NMR spectroscopy for Structural Biology NS
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Lecture: 18
2-D NMR or 2-D Co-relation Spectroscopy: General Concept 2

So, we discussed about 2 important experiments in the 2D series of experiments that was the COSY and double quantum filtered COSY. Now so, we will continue the discussion of that and then of course go over to other experimental schemes. So, in this context it is important to know some more concepts about the basics of the 2D experiments and that is one of the important things is the resolution in 2D spectra.

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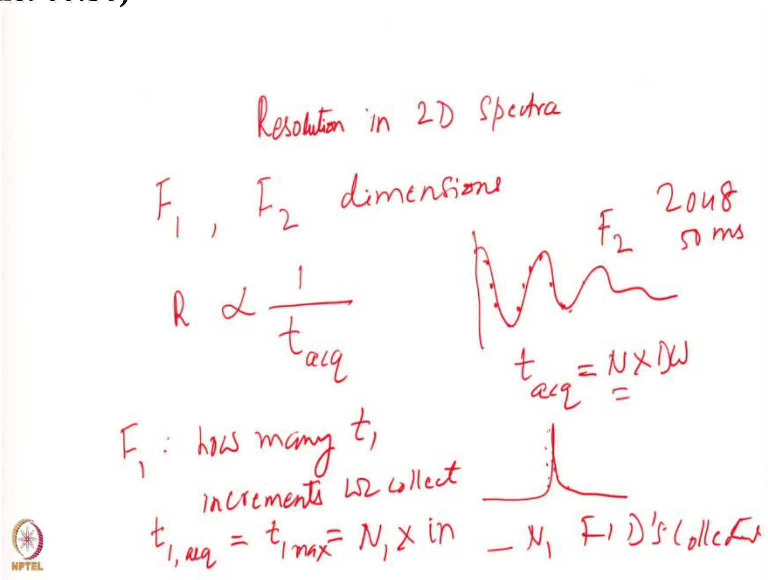

So, we should talk about the F_1 dimension or all sometimes is called the ω_1 dimension and the F_2 dimensions. So, we have to worry about the resolution in the spectra in both these dimensions what does the resolution depend upon. So, if you want to call resolution as R then this is proportional to the inverse of the acquisition time $R \propto \frac{1}{t_{aq}}$. So, this is the acquisition time if t acquisition is acquisition time then this is the resolution is inversely proportional to the acquisition time.

What does that mean? This is similar to what we are talked in the case of the 1D spectra suppose I have the FID here and I have various points which are collected here. So, many data points which I collect and then we say the $t_{aq} = N \times D_w$, D_w which is the dwell time this is the time between 2 consecutive points this is t_{aq}.

So, if you collect more data points you have better resolution in your spectra what does that mean then you have a line like this then you will have these peaks points coming appropriately at various places in your in your line. So, therefore the line will be represented better and the resolution will be higher if you collect more data points. In the case of the normal acquisition the one dimensional spectrum or even in the 2D the normal acquisition in the when you collect the FID it is simply the number of data points you collect.

So, therefore it is very easy to increase this resolution by increasing this number of data points. In the case of F_1 dimension the F_1 dimension resolution will depend upon this is this is for the F_2 dimension. Now for the F_1 dimension it will depend upon how many t_1 increments we collect acquisition time along the t1 dimension will be let us say if I want to write as

$$t_{aq} = t_{1^{max}} = N_1 \times increment$$

Now I call this increment as let us say "in" (increment) this is the same as the D_w is the same as the D_w except that increment this is these are collected one by one this is systematically that many of ideas are collected that means we have N_1 FID is collected. So, if you want to increase the acquisition time along the F_1 dimension we will have to increase the number of increments you have to increase the number of experiments number of FIDs you collect.

So, therefore it is a very time consuming process. So, for example if I collect here 2048 data points in the F_2 dimension. This may go for let us say about 50 milliseconds assuming hypothetically. So, that is given the certain increment dwell time it may go for 50 milliseconds. But if you want to increase from for this fourth 2048 to 4096 then it will go to 100 milliseconds but that is no big deal.

So, whether I collect the data for 50 milliseconds or 100 milliseconds or 200 milliseconds it is not going to increase my experimental time data collection time too much. But if I want to increase the same in the F 1 dimension if I want to collect from 50 milliseconds acquisition time to 100 milliseconds acquisition time I have to do twice as many experiments twice as many FIDs.

