One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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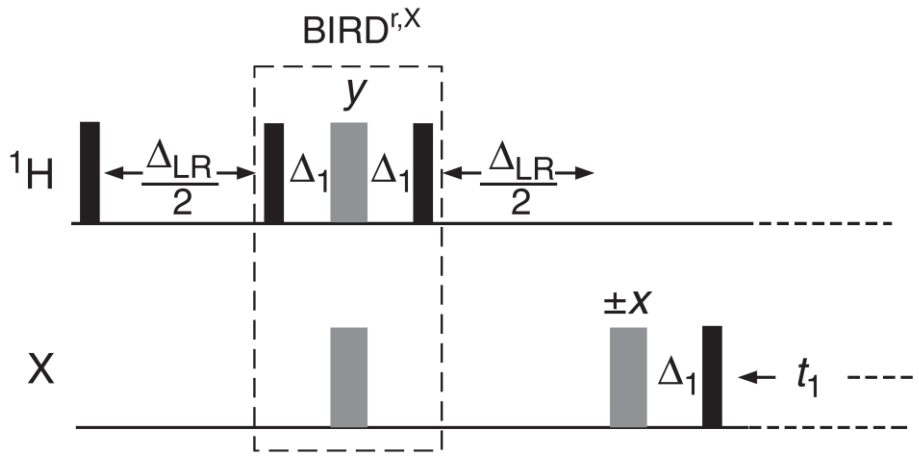
Lecture 47: HMBC-II

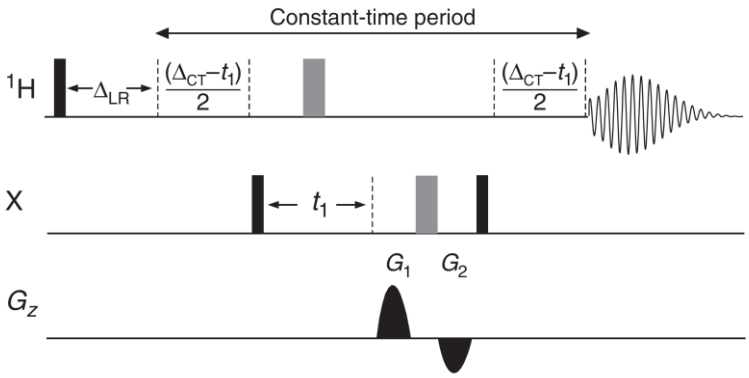
Welcome all of you. We have been discussing about hetero nuclear correlation experiments. We discussed in detail about heteronuclear single quantum correlation, where we are going to do an experiment, where hetero nuclear spins that are one bond coupled will be correlated. For example, carbon-13 directly bonded to proton or nitrogen-15 directly bonded to proton, such type of experiments, viz., carbon-13 proton HSQC or nitrogen-15 proton HSQC. We understood how we can do that, how we can interpret the spectra everything, why we are not going to see the signal because of the long range coupling which are present in the molecule in HSQC. We have calculated the intensity and saw that, in such a case, the intensity is too low to be detected in the HSQC. We understood that and we also interpreted lot of spectra. Then to go further, if you want to detect correlation, the long range correlations like proton coupled to carbon which are two bonds or three bonds away, two with the three JCH couplings, if present. There is an advantage in such cases, remotely bonded carbon to proton like quaternary carbons, CO carbons, they also can be detected. So, this type of experiment is called hetero nuclear multiple bond correlation. In hetero nuclear multiple bond correlation experiment, what we do is, we have a filter for long range correlation, delta LR whose value is set for the long range coupling of the order of 5 to 10 hertz. And then we will allow only those correlations coming because of the long range couplings. One bond couplings are suppressed and for that we use a filter. There are two types of filters in HMBC, one is filter, which is J accept and J reject. J accept is a filter like delta LR where long range correlations are allowed through, whereas one bond correlations like HSQC cross peaks are all suppressed. That is another type of filter. For this we have different types of filters, not just one filter. That filter can be utilized in such a way we can allow only long range correlations and suppress the one bond correlations. One such filter for that is a BIRD sequence. I told you about the BIRD sequence. The BIRD sequence is a very simple equence. We saw in the BIRD sequence what is going to happen. It has two 90 degree pulses separated by a delay and in between a 180 pulse on the proton channel and on the carbon 13 also we have a 180 pulse, and the delay is set to 1/JCH. In this case what is going to happen is, we understood with vectorial picture that this BIRD sequence has an effect only on the carbon 12 attached protons which would selectively get inverted. Whereas this sequence has no effect on carbon 13 attached protons. This is what we saw. It will selectively invert only that and this is the effect of the BIRD sequence. Effect of the BIRD sequence is carbon 12 protons are selectively inverted and carbon 13

attached protons will continue to evolve. And what we saw for carbon 13 attached protons, the chemical shifts will refocus and after a 2 delta period we saw the doublet vectors which were along the y axis of the 13C vectors, return to z axis. This is going to be detected by the final proton pulse. That is what is going to be detected for carbon 13 the BIRD has no effect. That is what you should remember, and this is a HMBC sequence with BIRD.



