One and Two Dimensional NMR Spectroscopy for Chemists
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Lecture – 56
Coupled and Decoupled HSQC and HMBC

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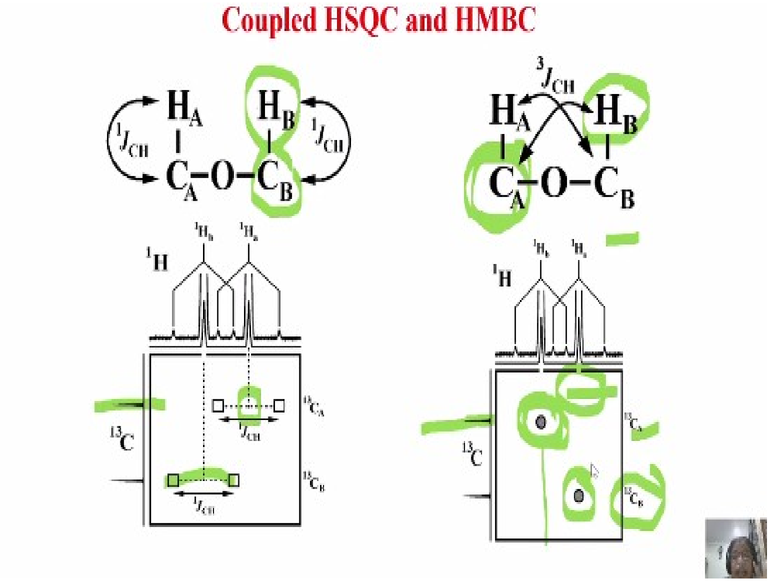

Coupled HSQC and HMBC

Welcome back in the last class we discussed about HSQC and HMBC; that is coupled HSQC and coupled HMBC, where I took the example of a molecule like this; where we saw in the HSQC spectrum there are two carbons for this hypothetical molecule which is chosen. At the chemical shift of carbon A in the carbon dimension you get a single peak. Whereas at the chemical shift position of carbon B in the carbon dimension you get a single peak.

There is no doubt in this molecule in the HSQC spectrum, in the carbon dimension there was a single peak, because in the coupled HSQC we saw that in the direct dimension that is in the detected dimension we saw two peaks. It is a doublet; for example at the chemical shift of A we got a doublet because of coupling of this carbon A with this proton A. Similarly, at the chemical shift of carbon B in the direct dimension we saw a doublet; this doublet is due to coupling between carbon B with proton B.

And remember this spectrum is shown; satellite spectrum is shown by enhancing the intensity of the proton spectrum. But in the HSQC spectrum, the central peak is not there, that is absent. You will only see two peaks; doublets due to carbon, proton coupling. This is what we observed, and this is what we discussed. On the other hand, in the HMBC spectrum we observed that carbon A has a long range correlation with proton B, HB. This carbon A has a long range correlation with proton HB. As a consequence, in the carbon-13 dimension, at the chemical shift of carbon A you saw a cross peak at the chemical shift of proton B. In the direct dimension you see it is chemical shift of proton B. And cross peak was seen at the proton position of B, at the carbon chemical shift position A in carbon dimension. Similarly, for carbon B the cross peak was seen at the position of proton A. This is heteronuclear multiple bond correlation wherein you will see that one bond correlations are efficiently suppressed. You do not see cross peaks arising due to HSQC; they are all suppressed. I hope you understand.

Thi is the major difference between coupled HSQC and coupled HMBC. HSQC gave me a direct correlation between carbon, which is attach to proton which is a doublet in the detection dimension due to its coupling with attach proton. Whereas, in the carbon dimension it is a singlet. I am repeating again. So, in the HMBC carbon A gave a correlation to proton B, which is 3 bonds away. You saw the cross peak; you can see it here for the carbon A; cross peak is seen at HB. Similarly, for carbon B cross peak is seen at proton A. Now that is so far was good.

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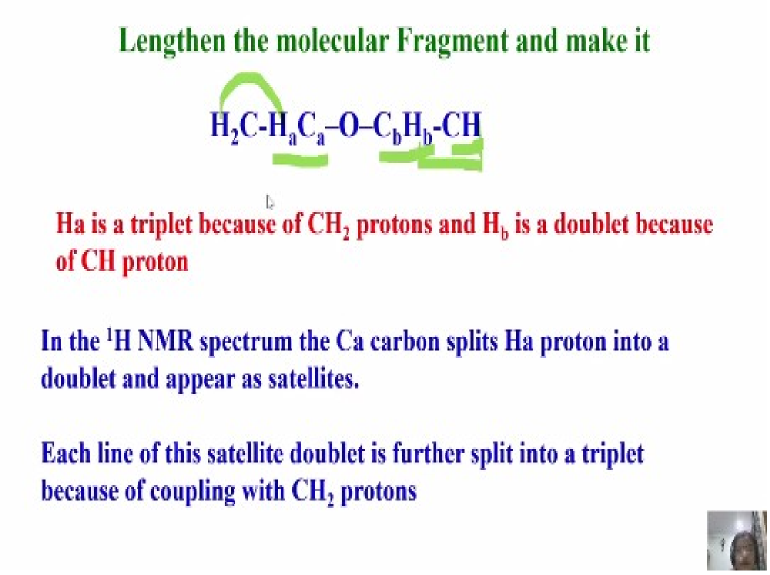

Lengthen the molecular Fragment and make it

$H_2C\text{-}H_aC_a\text{–}O\text{–}C_bH_b\text{-}CH$

Ha is a triplet because of CH_2 protons and H_b is a doublet because of CH proton

In the ^1H NMR spectrum the Ca carbon splits Ha proton into a doublet and appear as satellites.

