NMR Spectroscopy for Chemists and Biologists
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Lecture 47 – Two Dimensional Correlation Experiments III

Let us do a quick recap of what we did last time. We have been discussing two-dimensional correlation experiments. The COSY has been the most fundamental of all of these experiments which is the first experiment that was done with regard to the homonuclear correlation experiments and this is very important to understand and therefore we shall do a quick recap of what we did last time. We actually went through a detailed analysis of this basic experiment using a two spin system, weakly coupled two spin system.

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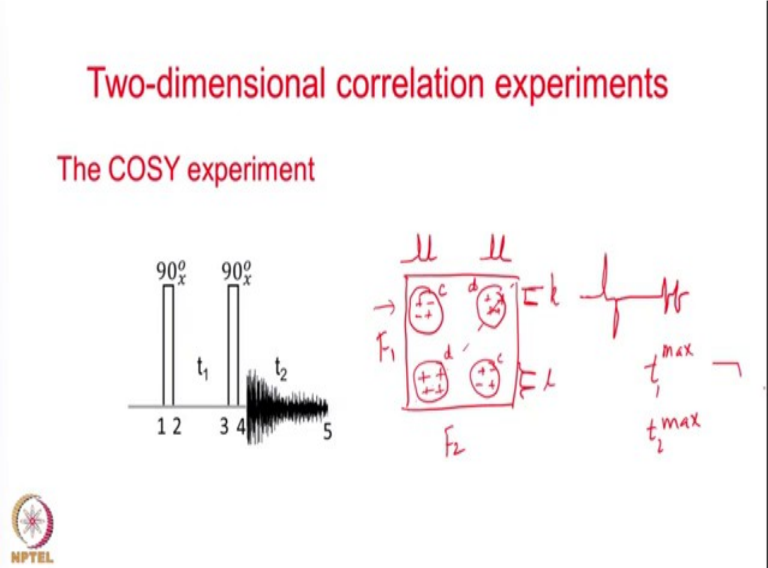

So let us do a quick recap of this what we said. So here the pulse sequence is given, it is a two pulse experiment and it generates a two dimensional spectrum of this type right here and we have the so-called diagonal here and if I have a two spin system I have one-dimensional peaks here, two doublets for a two spin system. So similarly I will have two peaks here and then two peaks here. This is a one-dimensional spectrum.

And in the 2D spectrum we said this will produce the peaks like this as this is a so-called diagonal peaks and they will have a fine structure which displays the coupling between the two protons and we also said that we have this sort of a display of science in the diagonal as well as in the cross peak. So this was arrived at by using detailed product operator formalism. And we said that the diagonal peak this is the so-called diagonal peak. If you call it as diagonal and this is the so-called cross peak if I call it as *C*, the same thing is true here.

This originates from one spin, we call it as *k* spin. This is the *k* spin and this is the *l* spin. So this results in a transfer of coherence from the *k* spin to the *l* spin, that results to this cross peak and the transfer of coherence from *l* spin to the *k* spin results in this cross peak. So this is also a cross peak and this is the so-called diagonal peak.

Now we also said that the diagonal peak has in-phase characteristics that means it is ¿ in all the four components and dispersive line shapes and the cross peaks on the other hand have anti-phase nature, that is a¿ fine structure and they have the absorptive line shape. In other words if I were to take a cross section at this point, then I will get a display of peaks like this and then you have the dispersive peaks will go like this.

So the dispersive line shapes are in the diagonal and cross peaks have anti phase absorptive lines shapes. The resolution in each one of these of course will not necessarily be the same because that depends upon how you collect the data, how much increments you use along the t_1 dimension to acquire the data. The resolution will be dependent on total number of increments, which will determine that so-called $t_{1\,max}$, this is a maximum t_1 and the resolution along the F_2 dimension or this dimension this is the F_2 dimension, this is F_1 dimension.

The resolution along F_2 dimension will depend upon $t_{2\,max}$, so maximum value of t_2. And generally we find that the $t_{2\,max}$ is always higher than the $t_{1\,max}$. So typically you collect about 2048 or 4096 data points along the t_2 axis and you may collect only about 256 increments or 512 increments along the t_1 axis because that determines the experimental time. So this is what we will get for a two spin system, this we arrived at by doing a detailed product operator formalism. Now, we also started looking at the three spin system.

And the general discussion that happened in the case of two spin system is also valid for the three-spin system as well. So let us try look at the three spin system.