So, if the 50 millisecond spectrum is going to take me about 10 hours then if I want to make it 100 milliseconds it will cost me how much did I say 10 hours 20 hours if it is going to collect 10 hours it is going to be 20 hours. So, therefore the number of increments is crucial here with regard to the acquisition in the F_1 dimension.

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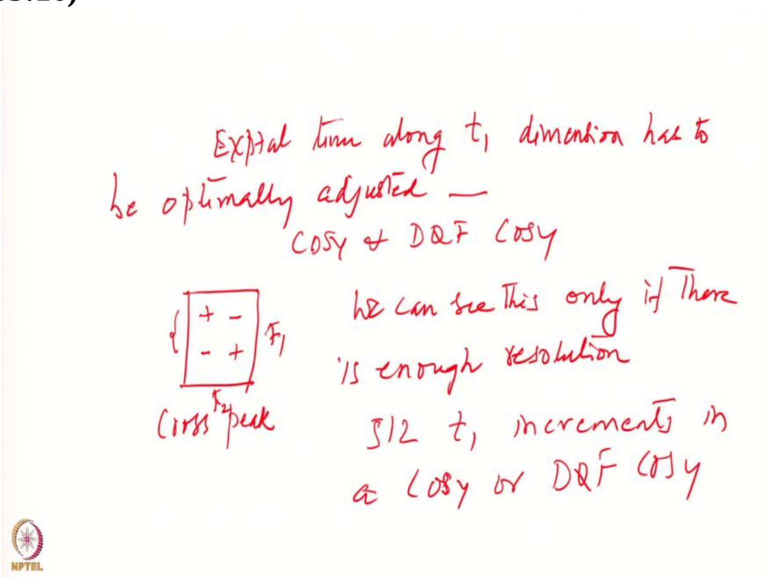

So, therefore experimental time along t_1 dimension has to be optimally adjust it for the resolution we want now why is the import why is the resolution important in the case of F_1 dimension. We have seen that in the COSY and the double quantum filtered COSY in the course in double quantum filtered COSY we let us say I have a cross peak like this, this is the cross peak. So, this is in for COSY and DQ of cosy we have seen that the cross peak for a 2 spin system has this kind of a structure right.

For a 2-spin system and the separation between them is the coupling constant now we will be able to see all these four components if only you have enough resolution if these lines are properly separated. So, we can see this only if there is enough resolution. So, therefore along the F_1 dimension it becomes crucial whereas along this, this is the F_2 this is the F_1. F_2 dimension is not a problem I can easily increase from 1024 to 2048 or 4096 data points no problem at all I can separate these 2.

But the separation along this axis this is the crucial point. So, therefore always the limiting factor is the resolution along the F_1 axis and this has to be optimally chosen. So, typically one does 512 t_1 increments in a COSY or DQ of cosy experiment. If the coupling constant is very small then you will have to collect more. So, what is the consequence if there is not enough resolution what will happen.

If there is not enough resolution then this positive and the negative ones the positive and the negative components these come too close and then to cancel their intensities they will cancel their intensity. Therefore overall your signal to noise ratio in the cross peak will get reduced even if you have good resolution here if these peaks are well separated along the F_2 axis if this resolution is not enough if they are not well separated then they will cancel and your cross peak will have very low intensity and sometimes you may lose it also.

If it is very small like 1 hertz or 0.5 hertz you may completely cancel it out and you may not see the cross peak at all therefore this actually is quite an important limitation for for the for those kind of experiments where you have COSY and where you have antiphase characters, antiphase characters meaning plus minus components along this cross peaks. So, this is. So, far as the 2 spin systems are concerned. Now we should go and see how the spectra look like if we have 3 spins or more.