Each line of this satellite doublet is further split into a triplet because of coupling with CH_2 protons

Now let us lengthen the molecular fragment. Let us see what happens? see up to this was known. We understood HSQC and HMBC spectrum for molecule up to this fragment. Now what we have done is we have extended the molecule; lengthen it by adding a CH group here and adding a CH2 group here. Now let us look at the proton spectrum. What is a proton spectrum, if I look at the proton a? That is a triplet because of its coupling with two equivalent protons. So Ha has to be a triplet. What about Hb? Hb has to be the doublet because of coupling with this CH proton. Of course, there is a CO here, and two bonds away and we assume that long range coupling between this proton and this are all not seen. So, we are seeing only coupling between this Hb with the CH proton; and this Ha with the CH2 proton. That is all we are assume. So as a consequence, this Ha is a triplet and Hb is a doublet. That we all know how to get the multiplicity pattern; you have already understood long back. So this is what it is.

But if I take into account the carbon coupling also in the 1H NMR spectrum what happens? Carbon a splits into a doublet because of proton a. Remember carbon a splits into a doublet because of proton a. So, at the proton chemical shift site what is going to happen? you are seeing a doublet; what are these doublets? These are nothing but the satellites. If I look at the proton peak this Ha peak is having a single peak like this because of C12 attach proton; and there are two small peaks on either side they are called satellites. This we know. We discussed in the previous HSQC. Exactly if I take carbon b that is also a doublet in the proton spectrum, which appear as satellites; that is fine.

Now we will understand more; we have lengthened the molecule. We have brought in CH2 here, and CH group here. Now what is going to happen for the satellite spectrum? because of this group. We know, we have already analyzed spectrum of satellites long back; I hope you remember. Now each line of this doublet, which are satellites, are further split into triplet because of coupling with equivalent protons.

Remember when we wanted to analyze AA'X spectrum, you broke the symmetry and we saw the coupling between protons in the satellite spectrum, which was invisible in the proton spectrum. Exactly similar now; this carbon is having coupling with this proton a; and split this into doublet, which appear as satellites at the proton chemical shift. Those satellitees, that is each component of the doublet will be split into triplet because of CH2 protons.

Now, similarly carbon at the proton position of b, because of split with carbon, it is going to be a doublet. So which again appear as satellites. What will happen to the satellites? It is a long range coupling; this proton has a coupling with this proton. So each line of the satellites is going to be a doublet. So now you are bringing in you are bring HH coupling, coupling between protons in the satellite spectrum. You understand. Now we are seeing two types of couplings. One is directly bonded carbon proton coupling, and also we are looking at HH coupling in the spectrum. Let us see how the HSQC spectrum appears in a molecule of this type.

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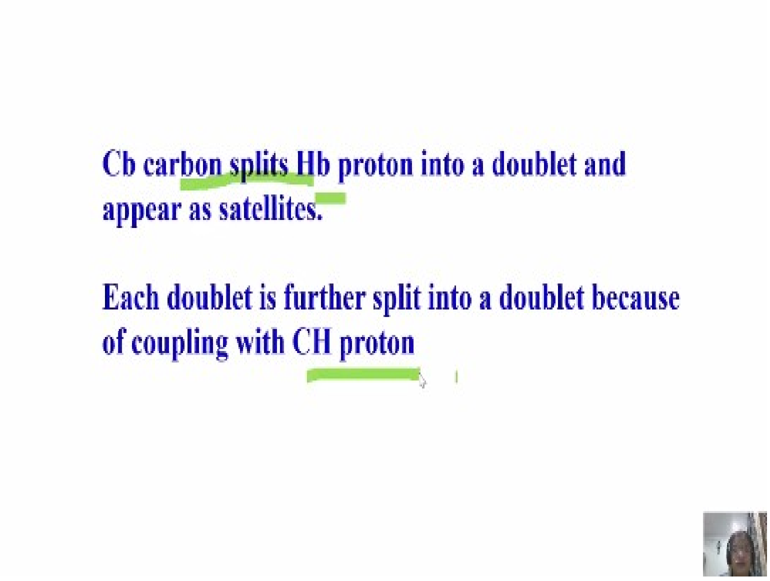

Cb carbon splits Hb proton into a doublet and appear as satellites.

Each doublet is further split into a doublet because of coupling with CH proton

So, as I said again carbon is split into doublet and appear as satellites; each doublet is further split into a doublet because of CH proton, that I just now discussed. Now carbon b, as I said, it splits into a doublet because of proton b, and each line of these satellites is split further into a doublet because of its coupling with CH proton. That is what we know from the 1D spectrum.

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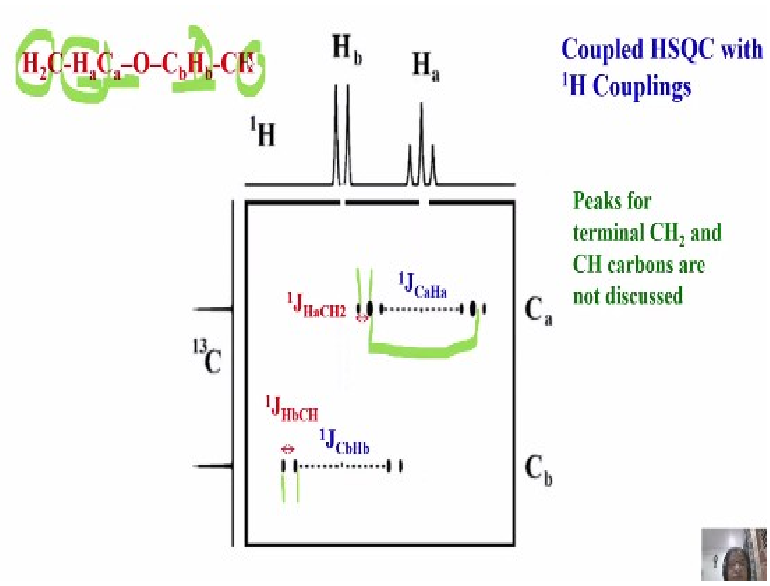

$H_2C\text{-}H_aC_a\text{–}O\text{–}C_bH_b\text{-}CH$

Coupled HSQC with ^{1}H Couplings

Peaks for terminal CH_2 and CH carbons are not discussed

Now let us look at the HSQC spectrum. Now we have taken the elongated molecule, where the CH group is added here and CH2 group is added here. How does the coupled HSQC and spectrum look? Before adding these two groups there was no central peak, and we were seeing only two peaks here. Remember in the previous HSQC spectrum we saw only two peaks, because of coupling of a carbon a with proton a.

