One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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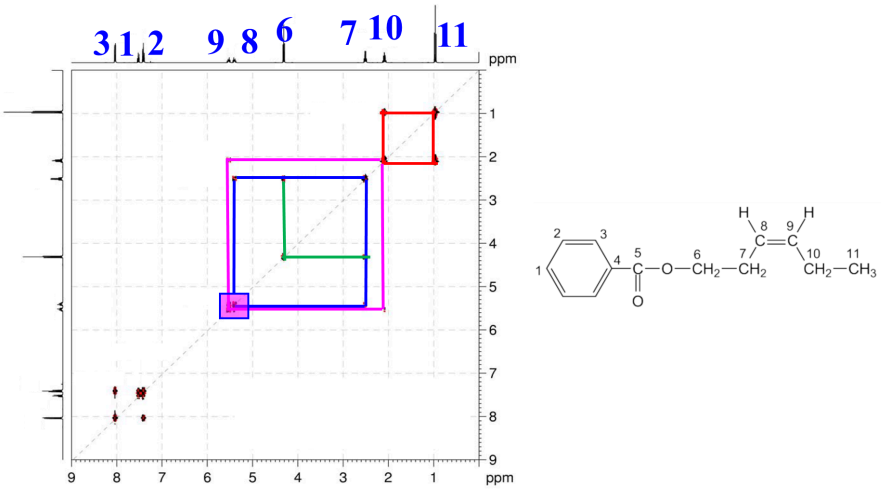
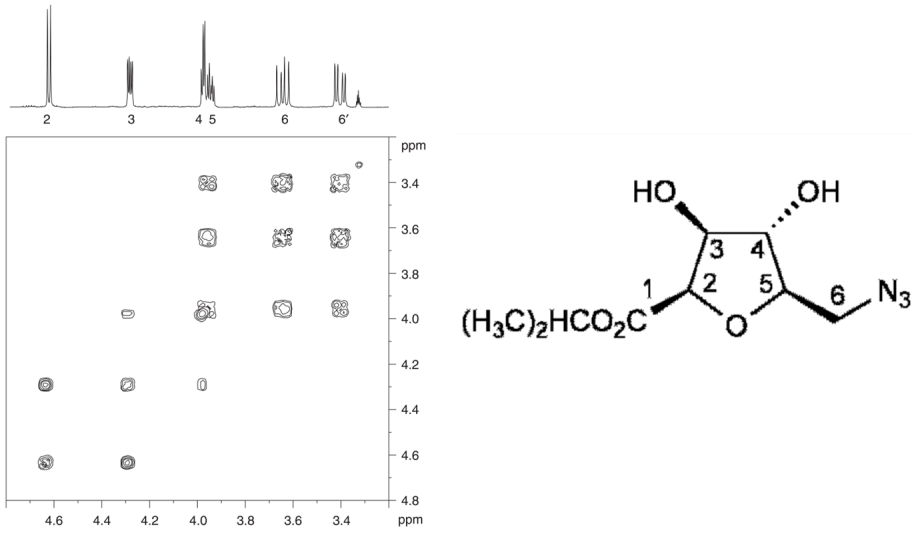
Lecture 41: Types of COSY Spectra

Since last two, three classes we have been discussing about the 2D COSY. That is the most important experiment for any chemist, if you have to make the assignment of peaks in all your molecules. If you synthesize the molecule, characterize it, take the 1D NMR spectrum, if the molecule is little bit bigger, there will be lot of peaks and how do you make the unambiguous assignment? you have to make COSY. That is the best way. And we took several examples with how a COSY works, what it does for a coupled spin, uncoupled spin, everything we discussed. And I said always one dimensional spectrum of a COSY appears on the diagonal. Take the spectrum of COSY spectrum of any molecule, sit on the diagonal, come vertically down, go horizontally and complete a square, then this diagonal peak and the other diagonal peaks are the coupled partners. Wherever you get the cross peaks, those diagonal peaks are the protons which have different chemical shifts, they give rise to cross peak. Like that from the diagonal, you can find out the other cross peaks if there are any. So, we can continue like that in a step by step manner and make the assignment for all the peaks in the given molecule. So, COSY is the simplest experiment to identify the immediate coupled partners. So, with that we will take another one or two simple examples, then go to a complex molecule. And today we will also see some examples of literally different types of COSY experiments, if there is time. So, I will take other one or two examples where complexity is there, to show how do you make an assignment. Now, that you have become very confident already, I can go little faster here to make the assignment of this one.

We will start the another molecule here, this is COSY spectrum of cis-3-hexenyl benzoate. This is the structure of this molecule and confidently I would always say this is CH3, start with CH3, proton 11 and then come down, you complete this, this has to be proton 10 and proton 10 gives rise to a cross peak here. So, this has to be proton 9 and here proton 9 has a cross peak, there is a multiplicity pattern here. You can see two groups here. Proton 9 is also giving cross peaks, it can give to this, this, etcetera. So, that could be for a proton 8. From proton 8, you have a cross peak to two things, one for 10, proton 8 to 10 also is there, and also it is giving rise to cross peak to 7, it can give rise to cross peak to 7.

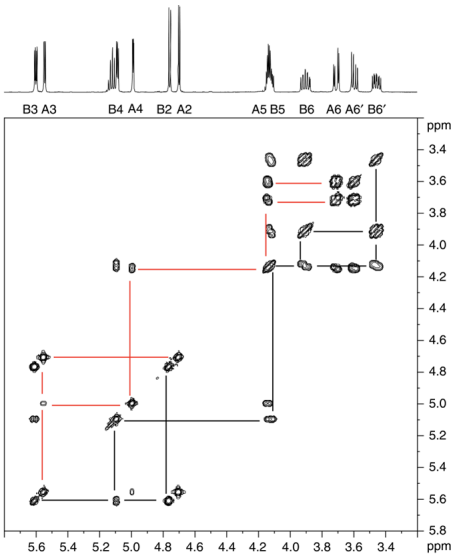


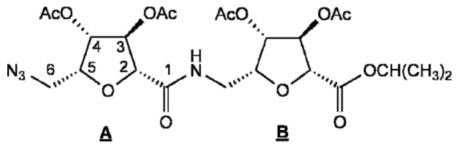
See, from proton 8, go here, complete this square and this is 7. And of course, there is another diagonal peak here, what is that diagonal peak? If you carefully see, do not get confused, we will start with11, 11 to 10, 10 to 9, 9 to 8, 8 to 7, you complete this square. From 7 diagonal come down, you hit diagonal and complete this square. So, this has to be proton 6, so that completely analyses this bunch, you can make the safe assignment without any difficulty. But what is left over? Left over is only phenyl part, in the phenyl group of course, 1, 2 and 3, I can simply make the assignment based on the intensity pattern, this and this protons are equivalent, these two are equivalent gives double the intensity and this is a single proton, para proton half the intensity. So, very easily you can identify this. I did not mark it there, that is fine.



