NMR spectroscopy for Structural Biology NS
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Lecture: 17
2-D NMR or 2-D Co-relation Spectroscopy: General Concept 1

So, we are now ready to go into the next class of 2 dimensional experiments or in general multi-dimensional experiments and these are called as correlation experiments or correlation spectroscopy 2D correlation spectroscopy. In fact this has been the most fundamental most crucial experiments from the point of view of structural biology.
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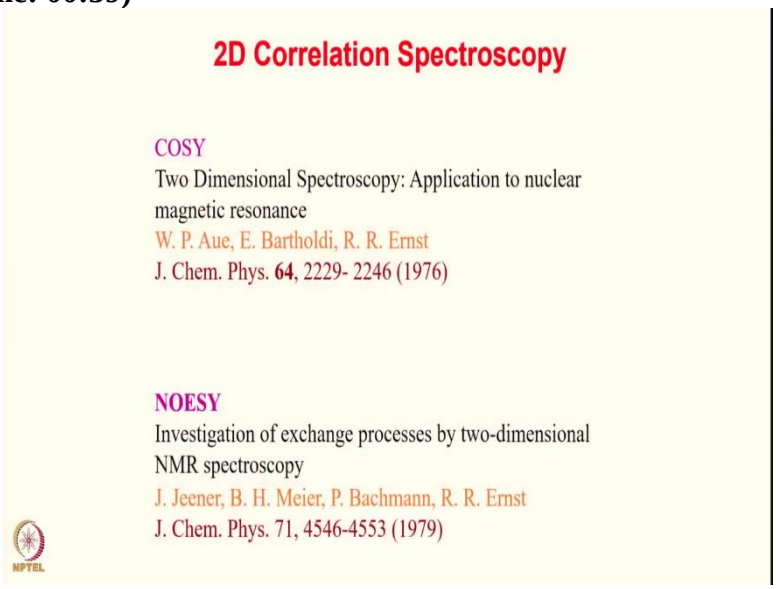

And this is the one which actually led to the Nobel Prize to the 2 persons one is Richard Ernst and then other one is K Wüthrich and we will go along those ones and the basic developments were made by Richard Ernst. We see here are Ernst those first was published in 1976 in journal of Chemical Physics and then again in journal of Chemical Physics 1979 two fundamental experiments which have been crucial in all applications in structural biology.

So, we prepared the ground to understand these experiments in the previous class because all of these involve very crucial magnetization transfers and that is the one which is the information carrier in these experiments.
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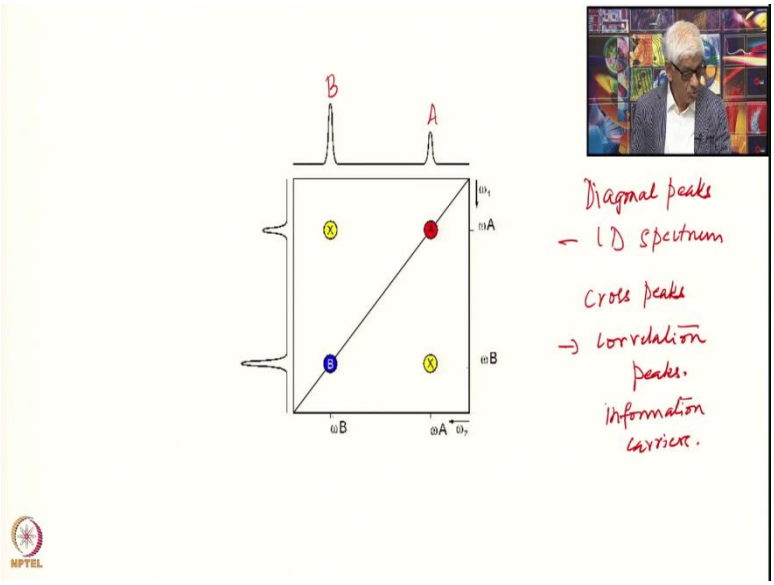

So, what does this experiment look like what is the correlation experiment. Correlation experiment is like this schematically this is an experiment which is indicated like this. So, we have the 2 dimensions here which are represented as omega 1 and omega 2. Of course we could have used F_1 and F_2 this is just taken a two spin system here there are only a single schematically indicated two lines there is one line here and another one.