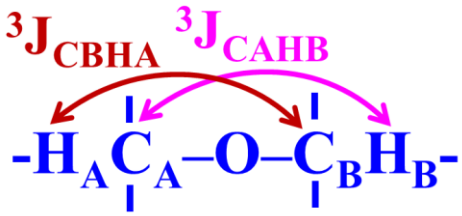
Everything remains same, we have a LR and in between these two LR a BIRD sequence is put. And BIRD sequence, as I said, selectively inverts only those protons remotely bonded to carbon 13, that is 2 or 3 bonds away, and does not affect the protons which are directly bonded to carbon 13. So, then this achieves the selective refocusing of 1JCH since only carbon 13 is experiencing a 180 pulse, with refocusing of all long range couplings because the delay in the BIRD sequence is set for 1JCH, that delay delta. So, it refocuses all the correlations coming because of $^{n}J_{CH}$ for which carbon 13 experience net 180 pulses, that is what it does. It refocuses 1JCH. Of course, 1JCH values can vary it is not constant 1JCH can be 150, 200, 250 Hz depending upon the type of molecule we are looking at. This can affect the efficiency of this approach because we set the delay for 1 value in the BIRD element. But if the value is different that can cause some issues, complications will come. So, addition of a subsequent 180 degree carbon 13 pulse followed by 1 over 2 period of delta 1, refocuses only 1 bond coupling that have passed through the BIRD filter. This is another way of doing it. What we do is we apply another 180 carbon 13 pulse followed by short 1/2JCH in the delta, which refocuses those 1 bond coupling which did not get filtered out because of the BIRD, that can be done. So, since 1JCH are refocused, and they no longer generate multiple quantum coherences and do not contribute to final spectrum. This is what happens. What is going to happen is we suppress one bond coupling which do not contribute to spectrum because we use the filters. There are varieties of filters. Another thing is the phase alternation of 180 pulse. That is another way we can effectively suppress one bond correlations. That is also

possible, we can do that. One more experiment, an another improved version of HMBC. It is called constant time experiment. Constant time HMBC, eliminating proton-proton coupling in F1. It is a very simple thing. How do you? dont allow couplings to evolve in the t1 period. Keep them constant. If they do not evolve then it is as good as decoupling, or removing them. The 2JHH for example, JHH evolution can be suppressed if the modulation of the signal in t1 is suppressed. How do you do that? t1 gets modulated. We get a modulated signal you know when we keep incrementing the t1. Instead of that we can keep it constant. How do we do that experiment? but we need to increment the t1 to get the FID. But we do in a very tricky way. I will show you that. If the evolution of JHH is there in the t1 dimension it gives peak broadening in both HMQC and HSQC, usually not visible due to low resolution, but that gives rise to broadening. But JHH evolution is due to modulation of the signal as a function of the t1 period. It evolves, because there is a modulation. What we do is we stop this modulation. Somehow we keep the evolution time constant, then we can prevent the JHH modulation. For example look at this one. Constant time period here. This is called constant time period, delta CT that is always kept constant. The delay is set to a maximum value that t1 period will attain in the experiment. As you know t1 keeps incrementing from a small value, we can increment to some value. That maximum value keep it constant here. That is what and then that is called delta CT, t1 maximum this one, and then this one, the 180 pulse in between we can move it forward or backward, either way, so that this time is always remained constant, you understand. So, we can keep this constant but we can remove this. Whereas this LR is constant this is before and after that is constant, this is after this one so that is always kept constant. This is a constant time, this is an experiment, delta CT minus t1, we can keep increasing it. As t1 increases the total period remains constant. you have to maintain this one constant and this one keeps changing. That is what we do.



And this variable delay can be reduced from maximum value to 0. First delta CT we keep it constant and this keeps on subtracting, this t1 delay so that it comes to 0 as t1 is incremented, from maximum of t1 value to 0 value. So, delta CT is the one which is

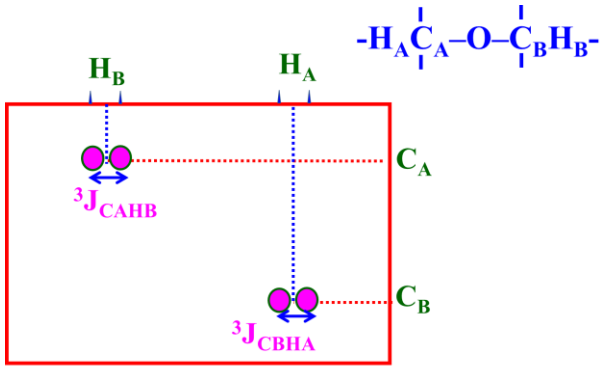
maintained. And during this process what happens the proton-proton coupling evolution do not get modulated at all. It do not modulate the detected signal. This is called the constant time experiment. Identically we can do this constant time experiment in HMBC, that is one way. So there is another improved version of this constant time. Alright there are so many such experiments, so many modifications are there, now several modified versions of there like IMPEACH is there, PENDANT is there, they are all different pulse sequences. Let us not discuss too much about those things, but please remember we can use constant time HMBC, we can also use BIRD element to selectively invert the protons attached a carbon-12. All these experiments are improved versions so that one bond 1JCH is always suppressed, and the long range NJCH are all allowed through. That is the filter which allows and the others are reject filters will stop that. This is the basic idea of any HMBC pulse sequence. With this now we will try to understand the interpretation of the peaks in HMBC. Start with the same molecule which we took earlier.