We go to the another molecule like this, very simple, 500 MHz COSY spectrum of a molecule like this. Here we have two protons, you have to assign which is the proton in which you are confident. Obviously, I look at the proton 2 here, this can couple to only

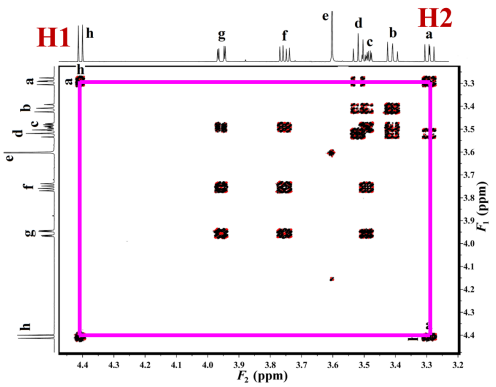
one of the protons and become a doublet, immediate coupled partner, I would say this is proton 2. And then go here horizontally, you will see proton 3, see here proton 3, from proton 3 I come here, go vertically up and then I will complete this square. This is 2 and 3, complete this square. This is 3 and 4, from 4 I can come here, of course, there is a bunch here, 4 and 5 cross peaks are there, but are unresolved. From 5 I come here, complete this, I complete this square, 5 is coupled to 6, but 6 has two protons, 5 is also coupled to 6 prime. There are two protons here, both are two bond

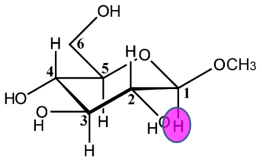




couplings or three bond coupling with proton. This is for H5, so we will have two cross peaks here, this and this, so if this is 6, other is 6 prime, so you can continue complete the assignment, very simple, which is 6 and which is 6 prime, you have to use the knowledge of coupling constants, alright.

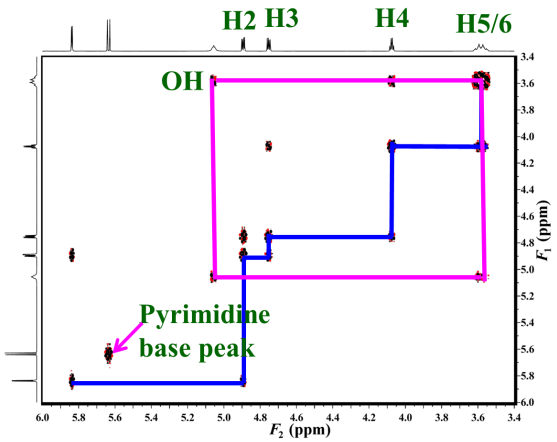
If there is a dimer like this, for a molecule like this. A dimer of two molecules here and here, how do I assign? The assignments have been very easily made, look at this molecule, go vertically up, they are all red lines and it forms one spin system, systematically go, and all these correspond to one of them. And all the other ones are for this molecule B. Start with one of them come here and then of course, go here, go here like that, you can assign for other molecule, even though it is a dimer with a bigger molecule, very slight difference in the chemical shifts are there, in each of the cross peaks, very easily you can make the assignment. So, I hope by now, you have already got the idea, how to go ahead and make the assignment,

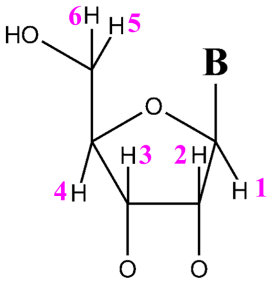




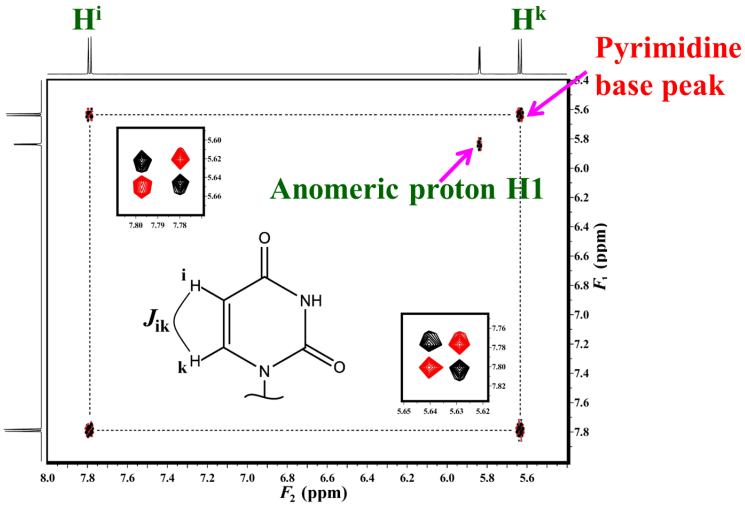
I will take the assignment of a molecule like this, a sugar like molecule, it is a glucoside. Always if you want to analyse the molecules of sugars, please remember, most of the time, it is an anomeric proton that comes down field, it is always between 4 to 5 or 6 ppm. So, you should look at the anomeric proton and that is the only proton, which can couple with another proton, which gives a doublet, what is that proton here, if you look in this molecule, this is a doublet coming in the region of around 4.4 ppm. Safely I would say that is anomeric proton, proton H1, you have to use the chemistry logic now, I use that as a H1, anomeric proton and using that anomeric proton, I will complete this square, that must be proton H2, obviously this is proton H2, H1 is coupled to H2. So, very easily you can make the assignment. Please remember, I am taking this example to show you that, in the case of sugars and others, always start with anomeric proton, that makes your job simpler to analyse. And then, this region is expanded here, this region, you will see what is going to happen, the entire region up to this is expanded and you can see what will happen, we already know this is H2, the anomeric proton. From H2, go here, come here, come here, complete this square. But why this central peak is missing, this is the anti-phase character, as a consequence what happens, instead of 1:2:1 , ir ia -1 0 1 intensity is coming. So, it can happen sometimes, the central peak disappear like that, does not matter. Drom this you start, complete this square. So that has to be proton H3. From proton H3, you can go down, go horizontal, come down like this, complete this square, then this has to be H4. We started with H1 in the previous slide, H2, H3, H4, then from H4 here, you can continue like this and complete this square, so this has to be H5, very easily you can make the estimate.

