NMR Spectroscopy for Chemists and Biologists
Dr. Ashutosh Kumar
Professor Ramkrishna Hosur
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay
Lecture 52
Total Correlation Spectroscopy

In the last class, we discussed about a concept called scaling in two-dimensional NMR. The idea was to see that the parameters like the chemical shifts and the coupling constants appear modified in the spectra. This is two particular advantages, You can make the J's values appear larger in which case some advantages will be there with respect to the cancellation of the intensities of the positive-negative signals which will not happen.

And we also talked about this down scaling of chemical shifts, it will decreases effective spectral width and therefore it will increase the dwell time between the two points and you can improve the resolution in the spectra. The resolution in the sense, it will improve the resolution in the fine structures of the cross peaks and therefore the cross peaks appear better resolved.

However ofcourse, the separation between two cross peaks in the spectrum along the F_1 dimension will get reduced because you are downscaling the chemical shifts and that ofcourse if one can afford it then one can use downscaling of the chemical shifts and get better result multiplet structures in your cross peaks.

So we will continue that discussion today but now we will discuss the up-scaling of chemical shifts. How to increase the separation between the cross peaks in the two dimensional spectra? This will happen along the F_1 dimension only as before because that is where we are going to do all kinds of manipulations of the delays and we will get better results. Because there is no constraint of the acquisition of data and we can manipulate the delays of the pulses and things like that in the indirect dimension.

So t_1 evolution is played with and that will give you the desired results. So today we are going to discuss the up-scaling of chemical shifts.
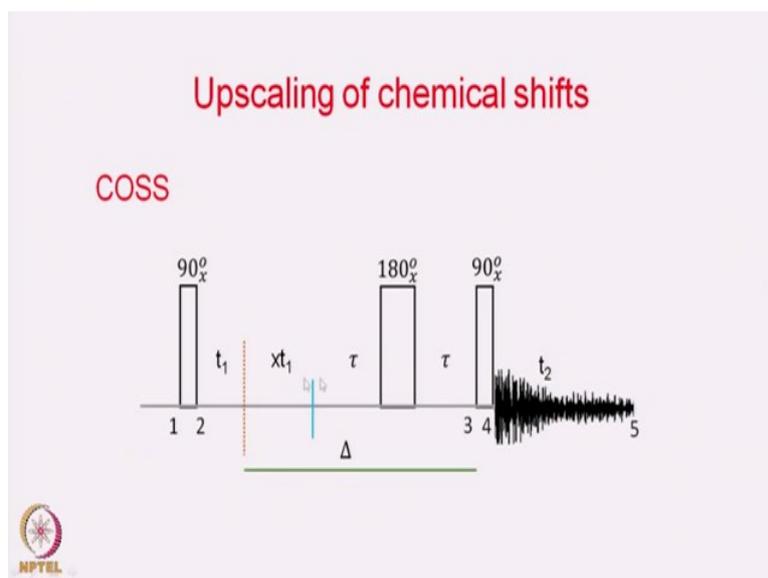
The pulse sequence for this experiment is shown here and this is called as COSS, this is a correlation with the shift scaling. So it is very similar to the previous pulse sequences except that we play around with the delays, the evolution periods of the different Hamiltonians okay. So here we start with to the 90x pulse again and here we have the t1evolution period. Now we have an extended t1 evolution period, here we call it as xt1 and then from this point onwards there is the so-called spin echo sequence tau 180 tau sequence from here to here.

Now we also keep this period from here to here constant and that period is delta okay. So therefore we can see, as you increase this period here this tau values will decrease and therefore this 180 degree pulse keeps moving along with the t_1 increments. So the net result should be that I should have the time period from here to here constant Δ.

Now what all things happen in this? So this we can see immediately that during this period during the tau 180 tau period the chemical shifts will get refocused because this is a spin echo sequence. Whatever evolution happens during this time period will get refocused during this time period. Therefore chemical shift will not evolve during this 2 tau period. But it will evolve during this t_1 and xt_1, so from here to here chemical shifts will evolve.