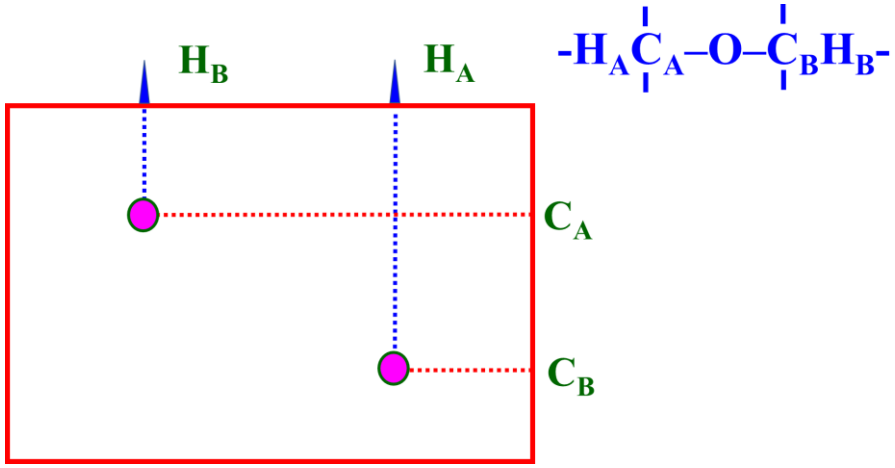
We will look at the F2 coupled HMBC carbon 13 proton coupled. Where does HMBC gives cross peak? we understood HSQC where does it give cross peaks both in coupled and decoupled versions. Where does HMBC gives, we will understand. Remember in the F1 dimension if the chemical shift of carbon A is there, it gives correlation to the remotely bonded proton. Not for the one bond, that is HSQC you know that. So we suppress that it gives correlation to long range protons. So in the HMBC experiment in the F1 dimension if you get a chemical shift for carbon A, in the F2 dimension it pertains to the chemical sorry chemical shift of proton B, not proton A. This is the difference between HSQC and HMBC. In HSQC if it is carbon chemical shift, the proton chemical shift is which is attached to it. Whereas in the HMBC if you look at the carbon chemical shift of A it gives correlation to proton which is 2 or 3 bonds away. Here $3J_{CAHB}$ is there. If you go to carbon B, in the F1 dimension chemical shift is carbon B, in the F2 dimension it is going to be proton chemical shift of A, not B. CBHB correspond to HSQC, but now carbon B chemical shift correlates to proton A chemical shift, which is $3J_{CBHA}$ that is the type of peaks we get if you do coupled HMBC.

Let us see the spectrum, here this is F2 coupled HMBC. Now I am seeing the carbon chemical shifts, here go across I am not seeing correlation here, that is one bond we are

suppressing that. That is HSQC cross peak come here, it correlates to the remote proton and this proton is a doublet, why? because of 3 bond JCH, carbon proton coupling and the center of this doublet correspond to chemical shift of proton HB.