The satellite which you see in the proton spectrum, was seen here, in the proton dimension. In the carbon 13 dimension we saw a single peak, that we analyzed. Now because of coupling of this proton with this proton, this proton is a triplet that is what you are seeing in the proton spectrum. This Ha is a triplet because of its coupling with CH2 protons. Now this coupling is going to be reflected in the proton spectrum; you know that.

When you analyze the satellite spectrum, in the satellite analysis I showed you, the homonuclear couplings are reflected in the satellite spectrum. This triplet with the HH coupling is seen in the satellites. So each component of this doublet is further split into triplets because of HH coupling, understood. The original HSQC spectrum we had only two peaks, because of coupling of carbon with proton. Now each line of this is split into a triplet because of coupling with two protons. Hence, it is going to be doublet of triplets. In the direct dimension it appears as doublet of triplets. Whereas in the carbon 13 dimension it is still a single peak. Is it clear? there is no change at all.

Now how does the carbon b coupled with proton and coupling with this CH proton appear. Of course, this in the proton spectrum at the site of chemical shift of proton b, this carbon splits this into a doublet. So we saw again two peaks will be there; in the previous HSQC we saw it is a doublet. But now each component of the doublet is split into additional doublet because of coupling with this proton. So, it appears like this. Exactly that is what we are seeing. In the coupled HSQC, now we have brought in proton-proton couplings also. But in the carbon 13 dimension it is still a single peak. Is it clear?

Now in the coupled HSQC, without proton-proton couplings we saw only a doublet. When the proton-proton couplings are introduced, they appear in the satellite spectrum, which are reflected in this HSQC spectrum. That is what I wanted to say. Now this one interestingly; this spectrum, center of this to this center is going to give me coupling between carbon to proton; this carbon to this proton, direct one bond coupling.

Now if I take the separation between this and this, what do I get? We get coupling between this proton and this CH2 protons. That is what we are going to see. And again in this case this separation gives me coupling between carbon and proton; Cb and Hb. And this separation gives me coupling between this proton and this proton. So this is the analysis, analysis becomes very simple now.

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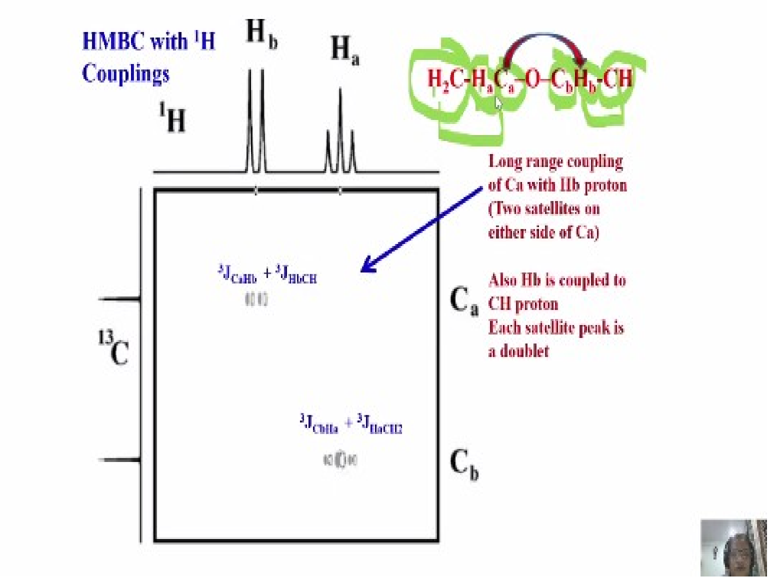

HMBC with ^{1}H Couplings

Long range coupling of Ca with Hb proton (Two satellites on either side of Ca)

Also Hb is coupled to CH proton
Each satellite peak is a doublet

Same thing now let us look at HMBC. This was HSQC. Now we will look at HMBC. What did we say in the HMBC spectrum without bringing this into account. At the site of carbon b we saw a peak because of long range coupling with this proton. At the site of chemical shift of carbon a we saw a cross peak because of its correlation with this proton; long range coupling. Exactly same.

So at the site of carbon a you must see a correlation with proton b; no doubt. We see at the site of a, we see the correlation due to proton b. Now this proton b split into a doublet because of this proton. So each line of this satellite should be a doublet. Very clearly we are seeing that. Each line of this is going to be a doublet. Are you all with me? I hope you are all with me. First now Ca is going to give a cross peak at the position of Hb. But this is a doublet because of this so each line of this will be a doublet. This is what we are seeing.

Now look at the carbon b. What is happening to carbon b? It is going to give a cross peak with proton Ha. We are looking at the similar old HSQC only; and now come to the extension part. Now carbon b gave a correlation peak at proton a; what is happening to proton a? at the site of carbon a? it is split into a triplet because of this H2 protons.

As a consequence what is going to happen? Each line of this doublet is split into a triplet. That is what is happening here. Additional splitting because of the long range coupling; in addition to seeing this Cb correlation to Ha; you are also seeing this HH coupling here. Very clear. What is that? simply look at this one; I have written. This splitting is going to give me 3JCbHa; 3 bond CbHa; 3 bond that coupling is there. Plus, the doublet is split because of 2J HaCH2. The Ha-CH2 it is 2 bond, 2 bond HaCH2 is what you are going to see, exactly here. Now look at Ca this is going to be Ca-Hb coupling, plus you are having coupling between Hb and this CH. So fantastic you know. The long range couplings of the protons are also reflected here. So, this is because of additional groups here. Addition of this groups, coupling between this and this is reflected, coupling between this and this is also reflected here.