Now H6 and other things are here, separately, up to this was expanded, we could get up to H5. Now H5 is known, from H5, what are the two possibilities you can think of, H5 is coupled to two protons here, H6 has two protons, so come here and complete this one. This one is H6a, another one H6b, why I said H6a and H6b, axial and equatorial you have to assign based on the coupling strengths. Always remember axial-axial coupling is larger, axial-equatorial coupling is smaller than that and equatorial-equatorial coupling is even smaller than that. Now you have to look at the multiplicity pattern and see which is larger coupling, so from that you can know whether it is axial proton or equatorial proton and then you can make the assignment. That is the usual way we have to start doing it.



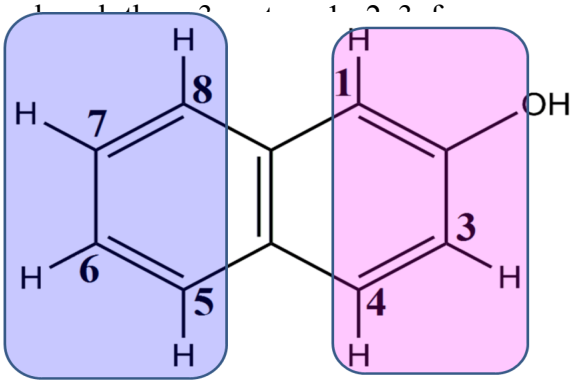


And we will go to the 2D spectrum of a protected nucleoside like this. And very easy in a protected molecule. Again start with anomeric proton, complete this square, keep on going like this, I go here and then complete and go to, I am not completing this square, I assume that you know by now, 2, 2 to 3, 3 to 4, 4 to 5 or 6, we do not know whatever it is, both 5 and 6 are indistinguishable, they are overlapped here, fine.



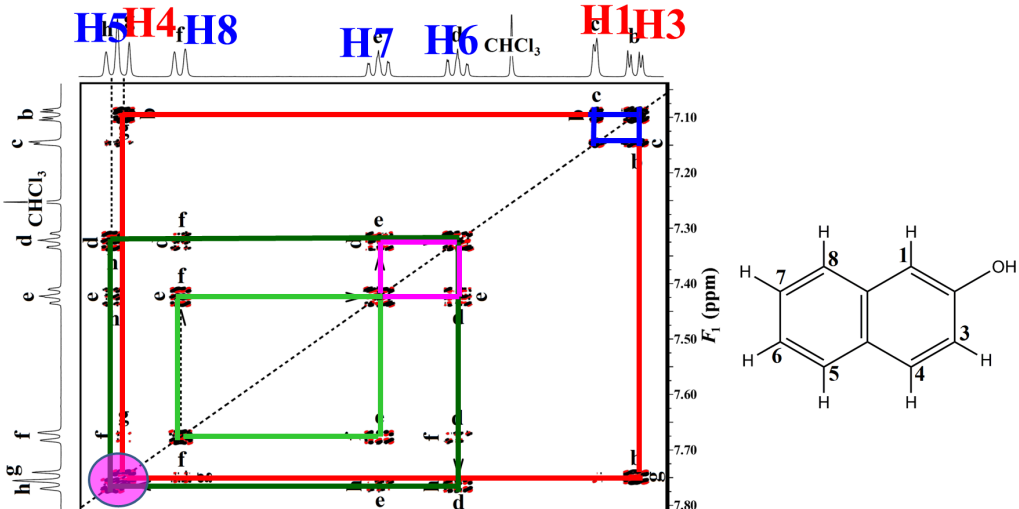
Also there is a, there is a pyramidine base group is here, with that we can take and make the assignment here with a pyramidine base group, that is also there, pyramidine base group is giving rise to cross peak. This is the anomeric proton and therefore this is the pyramidine base group. With that base group, with the anomeric proton here, you can make the assignment for these 2 protons, aromatic protons. So this is how we can start making this thing, some complications will come at times,

I will take the example of the analysis of phenyl groups, I have deliberately taken this molecule, remember here there are 4 protons, here there are 3 protons, from the multiplicity pattern I can tell you, this has to be a doublet of doublet, one meta coupling and a para coupling, this has an ortho coupling and a meta coupling, doublet of doublet, this is also similar, all those patterns you have understood already, we have discussed quite a bit about the multiplicity pattern of the phenyl protons. We will start with this, I will say this is one spin system, this is another spin system, there are 2 different spin systems, these proto system, this 5, 6, 7, 8 forms anotl