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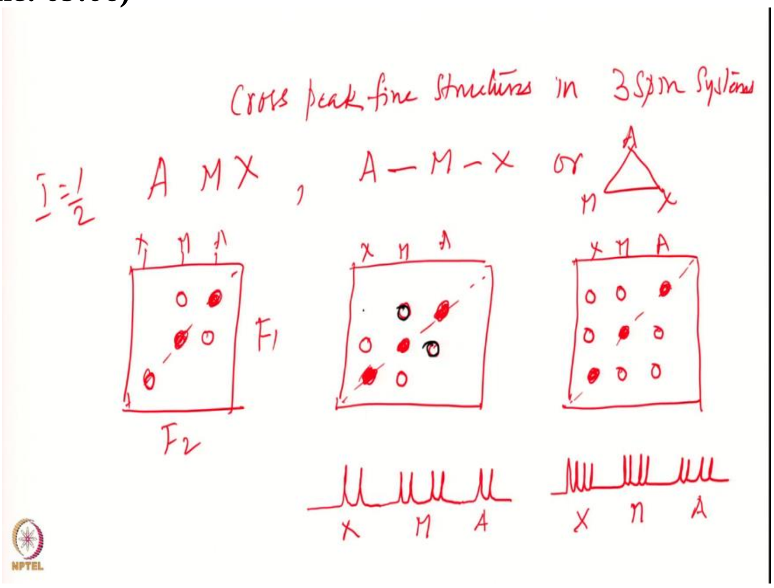

So, then we let us look at the fine structures cross peak fine structures in 3 spin systems. Let us say the 3 spins are AMX these all have spin half all of them are I = ½. So, we label them as AMX and of course these can be connected in 2 different ways AMX or this way also AMX. So, this is the way you can have 2 different patterns here. So, what will happen what will be the fine structure in the cross space. Let us look at once again the schematic here of the 2D spectrum.

So, here we have F_1 F_2 and I will have here 3 diagonal peaks let us say and let me call this as A call this as M and call this as X now what cross peak do we see. So, we will see a cross peak here and a cross peak here A to M in the case of if it were a linear system we will have 2 different things. So, let me let me draw that here and of course you will have for the linear system how to look let me draw that here for the linear system this we call it as a linear system.

So, AMX this is A this is M this is X. So, A to M there is a cross peak here and therefore there will also be cross peak here and M to X also there will be coupling there is also cross peak here there is also cross peak here because remember these ones are J coupled this happens through J coupling this should be in the same line. So, this happens through J coupling. Now in the case of this one this is the diagonal we write it as A here M here X here I will have the same as before here and here.

But now I will also have this because I also have A to X coupling right A to X coupling I will also have M to X this also will be there this will be there and this will also be there. So, the in the case of this triangular coupling pattern I will have a different kinds of structure and pattern overall pattern of the peaks of course in each of these peaks there will be a fine structure that is what we are going to see.

So, in this case depending upon which peak we are talking about which coupling constant is responsible for the peak that is important to see. So, now if you are looking at this each one of them of course there is what will be the 1D spectrum for such a kind of a thing if A M X let me draw the 1D spectrum of those that a has a coupling to M therefore it will be a doublet A will be doublet and what will be M?

M has 2 couplings therefore it has it will be doublet of a doublet let me go and this is M and what will be X and X has only one coupling therefore that will also be a doublet. So, this is X this is M this is A in the same order which I am writing here. So, how this will be here now each of them here has 2 couplings each spin has 2 couplings right. So, therefore each one of them will be a doublet of a doublet.

So, this is X this is M this is A and this find structure will show up in the cross peaks and the diagonal peaks as well. Now which peak which coupling is responsible for which cross peak suppose I take here I take the M am cross peak in the am cross peak this is arising from the coupling from A to M. If I see here M this cross peak I use let me use a different colour. Let us say take this cross peak or this cross peak it is coming from the coupling from A to M it is not coming from the coupling from M to X because the M, M to X peak will be here.

So, therefore there are 2 couplings in the M and these ones we distinguish them as active coupling and the passive coupling.

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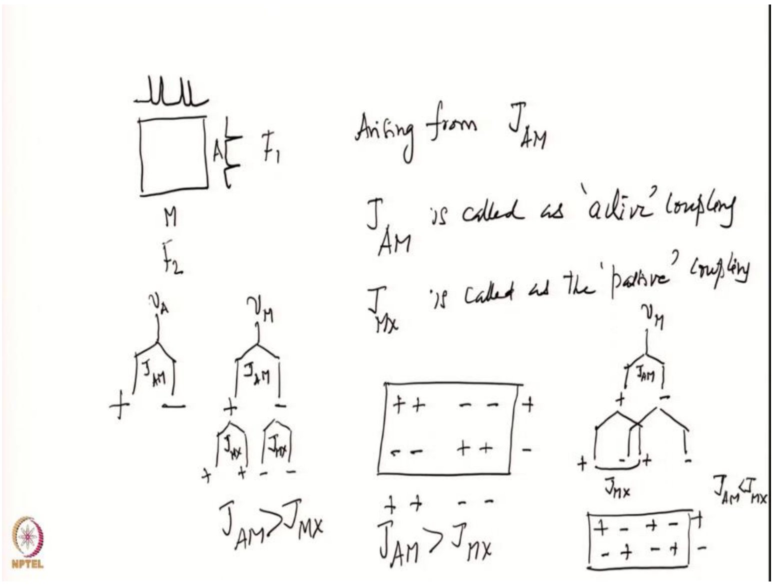