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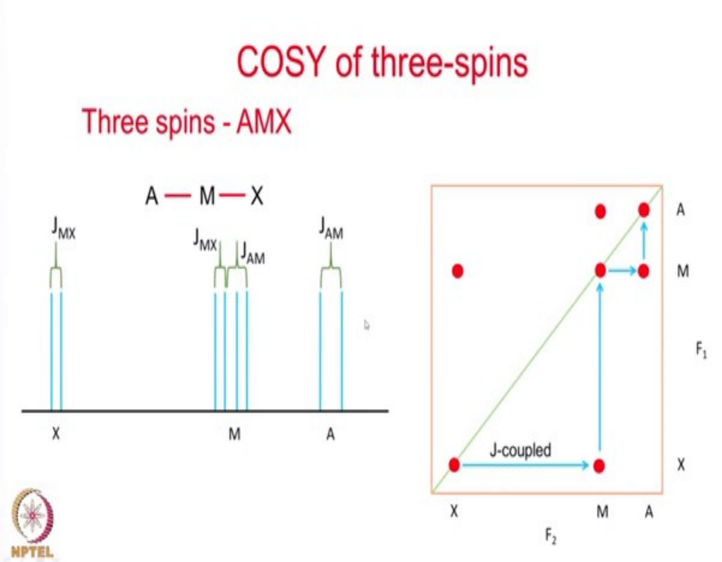

We said that the three spin system can be of this particular nature. This is called as a linear three spin system. There is *A-M-X*, *A* is coupled to *M* and *M* is coupled to *X* and there is no direct coupling between *A* and *X*.

Now, each of these spins will have different kind of multiplicity and that is indicated here, the *X* spin is split because of the coupling to the *M* spin and the *M* spin is a split because of the coupling to the *X* spin and the *A* spin and this will produce the so-called doublet of a doublet and this coupling information is present here. So this *M-X* coupling and this is the *A-M* coupling here and the *A-X* spin will be a doublet again, but this is showing the coupling constant *A-M*.

So this produces an overall COSY spectrum like this that you have a correlation from *A* to *M* here and this is a cross peak and these two are the diagonal peaks between *A* and *M*. And this cross peak here arises because of the coupling between *M* and *X.* So notice here, there is this one coupling, this will have a fine structure of the *M* along this axis and the fine structure of *X* along this axis. Each of these cross peaks will have the fine structure as indicated here. Depending upon on which axis you have this spin appearing it will show a fine structure along that axis. So we will see that in greater detail.

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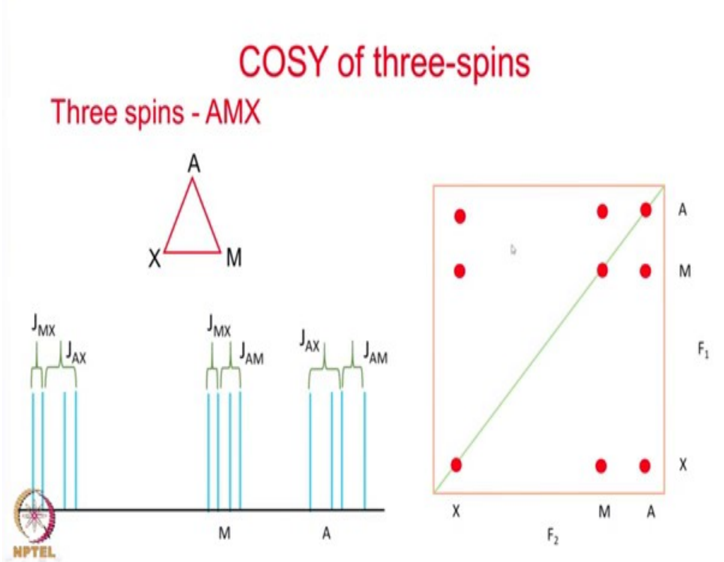

Now there is other type of a three spin system. This is so-called triangular *A-M-X* spin system and here each spin is coupled to the other two. So *A* is coupled to *X* and *A* is coupled to *M* and *M* is also coupled to *X*. Therefore all the three spins now appear as doublets of doublets, but their fine structure will depend upon the relative magnitudes of the coupling constants. So you have *J* for the *X* spins, you have the *M-X* coupling here and *A-X* coupling which is here indicated here.

And for the *M* spin, once again the *M-X* coupling which is the same as that and then we have the *A-M* coupling which is here and for the *A* spin you have the *A-X* coupling and the *A-M* coupling. So this results in a cross peak structure or the COSY peak pattern in this manner, you have the cross peaks from *A* to *M*, *A* to *X* and then from *M* to *A, M* to *X* and similarly from *X* you have to *M* and *A*. Now let us try and look at the fine structures now in each of these cross peaks. So what is important is that fine structures in the cross peaks.

So that is where you can actually measure the coupling constants and in the diagonal peaks, usually you cannot measure the coupling constants, because of the dispersive nature the resolution is rather low. In the cross peaks, these are the information carriers, we established once firstly the correlation and also measure the coupling constants from the cross peaks.