So, this is the A spin and this is the B spin; A spin and the B spin here. Now it is the 2 dimensional experiment the spectrum looks like this schematically. Schematically it is looking like this. So, you have the. So, called A peak here this is the A peak which has the same frequency along the ω_1 axis and ω_2 axis you look here this is ω_A here. And similarly this peak is ω_B here and ω_B here. So, therefore these are called as the diagonal peaks.

So, we have diagonal peaks. So, therefore the diagonal if you want to look at essentially it represents the one dimensional spectrum this represents the 1D spectrum then you have the. so, called x these ones here. Now along this axis we have the ω_A here and this axis it is omega B so, far as this peak is concerned. Similarly for this peak we have the ω_A along this axis on the ω_2 axis and ω_B along this axis.

So, these are called as cross peaks. Cross peaks are the correlation peaks. These are the information carriers. So, the cross peaks are the information carriers what do they tell you this will tell you that the spins A and B are somehow correlated what kind of a correlation it can be very different this can be different kinds of correlations it can be J coupling correlation or dipolar coupling correlation whatever it is there can be different kinds of correlations possible accordingly you have different kind of spectra.

So, therefore correlation peaks A and B and how do they arise this depends on the pulse sequence this depends on the x way you design the pulse sequence in you what are the pulses you use what are your mixing sequences etcetera and that is the important part of this is the schematic of the 2 dimensional correlated spectroscopy. So, you have 2 frequency axis and the diagonal of this correlated spectrum is a basically a one dimensional spectrum.

And you have off diagonal peaks or also called as the cross peaks. The cross peaks actually are the correlation peaks and these are your information carriers. Therefore whatever information is present in the one dimensional spectrum I mean if you take the one dimensional spectrum

certainly there will be these correlations are present already suppose you are talking about the J correlation J coupling J coupling is present in your one dimensional spectrum.

But you do not know how to extract it you do not know which spin is coupled to what you do not know that in a complex NMR spectrum of a protein for example you do not know which peak is connected to what which is which spin is connected to what spin where do these appear in your in our spectrum you do not know that. But when you do a correlation experiment of this type then you will see that you get correlations which are off diagonal and you can connect them to the diagonal positions then this will tell you that spin A and spin B are correlated.

They are coupled there is a J correlation or some other correlation therefore these are called as the information carriers and the entire plane is available to you to display this correlation that is why this became an extremely powerful tool for improving the resolution inward spectra and the information content in your spectra.

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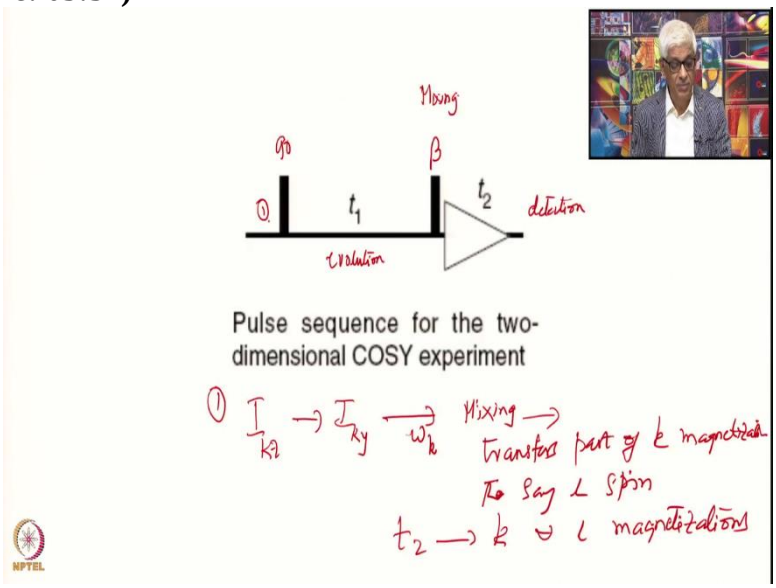

Now this is the pulse sequence for the COSY 2 dimensional COSY experiment. So, we start with a 90° pulse here this can be a 90° pulse and this can be a pulse of flip angle β or it can also be a 90° pulse. So, β can vary from one value to another value but it can be a 90° pulse also here you have the t_1 period which is called as the evolution this is the evolution and this pulse which is the β pulse second pulse which is there this also acts as the mixing this pulse acts as the mixing and the t_2 is your detection period FID is collected during the t_2 period therefore you generate a 2 dimensional Fourier transformation 2 dimensional spectrum after you do this the evolution time as I described earlier is incremented systematically from one time one FID to another FID.

