One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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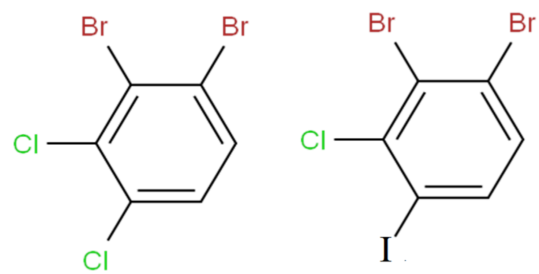
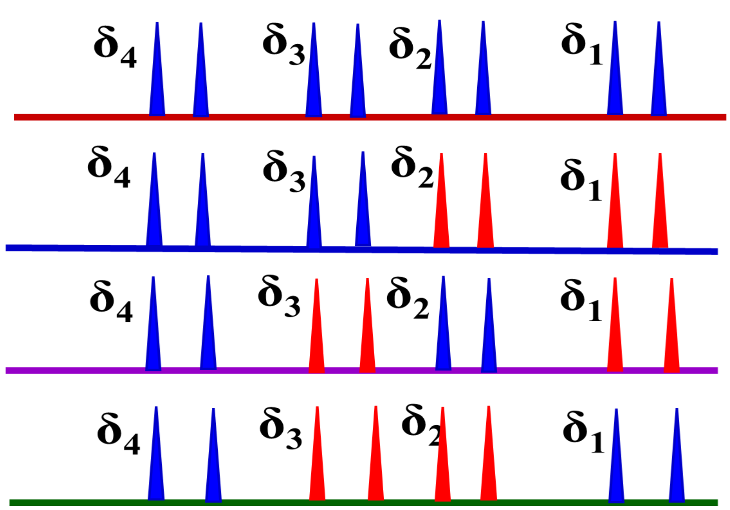
Lecture 40: 2D COSY-II

Since the last 2-3 classes we have been discussing 2D NMR. Especially in the last class I discussed about the COSY, the correlated spectroscopy. I mentioned COSY is nothing but the 2 pulse sequence 90-t1-90-t2. We have only preparation period, the evolution period and the detection period. The t1 period is the evolution period and we collect the signal in 2 dimensions, do the Fourier transformation you get a 2 dimensional spectrum. And what this 2 pulse sequence does? Especially when you consider a spin system. A single uncoupled spin, single spin without any coupling to anything, 2 uncoupled spins and 2 coupled spins, the various examples we took. I explained to you if you consider a molecule like $CHCl_3$ with 1 proton if you ignore the coupling with carbon 13 in natural abundance, then we get a single peak, it is only 1 chemical shift. When you take such an example of a molecule, in the COSY pulse sequence it evolves in the t1 dimension at the chemical shift of the proton and in the t2 dimension also, with the same chemical shift. And such type will give rise to a peak, when you do the Fourier transformation which comes on the diagonal. And if you go horizontally you get a frequency which correspond to chemical shift of proton in the t1 dimension. If you come vertically down you again get the chemical shift of the proton of $CHCl_3$ in the t2 dimension. So, the frequency of this one which is on the diagonal remains same in both the dimensions, it does not change. And I took the example of 2 uncoupled spins, then we had 2 peaks on the diagonal each of them correspond to chemical shifts in both the dimensions, identical chemicals of the same spin. If I have one proton here, viz., $CHCl_3$; in both the dimension it gives the chemical shift of $CHCl_3$. If I have another peak on the diagonal, in both the dimensionw it gives rise to chemical shift of some other molecule. I explained to you about 3 spins and then I took the example of 2 coupled spins. Interesting thing is 2 coupled spins will give rise to 4 peaks, 2 for A and 2 for X. When A spin evolves it evolves at its own frequency, that means at its own chemical shift in t1 and during the process of evolution it gives part of its energy to proton X for which it is coupled to. And then it evolves not only at its frequency and also evolves at the frequency of X. That means when it evolves at its own frequency it gives a diagonal peak, when it evolves at the frequency of X, it gives a cross peak. Similarly X will evolve at its own frequency and gives rise to diagonal peak and it gives part of its energy to A and evolves at the frequency of A spin, and give rise to cross peak. And I explained to you when we have 2 coupled spins, we have 4 peaks diagonal peaks in the square pattern for A. Similarly 4 for X and they come in the square pattern. And we also understood they are all autocorrelation peaks, the 2 peaks will correlate within themselves and the cross peaks are correlation of A and X. A gives its magnetization to X gives rise to cross peak. X gives magnetization to A gives rise to cross peak. Cross peaks are also 4 peaks in the square pattern, but the structure of diagonal and the cross peaks are different. The diagonal peaks both are in phase doublets in both the dimensions. The cross peaks are anti-phase doublets in both the dimensions. This is what we understood. We will go further now and see how we can utilize COSY in understanding many things.