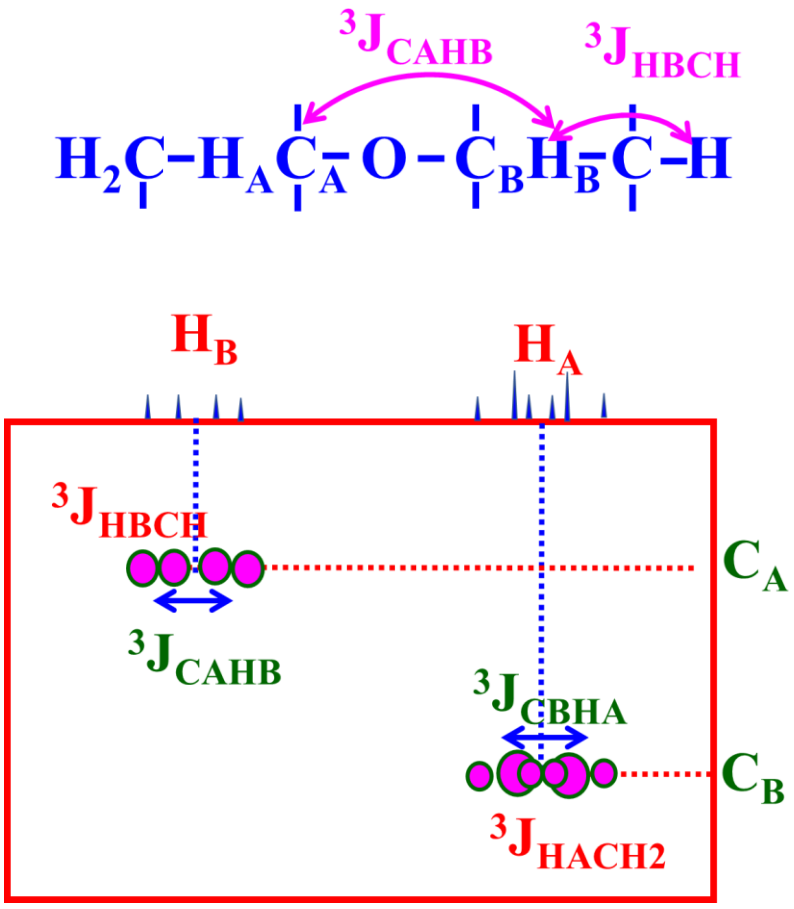


What about carbon B? where does it correlate? carbon B also is a doublet because this is correlated to proton A, proton HA. As a consequence long range $^{3}J_{CBHA}$ coupling is there. So, that is why that is also a doublet. So, along this axis you get carbon chemical shift, along this axis you get proton chemical shift. This is the F2 coupled HMBC spectrum of this molecule. Please see the difference here. In HSQC you are seeing the cross peak of CA to HA, CB to HB. In HMBC, CA is correlated to HB, CB is correlated to HA, remotely bonded 2 to 3 bonds away. This is what it is. The same thing if I do the decoupling what happens? I told you HMBC is always recorded in a coupled mode, we do not decouple it, I told you.



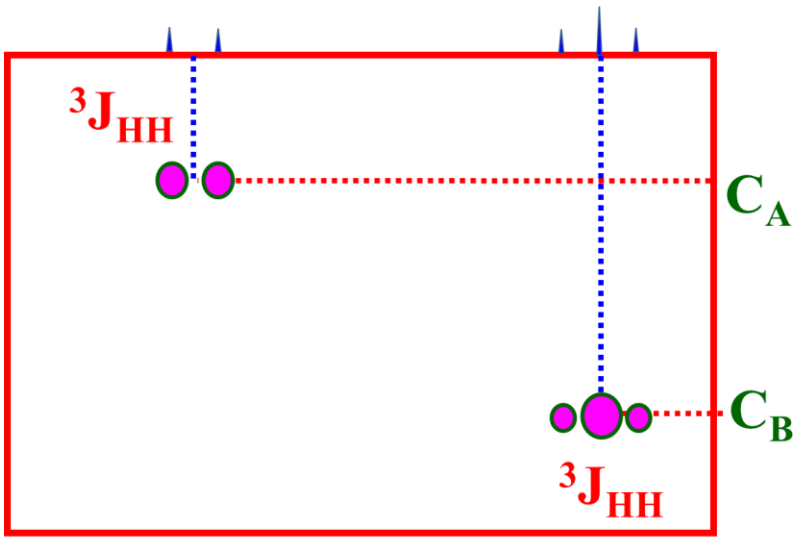
Even if you want to do the decoupling how do you understand that? Simply do break the coupling between 3 bond carbon proton coupling. So, it is going to be a singlet. So, if this axis is a chemical shift of carbon A, go vertically up you get chemical shift of proton

B, not A, remember. Similarly here chemical shift of carbon B in this axis, it correlates to chemical shift of proton A. So, they are cross correlated. See CB is correlated to HA and CA is correlated to HB in the decoupled spectrum. This is decoupled. What happens to proton-proton couplings in HMBC? can it come? Why not? exactly similar to what we saw in the HSQC.