So, therefore you generate a 2 dimensional information. Whatever is the frequency present in the t_1 period will appear along the F_1 dimension or the ω_1 dimension. Now what does this mixing do the mixing do it does it transfers magnetization for example if I have during this transfer. So, here I let us say I have starting with the k spin z magnetization to begin with to begin with of I have here at this point one.

Suppose I have z magnetization z magnetization of the k spin when I apply the 90°pulse then I will generate the y magnetization let us see because the 90° pulse rotates the z magnetization into the transverse plane. So, now the evolution period during this period the k magnetization

transverse magnetization evolves during this evolution period. So, when this evolves it revolves with the frequency ω_k.

Therefore you generate k magnetization and then it is frequency labelled as your function of t_1 it will be frequency labelled right because you are systematically incrementing the t_1 value therefore the evolution will follow this frequency. So, therefore you acquire various kinds of phases. Now what happens during the β period during the mixing transfers part of k magnetization to say l spin.

So, therefore I have initially I have a k then I transfer some of it to the l spin therefore what is present in the t_2 period. So, therefore in the t_2 period I have both k and l magnetizations right and this is the basis of this scheme here. So, during the t_1 period so, what is present along the F_1 axis the ω_1 axis if I consider the ω a frequency omega a frequency which is present during the t_2 period because of the mixing I have both A magnetization and B magnetization or the k magnetization and the l magnetization.

Therefore I will have both the frequencies present and because of that when I do 2 Fourier transformation I will have the diagonal peak and the cross peak what remained on the A spin remains as diagonal what got transferred to the B spin appears as a B magnetization and that appears as the cross peak. So, that is the philosophy of this experiment; and let us see how this is useful.

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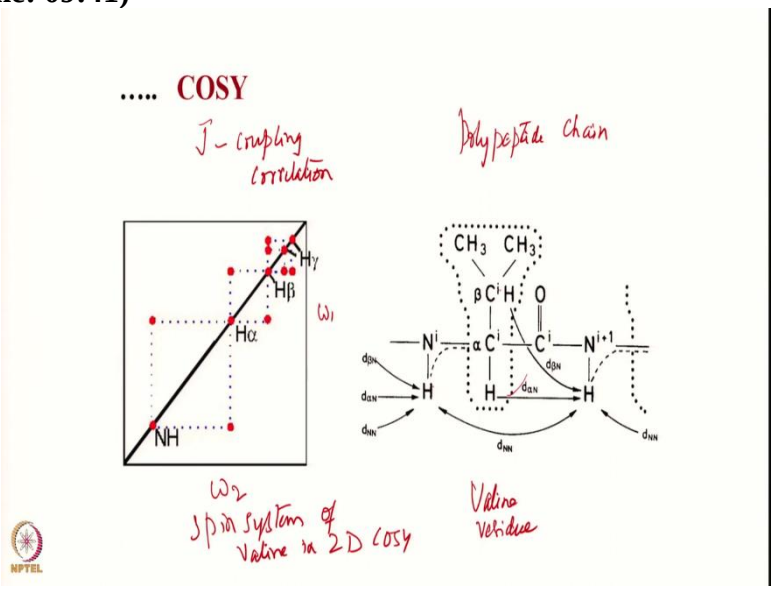

Let us take a particular molecule here and what is this molecule this is a peptide this is a dipeptide. So, this is a dipeptide here the NH C_αCO this is one amino acid it is written as i, i, i. So, I represent the magnetized of the residue in a polypeptide chain. So, this is the polypeptide chain and from N C alpha CO this is the residue I and the next residue is i + 1 and the previous ratio is of course i - 1.

So, now you see let us consider this the cosy experiment is designed for J correlations this will allow transfer due to J coupling J coupling correlation. Now what are the J couplings present here let us examine this molecule. If we examine this molecule let us say I have the NH when we are talking about the protons here. We are talking about the protons which are the protons present NH proton alpha proton the beta proton and the methyls.

And the methyls we call them as γ protons α, β, γ and so on so forth. Now there is a 3 bond coupling from NH proton to the α proton and therefore there is a transfer between NH and alpha NH and α transfer produces me this peak here it produces this one peak. Similarly α to the NH produces this corresponding peak on the other side. Now from the α proton I also have a coupling to the β proton this is the 3 bond coupling.

So, α to the β this is again a 3 bond coupling. So, therefore I have α to the β cross peak. So, this is the α to the β cross peak and similarly this is β to the α cross peak or either way one can say β to the α cross peak and this is α to the β cross B because the flow is going like this if I call this as ω_1 axis and if I call this as ω_2 axis.

