# One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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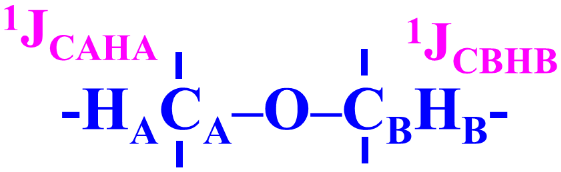
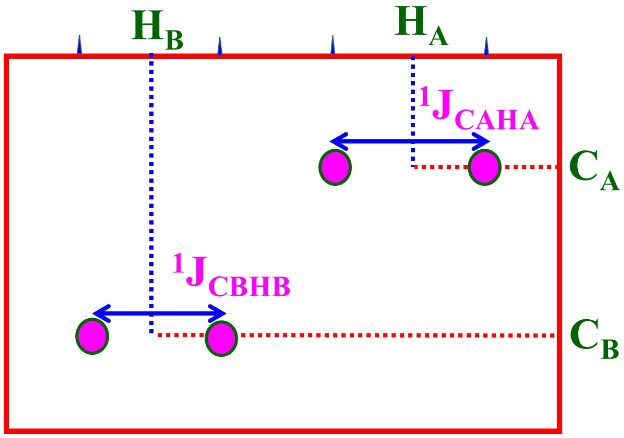
## Lecture 45: HSQC-I

Welcome all of you. In the last class, we started discussing about heteronuclear correlation experiments, where I introduced HETCOR experiment, direct correlation between heteronuclei and the proton. Since it is time consuming, I also said we can do inverse experiments. They are HSQC, HMBC, HMQC like that, several such experiments. What is done in these experiments is, the magnetization of proton is transferred to X nuclei or any abundant spin to dilute spin. It is taken back from the dilute spin to abundant spin, and the abundant spin is detected by decoupling the heteronuclear spin. In the process, what happens is, we will come to know the information about the nucleus or the dilute spin which is attached to abundant spin. We can get the correlation information. And this is fast, actually it is saving lot of time compared to the direct detection experiments, because the enhancement of the signal intensity goes by the ratio of the gamma. For example, if you do 15N-1H HSQC, about 10 times enhancement is there. In 13C-1H HSQC, about 4 times enhancement will be there. So, the 4 times enhancement results in the enormous saving of the experimental time. Direct detection experiment takes about, let us say 10 to 15 Hours, whereas the inverse experiments like HSQC, HMBC etcetera will hardly take less than an hour. In the present day spectrometers, it is all very well set. And also I told you there are several HSQC experiments possible. You can have the decoupling in the direct dimension or decoupling in the indirect dimension or decoupling in both the dimensions or not decoupling in any of the dimensions. Varieties of examples are possible. And I showed you how we can do that. In all these HSQC and HMQC or HMBC experiments, major drawback is the signal coming from 12C attached protons or for example, in 15N-1H HSQC, from the 14 attached protons. This is a strong signal which we need to suppress it. There are ways of suppressing this, either by using a phase cycling, where once we use a 90 degree plus X, and second time minus X experiment, I showed you how we can do that. And then we can do phase alternation like that. Otherwise, we can use gradients and I also showed you how we can apply the gradients, what should be the ratio of the gradients. Gradients ratio should be 1 is to 4 for proton carbon that we calculated and saw and for the 15N it is 1 is to 10. So, with this we have understood quite a bit about inverse experiments. But we need to know how we get the peaks, how do we interpret the peaks. We will start from that from today about the cross peaks in HSQC. We will discuss about cross peaks, what are the types of cross peaks, how do we get interpret the cross peaks in HSQC, etc. Consider a hypothetical molecule like this.



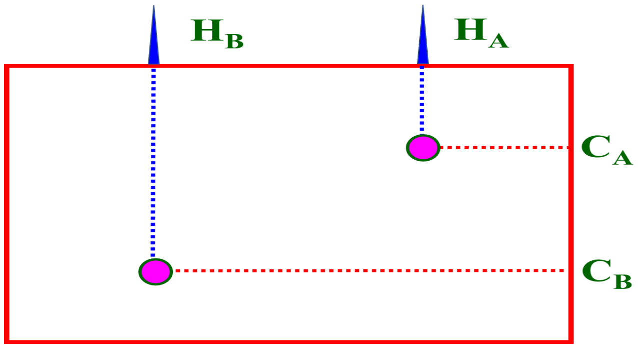
$$-H_AC_A-O-C_BH_B-$$

We have CA and CB are the two carbons. Carbon A is attached a proton A, carbon B is attached a proton B and this one bond coupling is $J_{CBHB}$, this is $J_{CAHA}$, this is very well known, it is a simple hypothetical molecule I have chosen. Now, we can think of two different types of isotopomers for this. One is a situation where this carbon A is in carbon 13 state and carbon B is in carbon 12 state. And we have only J coupling for this and it is not there for this. Whereas, consider another isotopomer, we have carbon 13 here and carbon 12 here and we get one bond carbon proton coupling we can see there in this isotopomer. And interestingly, in both these isotopomers, we can expect only in 1% of the molecule, for each of them. Remember, when we discussed about isotopomers, varieties of things, when we tried to interpret the carbon 13 NMR. So, each of these isotopomers is 1% of the molecules. Let us take a hypothetical molecule and see how does the F2 coupled carbon 13 proton HSQC spectrum is seen.