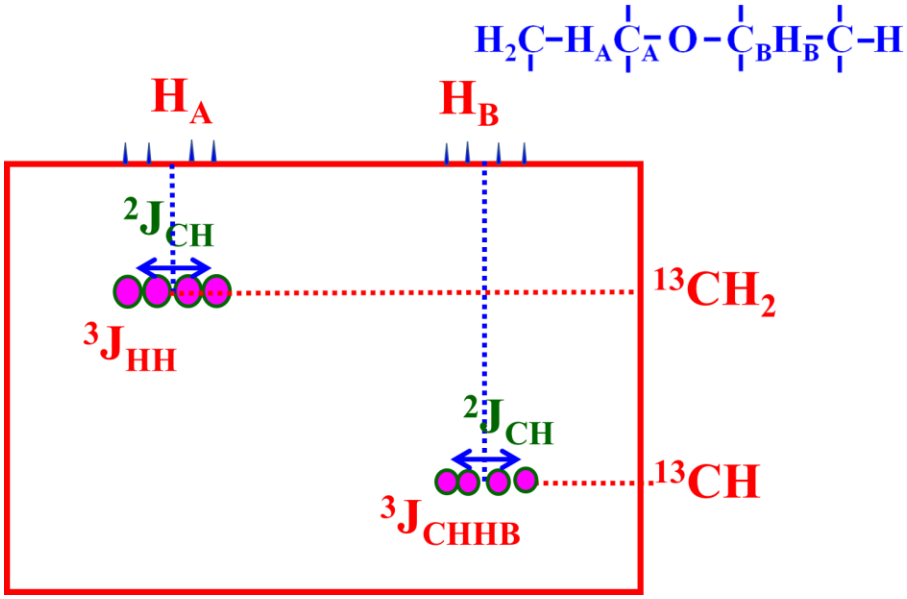


This CA correlates to HB but what is HB? it is coupled to this. When it is coupled to this what will happen? it will be a doublet, that will be seen. So, CA is a doublet because of 3 bond coupling with HB plus it is also a doublet. Each peak of the doublet is a doublet because of coupling with CH proton. This is what is going to happen. So, CA will be a doublet because of 3J coupling with HB plus doublet of doublet because of HH coupling, that is what it is. In the F1 dimension we are going to get chemical shift of A and F2 dimension it correlates to proton chemical shift of B, and it is a doublet of doublet with large coupling and small coupling, they are almost of the similar strengths. The 3J coupling of carbon proton and 3J coupling of proton proton, the pattern is a doublet of doublet. If you go to the other carbon, carbon B it can correlate to proton A, 3 bond.

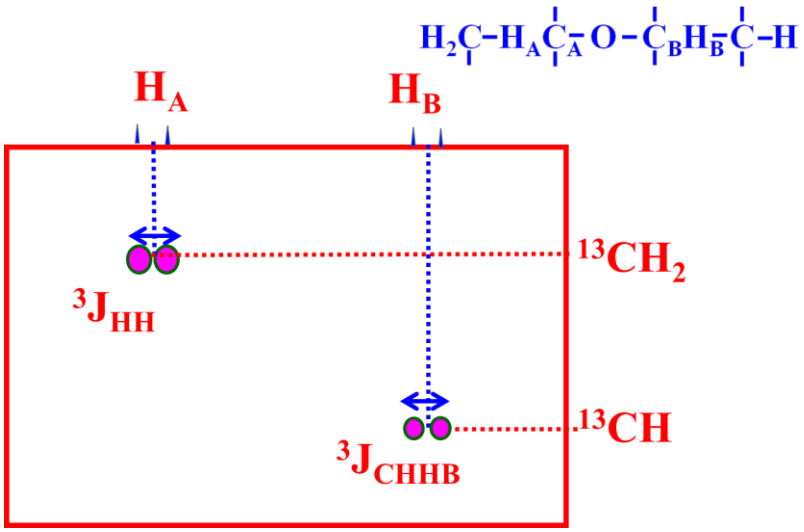
Then what about this proton? it is coupled to chemically equivalent CH2 protons then it will become a triplet. So, what will happen? first this carbon is a doublet because of long range coupling, 3J coupling, and then each doublet peak is a triplet, it is triplet because of coupling with CH2 proton this is what you are going to get. CB is a doublet because of 3 bond coupling and is a triplet because of CH2 protons. So, in the HMBC spectrum in the F1 dimension you get carbon chemical shift of B, but in the F2 dimension you get proton chemical shift of A, because of splitting of 3J and 3J HH one is a doublet other is a triplet and this is how the pattern you get, very easy. Look at this CA carbon it is correlating to HB proton. The center of this corresponds to chemical shift of proton HB, it is a long range coupling one of them may be 3JCH and then each of them is further split because of HA-HB. So, that is what Hb and CH proton, 2 protons are there, this is a doublet of a doublet. Whereas HA proton if you look at it this is correlating to carbon B and the center of this correspond to HA proton chemical shift. It is a large doublet because of 3 bond CH coupling, but each of this doublet is a triplet 1 2 1, 1 2 1, they are overlapped because it is coupled to CH2 proton this HA proton is coupled to CH2 proton. As a consequence it is going to be doublet of triplets. Some overlap is there and you will not be able to see all the multiplicity clearly. This is what you are going to see in the HMBC spectrum see the difference between HSQC and HMBC. In HSQC CA was correlating to HA, CB was correlating to Hb here, but in HMBC they are all different. CA correlates to HB and CB correlates to HA. But the multiplicity patterns what you saw in HSQC what you are seeing in HMBC are similar, only thing is in the HMBC we have a long range correlation and HSQC has a 1 bond correlation and in both the cases if you want proton coupling they all get reflected. This is what basically you have to understand.