So this is what happens if you bring in proton-proton couplings also in the HMBC spectrum and HSQC spectrum. I hope you understood. Now we have dealt with four cases. In this small molecule without these additional extra groups, we only saw this fragment in the coupled HSQC we saw we are going to see a doublet at the site of this because of this coupling, doublet at this site because of this proton in the direct dimension.

And the carbon 13 dimension gave me chemical shift of each of them. In the HMBC at this site of carbon a, we get the correlation to proton b. And at the site of carbon b, the correlation to proton a. Now in the next two examples we lengthen the molecule with this and this. Now exactly same we saw in HSQC; in addition to that in the satellite spectrum, coupling between this proton and this proton is seen at Ca site in HSQC. Coupling between this proton and this proton is seen at the site of Cb in HSQC. And on the other hand, in HMBC this carbon correlates to this proton; and this coupling is seen. This carbon correlates to this proton and this coupling is also seen. So we took four examples. Two examples without bringing in HH couplings in the satellite spectrum. In the remaining two examples of HSQC and HMBC we also introduced proton-proton couplings. I hope it is all clear for you. This is what it is.

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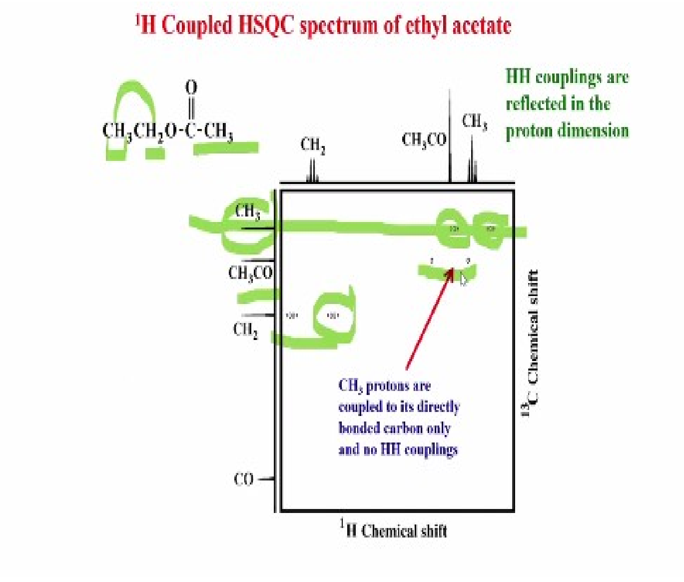
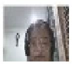

¹H Coupled HSQC spectrum of ethyl acetate



Now let us take the simple example of ethyl acetate and try to analyze the spectrum. Now this is 1H coupled HSQC. This is similar to the molecule which was seen before. There we saw that HH couplings and CH coupling, everything. Now what is the first one? We know. We are confident this must be CH3, which is at high field. It is coupled to CH2. In the proton spectrum this is CH3; and it is a triplet because of CH2. That is clear.

And this CH2 is a quartet, because of CH3, that is clear. Now in the HSQC spectrum what will happen? This carbon is going to correlate with these three protons. Exactly you get a doublet because of one bond CH coupling; you understand. But now each line of this doublet is a triplet 1:2:1 triplet, because of coupling with CH2 protons. That is what we observed in the previous example. In the realistic example you are able to see that.

But in the carbon 13 dimension you see only one peak. And that is the chemical shift of this carbon. All information you can get in one simple experiment. Look at this one, what is this one? This is CH2. In the HSQC you see a doublet because of correlation of this carbon with this proton, and it is a doublet because of 1JCH, and each line of this doublet is a quartet because of coupling with these three protons. Very clear. There is no doubt at all everything is very clear. Right !

Now CH3CO; if you look at this what do you expect for this CH3CO? In the proton spectrum it is a singlet and these two are carbon 13 satellites. And there is no long range coupling with proton for this. They are all far away. So, it remains a doublet in the HSQC spectrum. No other proton-proton couplings are seen. I hope you are all with me.

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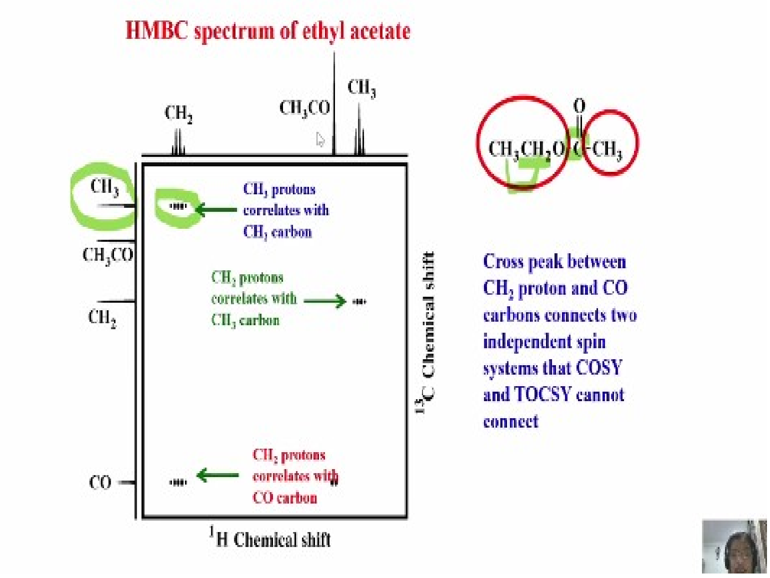
HMBC spectrum of ethyl acetate

Cross peak between CH_2 proton and CO carbons connects two independent spin systems that COSY and TOCSY cannot connect

Now the same thing if you look at the HMBC what happens? Interesting thing. Exactly at the site of CH3, you must see correlation to CH2 proton long range coupling, long range correlation that is what we see. Exactly at the site of CH3 you are seeing correlation to CH2 protons. But because of this coupling to this, what you should expect. You should expect each line of this doublet splits into a quartet. Carbon CH2 is coupled to three protons that multiplicity is seen here.