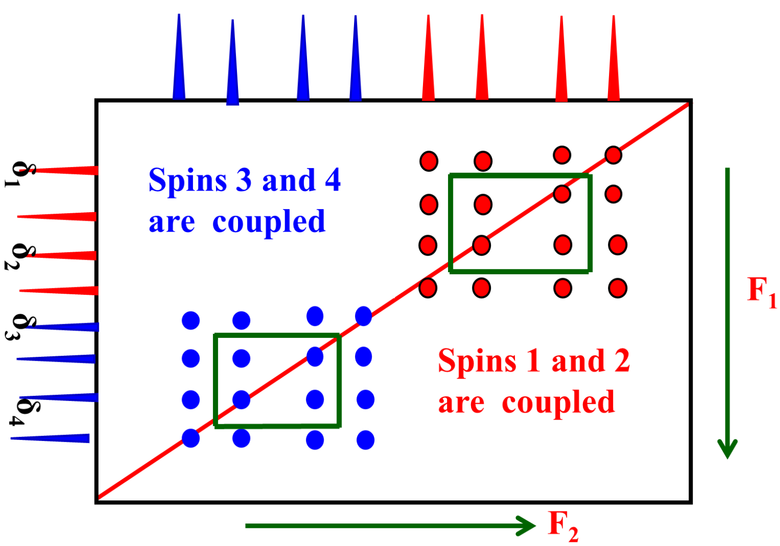
I will show you how do we identify different molecules, the coupled partners using COSY. I consider example of 2 molecules.



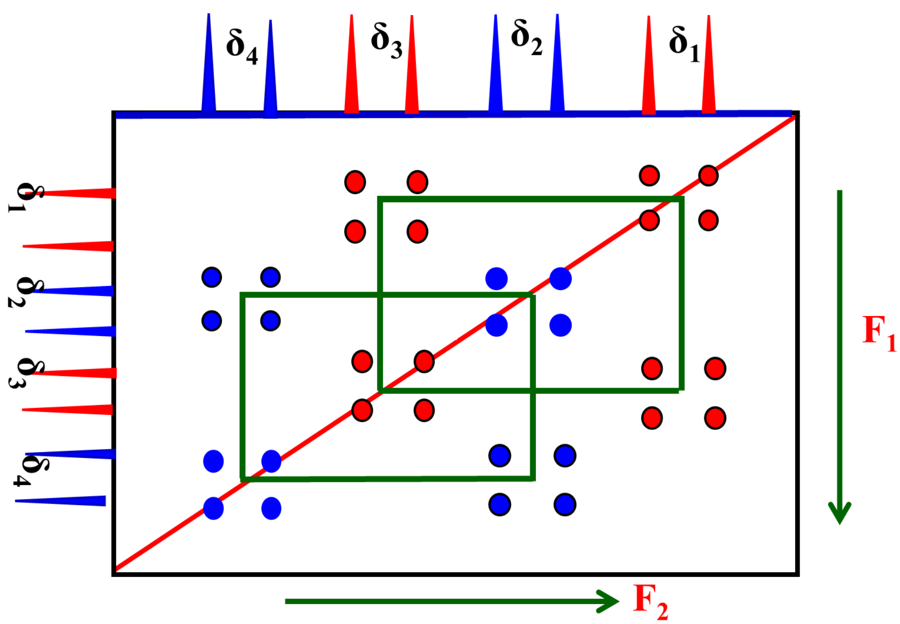
There are 2 protons here, 2 protons here, all are the completely different protons different chemical shifts, there is no symmetry here. And this proton and this proton are different, this and this are different. When these 2 are coupled between themselves I will say it is AX spin system, weakly coupled, the chemical shifts are far away separated. These 2 protons also form weakly coupled 2 spins, and give rise to 4 peaks, 2 for A and 2 for X. Four peaks we get here 2 for A and 2 for X, 4 peaks. So, each molecule give 4, 4 peaks. And this is the pattern you are going to get and each molecule will have 2 chemical shifts. Now, I mixed up I have taken the mixture of these molecules. Both these I have taken together. Now, each of them gives 4 peaks, for this 4 peaks for this. The 2 chemical shifts, 2 chemical shifts. This is the spectrum I am going to get.