So, as we are looking at the particular peak and blow up this here and this side is the A and this side is the M I have this 2 things here. So, what will be the structure of these how many peaks will be there. So, we will see that now the A is a doublet and M is a doublet of a doublet and therefore if I take this product here there will be that many peaks inside here. So, therefore each one of these there will be peaks to all the four each one of these from this also there will be four peaks here from with here also there will be four peaks there.

So, there will be total of eight components in this peak this is my F_2 axis this is my F_1 axis there will be eight components. Now what is the sign pattern here in the case of a 2-spin system we said + -, + - how it will be here. So, this peak is arising from J_{AM} therefore J_{AM} is called as the as active coupling and in the M there is also this MX splitting J_{MX} which will be present only along the F_2 axis this is called as the as the passive coupling.

This is not responsible for the cross peak but it will certainly contribute to the splitting. So, now if I want to write the splitting pattern of both the spins A and M suppose I have this a chemical shift here and this splits because of the am coupling as in the case of double counter filtered causing or the COSY and this plates this produces inside there because of the active coupling + and -.

So, therefore inside here there will be pluses and minuses this splitting will lead to plus and minus and what about the M this is the A this is the nu a let me write here ν_M, ν_M will also show the am coupling which is the active coupling. Active coupling leads to plus minus splitting as in the as we are seeing in the double quantum filtered COSY or the COSY. Now what does the MX coupling do because this will be further split because of the MX coupling.

Therefore this will be further split into 2 but now this will not produce me plus and minuses it will produce + +, - - and here this is J_{AM} this is also J_{AM} and this is J_{MX} this is J_{MX}. So, now using this; what I will do I will draw what will be the structure of this am cross B. Let me draw that once more here this is this side is A, A what is the structure I will have + - splitting.

Along this side I will have + +, - - because the M has this sort of a structure. So, now if I multiply these 2 what I should get here I will get here + +, - -. Now I multiply with the minus sign here there I will get here - - , + +, minus into minus gives me plus therefore inside here I will have this sort of a structure. This is provided J_{AM} is greater than J_{MX}.

What happens if the MX coupling is larger than AM coupling let us also draw that. So, if this is for that situation right. So, this is for J_{AM} greater than J_{MX}. If the other thing happens let us say ν_M, J_{AM} is smaller than J_{MX} this is J_{AM} this is plus minus now I have splitting MX is larger. So, now from here to here it is J_{MX}. So, what will be the structure here this will be plus plus here and this will be minus minus here.

So, what is the thing we will get. So, therefore if I want to draw that here below this side will be plus minus as before but now this will be plus minus plus minus and this will be minus plus minus plus. So, this is the case when J_{AM} is smaller than J_{MX}. So, this is J_{AM} is smaller than J_{MX}. Therefore it is very crucial to see what sort of a pattern you will get in your finds in your in the cross peak and this will tell you what kind of precautions one should take in doing your experiments.

So, to analyze analysis of the spectra has to take care of all of these factors when you want to measure the coupling constants from the cosy spectra and this is true for both cosy as well as double quantum filtered COSY. Now what will be the nature of the AX coupling in there is no AX peak here. We have talked about the am and similar thing will be for the MX also. MX also will be for in the same manner you will have in the linear system we are talking about the linear system here now this is for the linear system.

