you will get many of this doublets, you will get many of these septets. So which septet is coupled to which connected to which doublet, that information you will not get from a 1D.

But that you will immediately get from a 2D because the septet which comes at this position will be automatically or correlated with as doublet at this position. So if you have many doublets here, and many septets here , they will get individual peaks. This is called a peak, they will get individual spots here, for each septet doublet combination. So that is how 2D NMR is another example how it helps us to find out which protons are coupled to which protons.

So this the first point which we saw, advantages of 2D. Now let us see the next example of 2D which helps to resolve overlap. So this is what I was saying in the previous, that B is not coupled to any hydrogen therefore it is absent in the spectrum. So this type of experiment is called homonuclear, so two homonuclei are coupled to each other are are detected on both axis so it is a example of a homonuclear experiment.

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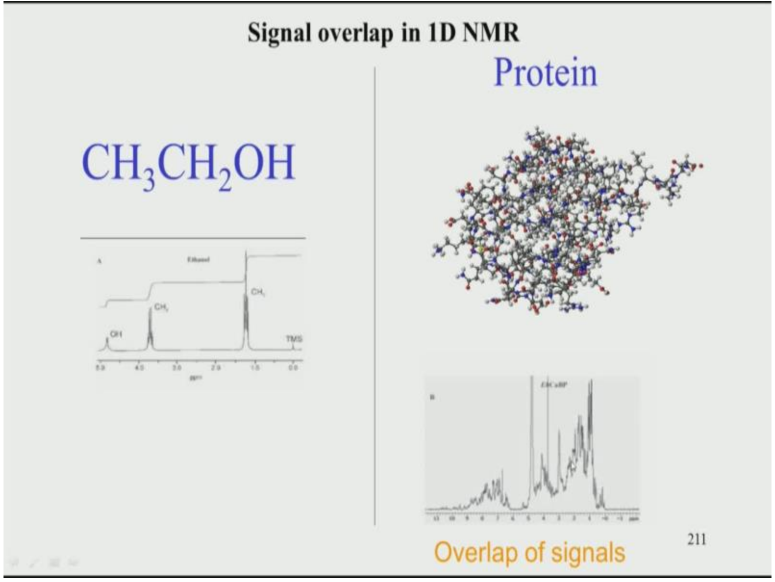

**Signal overlap in 1D NMR**

$CH_3CH_2OH$

Protein

Overlap of signals

211

So now in as I said in a simple molecule like ethanol it is very simple to say that okay these are the two coupled to each other because there is a only two hydrogen seen in the spectrum but look on this side. This is a complicated molecule, this is a protein molecule which is made up of amino acids and all the hydrogens which are shown here by grey color balls, all of them will get a pic here. So you see this is a very complicated spectrum. It is not very simple like this. So therefore for such complicated spectra spectrum, we cannot use a simply say which hydrogen is coupled to which hydrogen.

In fact, we cannot even see the hydrogen’s peaks properly.So to resolve overlaps, to get that correlation information, we have to do 2D NMR. There is no choice for us for a bigger molecule like a protein or a peptide. Even for a small organic molecule like a drug molecule which may be the molecular weight will be around five hundred or so even for those kind of molecules, we need to do a 2D homonuclear or heteronuclear spectra so that we can get the information of which coupled peaks are there here. Ok?

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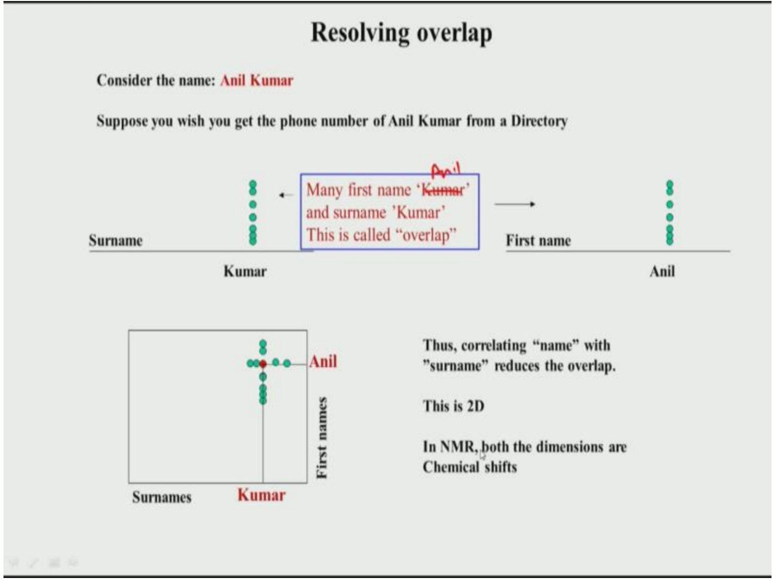