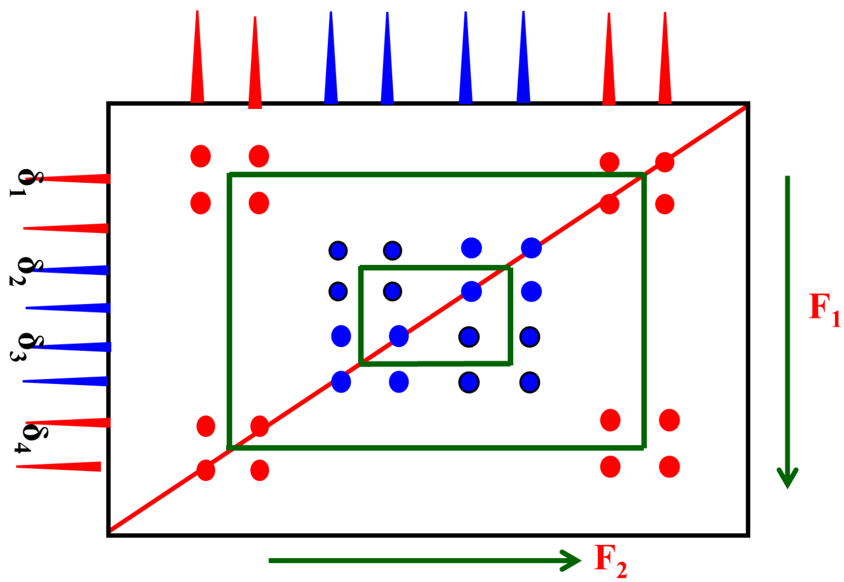
Now, our challenge is to identify which are the coupled partners. Which are the 4 peaks coming from 2 different protons of 1 molecule. The question is very clear the challenge is to identify what are the 4 peaks pertaining to each molecule. The 4 peaks if I identify I know the 2 chemical shifts. How do you identify that? Now, there are various possibilities if I want to identify the coupled partners. One whatever I have marked in red, these 4 peaks may belong to 1 molecule, the 2 protons coupled A and X will give 4 peaks, 1 and 2 could be coupled partners, 3 and 4 could be coupled partners, there is a possibility. So, this 1 and 2 correspond to one molecule, the 2 ortho protons of one molecule. And 3 and 4 correspond to ortho protons of other molecule, which I showed, this is one possibility. What are the other possibilities? There are other 2 possibilities; 1 and 3 could be coupled partners these 2 and 4 peaks may be coming from 1 molecule and these 4 peaks may be coming from other molecule, that is also possible. So, 1 and 3 and 2 and 4 are coupled partners. The 1 and 3 are coupled gives rise to 4 peaks and 2 and 4 are coupled give rise to 4 peaks. Then what is the next possibility? 1 and 4 are coupled that gives rise to 4 lines pattern. The 2 and 3 protons are coupled that is also another possibility. So, there are 3 ways we can think of coupled partners in 2 molecules. I can get simply I get a spectrum like this, but if I have to make an assignment I have to say these 2 peaks may correspond to 1 molecule these 2 may correspond to another molecule. these 2 peaks may correspond to other molecule. How do I do that. Now, I can utilize the COSY and understand and make the assignment very very easy way. Look at this one.



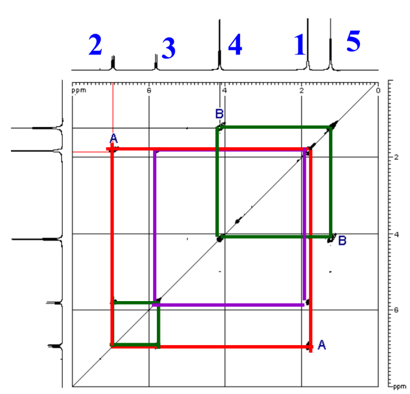
If I take the mixture of these molecules and run a COSY spectrum if I get a pattern like this, This is the COSY spectrum and this is the diagonal then I would say 1 and 2 are coupled. Why these 2 are coming from 1 molecule, because see as I told you 2 peaks of this will give 4 peaks diagonal in a square pattern in-phase doublets, both of these give diagonals 1 and 2, both will give diagonal peaks 4, 4 peaks, in-phase diagonal, in-phase doublets. And also 1 is coupled to 2 means there is a scalar coupling. Proton 1 will give a cross peak to proton 2 at this frequency, the 2 whose chemical shifts are here, give us the cross peaks to proton 1. Remember the peaks coming on the diagonal correspond to its protons chemical shifts. Now, this is the chemical shift of this proton, this is the chemical shift of this proton. So, from proton 2 if I go horizontally, here it gives a cross peak to 1 like this. From proton 1 come down here, go here it gives a cross peak to 2. It tells me 1 and 2 are coupled that is, they are from one molecule. The protons 1 and 2 are giving cross peaks from 1 molecule. Look at the other one there is another possibility 3 and 4 can come like this and coupled I will say these two are diagonal peaks correspond to chemical shift of 3, and the chemical shift of 4. Now 3 and 4 giving a cross 3 is giving a cross peak to 4. And the 4 is giving a cross peak to 3. That means 3 and 4 are coupled. So, there are 4 diagonals and we have cross peaks between 2 of them these 2 cross peaks tells me they are coupled, and are from 1 molecule. These 2 cross peaks tell me 1 and 2 are coupled and they are from another molecule. So, if I wanted to assign 4 peaks my challenge was which are the coupled partners. If I simply run a COSY spectrum of this mixture, if I get a spectrum like this, then I immediately say these 2 are from one molecule and these 2 are from other molecule. All right. This is how it is when spin 1 and 2 are coupled spin 3 and 4 are coupled.