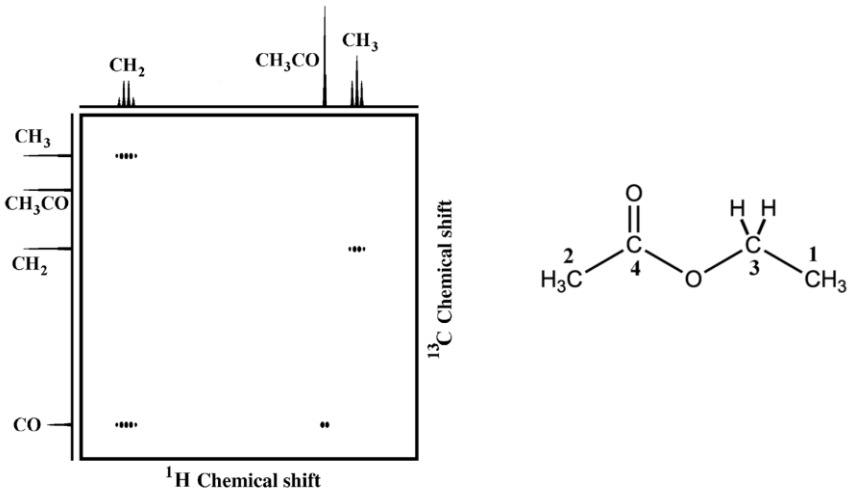
If you do the decoupling of the same thing what is going to happen you remove the carbon proton coupling, but HH coupling is present. So, at the CA chemical shift, it is correlating to HB which is a doublet because of this. You are removing this coupling, but this is present that is what happened, is a doublet. Same way here at the CB chemical shift which is correlating to HA that you are breaking, but this will be a triplet because of this that is seen here that is what is seen. This is the decoupled HMBC this is what we are going to see.



Then what about the terminal groups, like we saw in HSQC. We can try to interpret that. If you go to the CH2 is carbon, CH2 can correlate to CA carbon, and this is correlating to HA, this is the carbon of this one and it correlates to HA proton. And of course CH coupling is also there and because it is F2 coupled, you also have HH coupling. The HH coupling is also seen, HH coupling is a doublet because of this proton that you should remember we are seeing this carbon which is a long range correlation to this and also this has a CH2 carbon. The CH2 proton is a doublet because of HA this is what you are going to see. Similarly, HB along this axis you get carbon chemical shift, exactly from the center if you go, you get proton chemical shift. Again CB you are correlating with this proton we are considering this carbon, correlating this proton and there is a doublet because of that and that is what you are going to see. Of course, if you do the decoupling you will remove the carbon proton couplings, but you retain proton-proton couplings that is what happens. You are retaining the HH coupling here both the cases in both this carbon will be a doublet and this carbon is a doublet because of this. So, HH couplings are retained.

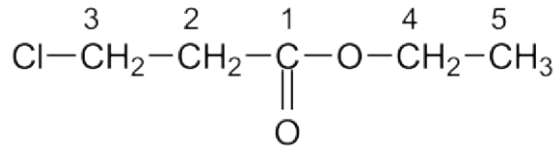


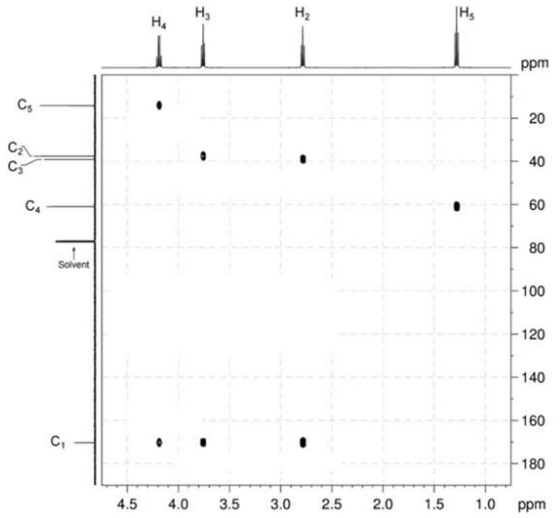
We will look at the realistic example of HMBC spectrum of ethyl acetate. This is simple molecule. Let us look at this. It is exactly like we analyzed HSQC of the same molecule. @e will analyze the HMBC spectrum. How do you analyze HMBC spectrum here? Start with CH3.