Now the coupling constants are not affected by the 180° pulse and they will evolve during the entire period $t_1 + \Delta$.

Product operator calculation for a two-spin system

$$\rho_1 = I_{kz} + I_{lz} \qquad \rho_2 = -(I_{ky} + I_{ly})$$

During the next time period of $t_1 + \Delta$ following evolutions will happen:

- Chemical shift evolution will occur for the period $(1+x)t_1$, as they are refocused by the spin-echo sequence during the period 2τ.
- Coupling evolution will happen for the entire period $(t_1+\Delta)$, where, Δ is a constant.

So that is what is indicated here and then we will actually do explicit calculation using the product operator formalism. It will illustrate this we will not go through in as much detail as we did for the COSY and other experiments. Because the same way the calculations can be done and we will illustrate this with one someone particular evolution and will give the end results. So product operator calculation for the two spin system k and l both are spin half systems.

So at the beginning the ρ_1 is the z magnetization, so it is $I_{kz} + I_{lz}$ and after the first 90°pulse I get $-(I¿¿ky + I_{ly}), ¿$ with a minus sign here because the pulse is applied along the X-axis. Now during the next time period $t_1 + \Delta$ the following evolutions will occur this is what explained you just now, the chemical shift evolution will occur for the period $1 + x t_1$ because these chemical shifts are refocused by the spin echo sequence during the period 2τ.

Coupling evolution will however occur for the entire period $t_1 + \Delta$ and Δ is a constant. So therefore the pulses move the 180°pulse especially it moves through the experiment and in order to maintain this delta constant the tau period has to be decreased and as t1 is incremented.

The observable part of density operator at the beginning of the detection period is given by

$$\rho_4 = (I_{ky}\sin \omega_k \alpha t_1 + I_{ly} \sin \omega_l \alpha t_1) \cos \pi J_{kl}(t_1 + \Delta)$$
$$+ (2I_{kz}I_{lx} \sin \omega_k \alpha t_1 + 2I_{lz}I_{kx} \sin \omega_l \alpha t_1) \sin \pi J_{kl}(t_1 + \Delta)$$

Where $\alpha = 1 + x$

$$\rho_4 = \rho_{4c} + \rho_{4d}$$

$$\rho_{4c} = (2I_{kz}I_{lx} \sin \omega_k \alpha t_1 + 2I_{lz}I_{kx} \sin \omega_l \alpha t_1) \sin \pi J_{kl}(t_1 + \Delta)$$

$$\rho_{4d} = (I_{ky}\sin \omega_k \alpha t_1 + I_{ly} \sin \omega_l \alpha t_1) \cos \pi J_{kl}(t_1 + \Delta)$$

Okay, now we will not go through the full calculations as we did for the COSY because we have already indicated how this calculation has to be done. And one can go through the same procedure and calculate the density operator remove the non-observable parts and keep only the observable parts of the density operator.

Now going through all of this calculation you arrive at ρ_4, this is the density operator at the beginning of the detection period and now here the calculation is done taking both k spin evolution as well as l spin evolution and only those parts of the density operator retain which are observable. These ones will evolve in the t_2 period to produce observable magnetization and so that is what is retained here. So at the end of the t_1 evolutions or we will say at the beginning of the detection period because the last pulse is already been applied soon after this one they will evolve in t_2.

Now what does this ρ_4 contain? The ρ_4 contains these terms

$$\rho_4 = (I_{ky}\sin \omega_k \alpha t_1 + I_{ly} \sin \omega_l \alpha t_1) \cos \pi J_{kl}(t_1 + \Delta)$$
$$+ (2I_{kz}I_{lx} \sin \omega_k \alpha t_1 + 2I_{lz}I_{kx} \sin \omega_l \alpha t_1) \sin \pi J_{kl}(t_1 + \Delta)$$

Now let us look at this carefully, now these first two terms here they have the J modulation for the period $t_1 + \Delta$ and Δ is a constant. Therefore t_1 dependence will happen with the coupling constants during the period t_1.