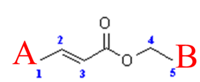


Alternately if I get a spectrum like this very interesting now sit on this diagonal come vertically, go horizontally here, and you get a diagonal peak. The 2 diagonal peaks are there and they give cross peaks. Go along this axis you get cross peak. So, 1 and 3 gives us a cross peak 1 is giving cross peak to 3 here, 3 is giving cross peak to 1 here, it gives you 1 cross peak. So, that means what I would say is if you go by this diagonal if I write then what I say 1 and 3 are coupled because it is giving cross peak and I have put one color and another one is obviously left over 2 and 4 are also coupled. If I get a COSY spectrum like this for this mixture then I will make the assignment of this doublet and this doublet for one molecule. This doublet and this doublet I will assign for other molecule.

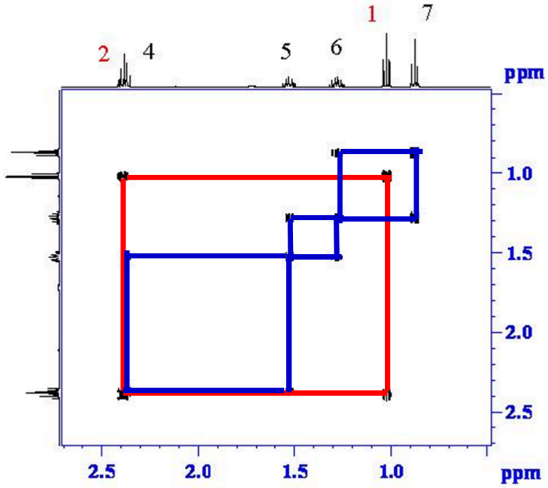


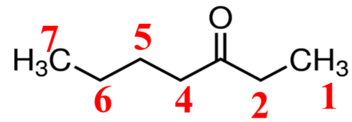
On the other hand, if I get a spectrum like this, this is another interesting thing. So, now from proton 1 come down you get a cross peak, go horizontally here you get a diagonal. Go vertically up and horizontally if you come if you hit 2 diagonal peaks, then they are coupled, that is the assumption, you have to understand that. So, if 1 start with proton 1 diagonal come vertically then go horizontally you meet another diagonal. And then this cross peak tells me, these 2 diagonal peaks are coupled. That means 1 and 4 are coupled exactly what I saw in the previous case, where 1 and 2, 1 and 3 are coupled; here 1 and 4 are coupled and also 2 and 3 are coupled. From proton 2 2 come vertically down go horizontally here there is a cross peak here between 1 and 2, 1 and 2 are the diagonal peaks and when you go you can complete a square going vertically down going up going horizontally and then going up and going horizontally you will complete a square. That means I will say spin 2 and 3 are also coupled. So, there are 3 possibilities simply take a COSY spectrum and looking at the COSY spectrum you will say which protons are coupled, which 2 spins are coupled and they are from which molecule, easily you can do that. So, we know how to use the COSY.