Remember, I am telling you F2 coupled, that is coupling in the detection dimension, indirect dimension is the other one. HSQC cross peaks pattern, how do they come? For example, if I take this isotopomer, I said there are two isotopomers, we will consider this isotoomer. For this isotopomer, in the F1 dimension, we get chemical shift of A. As I told you how to interpret the HSQC in the HETCOR experiment, there is no diagonal and is no symmetry here and see diagonal is not there, and the cross peaks are not symmetric.

Take one cross fix, go horizontally and vertically like this and you are going to get the proton chemical shift here, carbon 13 chemical or viceversa, one of them. So, let us consider this is the F1 dimension and this is F2 dimension. So, in the F1 dimension, I get the carbon 13 chemical shift for A. Same if you go vertically up, in the F2 dimension, you are going to get proton. So, this F2 is proton, this is carbon 13 here. Alright. And it is going to be a doublet because this carbon is in carbon 13 state. As a consequence, it is going to be doublet, which is given by one bond carbon-proton coupling. We will go to the next isotopomer. Next isotopomer is this. In this isotopomer, what happens? In the F1 dimension, we get carbon B chemical shift. In the F2 dimension, for the same cross peak, we get proton chemical shift. Again, it is a doublet due to one bond carbon-proton coupling because remember, I am discussing F2 coupled HSQC. We are not doing any decoupling and let us see how the spectrum is seen for this molecule. This is the molecule I have chosen. As I said, first isotope ammer. Remember, carbon 13 NMR spectra, I told you, it is superposition spectra for each isotopomer. First, consider the one isotopomer. For carbon 13, this is CA is carbon 13. As I told you, in the carbon 13 dimension, we get chemical shift. Draw horizontal line, you get a chemical shift of carbon A. As I told you, this carbon is a doublet because of one bond proton coupling. This is one bond doublet and that is going to give you, this separation, one bond $J_{HACA}$. This separation corresponds to one bond $J_{HACA}$. But remember, if this is the carbon dimension, this is proton dimension. Center of this doublet correspond to proton chemical shift. And this is what we have been seeing also, I have discussed also. This is nothing but the carbon 13 satellites in the proton spectrum, which is because carbon is coupled to proton. So, this separation gives you one bond carbon proton coupling. So, from the center of this, you draw a vertical line, you get carbon and proton chemical shifts, draw a horizontal line, you get carbon chemical shift. This is what the spectrum you get for this isotopomer. What about the other isotopomer? We will have to see other isotopomer. That is also a doublet because in the second isotopomer, this is carbon 13. So, that gives us a doublet because of coupling with proton B and this horizontal axis if you go, you will get carbon B chemical shift because this is F1, which is the carbon 13 dimension. And exactly from the center, you go vertically up, what are you going to get? You are going to get proton B chemical shift. This is what is going to happen now. And again, this separation correspond to one bond $J_{CBHB}$ coupling. This is the spectrum are going to get in the F2 coupled HSQC. Say I am doing the F1 dimension decoupling, that is okay, only in the F2 dimension they are coupled. Now, F2 decoupled we will see what happens.