CH3 protons which is marked 1, H1 it has a long range correlation with the CH2 carbon. These protons are correlating to this carbon that is 2 bond coupling. What will happen that will become a doublet. So, we are seeing this proton correlated to the CH2 carbon is a doublet. Then what will happen? Each line of the doublet is split because of this CH2 protons into a triplet. This is going to be doublet of triplet. But you see this is a doublet and you will see 1, 2, 1, 1, 2, 1, two triplets are there, which are overlapped here. We do not see that and because of that it appears like a quartet. You have seen this multiplicity pattern when you were analyzing the protons spectrum. So, it is a doublet and each of the

doublet is a triplet because of CH2 protons. You go to the next one. Next one is CH2 proton. This is H3. This CH2 proton can have a long range correlation to what? This carbon, CH3 carbon. It will make it a doublet. But then each of this doublet is a quartet because of 3 equivalent protons. So, you will get doublet of a quartet and 4 lines. See, it is doublet here and each line is a 1, 3, 3, 1, 1, 3, 3, 1, quartets overlapped here. It looks like a pentet. That is what happens. It is doublet of a quartet, peaks are overlapped and appears like a pentet. And also if you see this CH2 proton is also correlated to CO carbon 1, 2, 3 bonds, three bond correlation is there. What will happen here? This is going to be because of CH2 protons have a long range correlation to C4, it is a doublet. And then each doublet line is split into quartet because of the coupling with CH3 protons. See again, you get a pentet like you saw in the previous example, like here. Again it is a pentet because this is correlated to this and this is also correlated to this. In both the cases, this proton will split this into a quartet. Exactly what you are seeing. Again it is a quartet where some of the lines are overlapped here. What is the next one? Next one if you see, we have to look at the other carbon CH2 carbon. This CH2 carbon, and we have CH3 protons here. Let us consider these protons. It can have two bond correlation with CO carbon, that is all. When it is a two bond correlation what it will do? It will become a doublet. That is all and no other coupling is there. HH coupling cannot be seen because nothing is there. This is not split by any other protons. So, CO carbon because of this is going to be simply a doublet. CH3 protons have a long range correlation with CO carbon and it is a doublet. Simply by using all this information, we can identify all the cross peaks. So, now we know how to analyse coupled and decoupled HMBC spectrum of one of the molecules you have taken. We will take another example, a decoupled HMBC spectrum of this molecule.

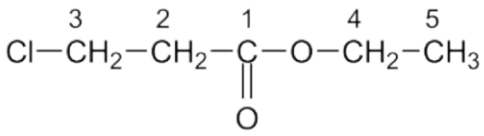




Now we will start analysing the decoupled spectrum of this molecule. This structure is given here. We will look at the molecule and we can even get the structure of the molecule. If you analyse HMBC cross peaks. All the carbons have been numbered. Look at carbon 2. This carbon 2 shows two bond correlation to proton 3. See it is carbon 2 here. Proton 3 which is proton 3 here. Carbon 2 is here. It is showing three bond correlation. That is what it is. And C3, carbon 3, shows two bond correlation to proton 2. That is all. Carbon 3 is showing to proton 2, carbon 2 is showing to proton 3. See very interesting. Carbon 3 is showing to proton 2, carbon 2 is showing to proton 3. There is a correlation between the two groups. Further you see C4 is correlating to H5, 2 bond correlation here. C4 is correlating to H5. Also, H5 is correlating to C4. These are isolated peaks. But if you look at, one more carbon is there which is not attached to proton that is CO carbon. This CO carbon shows three correlations. One is this one, C1 to H4, Other is C1 to H3, long range correlation, 3 bond and C1 to H2 here, 2 bond. This is a very interesting thing. What did you understand? We saw only two correlations for this C3 to H2 and C2 to H3. Similarly, C4 to H5 and C5 to H4. These correlations we observed. But in between there is a CO group. This CO group correlates to this proton, this proton and this proton. And there is no other correlation of these two, this carbon and these two to this carbon. All this observation what do you understand? It conclusively tells you that C double bond O, this group is sitting within H2 and H4 groups between this and this. From the correlations we observed in HMBC, we can arrive at this structure very easily. Check the correlations, simply place the correlation what we observed and we know this is the structure of the molecule. Because this has to give correlation to this, this and this. This has to be between these two. It cannot be for the terminal groups. And these two we saw this is correlating to this and this is correlating to this. Similarly, this is correlating to this, this is correlating to this proton. With all this knowledge, we can get the structure.

The H2-C1 (2 bond); H3-C1 (3 bond)
And H4-C1 (3 bond) correlations put

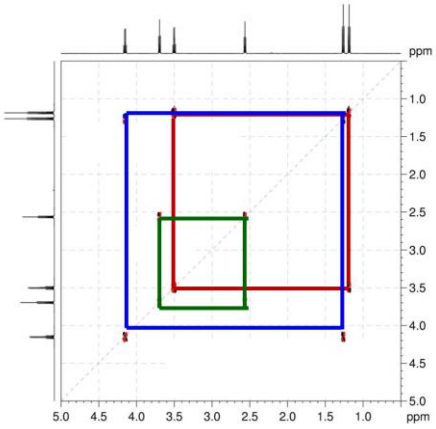
C=O-O between H2 and H4 groups

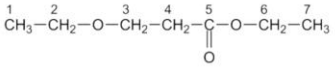


So, you remember how HMBC because of the remote correlations for the protons to the carbons which are not attached to protons. The CO, IPSO carbons are some carbons which are not attached to protons, quaternary carbons, we can even get the structure. We