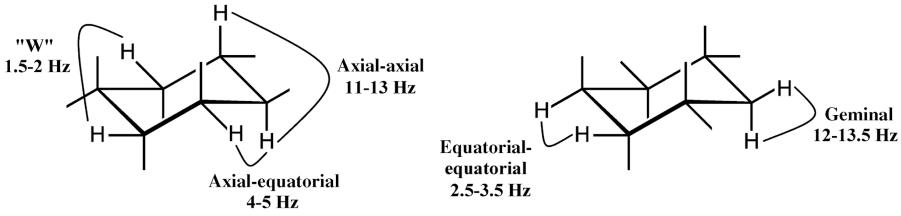


Spin System 2:
H8-H7-H6-H5

Spin System 1:
H1-H3-H4

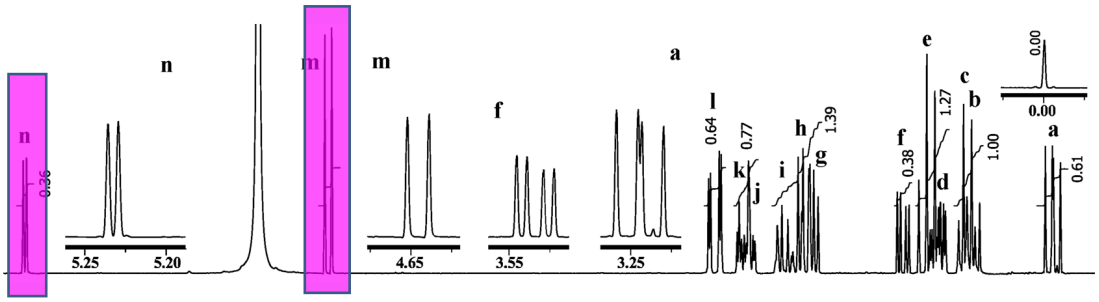


Now analysis of this one is very easy from the COSY, start with this H1, why I will say this is H1, this is the only proton, it can have meta coupling and a para coupling, if it is not resolved it is a doublet, otherwise it should have been doublet of doublet. The very weak separation, very small separation is there, you can find out, that has to be H1. And from H1, I will start with this and say this must be H3, because H3 can have a first ortho coupling, large and then each line is going to be doublet of a doublet because of this, that is H3. And H3 is going to give a meta coupling to other one, that has to be H4, this has to be H4. There is a doublet, it is not a triplet, but you know, this one doublet is here, other part of the doublet from other one is overlapped here from other phenyl ring. See there is a overlap here. You can see here, this peak corresponds to this doublet and this peak corresponds to this doublet, you can make the assignment very precisely here. We will start with this one, I would say this is proton 8 or 5, does not matter, there is a confusion, I will start with proton 5, proton 5 will couple to this one and here comes proton 6. And proton 6 has to be two triplets, because this is doublet of doublet and ortho couplings are nearly equal, it appears like a triplet and then further split because the meta coupling, it is triplet of doublets here. Same way for this proton, from 6 you will go to 7, and from 7 you go to 8, in this type of phenyl groups very easy to assign using the COSY that is why I took this example. But there is one ambiguity here, whether this proton is H5 or H8, because both of them will give doublets. See both of them will give identical pattern is doublet of doublet of doublet, both of them will give 8 line pattern. So, which is which, whether this is proton 5 or this is proton 8, I do not know. There is an ambiguity of the assignment of the spin system. Whether this is the pattern you have to assign or this is the pattern you have to assign, we do not know. For that what we do is, we resort an experiment called NOE, separate NOE experiment, which tells how we can assign by separately irradiating one of them. So, when I discuss the NOE, I will take the example of this and I am going to discuss at that time. So, there is no point in going for that now, because that will be a separate this thing, we have to discuss by doing NOE, but instead of that what I am going to do is, I will start analyzing the 2D COSY spectrum of D-Glucose. This is where another important thing, see that molecule I will come back, when I do the NOESY, because I have not discussed NOESY yet, when I discuss NOESY, there are two ways of doing NOESY, steady state NOE and transient NOE. And steady state is the simple one dimensional difference NOE, that is why I did not explain this to you, because when I explain NOE you know that, I will come back and do that. In the meantime we will go to the 2D COSY spectrum of D-glucose.



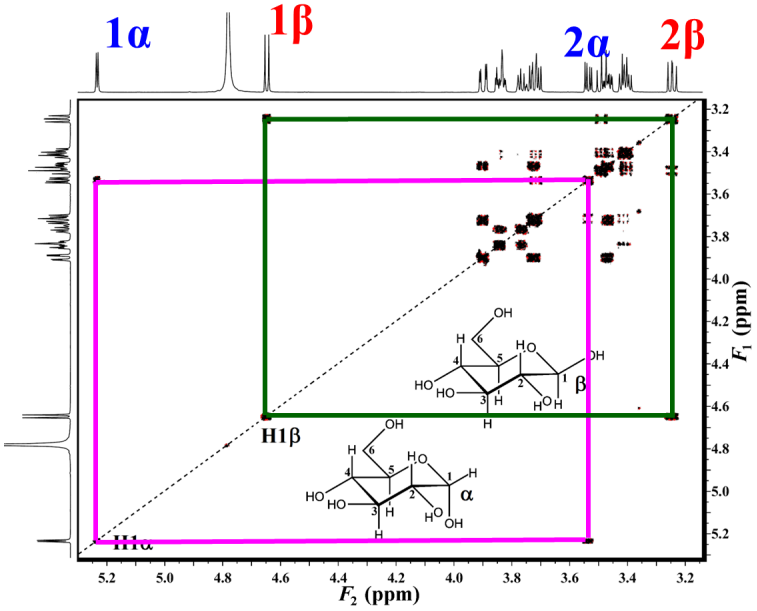
See glucose exists in two forms, conventional glucose what we take, it has got alpha and beta form, 64 percent beta and 36 percent is alpha, that is the mixture it exists in two forms. And this is the important thing, before you go to the analysis of the spectrum, you should know what is the strengths of couplings. As I told you axial-axial coupling in sugars are larger than axial equatorial, then equatorial-equatorial is much smaller. So always this is larger, this is next to that, then this is smaller than both. This information is needed. And of course, geminal coupling like this is quite large. Why I say this is needed, when we have ambiguity in the assignment of let us say groups like 6 and 6' like this or 5 and 5', which is the one which you have to consider, when both gives rise to the cross peaks, but we use knowledge of coupling also at that time.

So this is the 1D spectrum of alpha and beta D-glucose together. Look at this.

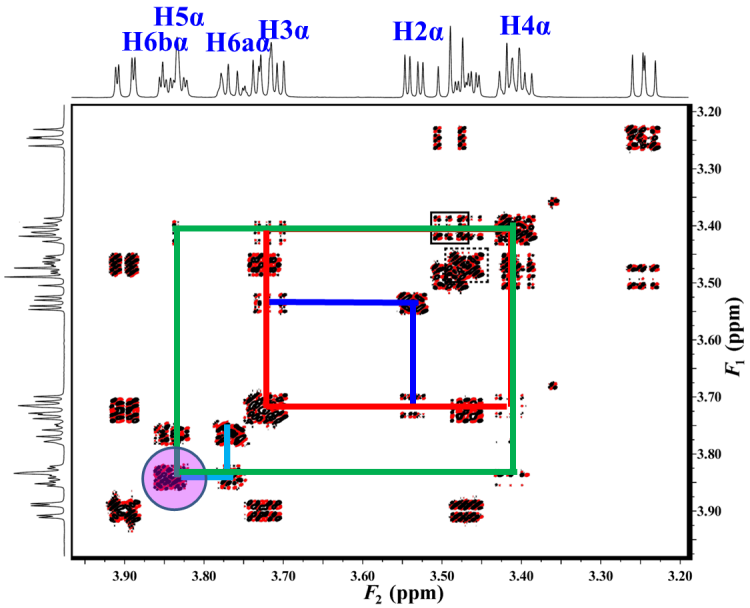


Always two anomeric protons comes to the down field. I told you this is anomeric proton of beta and alpha D-glucose also structure I have not written, this anomeric proton is coming. One important thing is this type of sugar molecules everything is recorded in water which comes at 4.786 ppm. What happens is we record the spectrum in D2O. D2O exchanges all OHs present in the molecule. They all come around so near water peak and get exchanged with water. You will not see OHs peaks. So only we will have to worry about anomeric proton and other protons. Generally OHs are exchanged in D2O