Now with regard to the chemical shifts here, you see this come from the chemical shift evolution $\omega_k \alpha t_1$ and $\omega_l \alpha t_1$. These come from the chemical shift evolutions of the k spins and the l spins. Now you see this is $\omega_k \alpha t_1$ and what is α? $\alpha = 1 + x$ okay, so because the chemical shift evolution has occurred for the period $1 + x t_l$ call it as αt_1.

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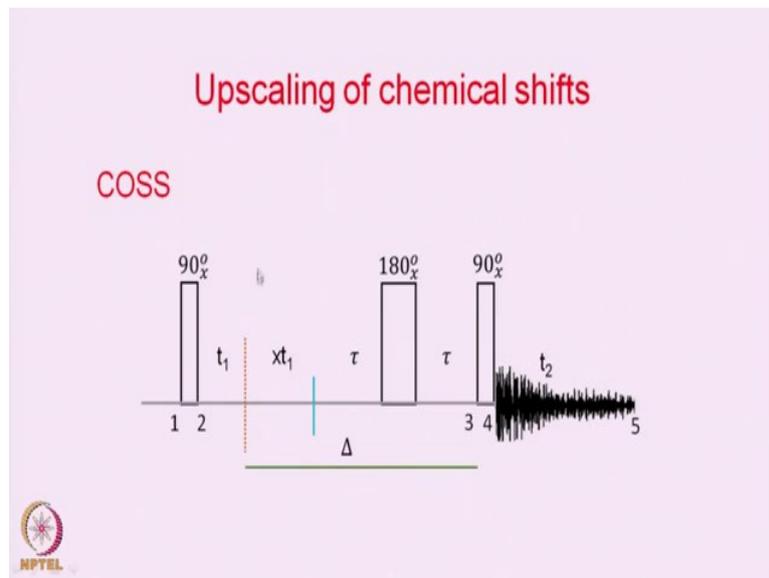
So therefore you can see in this pulse sequence this from here to here is $1 + x t_l$, the chemical shift evolution has happened for this entire period.

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And that is why it is appearing there as αt_1, $\omega_k \alpha t_1$ and what comes from I_{ly}, it is giving you $I_{ly} \sin \omega_l \alpha t_1 I$ okay and both these are in phase magnetizations, this is the in-phase

magnetization of the k spin and this is the in-phase magnetization of the l spin. So this comes from the k spin evolution during the t1 period therefore I have ω_k here and I have a I_{ky} here and this will evolve in t_2 period with the frequency of the k spin with ω_k right.

Therefore this will be responsible for one diagonal peak because in the t_2 period it will evolve with the k spin frequencies. Now this term $I_{ly}\sin\omega_1\alpha t_1$ this came from the evolution of the l spin during the t_1 period during the period $t_1+\Delta$. And therefore this as the frequency here ω_l and now this also is I_{ly} here, I_{ly} evolves in t_2 period with the frequency of the l spin. Therefore this also produces a diagonal peak. So these ones, these two terms produce diagonal peaks in the final 2D spectrum. But the chemical shifts are multiplied by the by the factor alpha.

So and that alpha is $1+x$, x is the number which you can choose whatever value you will choose if $x =1$, then it will be multiplied by a factor 2, chemical shifts will be scaled by a factor 2. If $x = 0.5$ then it will be scaled by the factor 1.5 and so on. This we can choose depending upon how much you want to increase the separation between the peaks. But notice if you want to do that your spectral width will be increased, so if your normal spectral width was 5000 Hertz, let us say total spectral width was 5000 Hertz.