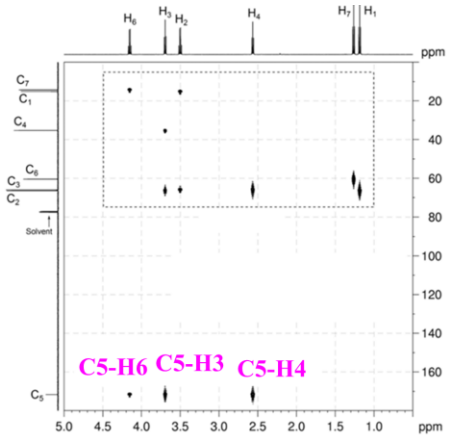
can completely place the carbon which are not attached to proton also. So, that is the advantage of HMBC. It gives correlation of carbons which are not attached to proton through remote protons.

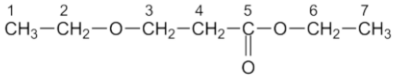
So, we will take a simple example and analyze a 600 megahertz COSY, HSQC and HMBC spectra of ethyl ethoxy propionate. This is a simple molecule, a COSY spectrum. COSY spectrum you know how to analyze, start with one doublet, one of the diagonal peaks, complete the square. Start with diagonal, complete the square. Start with this, complete the square. So, COSY identified three groups of spin systems, fantastic. Go to multiplicity edited HSQC, you get four groups of CH2, red color, negative sign.



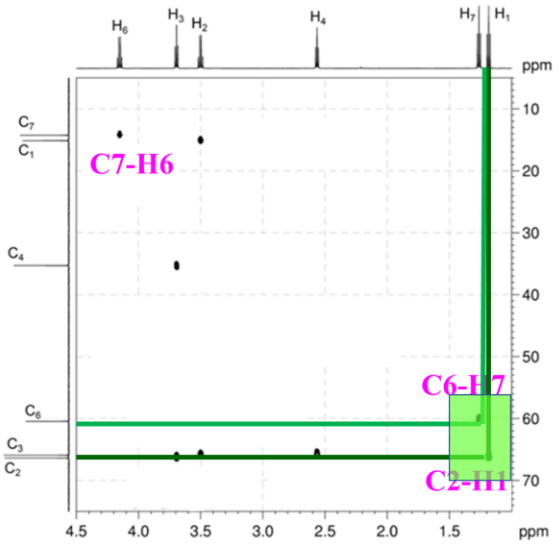


I think, four are here and only two CH3s which are odd protons with a positive sign. These are all negative signs, that also fine. You can assign these things very easily. Then, do the decoupled version of the same thing.





HMBC, interesting thing you will see. C5 carbon gives long range correlation to three different protons. C5 to H6, H3 and H4. C5 gives to H3, H4 and H6, very interesting. And C5, H6, 3 bond, all these three correlation we are seeing. If we expand this version of the same region and if you look at the correlation, we have C6 giving correlation to H7 and C2 to H1.



And remember, C6 giving correlation only to H7 and H6 correlates only to C7. This is very interesting thing, we should see that. This is what is happening because H6 is correlated to C7, H7 correlates only to C6. We can see carefully the peaks here. This puts CH2, CH3 as terminal groups. H1 correlates to C2 only, this H1 only to this and no other correlation. That gives me an idea that CH3 is a terminal group there. This C7, H7, I told you C6- H7, and C7-H6, that form one correlation and C2- H1 is also there. There are three correlations we get from this graph, which puts me, this is one group and this is another group. We continue this further because H2 correlates to C1 and C3 and H3 correlates to C2, C4 and C5. And with all these observed correlations like this C5-3 bond, C5-2 bond, C5-H4-2 bond and these two bond, three bond correlation, this is the structure we are going to get. We can place it easily. So, using HMBC, we can get even the structure of the molecules. Long range correlation puts the carbons which are not attached to the proton in a proper place in the structure by understanding the correlation of that carbon with the remote protons. This is what we wanted to see now. So, today we discussed in this class, lot about HMBC and HMBC pulse sequences and I told you HMBC gives rise to only long range correlations, suppresses one bond like HSQC cross peaks by using filters and we can have different types of filters and one such prominent one I told you is a BIRD filter. We can also do constant time experiment to prevent the evolution of HH couplings like that. So, with all these things, varieties of experiments are

possible. Finally, I just wanted to tell you by using BIRD sequence as a filter and we can allow only long range correlations. And we took lot of examples, we understood how to interpret the coupled and decoupled HMBC spectrum of some important molecules, how HH couplings are affecting the spectrum, how CH coupling can be removed by decoupling , and all those things. And we took simple example to show that one or two example of the molecules and by the looking at the correlations we can even place some of the functional groups like C=O or quaternary carbons in a proper place based on its correlation with other protons which are remotely bonded. So, we took couple of examples, I am going to stop here and we will continue with this HMBC little bit more and then go to altogether a different topic tomorrow, in the next class. So, I am going to stop here. Thank you very much.