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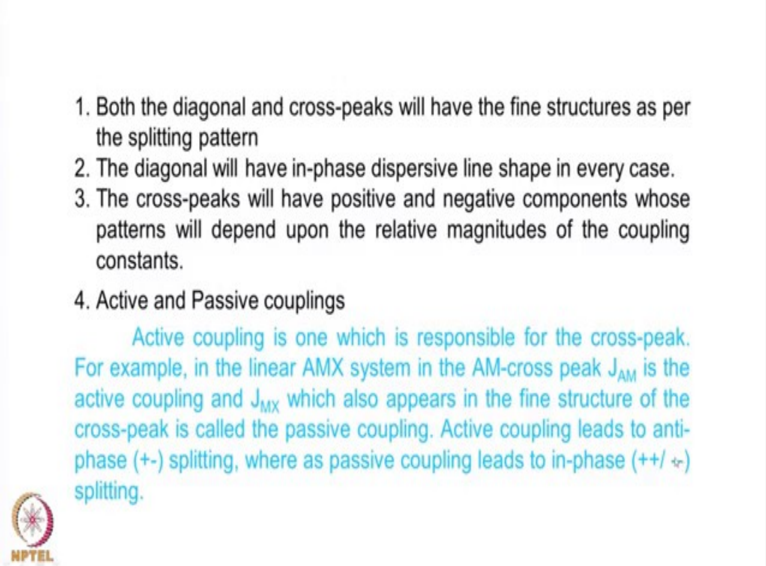

Now let us see what sort of fine structures we might have. Some generalizations here which are derived from experience with the two spin system and the same principles hold here as well.

See the both the diagonal and cross peaks will have the fine structure as per the splitting pattern which we discussed just now. The diagonal will have in-phase dispersive line shape in every case. The cross peaks will have positive and negative components whose pattern will depend upon the relative magnitudes of the coupling constants. Now there is one more concept which comes into the picture when you are actually looking at a three-spin system. There are two kinds of coupling one can distinguish here the so-called active and passive couplings. What are these? Active coupling is one which is responsible for the cross peak.

For example, in the linear *AMX* system in the *AM* cross peak J_{AM} is the active coupling and J_{MX} which also appears in the fine structure of the cross peak is called the passive coupling. Similarly it will be valid for the other cross peaks as well. Whichever coupling is responsible for the generation of the cross peak these called active coupling and any other coupling that appears there is called as the passive coupling. Active coupling needs to plus minus splitting.

So this is the anti-phase nature which we saw in the fine structures of the cross peaks, the plus minus splitting that arises because of the active coupling. Whereas the passive coupling leads to further splitting of these into in-phase doublets. So therefore this plus which is present here will be split into [+ +] and that will be the passive coupling constant here and the minus which is present here will split into [- -] and this is separation is again the passive coupling. Therefore, this is the principle which will be applicable for all the cross peaks.

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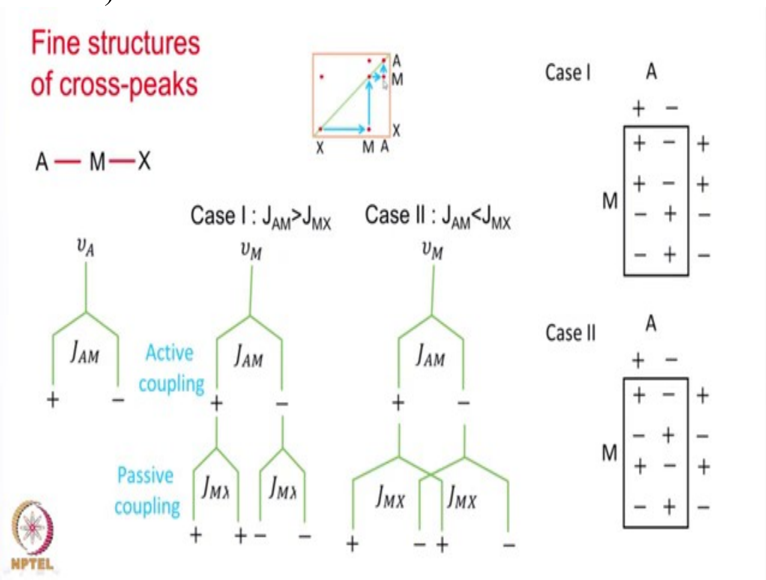

And let us see how this will manifest in the actual pattern in the cross peak fine structure. Let us take this example of the linear spin system once more. Okay, now this is the same pattern which I showed earlier. Now depending upon what is the relative magnitude of the coupling constants we will have two different situations. There can be one situation where the *AM* coupling is larger than the *MX* coupling and there can be another situation with *AM* coupling is smaller than the *MX* coupling. Now, let us look at one the particular cross peak here with case I and there is the *A* spin on here and the *M* spin along this axis.

This is my F_1 axis, this is my F_2 axis. So which cross peak are we looking at then? If this is the F_2 axis this is my F_2 axis if I am looking at the *A* spin here then that will be this and the *M* spin along this axis means that is this. So I am looking at this cross peak. Now this cross peak I am blowing it up here and I want to see what is the fine structure inside here. Okay, to see this, let us do a simple calculation as can be derived from our experience with the two spin system.