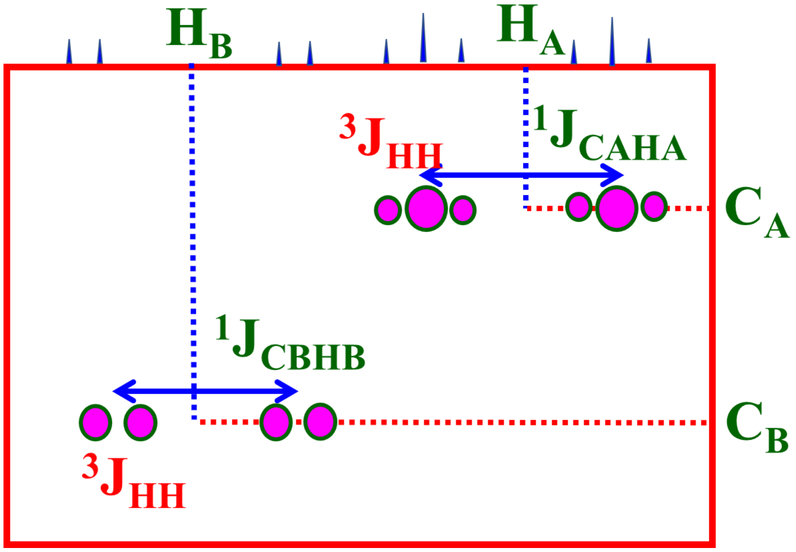


I am doing a decoupling. When I am doing the decoupling, you remember I told you in the last class, we are breaking in the coupling between carbon and proton in the F2 dimension. That means the satellites will disappear. We will get only a single peak, exactly at the center of the doublet what we saw previously. So, you get only one peak. So, this is a peak we are going to get, go horizontally, you get carbon chemical shift, go vertically, you get proton chemical shift. This is carbon chemical shift, this is proton chemical shift. That is all. What did we do here? We are breaking the coupling in the F2 dimension. Of course, in the F1 dimension already it was decoupled, there was no coupling. As a consequence, when you do the decoupling in both the dimensions, you get a single peak, single cross peak. Just go horizontally, you get carbon 13 chemical shift. If this is the heteronucleus, this dimension correspond to carbon 13 or any other X nuclei and in this dimension, if you go vertically, you will get proton chemical shift. This is the F2 decoupled HSQC spectrum of this hypothetical molecule.

Now, I can extend this molecule. I can do one more thing. I can also see proton-proton couplings in HSQC spectrum. Please remember when I discussed the proton analysis,

$H_2C-H_AC_A-O-C_BH_B-C-H$

I also told you, by looking at the carbon 13 satellites in the proton spectrum, I can also get homonuclear couplings. We took several examples of that. Similar to that, what we will do is to take the hypothetical molecule like this. What we did, we have extended the molecule by adding CH and CH2. Do not worry about whether this molecule exists or not, it is a hypothetical molecule. We have taken this molecule. So, what we are going to do now. If I look at the HA, what is the pattern I am going to get? If you look at HA, this carbon, like previous example, it will be a doublet because of



coupling with proton and carbon. But what about HA? Remember this HA is coupled to two equivalent protons. What does it give? This proton-proton coupling will make it a triplet. So, it is going to be a triplet. Similarly, if you go to HB, this will be a doublet because of this. CH proton coupled to this one and makes it a doublet. Let us look at the spectrum. How do we get? In the 1H NMR spectrum, if I take proton NMR spectrum, carbon A is split because of HA into a doublet, and appear as satellites. Look at the proton, carbon A splits this proton and we get satellites. That is what we saw when we analyzed the satellite spectrum. But this satellite also has coupled to this and each of them will be a triplet. This is what we have observed and we analyzed and got the HH coupling from the satellite spectrum. This is what happens. Each line of the satellite is further split into a triplet because of coupling with CH2 protons. This is the situation. What about the other one? If I look at this CB, the CB carbon splits this HB proton into a doublet because of one bond coupling and appear as satellites. But what happened? This proton is further split this into doublet because of this. This HH coupling appear in the satellite spectrum. So, this each line of the satellite is again a doublet because of HH coupling. So, each doublet is further split into doublets because of coupling with CH proton. This is how the pattern should come for this molecule. Now, let us look at the spectrum.

Exactly what we said. Remember, earlier in the coupled HSQC when we saw that along this axis, it was CA, that remains same. The center of this is HA chemical shift that is also same. From center of this to center of this was 1 bond JCH that is also same, there is no change. Additional thing is this peak is a triplet. Each line of the doublet satellites is a triplet because of coupling with CH2 protons. That is the only difference. As far as interpretation is concerned, center of this gives you a proton chemical shift, go along this axis gives you carbon chemical shift of this group CA. What about the other region? And of course, you can easily measure the couplings, HH coupling and CH coupling easily. Now, this center is HA and this separation is JHH and one bond separation is from any of this peak to identical peak. If you take another line of the triplet, this to this, this to this or this to this if you take it is going to be one bond carbon proton coupling. We will go to the CB. For CB from the center if you draw horizontal line, it is carbon chemical shift, and from the center you are going to get proton chemical shift. There is no difference at all. Only thing is the satellite is a doublet, but each line of the satellite is split into doublet because of coupling with CH proton. That is all what happened earlier. It was exactly at the center of this, we got two satellite peaks. Remember without HH coupling. Now, because of HH coupling, each of the satellites peak is a doublet and this is what is reflected here. So, tl