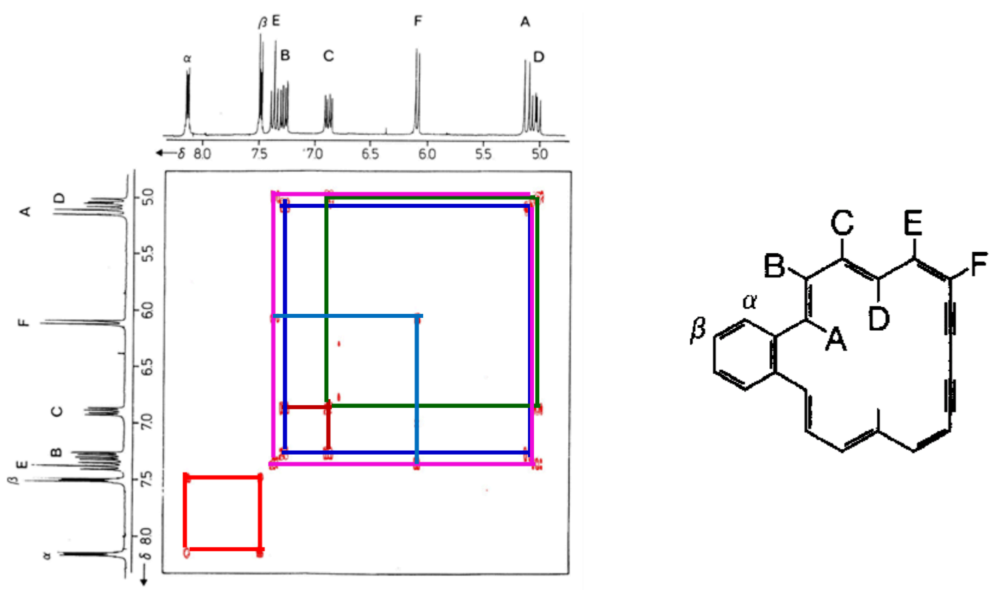


Let us use the COSY for analysis of the spectra of simple molecules. We will analyze the COSY spectrum of simple molecules then we get a feel for what is happening. So, with that we will become more familiar. I will start with a simple molecule ethyl 2 butenoate. This is a molecular structure. I have written 1 2 3 4 5 are the protons. The proton A is here, proton B is here, and A and B are the 2 letters I have given. But 1 is a CH3, 5 is a CH3 that is correct, because a terminal CH3 group, as I have been telling you, comes at the high field. One of them correspond to one of the CH3. Whether this peak correspond to this CH3 or this peak correspond to this CH3 I do not know. I have to identify. How do I do that what I am going to do is simply I will sit on one of the diagonal come vertically down, I hit a cross peak. Look at it I come vertically down here, I am hitting cross peak when from here I hit a cross peak go horizontally I get a diagonal. So, these two diagonal peaks are there. Yhat means this proton and this proton which comes on the diagonal are coupled. The protons 4 and 5 are coupled. And after there is no other thing to correlate. You can complete the square, that means these protons 4 and 5 are coupled. Any other coupling is there? you cannot see it. It forms a closed system, complete one spin system. So, we can safely say this peak is because of CH3 and this one is because of CH2. We could easily say that. Next what is the other thing we have to see, the other CH3. We will start with other CH3. This is another CH3, again terminals CH3. come down, go horizontally you complete a square that means proton one is coupled to proton two. At the same time you can see proton two is also coupled to proton three. You may ask me a question, why not this is three? and this why not this two? you can complete a square like this, you may say this is two and this is three, you can say that. But we can also use some multiplicity patterns. For example, this will be more complex because it will be split into a quartet because of this and each line of the quartet is further split into triplet because of this. So, it is a more complex pattern, whereas this will only be a triplet because of CH2. So, sometimes you have to use your idea of understanding of multiplicity pattern. See, this is more complex here than this one. It is not expanded, if you expand you will see. That is why I would say this is proton two. Now, from the proton two you can do one more thing, go horizontally and go vertically up, you see another peak here. So, two is coupled to three. As a consequence, these two are coupled and giving rise to cross peak here. This cross peak corresponds to one and two, whereas this cross peak corresponds to two and three.There is also a is also this one, this CH3 is also coupled to three. See all the three is there, you can see the cross peak here, cross peak is there from here to here, to here. This is coupled to this, is also coupled to this. It is a long range correlation. It shows correlation to both the protons. So, what you understand from this? This one is coupled to two gives a cross peak and two is coupled to three and that gives a cross peak. So, you can make an assignment of all of them, very easily you can make the assignment.