Let us say this is the chemical shift of the *A* spin without any coupling and when there is coupling this will split into two lines because of the coupling with the *M* spin and this is now the active coupling. So therefore this active coupling will produce me [+ -] pattern here. Okay, and now since there is no other coupling to the *A* spin, this is all we will have with for the *A* spin. Now, let us look at the *M* spin, the *M* spin has a coupling *AM* which will produce a [+ -] thing as in the case here but the *M* spin also has a passive coupling and that is the MX coupling.

So the MX coupling it will produce a [+ +] splitting of this peak and a [- -] splitting of this peak. So therefore if the *AM* coupling is larger than the *MX* coupling, then this will produce a pattern like this. You have *MX* here and *MX* here. So this will produce plus plus and minus minus. Okay, so on the other hand if the *AM* coupling is smaller than the *MX* coupling then what happens? This plus will split into [+ +] like this here. This will cross over into this and the minus one is split into [- -] like this, so this will now cross over.

So the pattern therefore along the *M* dimension will depend upon what are the relative magnitudes of the two couplings. In case I it will produce me a ¿ structure, in the case II it will produce me a ¿fine structure. So let us put that in here, so *M* spin I have this ¿and this one will have along the A spin I will have [+ -] here. So what it will be inside?

Therefore, this will be a product of these two, [+ -] you multiply by + so it gives a [+ -], again multiply by plus you get a [+ -] and you multiply by minus you get the [- +], multiply by minus here you get the minus plus. Therefore inside the cross peak therefore, I will have this sort of a fine structure. In the case II, then I will you have here [+ -] us as before and you will have here ¿.

Therefore if I now take the product I will get [+ -] here, [+ -] here, [- +] here and minus plus here. Notice therefore, there is a difference in the internal fine structure of the cross peaks depending upon the relative magnitudes of the coupling constants. Okay, so that was for this particular peak and how it will be if I were to look at another peak.

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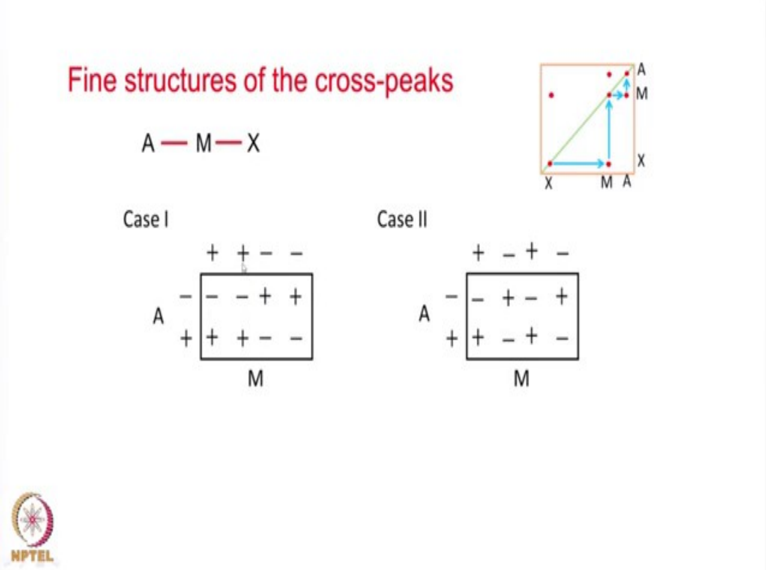

That is the same cross peak on the other side, that is this cross peak. This is also an *AM* cross peak, but now along the F_2 axis I have the *M* spin and along the F_1 axis I have the *A* spin. Therefore I write *M* here and *A* there.

So therefore for case 1, so I have this [+ -] here and *M* is ¿. So I multiply that here. So this is this gives me ¿, This gives me ¿ in this case. And with the case 2, I will get ¿ here. Notice the whole peak appears narrow along the *A* dimension and is broad along the *M* dimension because there is a doublet of a doublet along *M* axis and simply doublet along the *A* side. Therefore the appearance of this cross peaks will be different.

This will be like this and this will be vertical like this exactly the way I have shown in these cases. Now what can happen? Typically what generally happens is that you notice that what is the separation between these two and that will depend upon what is the separation between these two.

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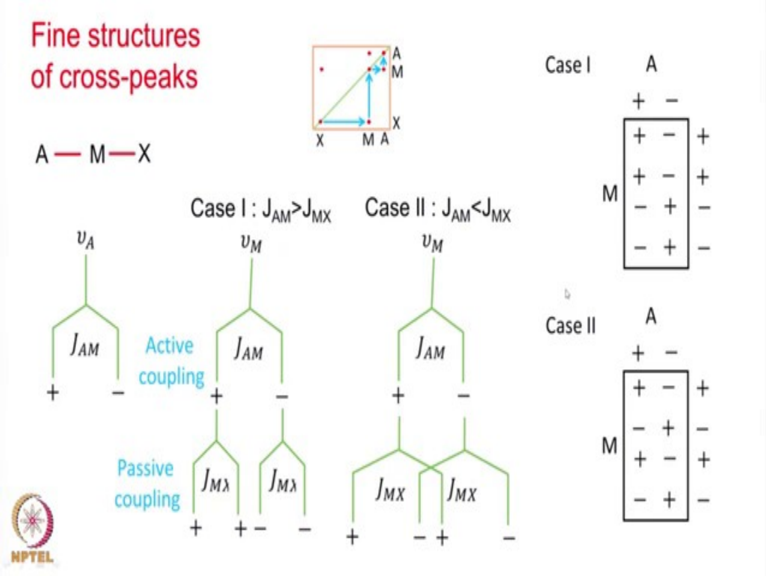