Now if you put $x =1$, $\alpha=2$ therefore your total spectral frequent width will be multiplied by a factor 2. Therefore 5000 Hertz will go to 10000 Hertz, so which means when you do incrementation, the t1 incrementation this will have to account for 10000 Hertz. So therefore your increment will be half of what it was earlier in this case. So this would mean that your t1 max will be half what it would be for the same number of t_1 increments as compared to the previous case without the scaling effect.

But the peaks will appear great more separated because of the increased chemical shift difference. Now what about the second term here? The second term consists of anti-phase magnetizations here, this is an anti-phase term and this is also an anti-phase term. So this will now is $\sin\omega_k\alpha t_1$, this came from the evolution of the k spin during the $t_1+\Delta$ period. But now it has this operator term lx which is l spin magnetization anti-phase to the k spin.

And therefore this will evolve during the t_2 period with the frequency of the l spin and therefore this will produce me a cross peak. To produce me a cross peak along the F_1 dimension it will have the frequency of k spin, F_2 dimension it will have the frequency of the l spin. But now because of this anti-phase operator here I will get plus minus terms in the fine structure in the cross peak along the F_2 period.

And this one is k spin magnetization and this came from the evolution of the l spin in the $t_1+\Delta$ period. So therefore this will also be modulated by this αt_1, this will also be modulated by αt_1. So and this term will evolve during the F_1 axis it will have the l spin chemical shifts and the F_2 axis it will have the k spin chemical shifts and therefore this will also produce a cross peak.

Therefore I separate this ρ_4 into two terms as
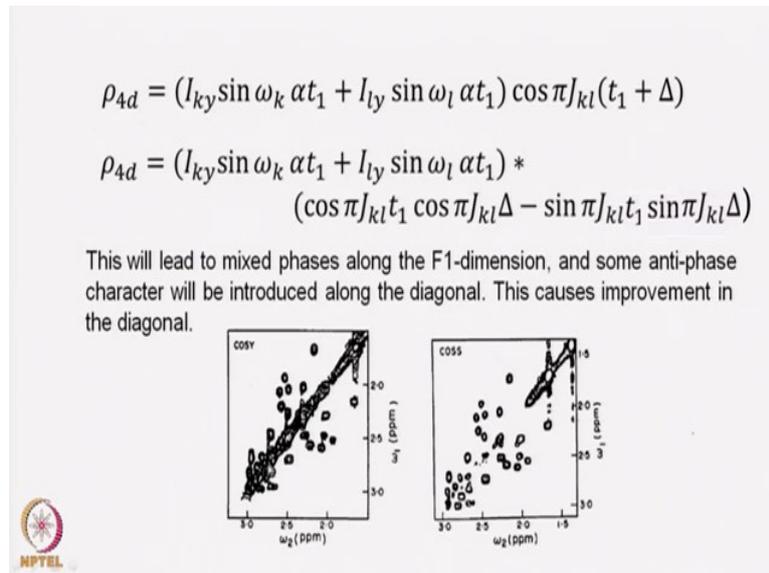
$$\rho_4 = \rho_{4c} + \rho_{4d}$$

ρ_{4d}this is responsible for the diagonal peaks in the 2D spectrum . ρ_{4c}this is responsible for the cross peaks in the 2D spectrum. So the diagonal peak terms we explicitly write it here

$$\rho_{4d} = (I_{ky}\sin \omega_k \, \alpha t_1 + I_{ly} \sin \omega_l \, \alpha t_1) \cos \pi J_{kl}(t_1 + \Delta)$$

And likewise the cross peak terms will be

$$\rho_{4c} = (2I_{kz}I_{lx} \sin \omega_k \alpha t_1 + 2I_{lz}I_{kx} \sin \omega_l \alpha t_1) \sin \pi J_{kl}(t_1 + \Delta)$$

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Now let us look at the diagonal peaks first, so here we have

$$\rho_{4d} = (I_{ky}\sin\omega_k\,\alpha t_1 + I_{ly}\sin\omega_l\,\alpha t_1) *$$
$$(\cos\pi J_{kl}t_1 \cos\pi J_{kl}\Delta - \sin\pi J_{kl}t_1 \sin\pi J_{kl}\Delta)$$

I have expanded it further this term especially the other thing is the same this term is expanded. So we have here these two terms though the sin term will be multiplied by this cosine term, so which will produce some kind of the two peaks and they will also be modulated by the sin multiplied by the sin term, okay . Now each one of these if you multiply this, so you will have one product here it is a sine-cosine and this will be sine-sine.