if you record in D2O. But two anomeric peaks are important as I told you it always comes between 4.5 to 5.5 or less than 6 ppm. We look at the peaks intensity. I told you 64, 36 is the percentage of mixture present in water. But when you measure the intensity of the peaks in the NMR spectrum, almost same ratio we got. Look at the anomeric proton spectrum here. This one and this if you take the ratio this is larger than this one. This is 64, this is 36. So clearly I would clearly say, because of the intensity this correspond to beta glucose. So anomeric proton you would look. From the anomeric proton peak we can start making the assignment. So anomeric proton if you know we can just based on the J values itself we can assign. Why? because remember in the case of the alpha glucose the doublet is axial equatorial coupling. Axial equatorial coupling is small whereas in beta glucose it is axial-axial coupling that is quite large. I will show you in the structure here. See look at in NMR spectrum look at the separation quite large. So obviously that as I told you has to be beta glucose. Using the coupling strength of anomeric proton splitting pattern which coupling of large value compared to alpha I can make the assignment. So I will start with this. This is beta, this is alpha, these are anomeric protons. Further we can start with the COSY pattern. How do you assign the COSY spectrum of this? We already know that we have to start with the anomeric proton. See anomeric proton one which has a small coupling is alpha. We complete this. So if this is alpha, this is proton two. If this is anomeric proton one, this has to be two. Now the structure of both of them is given. If you consider this anomeric proton, this has a axial-axial coupling whereas this one has a axial-equatorial coupling.



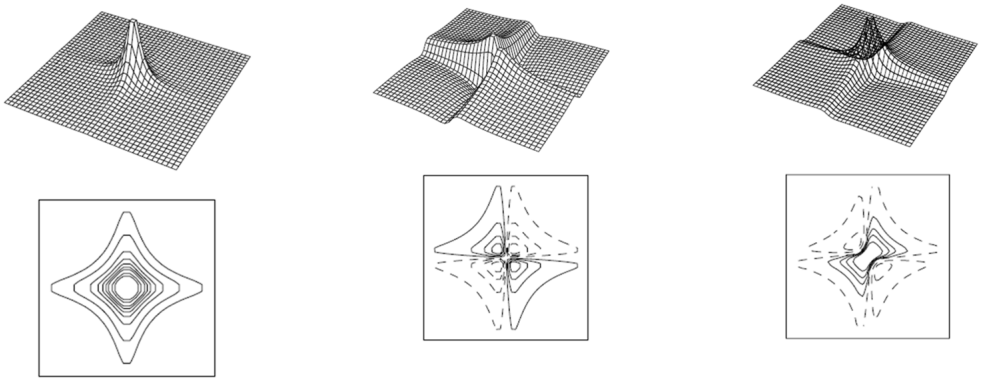
So that is a problem. That is why this coupling is much smaller whereas beta has larger coupling. I can make this assignment. Alpha if I know one, I know two very easily. I can make the assignment. Similarly for beta anomeric proton, I complete this square. I know then this is beta, this is one of beta, this is two of beta. So two protons, alpha anomeric proton one and proton two from alpha. Similarly anomeric proton one to two of beta, we can make the assignment. I use the knowledge of chemical shift anomeric proton and the couplings, the separation of peaks. That is why I said you should remember axial-axial coupling is larger than axial-equatorial. This is the important concept you have to use while making the assignment of all these things.



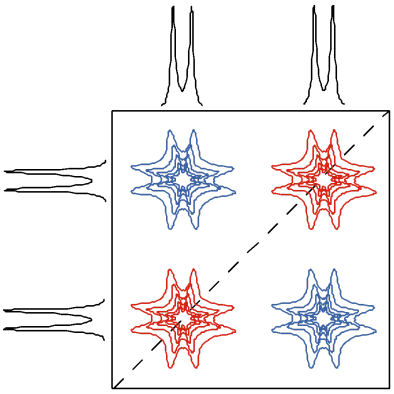
So now we know what is alpha, two, complete with two, go here, complete three and go completely and this is four and then complete this square, this becomes five and from this you have six and then six alpha and beta. Here the problem comes. Six alpha and beta, if you go for the structure, which is alpha and which is beta? How do I know which is alpha and which is beta? This is where knowledge of coupling we have to use. Looking at the splitting pattern and also generally, it is known that one of this equatorial proton, comes here, axial proton comes here. That is, there is always some chemical shift difference of 0.3 to 0.4 ppm. Similarly, beta glucose we can do the assignment. We start with two, complete this square, this is three and then this is four, five, six, alpha and beta. So the complete assignment of alpha and beta glucose can be done. This is a complete assignment of glucose we have done. This is where you can see the expansion. Beta, which is axial-axial coupling is larger and this is smaller alpha. We use that as a starting point and use the COSY to make the assignment. With this, most of the assignments we

have done and what I am going to do is, I will discuss something more about the different aspects of COSY experiments and what are its limitations. We have discussed about the COSY so far, but what are its limitations? We are comfortable by now. So many molecules we analysed, starting from simple molecules, mixture of molecules like glucose and varieties of things. And I gave you an idea where do you start in sugar molecules or in aromatic protons, when aromatic groups are there, where is the comfortable point for you to start. That is what I wanted to tell you.

Now let us see what are the limitations of COSY experiment. First thing is the polarization transfer between the coupled protons depends upon the strength. If the coupling is larger, cross peak will become stronger. If the coupling strength is very very weak, you will not be able to get the cross peak. Even if you get cross peak, it may be very weak in intensity. And all the 2D NMR peaks have a fine structure and they contribute to diagonal and cross peak. There will be coupling. So each peak as I told you in the two spin, each diagonal peak will be 4, 4 peaks with square pattern. Each cross peak will be 4, 4 peaks with square pattern, but in all these bigger molecules, the resolution will not there. But in principle, there are fine structures and cross peak contains both positive and negative signal, whereas diagonal peak contains only positive signal. That is what we also discussed. I discussed about the pattern of the diagonal peaks and cross peaks. But when the signals are broad, unresolved because of negative and positive signal close by, they cancel out. As a consequence, there is a drawback, you will not see the cross peak at all in COSY. When you do not see cross peak, it may be because there are two anti-phase peaks, anti-phase peaks are close by, so that will get cancel out and you will not see. And diagonals are in phase because it is a huge peak and it will mask the peaks close to it, the some of the cross peak very close to it. And sometimes diagonal peaks are very large, it gives rise to tails. Another thing, whenever record a spectrum, what is the line shape you are going to get? Of course, 1D spectrum has to be like this very clear. We have to do the line shape analysis to get a sharp peak. We usually take CHCl3 and measure the peak of height of the satellite and then at 1.1 percent and half of the satellite height, we measure the height of this. Very easily we can measure and get the line shape. Line shape analysis is done. This I have discussed extensively in one of the courses. I am not discussing this now. This course focus is different. So, I am not telling that.