So if I go back and look here, you see these plus and minus are coming quite close. That depends upon the relative magnitudes of the two couplings. If the two are similar, for example, if the two are similar the *AM* and the *MX* couplings are similar then this plus and minus will come on top of each other in which case they will cancel.

So therefore you may not see anything here or if they are very close then the separation between them will be very small and there is a tendency for them to cancel. The same thing will happen here too that on this case of course although it is minus plus here but, if this difference is small then the tendency for these two to cancel will be there. So often it is the resolution is not good enough, then you will have a one peak here and one peak there and one peak here and one peak there and the central peaks may vanish.

And that therefore depends upon the relative magnitudes of the coupling constants and how much resolution you have in your spectrum. Now, what is the effect of the resolution on the F_1 and F_2 axis? Notice here that if I measuring along the F_2 axis as I said, generally the F_2 axis resolution is very high because I requiring larger amount of data points, therefore the separation here will be better in whichever spin appears along this axis.

On the resolution along this axis is rather limited because of the limited number of t_1 increments we have, we have this smaller number of t_1 increments, then we will have a t_1 Max will be smaller and therefore the resolution will be poorer. Therefore if this [+ -] appears here the tendency for cancellation here will be much more than the cancellation along this axis. Cancellation along this axis will not be as much as the cancellation along this axis will be. So this may cancel this and this may cancel this and it would appear that these peaks are not appearing in your final spectrum.

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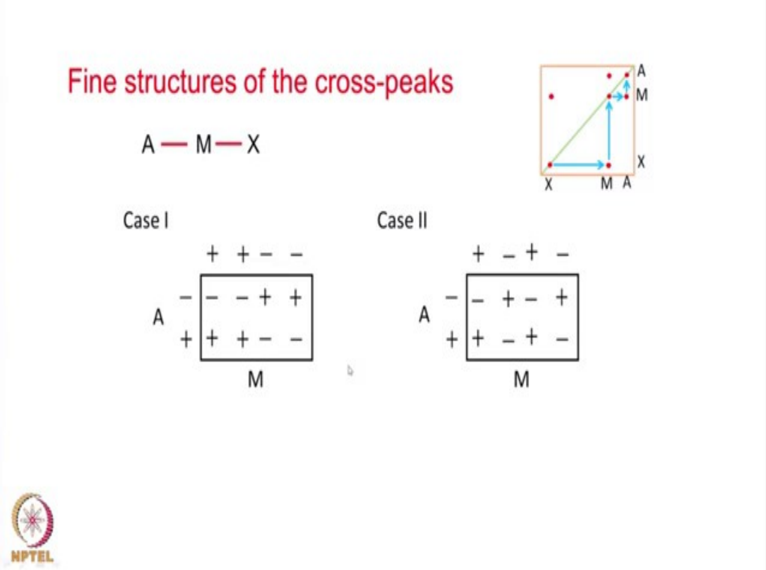

That is why the fine structures in these peaks may appear differently depending on the relative magnitudes of the coupling constants and which spin you are measuring. So here the *A* spin is along the F_1 axis, *M* spin is along this axis. If this coupling is very small there will be tendency for these ones to cancel and this plus minus and this resolution will be usually better but if the coupling constant difference is small then there may be cancellation here as well. So you will find cancellation of peaks appearing because of small differences in the coupling constants.

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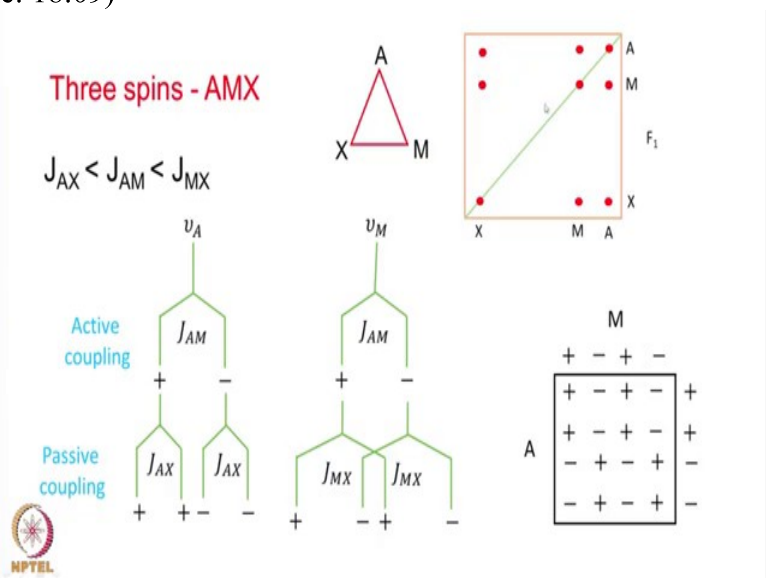