going to get? You are going to get 1 bc

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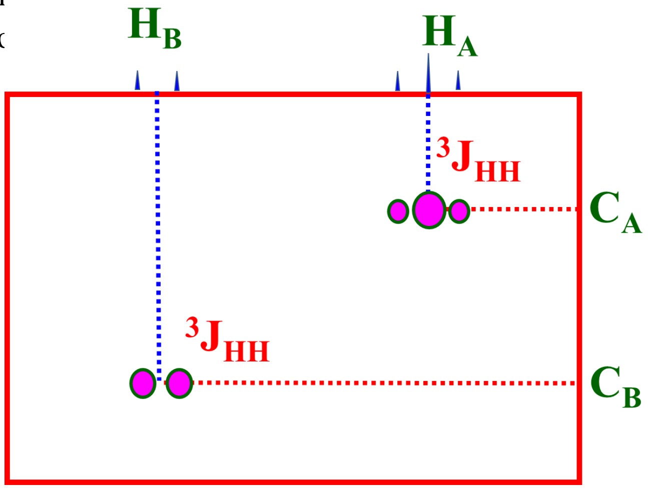
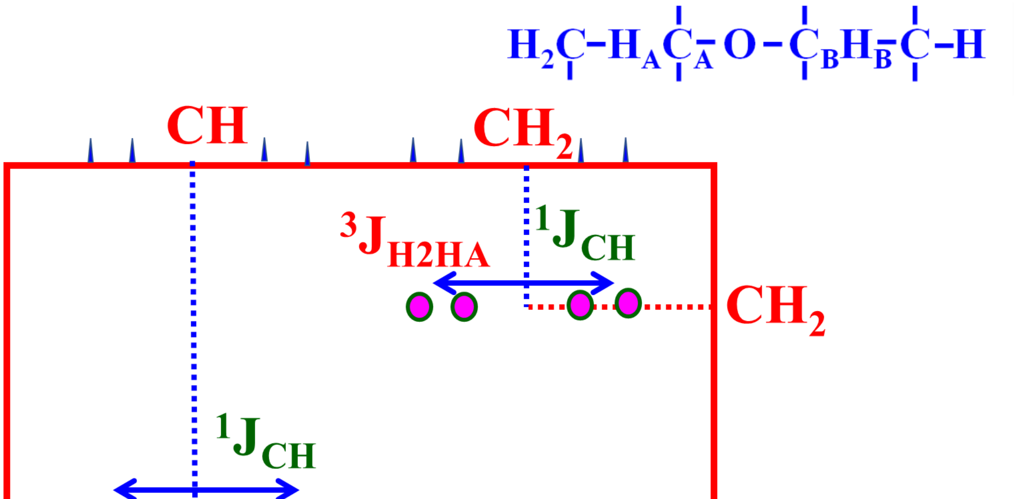
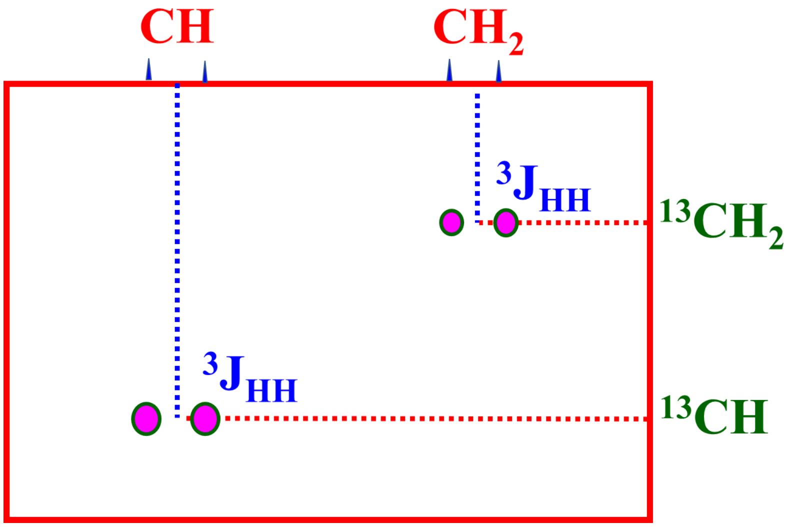


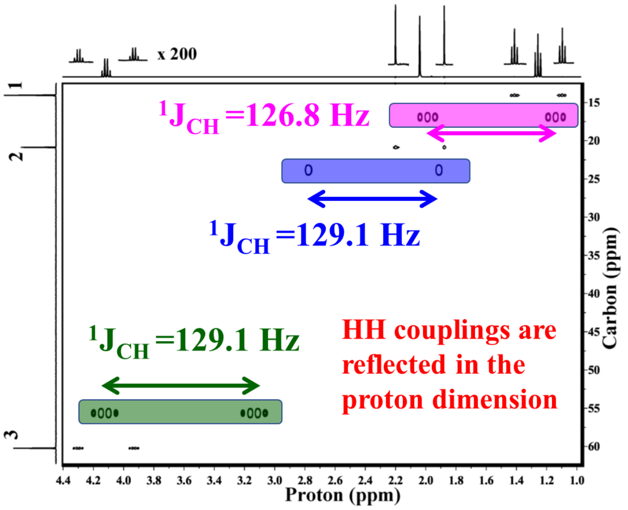
And if I do the decoupling of that, what you will get? I am breaking the CH coupling, but HH coupling remains same and this will give me HH coupling, CH coupling is broken from the center of this again is HA and other is CA. Here also, two doublets will disappear and you are going to get only one doublet, this will be a doublet because of coupling with this proton. This is F2 decoupled HHQC. Remember, I am decoupling here. This gives you 3JHH. And of course, you may ask me a question, what happened to these groups? I was only concentrating on these two groups. What happened to those groups? That also we can understand.