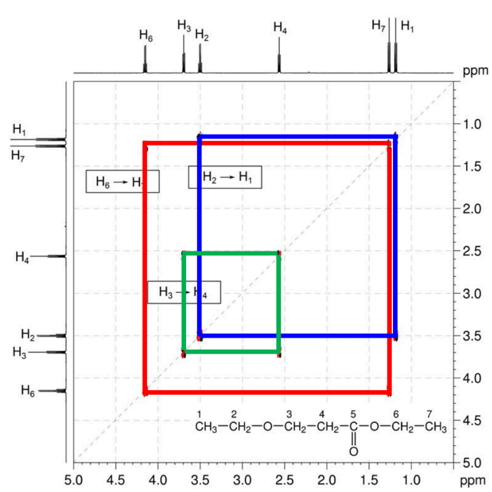
Go to a little bigger molecule instead of butionate we will go to 3-heptanane. This is three heptanone, this is this is the COSY spectrum. Now, where do we start with to assign. As always start with the one where you are confident of assignment. I would say these two CH3's come at the high field, true. And then both of them are triplets. Why CH3 is coupled to CH2, this is coupled to CH2. So, both the CH3's are triplets. So, obviously we can see both are triplets here. So, I can start with one of them I do not know which is which we will make an assignment. So, what we will do is, we will start with one of the peaks. Otherwise we can also do one more thing, we can also start with this CH2. We have used the other way these two CH2's. So, we can start with that CH2 and do the assignment. The reason is these two should be downfield because it is directly attached to C=O. That is one way. But I would go with this CH3, does not matter. This how it is chosen here. So, go with one of the protons come up, come here, you see horizontal and go up and complete the square. If this is proton 2, let us say if this is proton 2, and if this is coupled to proton 1, and it completes a square. Look at it, it is not coupled to anything else. This forms one square, right. But if we carefully see this is the only two coupled partners, nothing else. Of course you may say there is a peak here, but they are not same. There are two peaks overlapped here, it is from another proton. But this forms a complete square. So I would say if this is one of the protons CH3, it has to be 2 and this is the only isolated partner. Here you cannot see anything else here apart from this. These are the only two groups, and are isolated coupled partners. Then obviously there is another peak here. I can start with CH3, come down complete the square. If this is another proton CH3, then this completes a square. This has to be the next immediate coupled partner. I would say this is proton 6. That is an immediate coupled partner. What will happen? this 6 is further coupled to say 5. From 6 you come down complete the square now. Then what are you going to see? This 6 is coupled to 5. So this has to be 5. I can say this is 5, started with for example 7 I got a square so immediate coupled partner for this is 6. And from the 6 diagonal I completed a square then I know this is 5, this one. Further what I am going to do, I will start with 5 come down and complete the square. That means this has to be 4. So 4, 5, 6, 7 forms one spin system. Start with 4, go here 5, go with 6, and then meet 7. So step by step like a ladder you can go and make the assignment. So here, in this molecule, there are 2 groups of coupled spin systems. One is this group, and the other is this group. Both we could make the assignment just by looking at the COSY cross peaks. All you have to do is sit on a diagonal on one of the peaks in which you are confident of your assignment. With that start coming down go horizontally, if you hit another diagonal that means these 2 protons are coupled. And you should complete a square. From that diagonal you can continue like this further. See whether there is one more square, and then other one. Like that you can keep on extending like a ladder one after the other like here. Let us start with this go here, here, and here, like this. Keep going one by one and then complete assignment of all the coupled partners can be made.

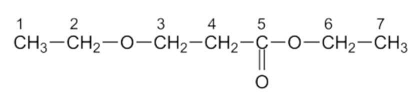


Now we will go to the bigger one. Slightly I am increasing complexity as the time goes by. We will analyze the spectrum of molecule annulene molecule. This has a symmetry here. Here if you carefully look at the structure of the molecule, you can immediately say these are phenyl protons. There is a symmetry, of course, there are only 2 protons. They form an isolated spin system obviously and it is not coupled to anything else, they are far away separated, not coupled to anything else. So obviously that is one spin system.

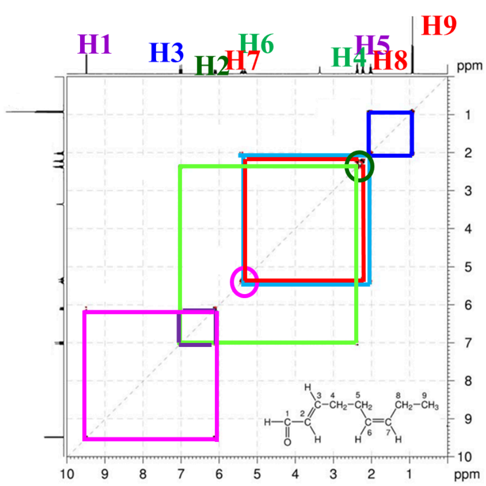
Next if I want to start the assignment for this one, I can go ahead and make the assignment. Here this phenyl group forms one complete spin system, can be assigned as alpha and beta aromatic protons. But further what happens if you look at it carefully this molecular structure you will see A and F are the protons which can split only by a single neighbor. This can become a doublet because of this, this can become a doublet because of this. And 1, 2, 3, 4 bond couplings are not there, let us say it is not there. So this is also

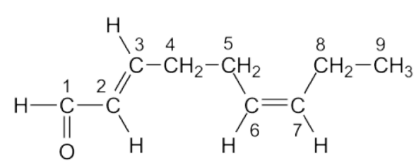
4 bond coupling, and it is not there. This A and F are the only protons, can be a doublets. They have to be doublets because each is split by only a single proton. So with that I have to identify 2 doublets here. Where are the 2 doublets here? in this group there is one doublet here, carefully I can see there is another doublet here. I can see both are there. Of course very clear doublets, fine. One important thing you should also carefully observe, A has a trans coupling with B. The E and F have cis coupling. Remember I told you already long back trans coupling is always larger than cis coupling. This could be about 12 to 15 Hz, and this could be a 7 to 10 Hz. So trans coupling being larger, the coupling between A and B proton is larger. So I would start with a proton A which is a doublet with a larger separation. I can see here there is a proton with a larger coupling. Look at this doublet this is smaller than this, obviously this is a cis coupling, the proton which has a cis coupling. Thus I will carefully say this is A and this is F. I can even make the assignment. We will start with that. Start with now A, come down here you hit a peak. Go horizontally you hit a diagonal, that means A is coupled to B somewhere here. So it completes a square. If this is A this has to be B, I know B. From B I can go further up complete this square. That means B is coupled to C. This is C. Now with C I can go like this I can complete a square. This is D. From D there is another coupling, from D to E. So you can if you carefully see from D one peak is coming here, there is a diagonal I will erase this thing to make it clear. So from D it is coming here and you see a diagonal here go vertically up and complete that, that means this has to be E. Further from E diagonal you are going to have coupling with F. This is a diagonal. So E and F are coupled. So very easily you can make the assignments. Start with the known one. I will repeat it quickly you can understand now. A to B there is a coupling, I will remove the markings first, A is coupled to B and forms a square. From B it forms a square that is C. From C it goes to D. From D it comes to E. From E we are going to get F. And this is a aromatic group. Very easily we can assign all the peaks by using the COSY spectrum of annulene.