Now they will both produce two peaks right. So therefore the retable superposition of two terms from here and two terms from here, so the sine-sine product will produce you cosine-cosine terms which are absorbed to your line shapes with plus-minus. And this will produce me sine cosine, this will produce dispersive line shape with plus-plus in phase therefore there will be superposition of this kind of a splitting plus-plus splitting with a dispersive line shape and plus-minus splitting with absorptive line shape.

Therefore this introduces as indicated here in the F_1 dimension some anit-phase character will be introduced along the diagonal this is in the diagonal peaks. Ofcourse this is the kind of modulation of the amplitude of these ones depending upon the value of delta you have these contributions of the two components different, okay. So depending on what value of $\cos\pi J_{kl}\Delta$ you have and $\sin\pi J_{kl}\Delta$ you have. The contribution of the dispersive line shapes and the absorptive lines shape from these two terms will be different and that although introduces the mixed phases.
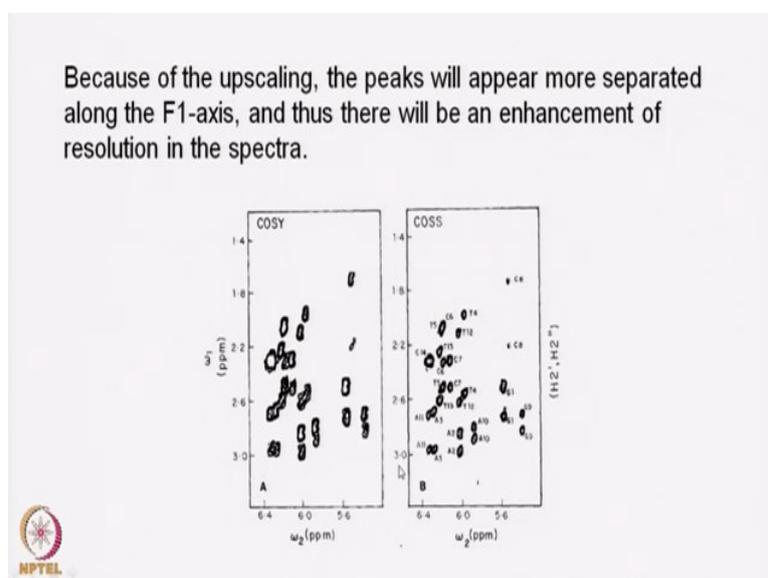
It will have some advantages as we can see here, similar arguments appear for I_{ly} as well, I_{ly} also will have two terms here, one product of this and other product of this. So this will produce you dispersive two components, this will produce you absorptive two components. This will be in-phase, this will be anti-phase and therefore there is a superposition of the peaks of dispersive and absorptive line shapes in the diagonal peak of the l spin as well.

And the contributions of the two will depend upon this value Δ, what value of Δ you can choose and how much one can choose? That will depend upon ofcourse your t_2 relaxations how much you can afford because delta you remember it is a fixed time period. It will be the same for every t_1 increment okay and therefore the signal will decay during that time period and that causes loss of signal.

So depending upon your system how much loss you can afford? How much decay of the signal afford you can choose a value of delta and in relation to $J_{kl}\Delta$ this product whatever the value that is that will determine the contributions of these two terms to the diagonal peak. And you can see here for a typical experiment in one of the samples which one has recorded and this you see is a significant improvement in the diagonal peak. The diagonal many of these diagonal peaks have vanished and all the peaks which are close to the diagonal are now becoming much more clearer.