**Resolving overlap**

**Consider the name: Anil Kumar**

**Suppose you wish you get the phone number of Anil Kumar from a Directory**

Many first name '~~Kumar~~ Anil'
and surname 'Kumar'
This is called "overlap"

**Thus, correlating "name" with "surname" reduces the overlap.**

**This is 2D**

**In NMR, both the dimensions are Chemical shifts**

So let us look at another example how we can see resolve overlap. So this is a very interesting example which I am going to talk about, this is just a hand waving argument. This is just a example from not from NMR. This is a non NMR approach. So let us say, that you have a name of a person called Anil Kumar. Okay? So this is actually the name of a, one of my colleagues, so I am just going to take that as example.

So let us say, I am trying to search the phone number of Anil Kumar from a directory. So I will, let us say I look at only the surnames. So if I look at surnames, obviously there will be a number of Anil Kumars, right? Number of Kumars. There need not be Anil Kumar, there can be many other Kumars. So you see this is what I have (sh) depicted schematically here, saying that there are many Kumars and they are all having in the line because that is called overlap.

They are all overlapped. So I do not know obviously which Anil Kumar there are many Anil Kumars. So I do not know which is many kumars so I cannot simply look at the kumar and get the phone number. Let us say I look at the first name, suppose I search in the directory by another approach. I start looking at the first name. Again there will be many Anils in the directory. So I will not be able to figure out which Anil is the one I am looking for. But if I combine the two, so this is what is shown here.

Many first names are Kumar, many surnames sorry many first names are Anil, so let me correct it. Here say Anil. Ok? And many first surnames are this one surnames Kumar. So they Overlap. Ok? But now if I combine the two, it is very obvious that there will be probably only one Anil Kumar or there may be many Anil Kumars but at least now by combining the two, correlating the name with the surnames. So you see now the correlation is coming out of this example.

So what I am trying to do, I am correlating or connecting the first names of people with their surnames. So when I do that correlation, I get a unique combination because there may be only one Anil Kumar in this directory or they will be even if there are several at least I have separated them out of the unwanted names which are having different first name but they may have surname as Kumar or they may have first name as Anil but different surnames and so on.

So by using this kind of a correlation idea, I have now reduced my ambiguity, means i have reduced my problem to a very simple problem that now I am focusing only on the combination. So this is basically what is idea of 2D NMR that it helps you to resolve that you can let us say resolve the peaks let us say I connect the chemical shifts in the earlier examples we saw, we use chemical shift in both the axis. We use carbon proton on one side, carbon on the XY axis.

So you see by combining the carbon and proton chemical shifts I was able to get the unique combination and I was able to resolve the overlaps problem by going from instead of I have twelve combinations which were possible, if you remember we saw that in case of three protons and four carbons, we had twelve combinations but instead of twelve combinations, I reduced the problem by going only to two combinations, so by three combinations.

So this is how was an example of how we can resolve overlaps. So similar is a example shown here that NMR 2D NMR helps us by correlating the chemical shifts from two dimensions using two dimensions.

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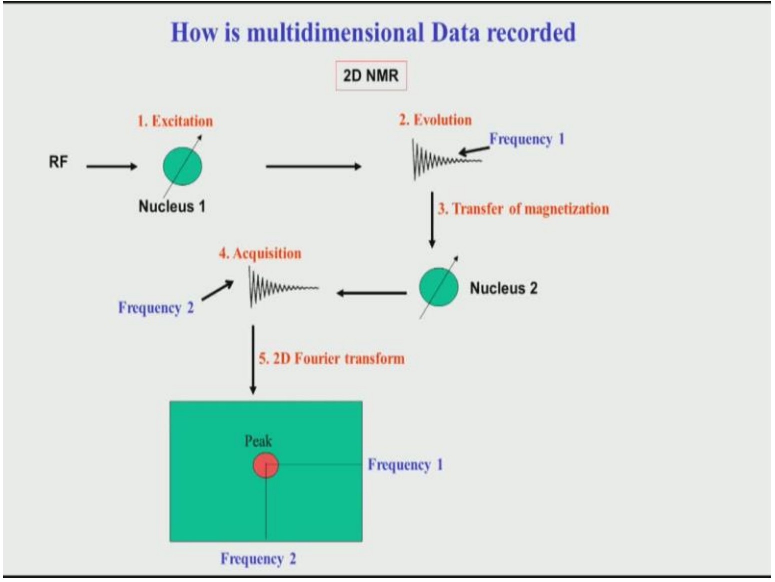