CH3 proton correlates with CH3 proton and here CH2 protons correlates with CH3 protons. And here CH2 proton has a long range correlation to CO carbon also. HMBC is the biggest advantage, you can get correlation to remotely bonded protons with carbon. If this carbon is there, it is remote bonding with this proton can be seen. Remote bonding of this carbon to this proton, if possible you can see. So it is only two bond. Three bond correlations can be seen. That is the example I gave for HMBC. We took the real example of ethyl acetate and we analyzed the spectrum. I supposed you all got the point as how to analyze the coupled HSQC and coupled HMBC spectrum. Remember, this is the one which you routinely use in your carbon 13 NMR.

If you want to synthesis the molecules and get proton- carbon correlation, proton and carbon chemical shifts, couplings and how to assign each carbon, and carbon chain you can get the proton-proton nearest neighbor by COSY, TOCSY etcetera. But if you want to find out which carbon is next to this proton, so you can do this type of experiment, the long range correlation to proton carbon like HMBC type experiment. So this is what it is.

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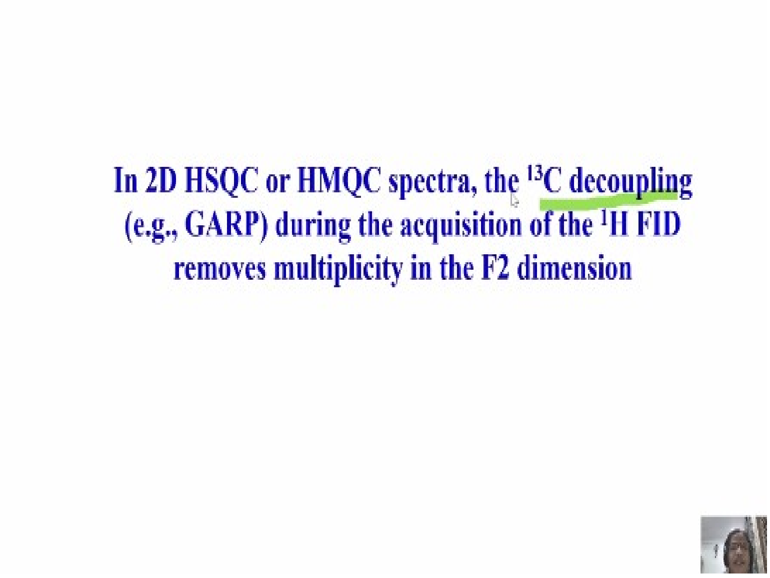

In 2D HSQC or HMQC spectra, the ^{13}C decoupling (e.g., GARP) during the acquisition of the ^{1}H FID removes multiplicity in the F2 dimension

Now next thing what I wanted to say is, in the same HSQC, HMBC, HMQC spectra we can bring in carbon 13 decoupling. So far, remember, we saw carbon coupled HSQC, carbon coupled HMBC. Now we can also do heteronuclear decoupling. Remember in in the carbon spectrum you can completely decouple proton and what you are going to see for each carbon a single peak.

In the proton spectrum you are going to see carbon 13 satellites. That is what we saw in HSQC. What we can do, we can do even the carbon decoupling in addition. While detecting proton you can do carbon decoupling. Then what will happen? the satellites will disappear and merge into a single peak. What you will expect in HSQC spectrum. The two doublet you saw as satellites, now merge and give a single peak at the center. Where we see only a single proton peak, we are going to see a correlation peak that is all. Only thing is splitting is removed. The coupling is removed, No more splitting; we will get correlation at the respective chemical shifts. Coupling information is absent. You do not see additional splittings in the HSQC spectrum.

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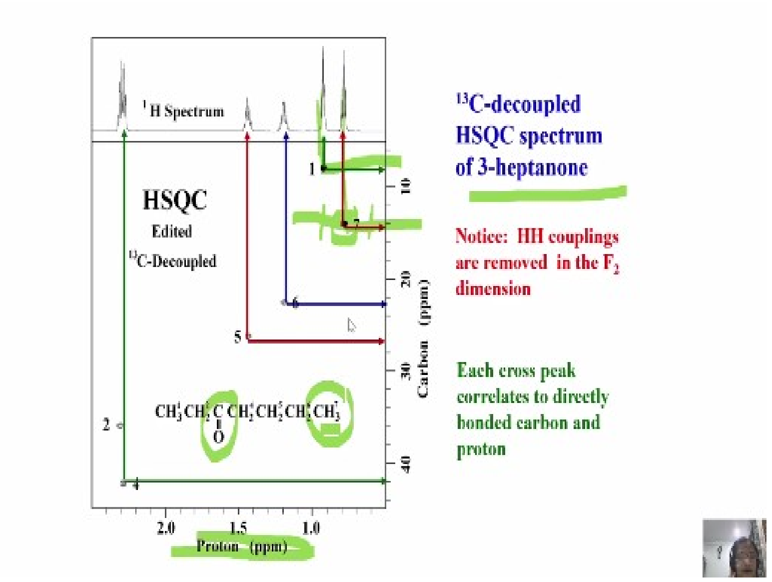

^{13}C-decoupled HSQC spectrum of 3-heptanone

Notice: HH couplings are removed in the F_2 dimension

Each cross peak correlates to directly bonded carbon and proton

Look at it now take a molecular 3-heptanone. In 3-heptanone molecule we have how many carbons 1, 2, 3, 4, 5, 6 different carbons. Of course, the CO carbon will not appear in the HSQC or HMBC spectrum because it is not attached to protons. Now this is a decoupled, carbon 13 decoupled, That, means when you are observing proton you are decoupling carbon 13.