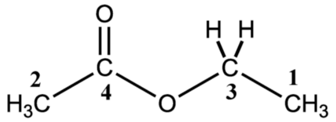


If I look at the terminal groups, we have CH and CH2. And what will happen to the CH2 in the F2 coupled HHQC? Very easily you can understand. Of course, you are going to get a cross peak, two doublets. Why two doublets? Because this CH2. I am looking at this will have one bond coupling with this carbon and proton, that is this one. Either of them, whether you measure from this peak to this peak or this peak is a one bond carbon proton coupling. Each of these satellites in the proton spectrum is a doublet. Why? Because it is coupled to this proton. So, CH2 appear in the F2 coupled HSQC, as doublet of doublets and the large doublet separation correspond to carbon proton coupling and the small doublet separation correspond to HH coupling. You go to CH group now, identical pattern we are getting here also. Why? Because I am looking at this carbon, this is a one bond carbon proton coupling and this proton is split into a doublet because of this. That is why this cross peak for this CH also appears as doublet of doublet. This is what it is. Separation of any of these peaks, that is from identical peak if you consider for each of this doublet either from this peak to this peak or from this peak to this peak, then we are going to get one bond JCH and this separation corresponds to three bond JHH either of them is possible. This is what it is. So, I have taken from center to center here, here also, that also we do, peak to peak, peak to peak you take for measuring CH coupling. So, this how we are going to get peaks for the terminal groups in F2 coupled HSQC.

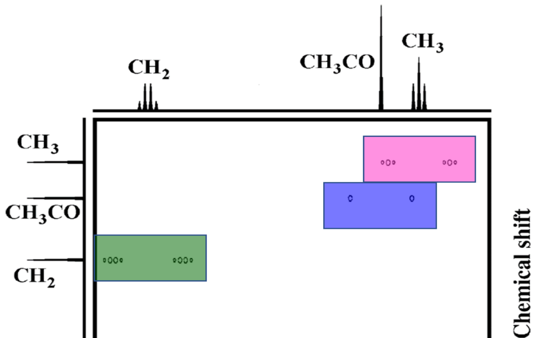


Obviously, next is F2 decoupled. What are you going to get for F2 decoupled? You are breaking this coupling. So, exactly these two will come a two doublet, a doublet will come exactly at the center like this for both of them because I am breaking the CH coupling, I am retaining the HH coupling. The center of this corresponds to chemical shift of CH2, center of this here corresponds to chemical shift of CH. Go horizontally here, you get carbon chemical shift, go horizontally here, you get carbon chemical shift of these groups, terminal groups. So, we know how to interpret the F2 coupled and F2 decoupled HHQC spectra. How we get the peaks in some hypothetical molecules which we took. And of course, F1 is always decoupled, I did not explain that, but otherwise, similar way we can get coupling between proton and carbon in the F1 dimension. In all these examples I took F1 is assumed to be decoupled, only for F2 dimension I took example of decoupled and coupled HHQC.