How is multidimensional Data recorded

2D NMR

So this is basically how 2D NMR spectrum is recorded. So you how do you actually practically do this experiment. So now we will look at the details of how 2D NMR is done in reality. So we will go through several different ways of explaining how 2D is done. So I would suggest that you have a look at all the possible explanations so we will see the first one, how we how it can be done? So the first thing that we do is that we start with an RF pulse. So this is similar to what we do in 1D NMR, we apply an RF pulse. Ok?

That is called excitation, so we excite a nucleus from alpha state to beta state by applying an RF pulse. So that is the first thing which we do in any NMR experiment so same thing starts here also in 2D we start with that. Then what we do is at the moment you excite a nucleus, then you leave the RF pulse, you remove this RF pulse, then the nucleus starts relaxing and that is called FID. During that period remember, we said in 1D you generate an FID, you induce a signal in the coil and that generates FID, but in 2D, we do not do that.

In 2D what we do is we allow it to evolve, this is the word used evolution. Remember please do not confuse this with the evolution we use in biology. That is called Darwin’s evolution. That is a completely different concept. This is just the word is somehow used for evolving a chemical shift. That means this is the FID in the chemical shift oscillation will start after you remove the pulse and this is what is called this is because of the relaxation and chemical shift.

Now during this process, when it is going relaxing, during that process, that means from here to here, this is remember a time axis. Ok? So this is the x-axis, here is a time. So during that process it what it does it does this nucleus, this nucleus one transfers its magnetisation to another nucleus while this is happening. Ok? So that is what you have to keep in mind. We will again as I said we will go through this exercise of understanding 2D in different ways.

So this is one way to understand is that we have nucleus which is excited, what it is now evolving or moving in chemical shift because of which you get this oscillations and during that process it is also relaxing that why it is showing a decrease. During this process, when it is moving in chemical shifts, as well as relaxing it transfers the energy to another or transfers the magnetisation to another nucleus and why is that so? Because it is coupled to this nucleus.

So let us say these are two hydrogens coupled by J-coupling or some other coupling to each other. Because of the coupling, they interact with each other and because of that interaction, they transfer energy to each other. So nucleus one will give magnetisation to two, two will give it to one. So we are only looking let us say one side. So the nucleus one gives its magnetisation to two, during this process. So remember we are not doing any extra work here.

All we are doing is allowing nucleus one to simply move in chemical shift and relaxation . During that process, it starts transferring the polarisation to (())(28.56). Now that transferred polarisation is now detected by exciting this nucleus. Remember this nucleus it has only transferred, but we have not excited this nucleus. So we have to excite this nucleus. When we do that excitation, that nucleus will now start moving in its chemical shift but it will carry the information of the chemical shifts coming from here.

So these two informations are coupled to each other and what is done is this is what is detected physically by using a receiver. So this is like the 1D NMR where we detected the signal but here we did not detect, we have simply allowed it to evolve, we say chemical shift. During that process, it transferred, we then excited this nucleus and detected its signal but that signal what comes out is having the information of this. So finally what we do is, we do the fourier transform of this two spectra.

Remember this is not two different spectra, they are within the same experiment. So this is can you, you can call it as one frequency dimension or axis and you can say this is another frequency because another chemical shift is there and that is frequency two. So by doing this fourier transform, what we are going to get is that we are going to get this information of this chemical shift directly connected to this chemical shift, that is frequency.

And that connection is not possible if I do a separate 1D here and a separate One D here. But because of this connection, because I am directly going from one nucleus to other, I have actually got them connected to each other by J-coupling and that connection shows up in the 2D spectrum which is shown here. So with this idea, we will again go through this exercise in the next class and we will continue with the 2D NMR and we will see how further we can understand how 2D NMR experiments are recorded and how this frequency is 2D axis can be interpreted.