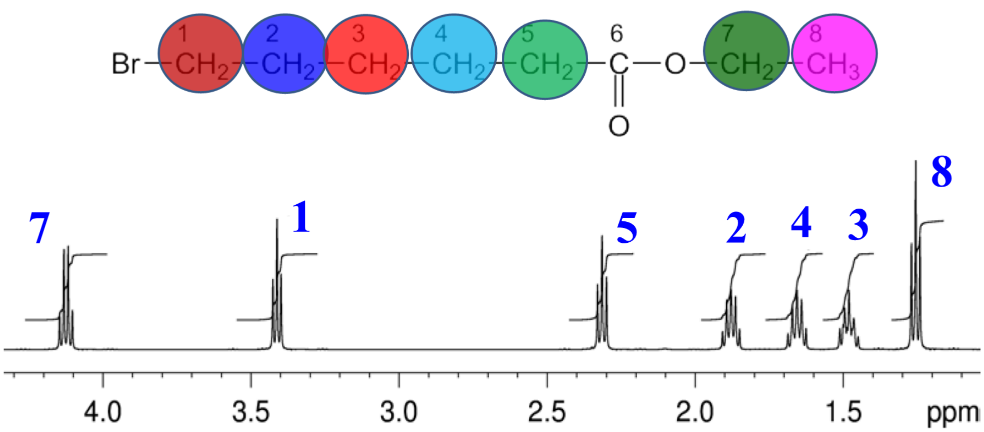


You go to another bigger molecules little lengthier now, we will make the assignment. Where do we start with? As always look at the molecule carefully, the molecule has terminal groups which are CH3. And each of the CH3 is coupled next immediate neighbor, that is CH2. Obviously these two peaks have to be triplets. There are two triplets here. So we will start with one of the triplets and then start making the assignment. Now you know how to go. You go first sit on the diagonal with one of the peaks and then complete a square like this. so this is CH3, you come down horizontally, then vertically, go horizontally and complete the square. And there is no other coupling, no other cross between these two. That means this and this are coupled partners. If this is proton 7 this is proton 6. What is the next one? The next one, of course I can see one more thing here, and this forms another pair because this is another triplet. If that is the triplet, what else it should be? This has to be 1 and this is 2. We start with this, you see look at this, you start with this, this completes the square. 1 come down horizontally complete, and between this and this there is a cross peak. That means this and this are coupled. So this has to be 1 because we already have seen 7 this one, this and this have been assigned. So this has to be proton 1 so when you complete the square this is proton 2, you understand. So we have assigned protons 1 and 2. Obviously next leftover is this group, this is two CH2s. One which is attached to oxygen directly will come down field, so I would say that is proton H3, the proton 3. Sit on the diagonal and then go horizontally and vertically like this you complete a square. So that means if this is 3 this has to be 4. This is 3 and this is 4. That means this COSY spectrum identified 3 groups of coupled spin systems in this molecule. Molecule looks little lengthier and bigger but if you know how to assign the COSY spectrum, it is a matter of 5 minutes you can assign all the peaks. Simply you have to sit on one diagonal where you have a confidence of your peak, complete the square. And if you hit another diagonal these two diagonal peaks, two chemical shifts, you know those two are the coupled partners. Then you can keep extending the logic in a stepwise manner.