So, let us take this example of proton 7; this is CH3 proton. In reality in HSQC; in the coupled HSQC this was a doublet. But now because you are removing the carbon proton coupling you are seeing only one peak, at the center, Very clear right. This is a decoupled HSQC, you directly get the correlation with this. Now what you do is, go along this axis you get the carbon chemical shift; go along this axis you get proton chemical shift. It directly gives you carbon and proton chemical shifts in the corresponding carbon and proton dimensions. That is all no coupling information.

Now look at this proton one; go here you get proton coupling, go here you get carbon coupling. So all this things can easily be obtained. So coupled carbon HSQC, and decoupled HSQC, the only big difference is multiplicity is reduced. And in decoupled HSQC only correlation of chemical shifts of heteronuclear spins directly coupled carbon-proton or carbon-nitrogen such information only we can get. Nothing else. So as I said, HH couplings are removed then this is how you get the correlation.

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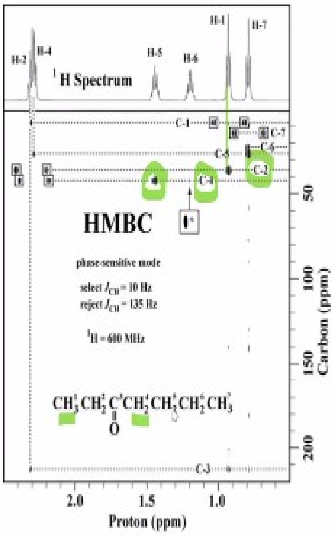
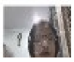


HMBC spectrum of 3-heptanone

Strong HSQC correlations are also seen (marked in squares)



Now the same thing if you do the HMBC what happens? At the site of this proton CH3, this correlation is seen at CH2 carbon. For example, CH2 carbon can give correlation to this proton or this proton. Look at this one, proton 6, we will start with simple one which we very well know. Here itself carbon 1; this one. The carbon 1, you see it will go along this axis you get a carbon chemical shift. You can see the correlation for this here H2. This carbon 1 is correlated to this proton 2, as you can see the peak here. This carbon 1 is giving a correlation to proton 2. Then you may ask as what are these things coming? That is HSQC correlation which are not completely suppressed here. In this experiment, in fact in the real experiment we have to suppress these things, but it is not suppressed here, that is why they are marked in squares here. This is because of HSQC correlation. You ignore those marked in squares, do not worry. Now you will see for carbon 1 there is a strong correlation to proton 2. Now carbon 2 if you take, where is carbon 2 it is here, okay this one. This carbon 2 should have strong correlation to proton 1 with this one; where is this proton 1, somewhere here. C1 should be there these are all hidden here. Somewhere there you must see. Or we can see carbon 2 is here, I am sorry, carbon 2 is here. Carbon 2 you see there is a strong correlation to proton 1. I am sorry I was not seeing this properly. Carbon 2 is here, I was looking elsewhere. Now it has a strong correlation to proton 1. This has the long range correlations. Now look at C4 carbon 4, it has a correlation to 5, the long range correlation. It can correlate to only to this proton. So like this HMBC spectrum gives you coupling to two bonds or three bonds away protons. The correlation to protons which are two bonds or three bonds away. Again it is a decoupled HSQC.

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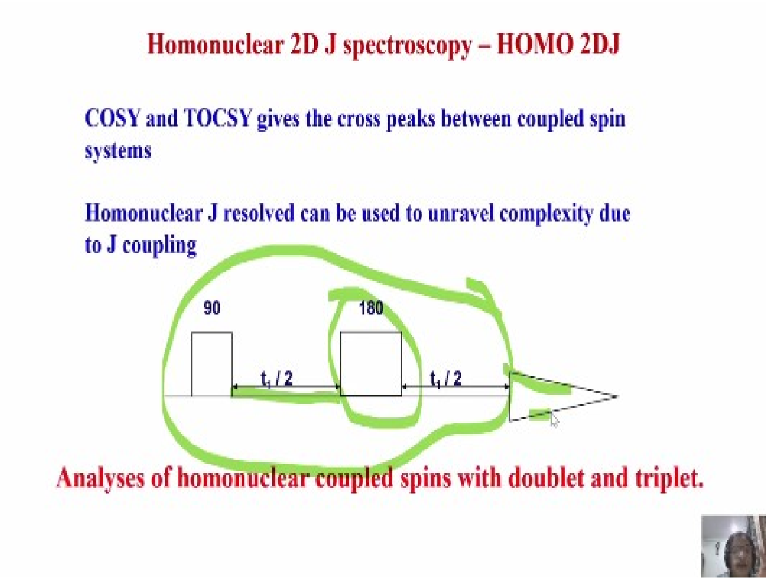

Homonuclear 2D J spectroscopy – HOMO 2DJ

COSY and TOCSY gives the cross peaks between coupled spin systems

Homonuclear J resolved can be used to unravel complexity due to J coupling

Analyses of homonuclear coupled spins with doublet and triplet.

So now we will go to the next topic. I think you are all familiar with a HSQC, HMBC, TOCSY and couple of 2D experiments I have introduced. Now I will introduce one more thing which is called homonuclear and heteronuclear J resolved experiments. Now we will see resolved type experiments, both homonuclear and heteronuclear. See in this case it is simple like spin-echo sequence.

How spin vectors behave, we understood this by vector diagram long back. It is the pulse sequence. You have a 90 degree pulse and then we have time delay t1 / 2. At the mid of t1 period you apply a 180 pulse and then after the t1/2 period, you start collecting the signal. So this is a simple homonuclear 2D J resolved sequence.