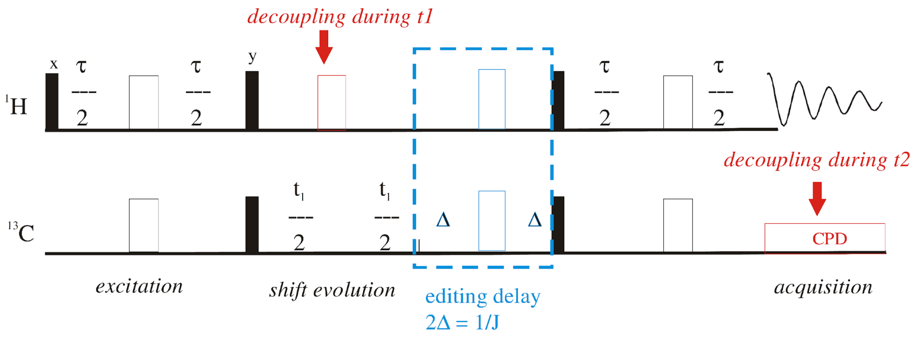


We will take realistic spectrum of this molecule, this is spectrum of ethyl acetate , it is a realistic spectrum and each of this peak is expanded here, this peak is expanded here and these two is expanded here. Now, our challenge is to identify and assign which is which. And this separation is $^1J_{CH}$ and this separation is $^1J_{CH}$, this separation is $^1J_{CH}$. How do you assign these peaks? Of course, obviously, you know that the terminal groups must be a CH3, it is a triplet, because of CH2. And this CH3 is not coupled anything else, it is a singlet. So, easily I can assign the proton peak, there is no doubt about it. But if you see carefully, and if you analyze the multiplicity pattern, it is the F2 coupled HHQC. Now, when I expand this, I will see this peak is doublet of triplets. Why it is doublet of triplets? The doublet large separation is coming because of 1 bond carbon-proton coupling that is huge about 126 Hz that is a doublet. But each line of this doublet is split into a triplet because of this proton bcause this proton split this CH3 proton into triplet. As a consequence, satellite pattern appears like 1:2:1 triplet, you see 1:2:1 triplets here. So, this pattern is doublet of triplets. Fine. What is the next one? If you see the next one, if I consider this one, very easy to interpret last one, green one. Why? I have chosen this to be CH2 group because from multiplicity pattern I know this is CH3 and this is CH2, this is isolated CH3. What is this pattern? How do you interpret that pattern? It is very easy. Of course, this CH2 1 bond coupling will be a doublet. This is what you are going to see 1 bond coupling. Further, these two CH2 protons are split into quartet because of the CH3 protons. So, each line of the doublet, the satellite will be a quartet of intensity 1:3:3:1. Exactly, what you are going to see here, if you carefully see here, you get 1:3:3:1 and 1:3:3:1 two quartets, very clear. So, you can interpret that. So, this separation give you 1 bond, CH coupling and this separation of any of this adjacent peaks in the quartet gives me HH coupling between this and this. Same thing of course, we get here also. Then what is left over? Left over is only this peak. This peak is a doublet, simply just a doublet and expanded version is seen here, it is a doublet. Why? This carbon has 1 bond coupling with proton and there is no other proton nearby to couple to it. These are all very far away, 1, 2, 3, 4, 5 bonds away, there is no coupling. As a consequence, this CH3 attached to the C=O is simply a doublet, this CH3 group. You may ask me a question, why I am not seeing the peak for this? Remember in all the correlation experiment, carbons which are attached to a proton only give correlated peaks, cross peaks, quaternary carbons, CO carbons like this will not give any peaks. As a consequence, you are seeing only 3 peaks and this carbon is missing. How do we get that carbon? That is another experiment HMBC we will discuss today if possible after some time. So, this is how we can get all the coupling information, very easily.

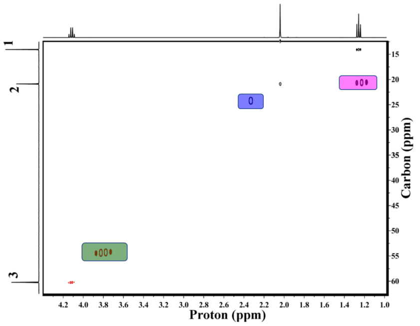


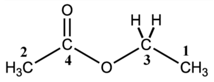
The same thing if I do the decoupled HSQC, what are we going to do? We are removing C-H couplings and only HH couplings are retained. What will happen now? The realistic example, there were doublet of triplets we saw earlier. Now, that doublet, large doublet is a 1 bond C-H coupling that is broken, we removed because of decoupling. So, what we are going to see only HH coupling, this one, this C-H is a triplet because of this. Similarly, this is a triplet because of this. You can identify that. Similarly, this is just a singlet, C-H coupling is broken, no HH coupling here. Very easily, you can interpret both coupled and decoupled HSQC spectrum of this molecule. I hope you are all with me and you are understanding what I am telling. Very easily, you can understand. It is a realistic example of a molecule where we have both coupled and decoupled HSQC and I said, in decoupled HSQC, you get only HH coupling if there exists.

In the coupled HSQC, you will get both C-H coupling and HH coupling. if there are HH couplings and this is what it is, you get only that. And the HSQC experiment, we can also do what is called multiplicity edited HSQC. What do you mean multiplicity edited? Remember, if you know the DEPT experiment, if you remember what we discussed, your DEPT45, DEPT90, DEBT135. Remember, I said DEPT135 if you do, you can make, we can see that CH2 protons are all negative, CH and CH3 are positive. There we could identify all the CH2 carbons in a crowded region. Why cannot we do that? Why cannot we bring in this HSQC, the DEPT sequence. Apply the DEPT135 pulse in the middle so that we can also get this correlation plus identify because of the sign, whether it is carbons attached to odd protons or the carbons attached to even protons. We can do that. Especially for identification of the CH2 carbons. It is very easy because carbons attached to even protons in DEPT135, we saw that it is going to be negative in intensity, whereas CH and CH3 are positive in intensity and this is how it is. So, in the basic 2D HSQC, always produces cross peaks which are in phase and positive. They are always in phase signal in the HSQC experiment. The cross peaks are always in phase and positive signal. If you combine this with DEPT135, then we can have cross peaks for the carbons attached to protons, like CH3 and CH gives positive peaks, whereas CH2 carbons gives negative peaks. This is what you are going to do and this is what is called a multiplicity edited HSQC experiment.

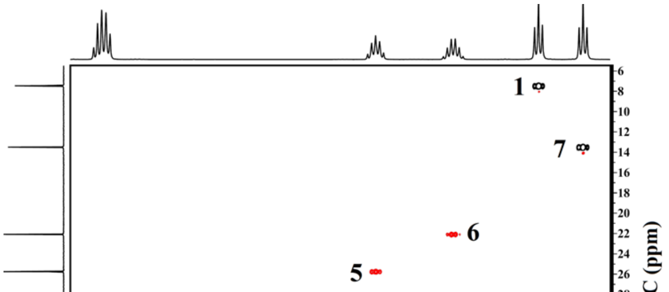


We can bring in multiplicity editing in the HSQC. This is what we are going to do. This is the editing delay we have to adjust according to 1/J, that is all. We know delay we have to adjust for 1/J and finally when we are going to this editing, we are going to observe all CH2 are negative, all CH and CH3 are positive. Rest of the things remain same. This is polarization transfer, proton to carbon taking back from carbon to proton and then detecting that proton while doing decoupling of carbon. Other only this portion is extra in this. So, we will do that. The same experiment, same molecule will take.