These peaks are much more clearer compared to what they are present here. So this is a particular advantage of recording the shift scale spectrum.

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Because of the upscaling, the peaks will appear more separated along the F1-axis, and thus there will be an enhancement of resolution in the spectra.

Another example is shown here because of the up-scaling then you have cross peak separation is getting increased and you see here this is the particular region of the spectrum of the DNA molecule and therefore this is a so-called 1 prime, 2 prime, double prime region of the sugar rings in the DNA. And you see here a significant improvement in the separation of the cross peaks in this area. This cannot be resolved very well but these ones are got resolved very well.

Similarly, you can see here there is a better separation of the cross peaks. Overall the peaks appear more compressed here compared to this and that is because of the increased overall spectral width in this. But the important point is that the cross peaks are looking more resolved in this spectrum okay. So therefore that is the advantage of doing up-scaling of chemical shifts.

Now, okay so now we will take up another experiment which is called as Total Correlation Spectroscopy it is called TOCSY, okay. So this is an experiment which is very commonly used in assigning spin systems and this is a very popular experiment which has very important sensitivity advantages and we will see as we discussed further. So the pulse sequence is very simple here, so you have the 90 degree pulse that is the excitation pulse magnetization is excited and it evolves for the period t1 and after that the magnetization is locked.

Now you remember this magnetization is in the transverse plane when you apply $90x$ pulse, you get magnetization in the transverse plane in the Y axis. Because it will evolve during this period but now you apply a spin lock. Spin lock is applied along the X or the Y or whatever axis and then the entire magnetization is locked along that axis okay. So here this is a very complex spin sequence here, spin lock it will consist of several pulses there are many-many different schemes for spin locking and there are many different pulses inside here.

So we will not go into the discussion of the details of the spin lock how it is achieved, but suffice it to say that during this period the chemical shift evolution is completely removed. The Zeeman Hamiltonian is completely removed and the only Hamiltonian that is present is the coupling Hamiltonian. So therefore the effective Hamiltonian during this period is the coupling Hamiltonian.

Now since the chemical shifts are not there we will have to consider the full Hamiltonian not just I_{kz}, I_{lz} as was done in the previous cases where you have a weakly coupled spin systems.

So when you have a weakly coupled spin systems coupling Hamiltonian will be I_{kz}, I_{lz} here $2\pi J\, I_{kz} I_{lz}$. But when the chemical shifts are removed the J coupling becomes the only part of the Hamiltonian and then you will have the full Hamiltonian that has to be considered and that is the effective Hamiltonian represented here.

The effective Hamiltonian is

$$\mathcal{H}_e = 2\pi J I_k.\, I_l = 2\pi J (I_{kz}I_{lz} + I_{kx}I_{lx} + I_{ky}I_{ly})$$

So we have seen the effects of this early in course, when we talked about the analysis of spectra. This was discussed by Professor Ashutosh Kumar and you see when there is a strong coupling you have to include all these terms in the coupling Hamiltonian. If there is a weak coupling then you will have only this term in the coupling Hamiltonian and the product operator calculations were done for the weakly coupled situation here.

You can also do a detailed calculation of the evolution of the density operator for a strongly coupling Hamiltonian. But certain tricks are to be used and this is more complex and we will not go into that detail discussion here and we will simply accept the results that have been obtained.

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Evolution of the magnetization components under the influence of this Hamiltonian is given by

$$I_{kx} \xrightarrow{\mathcal{H}_e t} I_{kx}\left(\frac{1+\cos 2\pi J t}{2}\right) + I_{lx}\left(\frac{1-\cos 2\pi J t}{2}\right) + \left(2I_{ky}I_{lz} - 2I_{ly}I_{kz}\right)\sin\frac{2\pi J t}{2} \rightarrow (1)$$

Diagonal   Cross-peak

Complete transfer of magnetization will occur for time t=1/2J. This is in contrast to INEPT transfer of coherence where complete transfer requires a time t=1/J.