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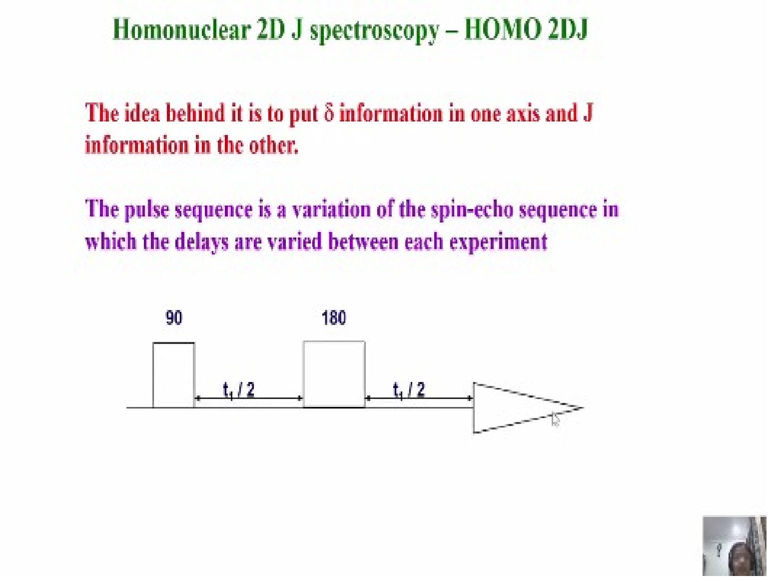

Homonuclear 2D J spectroscopy – HOMO 2DJ

The idea behind it is to put δ information in one axis and J information in the other.

The pulse sequence is a variation of the spin-echo sequence in which the delays are varied between each experiment

In this case idea is what it does is; it puts the chemical shift information along one axis and coupling information on the other axis.

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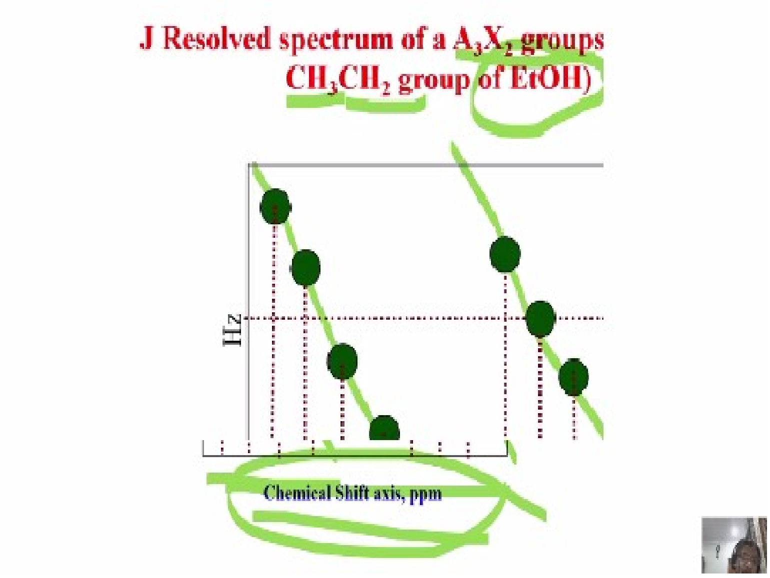

J Resolved spectrum of a A_3X_2 groups

CH_3CH_2 group of EtOH)

In the real spectrum what happens is, in this dimension not only you get chemical shift you also have the coupling information, because it is a direct dimension both are there. Chemical shift and coupling both are there, because in the detection dimension you are not doing anything, you are simply collecting the regular spectrum. So, all coupling and chemical shift information are present. Whereas in this direction, we have only coupling information that is all.

The J resolved experiment is simply a two pulse sequence like 90-t1/2-180- t1/2. That is the sequence. That is all you have to do when you do an experiment. This is an example for A3X2 type CH2 coupled to CH3. They may be CH3, CH2 groups of ethyl alcohol. Now because of the J coupling it appears it is as if it is titled by 45 degree here. So this is because delta and J, both are present. So now what we can do is, this one because both delta and J are present, we can tilt the entire spectrum by 45 degrees like this.

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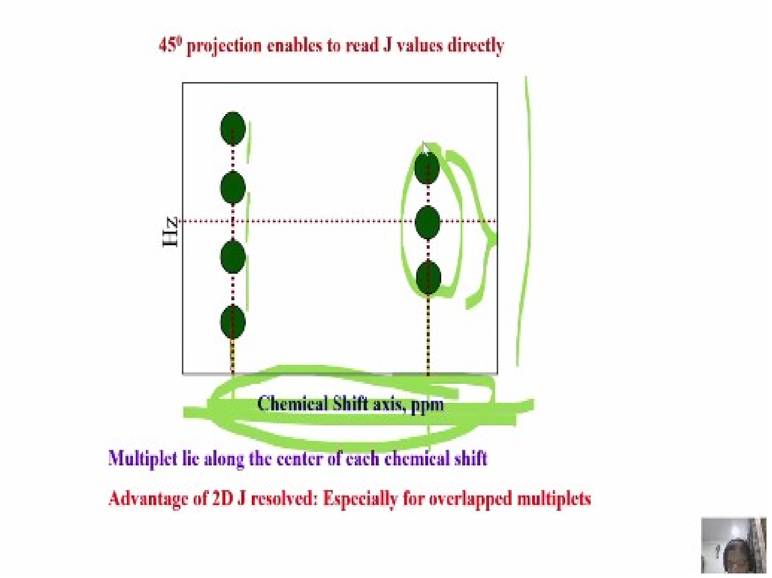

45⁰ projection enables to read J values directly

Multiplet lie along the center of each chemical shift

Advantage of 2D J resolved: Especially for overlapped multiplets

This is called 45 degree projection. Now what are we going to see? This is a chemical shift of CH2, this is chemical shift of CH3. And what you are going to get in this direction? In this dimension the separations will give you JHH, this is JHH, this is JHH, this is JHH. So multiplets lie along the F1 dimension, that is the indirect dimension. Whereas, the chemical shifts are now along direct dimension; that is t2 dimension. Look at it.