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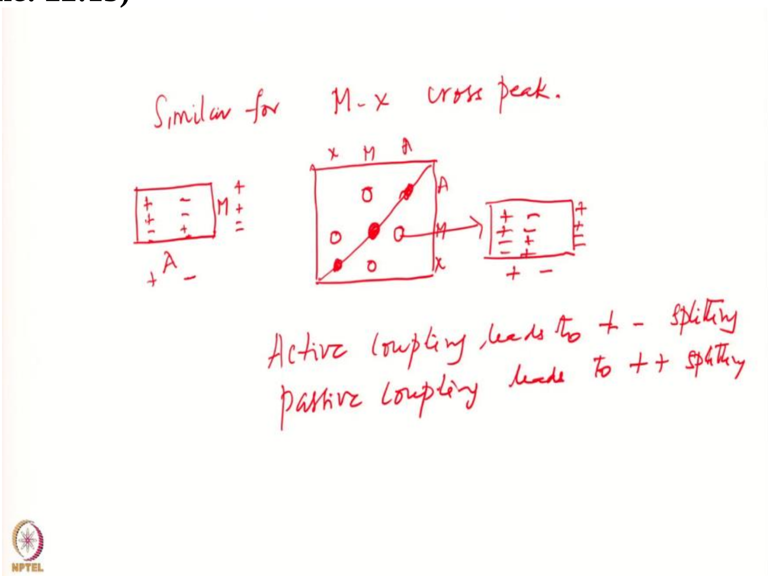

So, the pattern will be similar for MX cross peak ok and what about this peak let us look at the peak where this side is M this side is A and what will be that here we have a doublet. So, this will be a doublet and here we will have plus plus minus minus. So, therefore and this will produce me plus minus plus minus minus plus minus plus. So, therefore you see the symmetry is broken the peaks do not look identical.

In other words what I am trying to say here is. So, if I look in the 2D spectrum this is my M and this is my A this is my X. So, I had here A M X. So, I have the cross peak here the cross peak here and the cross peak here and the cross peak here. So, this is what we are looking at right. So, I showed you what is the structure for the am cross peak and that was A was on this side M was in this side.

In this case this case is the case what is shown here and this is M. So, this side is plus plus minus minus this is plus minus. So, I will have plus plus plus plus minus minus minus minus plus minus plus minus minus plus minus plus. So, this will be the structure for the other peak yeah. So, therefore depending upon which peak you are looking at you will have the final structure.

So, point to remember therefore is the active coupling leads to plus minus splitting a patio coupling leads to plus plus splitting. So, this is. So, far as the linear spin system is concerned.
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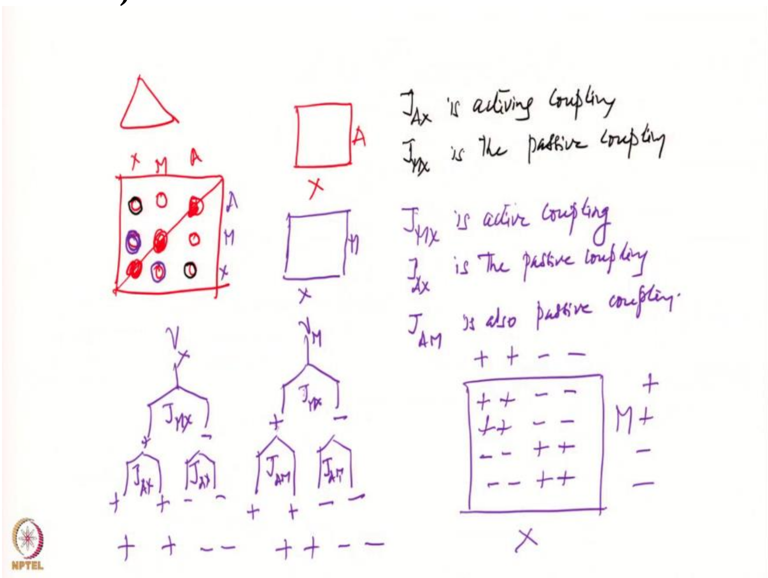

Now if I want to take a triangular spin system this sort of a thing. Now I will have here this is the diagonal this is AMX I will have a cross peak here a cross peak there cross peak here from peak there cross peak here cross peak there. So, all these are cross peaks now in each one of them we will have one active coupling and one passive coupling in the same line. So, each one of them will have let us say I have this is AX that is this peak. It is this peak AX.

So, what will be the active coupling here, here J_{AX} is active coupling because this cross peak is arising as a result of coupling between A and X this is the same here and the X is also coupled to M. So, the X multiplied will have A coupling and also the M coupling and J_{MX} is the passive coupling accordingly one can draw the various structures. Now suppose I take a different cross peak let me say I take this cross peak.