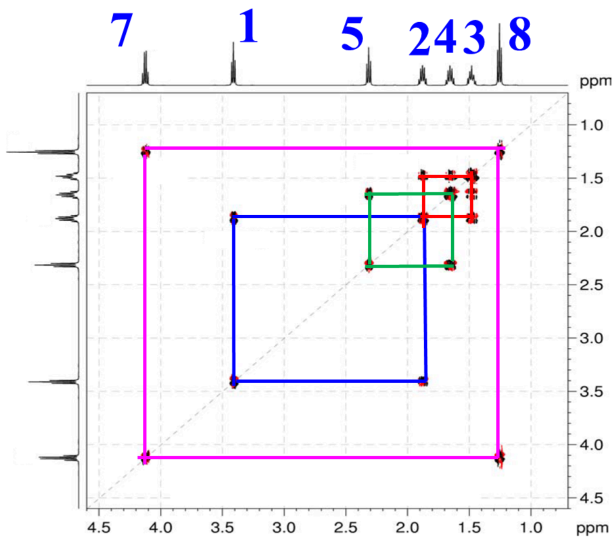


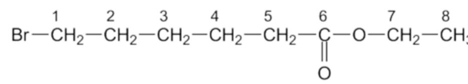


We will go to the little bigger molecule, bit more complex how do you assign this one this is a spectrum of trans-2, cis-6-Nonadienal. Again, if you carefully see this is CH3 I have to start with, this is a triplet. So I will start with that and then if you come down there is no other cross peak, only this. And go horizontally and complete the square. So if this is CH3 then this has to be CH2, I would say H8. Easily I can make the assignment for that. What next? come from 8, see there is a cross peak here. If you carefully see there is a cross peak there, complete this, go horizontally complete this square. So I have completed their square, so then what is other one? It has to be 7 and then next with 7 there is also coupling with something else which is unresolved here. If you look at it carefully here there are two peaks overlapped, unresolved. As a consequence, 6 is also there. There is a cross peak between 6 and 7, but because of poor resolution we are not able to see that. Alright with 6 I can go further, complete the square, that has to be 5. Then 5 has a cross peak coupled to 4, this has a cross peak here. And these two are not resolved, again it is poor resolution. You can carefully see there is no resolution at all. Here there are two peaks overlapped and you are not going to see. As consequence you do not see it but there are peaks here. So that must be 4. The 5 is coupled to 4. What next? From 4 if you complete squar, it is coupled to 3. I complete the square and from 3, I can sit on the diagonal of 3 go complete another square. Then this has to be 2. From the 2 diagonal I sit come down and complete the square. Then obviously that has to be 1. So molecule looks fairly complex, but if you know how to analyze the COSY spectrum, very easily we can make assignment of all the peaks without any difficulty.



Let us look at this molecule bit more complex lengthy molecule but I tell you it does not take much time at all to analyze. Obviously these two are the isolated groups. This is here 3 and there is a one more quartet. Where is the quartet we can think of only this. So this is 7 this is 8. The remaining things are here and of course this is a bromine, proton which is the attached to bromine comes down field. This is 1 and it has to be triplet, because it is coupled to only one of them. And now all other things, this is coupled to 2 CH2s some complex multiplicity pattern. This is coupled to 2 CH2s again a complex multiplicity pattern. This is coupled to 2 CH2s complex multiplicity pattern. But only this CH2 is coupled to this and is a triplet very easily we can make the assignment of all the things. Even I do not need a COSY. But nevertheless we will use the COSY and then make the simplest assignment for this thing. I can assign all those things. We will go to COSY now.





In the case of COSY start with the 8, come here and complete this square. Here the problem is to assign 2, 3 and 4. Which is 3? which is 2? and which is 4? we do not know because each of them are coupled to 2 CH2s. On either side there are CH2 groups. So the identical multiplicity pattern is there, which is 2 which is 3 which is 4 you do not know. For that COSY helps. You start with proton CH3 at high field 8, you complete a square. This is 7 and 8. And then this is 1 because down field CH2 I know, that complete this square. Then this has to be 2 I know. Carefully without any confusion I can assign what is 2, and from 2 I can go like this, and complete this. Then that is 3 see in the 1D there was a confusion due to multiplicty pattern was identical. I do not know which is 3, which is 2, and which is 4. Now I can reason out by just looking at the cross pick in the COSY and this is obviously 3 is coupled to 4 here, and that is 4. Complete this and gives 5, everything can be assigned. And this is the pattern here, so like this we can start assigning this thing, and lot more things we can do. Znd few more example of complex molecules especially biomolecules I will take to make you more comfortable. COSY is the one thing which everybody uses if you are a scientist using NMR spectroscopy you cannot do away with COSY. To make you more comfortable because this course is specially designed for the analysis of the spectra, in the next class I will take few more examples to make you understand COSY. And then we will go to few other example of different types of COSY experiments we have and everything later. So I am going to stop here. Today we discussed several examples of analysis of COSY spectra starting with the two molecules and then if you take the mixture how the pattern comes like that then the real examples of COSY spectra, several simple molecules to little bigger molecules we took, and we know how to analyze. All you have to do is simply identify one of the peaks in which you are confident of assignment, sit on a diagonal of that come down see the cross peak you should complete this square so that cross peak should be in pairs. And then from that diagonal go to another cross peak if it is there, complete that square. From that diagonal continue like this, you can go one by one, like a ladder and then make assignment for all the protons in a given molecule. So with this I am going to stop. We will continue with the other things in the next class. Thank you very much.