So what is advantage of it? You have resolved both the information; chemical shifts and couplings are separated out in two dimensions. Remember, this is called J resolved experiment. The J resolved experiment is simply a 2 pulse sequence 90- t1/2-180-t1/2 and you start collecting the signal, It is like a spin echo sequence, and we understood how the spin vectors behave for CH vector, CH2 vector and CH3 vector, when we were trying to understand APT. Remember, we understood spin echo. exactly. So in this real spectrum it is actually there is a tilt, and if you take the 45 degree projection of that now you get only chemical shift along this direction. In the t2 dimension you get only chemical shifts. In t1 dimension you get only J couplings, nothing else. So you have separated the information.

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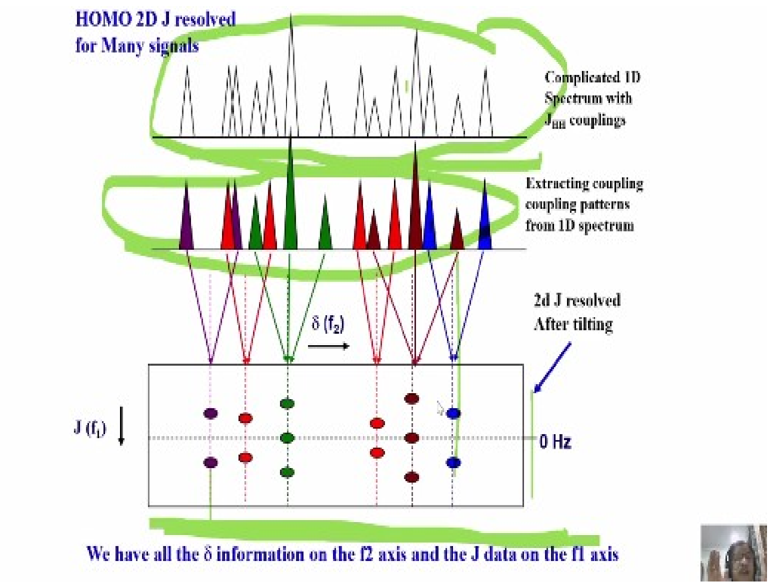

Now I give you an example like this. I have a complex spectrum like this. Now this is a multiplicity pattern. I do not know which is triplet, which is quartet, which is doublet. How do I do that? If I have to analyze this complexity, it is a difficult job. As I said why cannot we now separate out the information of chemical shifts and J coupling in two different dimensions. Let us try that.

What I will do is I am doing the coloring. Now I know this and this is a doublet, this and this is a triplet; like that this and this is a doublet, because I know this I am coloring. How did I know that I took the J resolved spectrum; and titled by 45 degree. So this axis gave me J coupling, and this axis gave me chemical shift. Exctly at the center of this you get chemical shift, then if I take 45 degree tilt this and this are coupled. If I take 45 degree tilt I know this, this and this are coupled. So like this we can make the assignment of all the multiplets in a complex spectrum, by taking the J resolved spectrum. So you are separating out information of J coupling and chemical shift in two different dimensions; this is what it is. One interesting thing you must see, when I take the tilted spectrum, 45 degree tilted, you are getting only chemical shift here. What does it mean? See in the normal spectrum it would have been a doublet and this would have been triplet. So now what did we do. In fact, if we take the 45 degree tilted J resolved spectrum, it is as good as telling in this dimension you have done the broadband proton decoupling, analogous to decoupling of one proton coupled to all the other protons, for all the coupled partners you are completely removing their couplings.

So what are you going to see only singlet peaks. It is similar to carbon-13 with a broadband decoupling where you got only single peaks for each carbon. Exactly analogous to that. So, if

I take the 45 degree projection, each peak in the J resolved spectrum pertains to a single proton. It is like telling this is a spectrum with broadband proton decoupling or in other words it is a proton decoupled proton spectrum; broadband proton decoupled proton spectrum, similar to broadband proton decoupled carbon spectrum. So the broadband proton decoupled proton spectrum is what you are going to see when you take 45 degree tilt.

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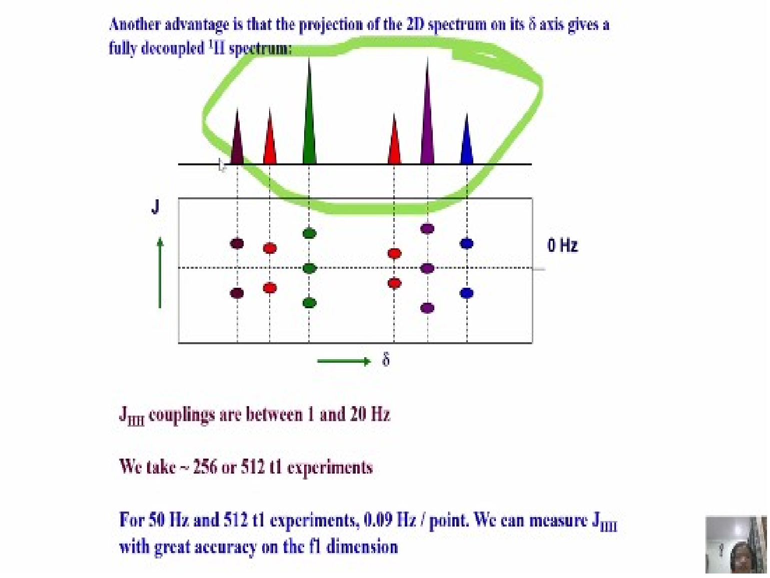

Another advantage is that the projection of the 2D spectrum on its δ axis gives a fully decoupled ${}^1H$ spectrum:

J_{HH} couplings are between 1 and 20 Hz

We take ~ 256 or 512 t1 experiments

For 50 Hz and 512 t1 experiments, 0.09 Hz / point. We can measure J_{HH} with great accuracy on the f1 dimension

See this is the advantage. Fantastic spectrum now I have removed the coupling. I am showing you this is like a completely decoupled spectrum. This is a one chemical shift, one chemical shift all the six chemical shifts are seen, and in this dimension we are getting the J couplings.