So, this peak is coming from β to the α transfer and this peak is coming from α to the β transfer this peak is coming from alpha to the NH transfer and this peak is lower one is coming from NH to the α transfer all right. So, now what happens next from the beta do I have any other transfer. Now look here the β proton is coupled to the methyl groups CH_3 protons. So, therefore there is a coupling there also.

Therefore from β I will have a coupling to the γ and there are 2γ and if these are non equivalent if these 2 CH_3 groups are non equivalent in terms of chemical shifts I will have 2 peaks there from the same β I will have 2 peaks. So, therefore this is of one γ to the β same β this is the second γ to the same β transfer and this is from β to the two γ these peaks are appearing from β to the 2γ.

And what is this residue see those of you who remember this amino acid structures this residue is valine NH C_α C_β H CH_3 $2CH_3$ groups and this is valine residue. So, therefore whatever is enclosed in the dotted box on the right hand side all these are exhibiting J coupling there is a J coupling between them. Therefore the cosy spectrum here is displaying a pattern displaying a pattern of cross peaks which are very characteristic of the coupling network in this amino acid residue.

So, we have an h to the αα to the ββ to the 2γs notice there is no γγ coupling γγ coupling is four bond coupling this is four bond and you do not have that coupling that coupling is very small therefore you do not see that. So, this is typically the kind of a structure what you will have at a low resolution spectrum you will have a pattern like this.

This is the very important information with respect to identifying the spin system. So, this is called as the spin system spin system of valine. So, this is we say the spin system of valine in 2D COSY all right. Now of course there is no coupling from any of these protons in a particular residue I to the next residue is there a coupling to the NH of i + 1 NH of i + 1 is for see there is no coupling from here to here of course don't look at the thick arrows there.

Now that is that is related to the another experiment but there is no coupling J coupling from this proton to this proton likewise there is no coupling from this proton to this proton or any of those ones therefore these cross peaks do not appear in this do not appear in the cosy spectrum of this if you are looking at the NH of this there is only correlations appearing for within the same amino acid residue there is no connection from one amino acid residue to the next amino acid residue neither here neither on the i - 1 side.

So, therefore these are within the amino acid residue you have the J coupling correlations therefore this causes spectrum is extremely useful to identify the spin systems of individual amino acid residues.

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Let us look into the this in a more detail a finer structure I showed you that there is a each one of them is a cross peak here there is a cross peak let us consider this cross peak here I said this is one full dot which is actually indicated is it just that is it just a dot like this or it has any structure if you look at the 1D spectrum of this NH, NH is coupled to this proton right. So, therefore NH will be doublet and this doublet going to be reflected in this or not or in this or not whether the doublet pattern of this NH is it going to show up here or not.

And it must show up because where it will go you are not doing anything with respect to that it must show up and similarly in this peak as well. So, if I consider this as an AX spin system. Now an h to the alpha let us forget these ones which are considered only those 2 spins are considering a 2 spin and look at this cross peak. So, I have here a 2 spin system for some molecule the this is 2 3 dibromothiophene.

This is a simple 2-spin system it has no other coupling available no other process only one coupling available. So, therefore the 2D COSY spectrum of this will have. Now you see this is the 1D spectrum is a doublet and a doublet here this is the doublet this is the doublet the same thing here as well. Now I have the diagonal here diagonal these 2 peaks are the diagonal peaks and these 2 are the cross peaks. So, there is a transfer from here to here and transfer from here to here what else.

This file structure how does it appear in the spectrum what is done here is you take cross section through this spread through the spectrum at this point at the next point then at this point here and at this point here you take cross sections and plot those cross sections here and these are the various cross sections. From the top to the bottom these are the four different cross sections taken through the spectrum.

And what do you see you see here positive negative. If this is positive the next one is negative or if this one is negative the next one is positive kind of an interface term which I talked to you earlier and this one and these are both absorptive signals this negative positive absorptive signals. You continue in this one you come here see here these are dispersive signals. These

dispersive signals it goes like this and then the row again goes like this therefore these are in phase.

So, if I want to write here these are inphase dispersive and what are these, these are antiphase absorptive. So, therefore this is the cross peak has this feature and the diagonal peak has this kind of a feature. And similarly the next one if I see the next line if you take the next line here and what do you see positive negative. Earlier it was the top one was negative positive and this one is positive negative and this one is all positive again.