Same thing will happen here and this will happen even in the case of three spin systems. Now the three spin system of a triangular nature, we are talking about the three spin system only, if I have a triangular type of coupling network, then I will have peak structure which is like this as I already indicated you but now let us look at the fine structure. Now start with the *A* spin once more. Now as soon this particular situation *AX* coupling is smaller than *AM* smaller than *MX*, so accordingly we will do a calculation but if the magnitudes of the coupling constants are different of course it will make a change in the fine structure accordingly.

Now, so let us start with the *A* spin, the *A* spin will produce me if splitting due to the *AM* coupling and that is which spin am I looking at? *AM* cross peak I am looking at. *AM*, that is *A* is here and *M* is here. So that is *A* is here and *M* is here. Therefore I am looking at this particular cross peak. Okay, so this particular cross peak let us look at the fine structure. So the *AM* gives me [+ -] splitting and the *AX* coupling which is smaller than the *AM* coupling will produce therefore [+ +] and [- -].

And what about then *M* spin? The M spin will be split into [+ -] because of the *AM* coupling and now the *MX* coupling now *MX* coupling is larger than the *AM* coupling, therefore this will produce splitting like plus and plus here and minus and minus here. And this will be there for ¿. Now you put this together here on this cross peak, so M spin has ¿ and *A* spin has ¿.

So you take the product here, so this will produce ¿because of this plus and this plus produces me again ¿and this minus produces ¿ and this minus produces ¿. Now the once again, we have to be concerned about the resolution and where are the cancellations possible. Now *M* spin has a ¿and that is depends upon the relative magnitudes of this coupling. If these two are too close then there is a chance that the central ones will cancel and all of these peaks will disappear.

If this is larger and then the resolution that is available to you, then of course it will appear ¿. Now on the other side you have the *AM* coupling, *A* is multiplied by the fine structure and the plus minus appears here. And that is this operation here. If this difference is small then you will have this plus minus coming too close and then there will be cancellations in this. So this row of peaks will then cancel.

Okay, now typically as I mentioned you have the resolution along the F_1 axis much smaller, so therefore along this axis you will have a poor resolution and that may result in cancellation of these cross peaks here. So often you will find that you will have this line and you will have this line and some the central peaks have cancelled out. Or even if they are present they present as a residuals after the cancellation. So this is the kind of a structure you will get for the three spin system in a triangular shape.

Now similarly one can calculate for the different cross peaks. If I take this cross peaks, then I will have J_{AX} this is the *AX* will be the active coupling and all other couplings will be passive couplings. What other couplings will be there? For the *AX*, I will have a *MX* coupling because the *AX* coupling and *MX* coupling for this cross peaks *AX* coupling will be the active coupling and *MX* coupling will be the passive coupling. Now if I take this cross peak, this is the *MX* cross peak, so *MX* will be the active coupling and the *AM* will be the passive coupling.

So similarly in all the cross peaks, you will have one active coupling and other one will be the passive coupling. Passive coupling leads to a [+ +] splitting and active coupling leads to [+ -] splitting, accordingly all of them will have different fine structure.

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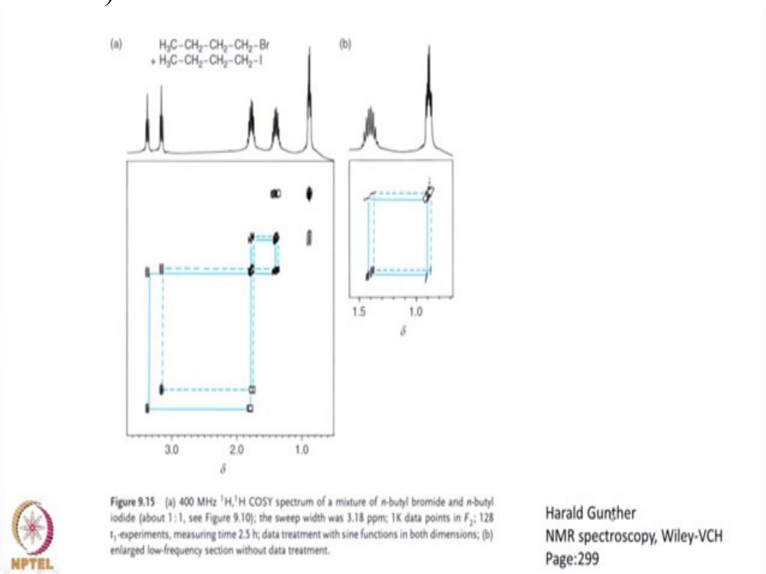

Okay, so I suppose this is now clear and I have repeated couple of times the same concepts and therefore this should be very clear now. Now let us look at some experimental situation. So here is an experimental spectrum.