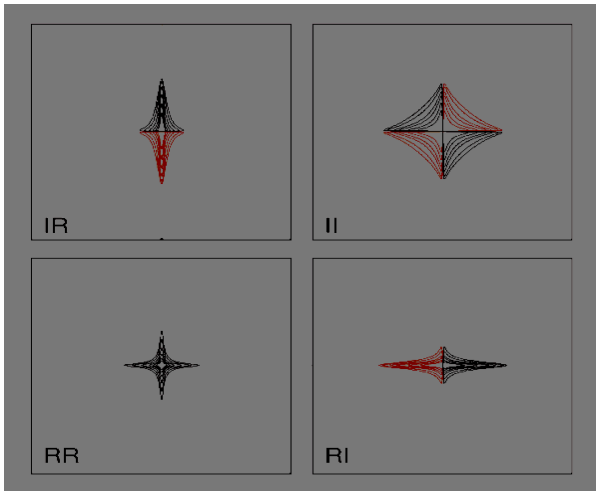


But there are three different types of patterns you are going to get, different peak shapes in the COSY or in any 2D NMR. One is pure absorption line shape like this. It is like a star picture. Other one pure dispersion like this, and this is phase twisted line shape. These are the three different types of spectra you are going to get depending upon the type of experiment you do. That is whether you are doing the magnitude type experiment or phase sensitive experiment or whether you are processing to get the double absorption line shape. That is what matters. So, always the spectra are represented in two ways. The 2D spectra are represented in two ways. One is phase sensitive mode, other is magnitude mode. Magnitude mode means there is no phase information, that is last. No phase information. Everything is discarded. Phase sensitive is always important. The magnitude mode is not good for high resolution, because you cannot distinguish between dispersive and absorptive peak. They are mixed up. Phase sensitive on the other hand is very good. You have line shapes of absorption dispersion both are separated out. And generally absorption mode is preferred for high resolution. That is what we do. Look at the COSY spectrum cross peak. If you do the magnitude mode COSY, you get a star like pattern like this.

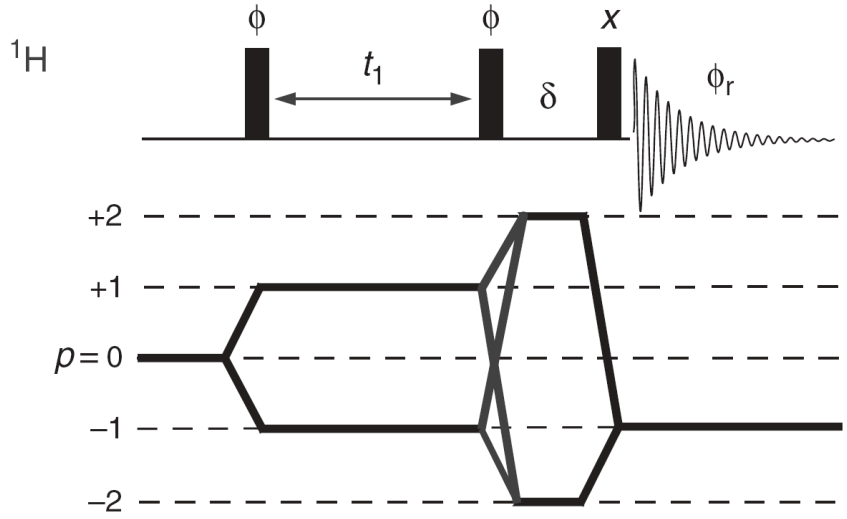


Usually you should get sharp peaks with a double absorption means very sharp peak, but we get star like pattern. This is because of mixture of absorption and dispersion like this. This is a magnitude COSY recorded in the magnitude mode not phase sensitive mode in both F1 and F2 dimensions. Of course one important thing, when you do Fourier transformation you have real component and imaginary components in each dimension. In both dimensions if you do, you have two real, two imaginary components. There are

four possible combinations then. Real real, imaginary real, real imaginary and imaginary imaginary. There are four possible combinations.



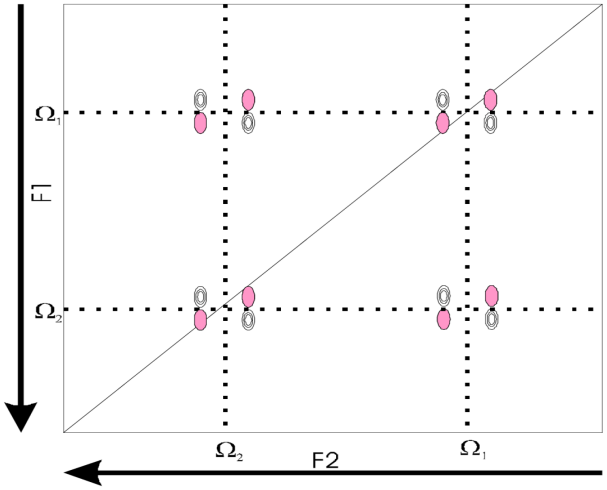
Accordingly, we have four different types of line shapes. For example, look at this. This is in one dimensional imaginary, other dimension real. Here you have both the dimensions imaginary imaginary. Here real and imaginary and her you have both are real. And look at it. This is the sharp peak. Look at this, it is very broad imaginary imaginary. In this, one side broad and other side is sharp. But this is what we have to consider. Always double absorption mode gives a better resolution. Please remember when you want to record the spectrum of COSY and others always do the phase sensitive detection with a double absorption mode. The data has to be in pure double absorption mode line shape. This is the double absorption mode line shape. Lines are very sharp and this is the contour plot. This is the stack plot like this. So, double



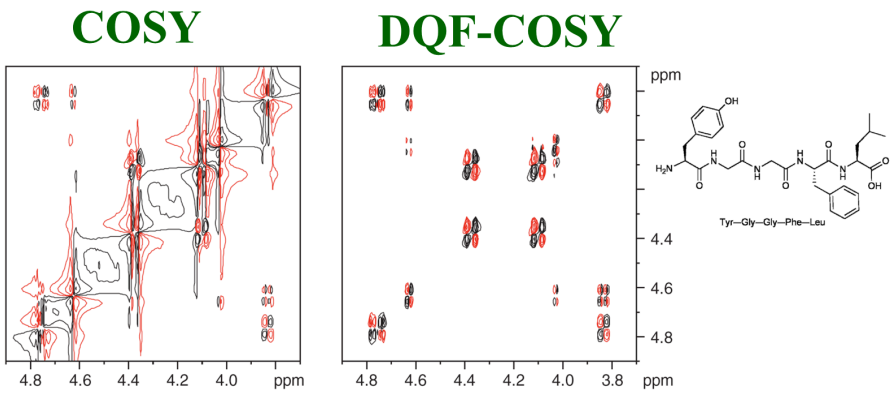
absorption mode is always preferred for better resolution. That you should know.