What is this cross peak this cross peak is M on this side because this is all A M X this cross peak if I am taking remember I am not taking this when I draw here if I draw here M if I draw here X then it is this cross peak active and passive couplings will be the same in both cases but the fine structures will be different depending upon which cross peak you are taking. If I take M here and X here then which is this active coupling J_{MX} is active coupling.

And what is the other coupling it has X along this side I have the MX coupling and AX coupling now J AX notice this side this side I have X multiplied this side I have multiplied. So, therefore all the 3 couplings are appearing here right. So, AX is the is the passive coupling and J AM is also a passive coupling let me demonstrate that here. Let us draw the M multiplet for this particular case.

The M multiplet is plus minus from J_{MX} and let us say MX is greater than am this is plus plus minus minus and this is J_{AM} coupling now this is for M I draw let me draw the same thing for a for the X and X again I will draw here J_{MX} is plus minus because this is the active coupling number those now this will have a different coupling here and what is this one here this is J_{AX} here it was AM here it is AX.

Because we are talking about the multiple, multiplet structure of the X spin therefore the patterns will be can be different depending upon what is the relative magnitudes of the 2 couplings therefore the fine structure of this is plus plus minus minus and this is also plus plus minus minus. So, if I draw that here now this is this side is X and this side is M and here we wrote as plus plus minus minus and this side also was plus plus minus minus.

So, what will be the structure multiply these 2 once plus plus minus minus once again plus plus minus minus minus minus plus plus minus minus plus plus. So, 16 components so each of the cross peak here will have 16 components. Unlike in the previous case depending upon how many couplings are present in each multiplet structure you will have you have to multiply that by that many components.

So, if one side has 4 components other side has 2 then there will be 8 components in the cross peak if both sides have 4 4 then you will have 16 components in the structure and the combination of the plus or the locations of the pluses and the minuses will depend upon relative magnitudes of the coupling constants.

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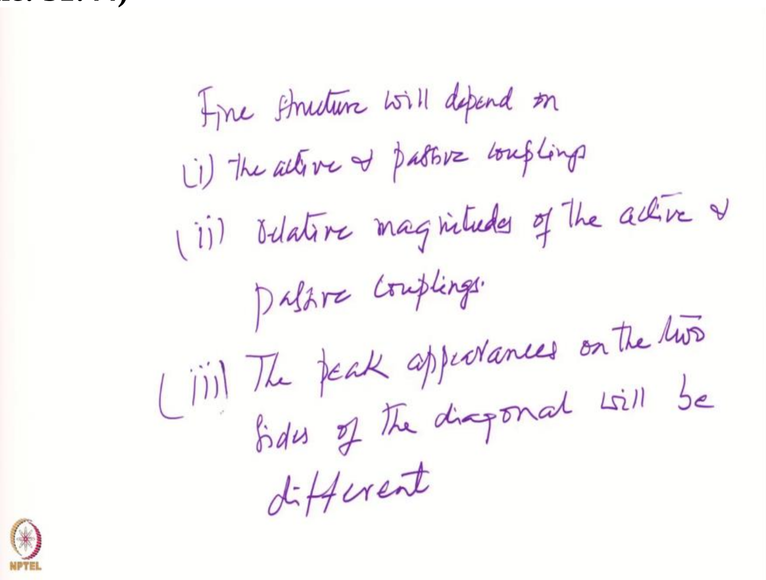

The fine structure will depend on 1. the active and passive couplings and 2. relative magnitudes of the active and passive couplings, 3. the peak appearances on the 2 sides of the diagonal will be different. So, these are the important features of COSY and double quantum fielded cosy spectra . I mean there can be more combinations of course if you have four spins there will be further splittings.

If you are more spins there will be further splitting but the active coupling will always be one remember this no matter how many spins are there how many couplings are there the active coupling will always be one all others will be passive couplings. So, therefore that is what is important in calculating the fine structure in each of the cross peaks. These will be directly used I am going through this so, much detail.

Because this will be directly used when you actually look at the splitting patterns in the spectra of nucleic acids and proteins and this is our ultimate aim is that we need to see how we can analyze the 2D spectra and the 3D spectra of proteins and nucleic acids which are very learned from the point of view of structural biology calculating the structures of the molecules this becomes very important. So, we will stop here.