All positive and this has dispersive line shapes again. So, the dive for the diagonal has dispersive line shapes and the cross peak has absorption line shape but the sign patterns are different. You come down to the third one here. Now this is the diagonal once more the diagonal is again have cross sections like this disperse you in phase absorptive antiphase. And you take the next one the last one here.

Last one is again dispersive inphase and this is absorptive interface but this send pattern is different from this what is negative here is positive here what is positive here is negative here. So, therefore typically what we will say here is if I were to blow up if I blow up this peak if I blow up this peak I show here this is what we get this is what we have. So, if I drawn to draw the sign here what is the sign is this is negative this is positive.

This is positive this is negative absorptive line shape these are positive negative components and these ones they are all positive but dispersive this is positive, positive, positive and positive all are inphase that is why I say positive or positive, positive, positive but they have dispersive line shapes. These dispersive line shapes actually creates a very ugly shape here what is plotted here this is the contour plot these are called as contour plot.

These are the cross section the top what is plotted is the cross section but when you display a 2 dimensional spectrum you represent it as a contour plot. So, what you do you have a spectrum like you should take cross sections at various heights and plot the contours how the things are going. So, that is the contour plot. So, therefore in the contour plot is usually easy to analyze a contour plot here like this.

This is the contour spectrum and you see various contours drawn at different heights of the peaks and the center of the peak is of course the center of the line. The chemical shift center of the chemical shift which indicates the chemical shift. So, therefore and you can measure the separation between these and the separation between this is what is the coupling constant. So, therefore the coupling constant information is present in the fine structure of your cross peak.

It is also present in the fine structure of the diagonal peak but you cannot extract from the diagonal peak because the line shapes are very ugly. And this very difficult to find the center here therefore the information carriers are the cross peaks which have absorptive line shapes and beautifully separated components here and you can see you can directly measure the coupling constant along both axis here as well as here it is the same coupling constant J coupling constant is present here.

Therefore in the correlated spectrum which is recorded with high resolution a COSY spectrum recorded with high resolution you will be able to see such fine structure if you do not do with high resolution then there can be possibilities of cancellations. Notice also that because of this intensity patterns because you have the positive negative peaks here. Suppose you do not have

enough resolution along this axis here have clear resolution along this side these peaks are well separated therefore I do not have any cancellations here.

But suppose this resolution is not good sufficient then there is a possibility of cancellation of these intensities this sum of this portion may cancel with some of these intensities. So, that actually can be a problem that you do not get suppose the coupling constant is very small because what does it depend upon? This separation depends on the coupling constant. So, if the resolution is not sufficient to resolve this coupling constant is not enough then there can be cancellation of the intensities.

Then there can be some difficulties in identifying your cross the pigment disappear in that case that is why you do not see the four bond couplings why you do not see four bond couplings because coupling constant is very, very small. If it is 0.1 hertz or 0.2 hertz or less than that then the positive negative are so close that you will not be able to separate them and they will cancel out intensities.

And that is that leads us to a new experiment which is called as a double quantum filtered COSY. Double quantum filtered COSY this is the particular improvement over here.

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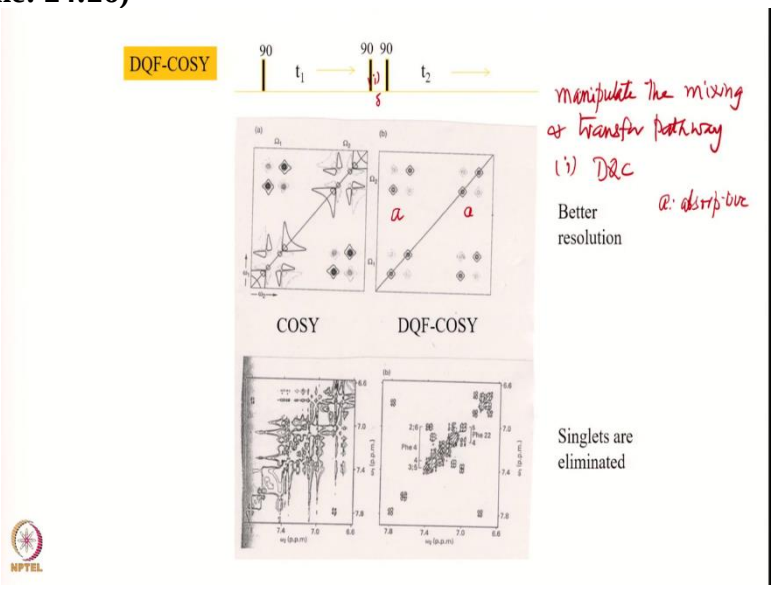

So, I have without going into the theory of this one we will see what is the final result? What is the result that comes out here? See here is this is the pulse sequence of the double quantum filtered COSY. So, you have the 90 t_1 90 of course here you can use the 90° pulse here this is up till here it is the same portion as the COSY. But we have introduced a small time delay here someone due to some small time delay that is a very small small very small time delay.