If we do multiplicity edited, ME means multiplicity edited, multiplicity edited have to decouple HSQC if you consider see here is a quartet because CH coupling is broken, we are going to get HH coupling for this CH2 is a quartet because of this but because also the CH2 carbon attached to even number of protons, this is negative intensity. See this is positive means black color, it is positive, red color is negative that is a convention we are following here. So, that means look at the CH3 carbon here, this is positive, this is CH carbon here, this is also CH3 carbon that is also positive where this is CH2 carbon is negative in intensity. So, you can identify CH2 carbons here very easily just by plugging in editing sequence in the HSQC. That is what we did here and this is what it is. These two are positive and this is negative. And if I take the decoupled HSQC spectrum of the 3 heptanonen other molecule, remember this was ethyl acetate, we will take it heptanone, this is the molecule. What do we expect if you do this one? Very clearly you can identify.

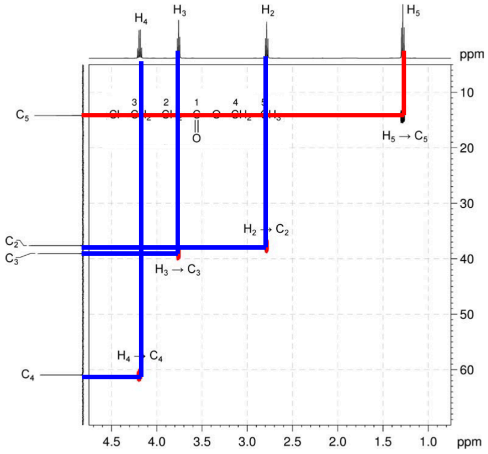


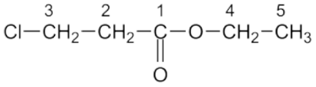
This is of course, terminal group H7 that is a triplet and this is triplet, this is quartet of triplet, a triplet of triplet, triplet of quartet, triplet, all those things just by looking at the molecular structure, you could assign all the protons here. Remember we analyze this also in the proton NMR spectrum. So, what do we do here? We know a is proton here, go along this axis of course, this is where you identify, go vertically along this axis, you get proton chemical shift, come horizontal you get carbon chemical shift for this. For this CH3 carbon, it is here, this is the proton chemical shift, this is carbon chemical shift. So, carbon and proton chemical shift for this molecule can be assigned very easily, you can obtain it very easily. But then we will expand this in the decoupled version, then you will see very clearly all this multiplicity. Here you see, this is a triplet, this is a triplet, this is a quintet, this is a quintet, this is a quadrate and there are two peaks overlapped here H1, H4 and H2 is a triplet, Hw is a quartet and this is a triplet H1. See this quartet is there H2 and this is the well separated out. Remember, if I look at the proton spectrum, it looks jumbled up, whereas the carbon 13 dimension, using carbon 13 chemical shift, I could separate out the multiplicity, I can resolve the multiplicity, very easily. I know what is quartet here 1, 3, 3, 1, which is difficult to extract from this. I can easily measure the J coupling here very easily. So, this is a expanded version of that and you go along this axis very easily like I said earlier, you can get carbon chemical shifts here, you go like this, you get proton chemical shifts, everything is done. Same 3 heptanone, if you do multiplicity edited me-HSQC, what do you expect here? 1, 2, 3, 4 CH2s are there. That means 4 peaks should be negative in intensity and these terminal CH3s, two of them should be positive. There are only 7 carbons, CO carbon does not give signal. Out of the 6 carbons, 4 should be negative intensity and 2 should be positive.

Look at it, this is red, red, red and red. This tells me all CH2 carbons are here and these must be CH3 carbons here. This is proton chemical shift, this is carbon chemical shift. So, CH3 carbons are identified and CH2 carbons are identified based on the relative signs, one has negative intensity, other has positive intensity. You may say color code is like this, that is the way it is followed. If you take the cross section here in the HSQC experiment and plot individual cross sections like this, each of them I can take this cross section and plot it. You see this is positive intensity, this is negative, all are negative. This tells me these are all CH2s. So, in the contours the color is different. These are all positive, these are negative colors. That is what we are going to see. We can look at this molecule, another molecule 400.13 megahertz, multiplicity edited HSQC spectrum of 2 methyl, 1, 3 heptanone. The same heptanone, another methyl group is added here.