Both diagonal and cross-peaks have the same phase.

So once that is done, evolution of the magnetization under the influence of this effective Hamiltonian is simply given by this expression here. So if you start with the I_{kx}, I_{kx} evolves

during the spin lock period, how does it evolve for the period t? And this is the period of the spinlock, so during this it will produce me

$$I_{kx} \xrightarrow{\mathcal{H}_e t} I_{kx}\left(\frac{1+\cos 2\pi J t}{2}\right) + I_{lx}\left(\frac{1-\cos 2\pi J t}{2}\right) + \left(2I_{ky}I_{lz} - 2I_{ly}I_{kz}\right)\sin\frac{2\pi J t}{2} \rightarrow (1)$$

And notice so during the t we are starting here from the kx because this is what came from the evolution during that t_1 period is started from the kx and give me kx and it also gave me a lx. That is the interesting part, so compared to the COSY you had this kx here and the lx part that why what this was 2 I_{ly} I_{kz}. This was producing an anti-phase term in the case of the COSY and here we have the in phase term for the I_{lx} as well. So we have a kx which is in-phase k magnetization I_{lx} which is in-phase kl magnetization and now these are multiplied by different coefficients.

Then you have a anti-phase term which is multiplied by another coefficient. Notice here, $t = \frac{1}{2}J$, what happens? If $t = \frac{1}{2}J$ and this becomes $1 - \cos\pi J t$, $1 + \frac{1}{2}$ this is 1 whereas this 1 goes to 0, this 1, $1 - \frac{1}{2}$ this goes to 0 which means there will be complete transfer of magnetization from I_{kx} to I_{lx} and this becomes 1. So and this anti-phase terms will be there okay.

But there is a complete transfer of in-phase magnetization from kx to lx. So now another important point to be noticed here is that you have both are the same phase. This is the diagonal peak which will has the X along the X axis and this is also I_{lx}, I_{lx} both are x. Therefore these have the same phase and therefore they will produce the same phase in the final spectrum. Therefore both the diagonal and cross peaks have the same phases.

$$I_{lx} \xrightarrow{\mathcal{H}_e t} I_{lx}\left(\frac{1+\cos 2\pi Jt}{2}\right) + I_{kx}\left(\frac{1-\cos 2\pi Jt}{2}\right) + \left(2I_{ly}I_{kz} - 2I_{ky}I_{lz}\right)\sin\frac{2\pi Jt}{2} \rightarrow (2)$$

Addition of 1 and 2,

$$\left(I_{kx} + I_{lx}\right) \xrightarrow{\mathcal{H}_e t} \left(I_{kx} + I_{lx}\right)$$

- This implies that the total x-magnetization is conserved through the mixing sequence and there is in-phase transfer ($I_{kx} \rightarrow I_{lx}$ and vice versa), retaining the phase of the magnetization; i.e., $I_{kx} \rightarrow I_{lx}, I_{ky} \rightarrow I_{ly}, and\ I_{kz} \rightarrow I_{lz}$. Therefore, this mixing is termed as isotropic mixing and the Hamiltonian is termed as isotropic Hamiltonian.
- After the mixing the magnetization components are detected in the t2 time period. Two-dimensional Fourier transformation of the collected signal results in a 2D-spectrum.

Okay, now if you started with I_{lx}, suppose you start with I_{lx} and do the same evolution here. So which means after the given evolution you are having the I_{lx} term and you are considering the evolution of that under the influence of the spin lock. So you get