There are several variants of the COSY experiment. COSY can be of several types. One is DQF COSY. The double quantum filtered COSY is something, where the advantage is, you will remove all the peaks that are masked by the diagonal. And it is very very easily you can get positive and negative peak signals very easily you can resolve it. And singlet like the reference, solvent peaks they all get suppressed, because we take the magnetization through double quantum pathway and then bring it back for the detection. This is the way we have to select the double quantum pathway. Similar to COSY, only thing is second 90 degree pulse generates multiple quantum coherence which is not detected by the receiver. The double quantum is always converted to single quantum. As I told you in the selection rule, anything other than delta M equal to plus or minus 1 is not detectable. They are all multiple quantum. We always detect single quantum NMR. I told you delta M equal plus or minus 1 is single quantum, that is what we detect. So, we do some process of selection of this double quantum, you can pass it through that by using phase cycling and then convert into single quantum. This is the way we do this by applying a 180 pulse, and how do you do the coherence pathway selection. And we have to apply different pulses. This is a more detail you do not worry about it. But DQF COSY experiment with coherence pathway selection is always like this. It is a simple experiment. Similar to COSY, but little modification is there, you can see here. After 90 pulse, even this is not 90 lot of modification is there, and also with a gradient selection. I said in the coherence pathway you can use different pulse phases, you can do by this is phase selection. Alternately you can use gradients. The phase cycling can be done or gradients can be applied. Generally in the phase cycling version DQF COSY both the coherence pathways are retained. In the gradient only one coherence pathway is retained. There are two different coherence pathways always. It can go here come back to 1, come back to here. There are two coherent pathways in the phase cycled version of DQF COSY, both are retained. Whereas in the gradient version only one is retained. Obviously that tells you the sensitivity of this is less compared to phase cycled version.

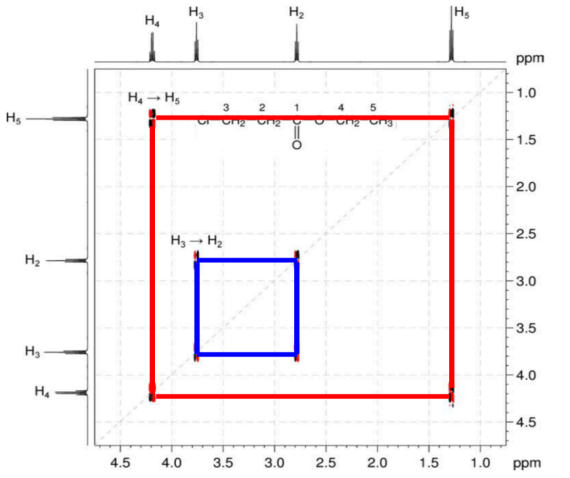
And the diagonal and cross peaks both possess anti phase double absorption mode line shapes, and they are not affected by double quantum filtration. Advantage is whatever the tailing we get, that is the severe tailing near the diagonal are all removed. So, it gives better quality spectrum. The double quantum filtered COSY gives you better quality spectrum. The singlet from uncoupled protons, solvents, reference all are suppressed you would not see it at all. There is a biggest advantage of that. So, major advantage of DQF COSY is removal of dispersive component of the diagonal peaks. As a consequence, the spectrum which is much much better. Here is a simple typical example of a DQF COSY spectrum.



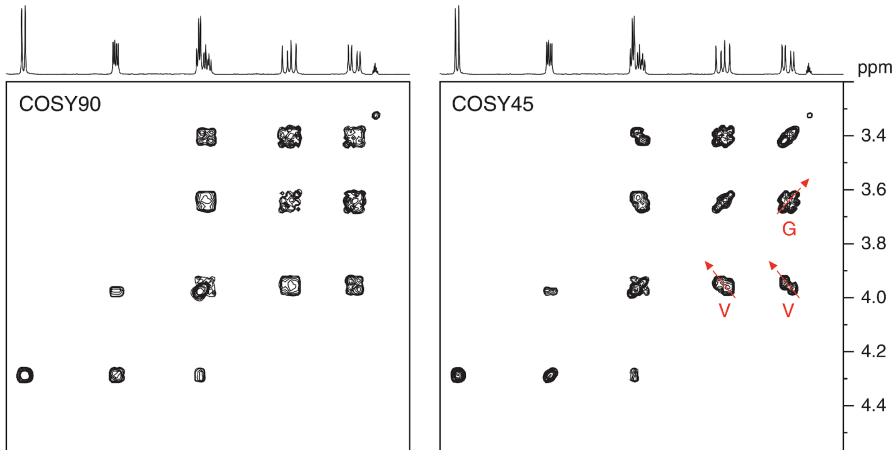
Look at this one diagonal both positive negative peaks are present. Here both are positive negative. Earlier, in the case of COSY both were you know positive peaks in both the dimensions. Here they are anti phase doublets. In the conventional COSY only cross peaks are anti-phase diagonal or in-phase, but here it is different and they are double absorptive, gives better resolution. I told you for the representation of the cross peak in the COSY, always double absorption mode is preferred. And the typical DQF COSY spectrum always there are no diagonal peaks will mask the cross peaks.If there are cross peaks nearby, here they will not be masked. Whereas you may remember in the COSY huge peaks will be there because both are in phase diagonal peaks and that will mask the peaks nearby. Sometimes you will not be able to identify. Here is a comparison.