Okay. So here is a spectrum of a mixture of two molecules CH_3-CH_2-CH_2-CH_2-Br, CH_3- CH_2-CH_2-CH_2-I, this is the same proton network, but I have a substituent which is different here. The reason why I am showing this is to show the power of COSY experiment, how useful it is in identifying correlations when there is a high overlap of peaks. Now, these two CH_3 both appear here in the one dimensional spectrum. And then you have 3 CH_2 groups.

So the 3 CH_2 groups appear here, but then you have one more here and this appears because of the difference here, you have bromine here and then iodine here and therefore you will have a you have a different peak at this point. So this is close to that. Now, let us see how we understand that. The methyl peaks these are the methyl peaks, the methyl peaks appear here. So on the basis of the chemical shift the methyl peak spectrum looks like this. So you have obviously the fine structures from the two molecules are not resolved here.

Okay, but then how do we know there are two methyl groups here? But look at the correlation here. If I look at the correlation I see two peaks here. Okay, so these two peaks and the same thing is displayed here. So this is the methyl, this is the blowup. This is the blowup of that particular portion. Okay. So this is a blowup of this particular region here. This is the methyl is appearing here and then you have a cross peak fine structure appearing here. This is the fine structure of this and you have these two peaks, which are slightly separated along F_2 axis.

But here in the one dimension$_a$l spectrum, you cannot see the difference but in the two dimensional spectrum you are seeing these two separate peaks. At a higher resolution here this is plotted here and we can see that there are two peaks which are appearing here and they both appear on the diagonal at this, the corresponding diagonal peaks are here, the corresponding diagonal peak is here. Now each of these peaks are now connected to the next CH_2. So that this was therefore this CH_2 that the two CH_2s here and these two CH_2s are connected to the next two CH_2s and those ones are these here. So these are connected to these two CH_2s.

See once again, you see they are not resolved in a one dimensional spectrum here, but they get resolved here in the correlation spectrum and you see slightly shifted cross peaks and in the diagonal they are here, in the cross peaks they are slightly shifted. Okay. So therefore this allows you to identify the correlations of the two different molecules distinctly and now you get the two CH_2s here corresponding to this. Now these two CH_2s are coupled to these two CH_2 which are distinctly different here.

Okay, now they are much better resolved here and therefore they are also resolved here on this axis. So one which is present here goes to this because prior you do you not know which one is this here, whether the CH_2 of the Br is this or the CH_2 of the I is here. Now it establishes the correlation which was on the top here is going to the top here which was on the bottom here is going to the next one here most downfield shifted one here. Therefore this establishes that network of couplings.

So you go from here to here, here to here, here to here and then here to here you see this. So as you are closer to the Br and the I the chemical shift difference between these is larger and therefore they are better separated here and the other one the separation is much smaller and therefore they are not so well separated here. So this is the power of the COSY and this allows you to display correlations in the two-dimensional plane in resolvable manner. Although you see here, these fine structures are not so well resolved because of the various cancellations which I mentioned to you, there will be cancellations in the fine structures and therefore you will see only the terminal peaks appearing and the central patterns will not be appearing in this sort of fine structures. And this picture is taken from this book Harald Gunther, NMR Spectroscopy particular page number is given.

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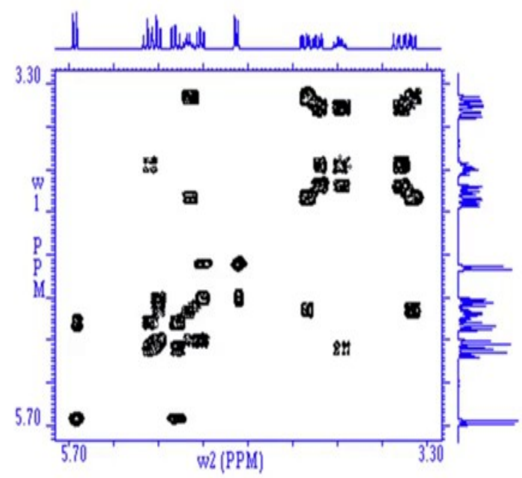

I will show another example here, how this COSY spectrum is extremely useful? Here is one dimensional spectrum or a particular molecule, it does not matter what molecule it is, this is taken from the net. So and I have here the corresponding one dimensional spectrum here as well.

And therefore I have this diagonal which is representative of this crowding of peaks and diagonal does not clearly show any resolution because the dispersive nature of the peaks and now I start seeing correlations here. What is correlated to what? The one which is the most of uphill is connected to this peak here, so therefore it is connected to this diagonal peak, this particular proton. It is also connected to this particular proton here and see that is this diagonal on this one. The one which is on the lower on this here is connected to this one as well as to this one.