So, we call it as let us say delta extremely small time this kind of order of a few 100 microseconds can be that much time delay small time delay. Now what you do is you manipulate the transfer pathway you transfer you transfer the magnetization transfer pathway what you do here is manipulate the mixing and transfer pathway. So, therefore what you what you get you adjust it in such a way that at the end of the second 90° pulse.

I will have double quantum coherences here at this point. So, let us say if you call this point as 1 I will have at point 1 I will have double quantum coherences this is the manipulation will not go into how it is done but this is the manipulation that is done. So, that is why it is called as

double quantum filter. So, you the process of mixing at the creation of the first 90° pulse creates all of these pathways it creates single quantum coherences double quantum coherences it will create all of those.

This 90° pulse will create all of those but then you do a kind of a filtering technique by doing this filtering techniques we keep here the double quantum coherences here and then the double quantum coordinates as I mentioned are not directly observable. So, therefore to observe that we need another pulse and this actually converts these double quantum coherences into single quantum coherences and therefore after that you can measure it before that you cannot measure it.

You measure it after this is second 90° third 90° pulse and then you will be able to measure the signal before that you can because you have done a trick here during this 90 t_1 90 and you do a trick to filter out a particular kind of a coherence the transfer pathway when I apply this second 90° pulse here it will the various kinds of components of magnetization will be present.

There will be single quantum coherences there will be double quantum coherences there will be z magnetization all components will be present at this point. But to do a filtering technique which we will not go into the detail by which you only retain here the double quantum coherence pathways double quantum coherences and then when you apply the next 90° pulse this will be converted into single quantum coherences and that is what you can measure.

What is the advantage? What is the advantage of this? Here is a comparison of the Cosy spectra and the double quantum filtered COSY spectra. So, these are contour plots. So, look at the again for the single 2-spin system here this is the cross peak for the cosy and this is the diagonal peak in the cosy. This is looking very ugly this COSY is looking very ugly here this one is clean of course but this one is looking very ugly.

Now look at the double quantum filtered COSY this and this are identical see I have here plus minus plus minus I also have your plus minus plus minus what absorptive shapes. So, therefore I have absorbed you here and also absorbed to be there. So, so therefore if I want to call that as absorptive as A this is also a absorptive A for absorptive line shapes.

So, therefore you the all that free information that was lost because of this kind of ugly line shape can be recovered there is an example here. See this is the COSY this is the diagonal, the diagonal showing you all kinds of tails and any peak which is under this here you cannot measure it at all you cannot identify that at all. And in the double counter filtered cosy you can see this clear resolution and peaks which are extremely close to the diagonal.

See this cross peak here this cross peak this cross peak and another one which is very close to that all of them can be resolved they can be identified and things which are close here they can be identified these are all of these becomes a beautiful well resolved spectrum better resolve spectrum. And secondly if there is any singlet in this spectrum the singlet will appear in the COSY spectrum as a diagonal it will not have any cross peak.

Because the singlet has no coupling therefore the singlet will not produce a cross peak but it will produce a singlet along the diagonal and that can be nuisance like the water. And it can be used it produces a huge line and it can mask all your peaks which are in the close vicinity of that. So, that will be very destructive. And here in the double quantum filtered COSY the singlets will not appear.

And this process of filtering technique here which eliminates the singlets and therefore singles will not appear and in the double count of filtered COSY you have beautiful resolution and the fine structure is very clear. And that is the advantage of double counter filtered cosy experiment. All of these are employed in application to all kinds of biomolecular system peptides proteins and you need this to identify the individual spin systems of the amino acids.

Because many amino acids will have very complex coupling patterns and you should be able to identify all those coupling patterns you should be able to identify the cross peaks in the individual amino acids then you can identify the spin system and so, that you can see the correlations in other spectra. So, this is the power of the double quantum filtered COSY over the COSY. So, I think we can we can stop here and we can continue in the next class.