It is similar to the previous molecule. Now, what is that you are going to get? Earlier we had 4 CH2 groups, now we have only 3 CH2 groups. So, 3 CH2 groups are negative, and the rest are all CH3, 1, 2, 3, these are all positive, odd proton attached. This is a multicity edited HSQC. For the previous molecule, one CH3 group is attached. That is all. See, now you can identify which are CH2s and which are CH3s easily.

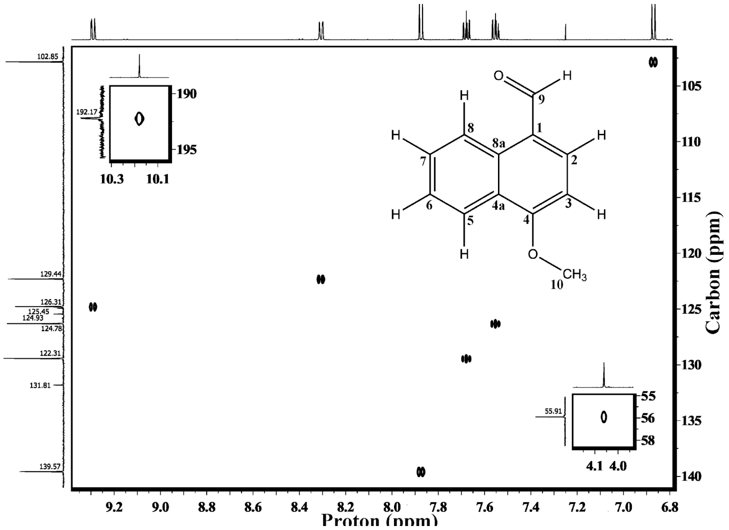
Let us start analyzing, so one or two decoupled HSQC spectrum, multiplicity edited.





$\mathrm{Cl{-}\overset{3}{CH_2}{-}\overset{2}{CH_2}{-}\underset{\overset{\|}{O}}{\overset{1}{C}}{-}O{-}\overset{4}{CH_2}{-}\overset{5}{CH_3}}$

Take this ethyl 3 chloropropionate molecule. This is decoupled multiplicity edited HSQC. And of course, you can find out 1, 2, 3, 4 protonated carbons, one quaternary, one CO carbon that will not give signal. You will see only 4 peaks are there. Out of which 1, 2, 3 are CH2 and one is CH3. Obviously, in a multiplicity edited, you are going to get 3 negative and 1 positive, exactly what it is. And of course, from this, you go up and identify that was CH3 and of course, this is CH2 which is attached to Cl, you can get this proton chemical shift and carbon chemical shift like that. Very easily, you can identify all the protons, this is the proton which is C4 attached to C=OO. This is the CH2 which is attached to chlorine is here. So the assignment of the HSQC spectra is fairly simple. All you have to do is if you get the decoupled experiment, do preferably multiplicity edited experiment where you can identify all the carbons and the chemical shifts of protons and carbons you will get. Also, you will identify CH2 carbons like this, very easily you can do that.



So, this is a 600 MHZ HSQC spectrum of 4-methoxy-1-naphthaldehyde. Look at this one very easily. This is a phenyl group I have taken. There are 2 protons here and of course, these are all the quaternary carbons. Looking at the multiplicity pattern, each of them is a doublet. I know these are H2 and H3 protons. Remember this molecule we also analyzed when we analyze the proton spectrum. In the proton spectrum of the aromatic carbons, we analyze that. And from this you can see that, this is proton chemical shift and this is carbon chemical shift. We have the knowledge of the assignment from proton spectrum. This is proton 2, this is proton 3. Similarly, for other group we can assign. So, like this we can keep on assigning n number of spectra. We will take another one or two example in the next class. Now, the time is getting up. What I am going to stop here, I will explain some more examples in the next class and then we will do something more about HSQC and then switch over to HMBC. So, what we did this today, we understood how do we get the cross peaks in the HSQC. We took hypothetical molecules with 2 different carbons attached to CH2 or CH. We saw that one- bond coupling in a coupled experiment. In the coupled experiment, we get one bond CH coupling in the F2 dimension and then in the decoupled experiment at the center, you are going to get a single peak. CH couplings are broken. Go vertically up, you get proton chemical shift, go horizontally, you get carbon chemical shift. That is how we analyze everything. Of course, we extended the molecule and we saw that like we saw in the proton NMR spectrum, when we analyze proton NMR spectrum of satellites, we also get HH coupling that get reflected. So, in the decoupled version and coupled version of a molecule containing different protons, more number of protons, the carbon which are remote coupling CH, that is carbon attached to proton. Those protons are coupled to remotely bonded to other protons, that coupling is also get reflected in the satellite spectrum. That also we saw, and we took the realistic examples of the molecule to analyze the spectrum, especially multiplicity edited, where we can put in DEPT 135 sequence in the middle to identify CH2 carbons which are negative in signs, whereas CH and CH3 are positive and we took one or two example to interpret the spectrum. So, with this, I am going to stop here. We will continue in the next class. Thank you.