Okay, now how do I know they are the same proton and they belong to the same network and not two different ones? Because when you go down here, you see coupling between those two as well. Okay, therefore these belong to the same network, So you have, this like an *AMX* system. So you have this *A* connected to *M* and *A* connected to *X*. And therefore and then you also have an *MX* coupling here. So therefore this is the *AMX* system and you have this fine structure here. Similarly another *AMX* system, this one is connected to this and also connected to this.

Now the diagonal of that one is here and the cross peak is here, once more cross peak here. So if this is the *AM* cross peak and this is the *MX* cross peak here and this is the *AX* cross peak or this is *A* to *M* and this is *M* to *X* and then I have *M* to *X* here as well. Therefore this connects to the triangular *AMX* spin system. So this is to illustrate how you can display the correlations in complicated spin networks. Similarly, you will see from here this extends further. It is not just an *AMX* until here it was so-called *AMX*, but then so this particular spin is coupled to another one here.

Okay. So you have another spin here, which is this one is coupled to this and that diagonal is at this point. But once again, this fellow is coupled to one more here. So this is coupled to this and this produces a cross peak here. Okay. So this cross peak this is coupled to this one here. Okay and you have a network of coupling spins. Therefore you can establish a complete network of couplings in complicated spin systems. You have so many spin systems here.

And that is power of the COSY and it allows you to identify each one of those. So this is the power of the COSY experiment and that is extremely useful and that is that is why we call this as a revolution in two-dimensional spectroscopy, because one then was able to looks at complex molecules which were not possible earlier. Now however there are also disadvantages and we should look at this, one can improve upon this and this has become possible and large number of two-dimensional experiments have arisen as a result of improvements in the COSY experiments to remove the shortcomings.

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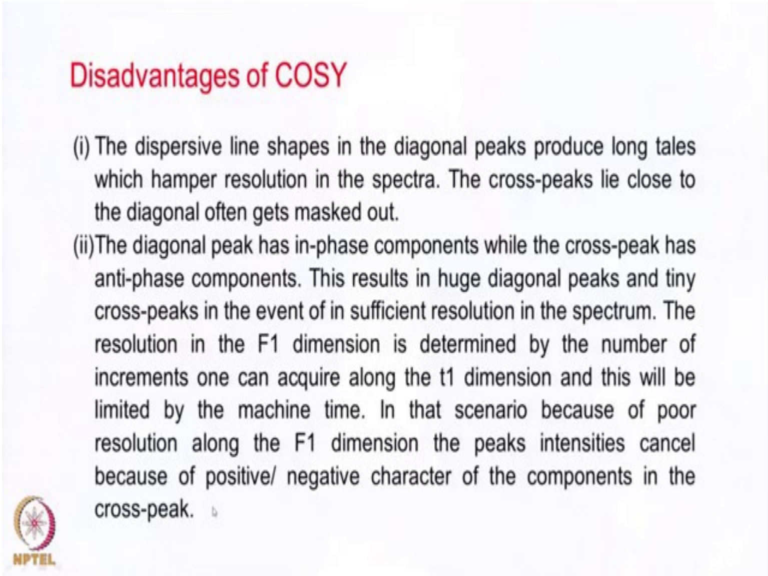

What are the shortcomings? The dispersive line shapes in the diagonal peak produces long tales which hamper resolution in the spectra. This is shortcoming because if there are cross peaks which are close to the diagonal you often miss them out because they get masked, you will not be able to see them. And then the diagonal peak has in-phase components while the cross peak has anti-phase components. This results in huge diagonal peaks and tiny cross peaks in the event of insufficient resolution in the spectrum.

The resolution in the F_1 dimension is determined by the number of increments one can acquire along the t_1 dimension which I mentioned and this will be limited by the machine time because every time you want to increase the number of increments in the t_1 it will be a separate experiment each t_1 increment is a separate experiment, therefore the experimental time will go up and that will produce lot of restrictions on your experiments. So in that scenario because of poor resolution along the F_1 dimension the peak intensities cancel because of positive negative character of the components in the cross peak.

So this is a disadvantage and how to get over this problem has been a subject of discussion, subject of development and that we will discuss as we go along. So let me summarize and the two dimensional experiment the COSY is the most fundamental, all the basic principles are contained here. We calculated the structures of the cross peaks and of the diagonal peaks using detailed product operator formalism, we demonstrated this with the two spin system and we learnt the principles from that and we used these principles to calculate the fine structure in cross peaks in more complicated spin system, especially a three spin system and we can extend this further because then every time we will have to calculate the evolutions under the different couplings.

But these principles which you have learned from the two spin system is sufficient to calculate the complex fine structures in the complex spin systems. And we also showed how the patterns of the cross peaks can be affected by the relative magnitudes of the coupling constants. And the cancellation of the peaks can occur if the resolution is not enough along the t1 axis and typically this will be the situation because you do not have enough number of increments along the t_1 Dimension and because of the limitation of the experimental time and that will be a disadvantage and one has to get over these things, these disadvantages by making improvements in your experimental sequences. I think we will stop here. We will continue with the developments later.