Look at the COSY spectrum of this molecule, it is a pentapeptide. Look at this molecule look at the COSY spectrum how are trials are there peaks nearby you will not be able to see they are all masked. It is a clumsy spectrum very you know not good at all. On the other hand look at the DQF COSY spectrum here, fantastic, you know it is a double absorptive line shape and both the dimensions, you can see anti phase character is there for all the cross peaks and diagonal peaks. Look at the resolution here look at the resolution here. So both give the same information there is no difference at all. But look at the better quality of the spectrum. So I have suggestion always I tell for the participants do not ever do COSY spectrum. Always do DQF COSY that gives you a better spectrum. So COSY spectrum like this is very clumsy and you will not be able to get the good resolution. This is the simple example to show how the DQF COSY spectrum is obtained in a molecule like this.

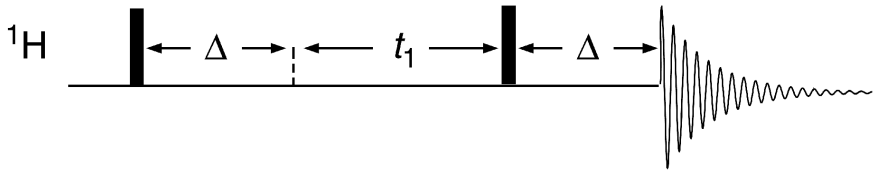


Look at this molecule, this is the molecule structure. And you can see one peak, another peak, two spin systems are easily identified, and this is the structure of the molecule. You can clearly see the structure of the molecule. From the structure you can make out there is one group here and one group here, that is all. But look at the resolution here, diagonal and cross peaks, fantastic resolution you are going to get compared to cross peaks. So the peaks near the diagonal are also very well resolved. This is the advantage of DQF COSY.

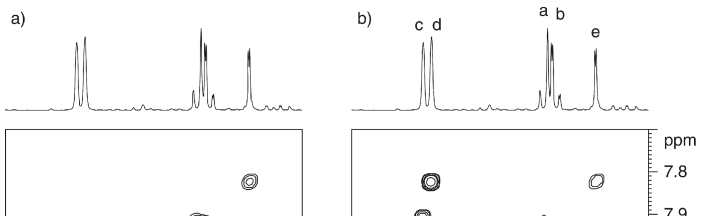


And about other variant of the COSY are, COSY 45 is there, COSY 90 and delayed COSY is there. And of course, let me see how much is there to finish today. I think there is a little bit is there I will finish it. So that tomorrow I can start with another one. In few minutes I will finish this one. COSY 45 is the simplest experiment, instead of 90 degree here we use the 45 degree. What is the advantage of it? the intensity of the cross peak near the diagonal becomes weaker and the diagonal becomes narrow. But another thing is cross peak appears tilted like this, and the direction of tilting gives me relative signs of the couplings. Instead of 90 degree if you use second pulse as 45 degree, not 45 any small angle, soft COSY we call, we can use 20 degree, 30 degree, 50 degree whatever the angle you want. But then what will happen is compared to this COSY 90 in this COSY 45 some of the peaks are missing and some of the peak appear tilted and the direction of tilting gives you the sign of the coupling. Usually if you look at some of the molecule vicinal couplings are usually positive and geminal couplings are negative and you if you want to find out the relative signs of that, do low flip angle COSY where for the second pulse use 45 degrees.

You can also have a long range COSY also called



delayed COSY. What we do is after the t1 before after the 90 pulse before t1 you apply one delay here and after another pulse you give another delay. Additional fixed delays there given before and after second pulse. This will enhance the resolution, but the total evolution time is constant that is always retained to get the better digital resolution. And then what will happen small couplings which are not possible to detect, also can be detected. Only thing is you have to set the delay of 50 to 500 microseconds. Remember this is nothing but a 2 pulse COSY, but only thing is small delay is given after first pulse and after the second pulse. That is all. And then we are going to get the long range COSY. That means we are going to get the coupling constants which are greater than 4 to 5 bonds, which is generally weak and we do not see it, that can be detected.



So this is a comparison of the COSY 90 and the delayed COSY see how about the peaks here which are not there you are not seeing, we are able to see it because there are small small couplings here which are not resolved in the COSY. This is a delayed COSY. So correlation due to small long range couplings of less than 1 Hz or 2Hz can be easily detected. So this is what I just wanted to tell you. I am going to stop here, but remember with this I will finish the interpretation of COSY and discussion about the COSY. In this class what we discussed is we took lot of examples of the analysis of the COSY spectrum starting from simple molecule to big molecules. I told you what are the tricks involved in the analysis; start with a confident peak on a diagonal and then go in to complete the square, go in step by step manner and for all the coupled partners you have to do. So each coupled partners is going to give rise to cross peak which are symmetric with respect diagonal. So you can identify all the coupled partners and make the assignment very easily. Phenyl group what to do, I discussed. If you have sugar sugar molecules I told you have to always start with anomeric protons which generally comes down field. And if it is alpha and beta depending upon beta which has axial axial coupling, quite larger and alpha is axial equatorial coupling which is much smaller, from you can identify whether isomer is alpha or beta, that also I said. In the phenyl group usually identify whether the singlet is there doublet is there triplet is there and choose one of the confident protons of the phenyl group and then make the assignment, continue it in easy way. And we also took lot of examples of big molecules to make the assignment and of course COSY spectra, as I said, are always recorded in the different modes magnitude mode phase sensitive mode etcetera. But always you have to record in the phase sensitive mode and double absorption mode is preferred for better resolution. The magnitude mode and all those things you do only in exceptional cases, where you cannot do phase sensitive detection. otherwise it is not done. Usually you have to record the spectrum in double absorption mode to get better resolution. That is what it is. And I showed a variations of the COSY like DQF COSY. Double quantum filter COSY gives rise to the anti phase character of both doublets and cross peaks, means both diagonal and cross peaks give better resolution. The peaks near the diagonal are not masked, get very

beautiful clarity. And then further you can have a long range COSY or a small flip angle COSY. Small flip angle COSY gives rise to a reduced number of peaks and the tilting of the cross peaks gives you the relative signs of the couplings. If you know the direction of tilting of two cross peaks relative sense of the coupling you can obtain. Then the delayed COSY, also called long range COSY, give a delay after the first pulse and after the second pulse. Then long range coupling which are smaller also evolve, and that also can be detected, gives rise to correlated peaks. So, there are number of such things. In COSY itself there are hundreds of experiments, lot of experiments you can do not hundreds maybe that is a huge number several experiment like AE COSY PE COSY soft COSY you know and say small flip angle COSY lot of things are there. So, it is not possible to discuss everything, but idea is same each of this developed methodologies are only the improved versions to get the better resolution and better way of assigning. With this I am going to stop. From next class we will start with a different experiment called TOCSY. Thank you very much.