$$I_{lx} \xrightarrow{\mathcal{H}_e t} I_{lx}\left(\frac{1+\cos 2\pi Jt}{2}\right) + I_{kx}\left(\frac{1-\cos 2\pi Jt}{2}\right) + \left(2I_{ly}I_{kz} - 2I_{ky}I_{lz}\right)\sin\frac{2\pi Jt}{2} \rightarrow (2)$$
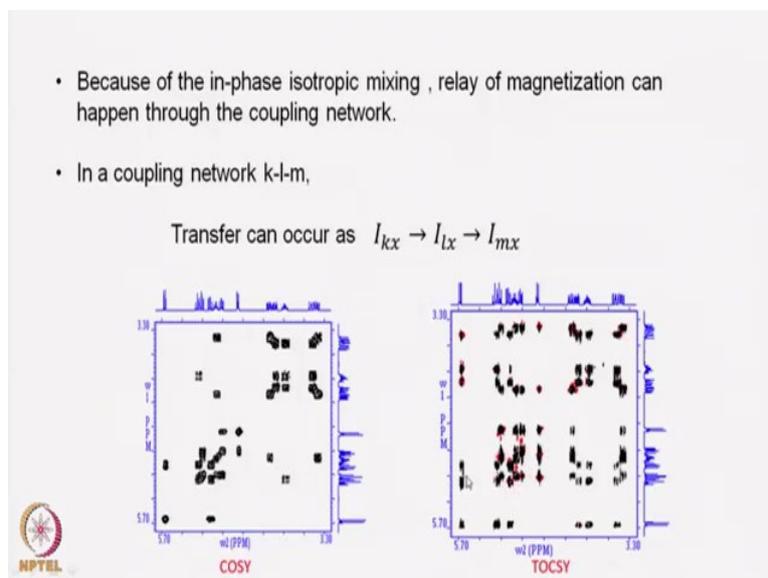
Now once again I_{lx} in phase transfer to k that is the important part in the case of COSY and double quantum related COSY's we have anti-phase terms for both.

In the COSY you have the anti-phase terms from the cross peaks, in-phase for the diagonal peaks and the double quantum filtered COSY you have anti-phase terms in both diagonal and cross peaks. And here you have in-phase components for both the diagonal and cross peaks and they have the same phases because you have both lx and kx for the two cases. Now if we added these two equation, this is the equation 2 and the earlier one was equation 1 which originated from k spin. If we add this you notice here, this term will completely vanish and you have $I_{kx} + I_{lx} \rightarrow I_{kx} + I_{lx}$. That means the x magnetization is completely conserved.

So it is just distributed between the 2 spins $kx + lx$ and $kx + lx$ here that after the spin lock it is just the same. So therefore nothing has happened $kx \rightarrow lx$ and $lx \rightarrow kx$. Suppose you started with ky, same thing will happen $ky \rightarrow ly$ and $ly \rightarrow ky$. If you started with kz same thing will happen it will go from $kz \rightarrow lz$ and $lz \rightarrow kz$. So therefore, this mixing by the spin lock is called as isotropic mixing because it retains the phase and that is called isotropic mixing and the Hamiltonian is termed as isotropic Hamiltonian.

After the mixing, the magnetization components are detected in the t_2 time period and two dimensional Fourier transformation of the collected signal results in the 2D spectrum.

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- Because of the in-phase isotropic mixing , relay of magnetization can happen through the coupling network.

- In a coupling network k-l-m,

Transfer can occur as $I_{kx} \rightarrow I_{lx} \rightarrow I_{mx}$

COSY   TOCSY

So what is the consequence of this in phase isotropic mixing? There will be relay of magnetization. We supposed during the mixing period you had a multiple spins which are connected, so you had let us consider a 3 spin network here k, l and m, k is coupled to l, l is coupled to m there is no k-m coupling okay. But now so during the mixing period we saw that if you start from the $kx \rightarrow lx$ when you had only 2 spins lx starting with $lx \rightarrow kx$.

But suppose there is a third spin here, the lx is coupled to the m therefore what stops it from going to m? Therefore during the spin lock period what originated from k came to l and then from l it can go to m. Because here is no chemical shift evolution here anyway. It is only coupling evolution all the spins are essentially lost their identity. Therefore it is called as the only the coupling part.

The individual chemical shifts are not are not there therefore what came from here went to l and $l \rightarrow m$, so there can be transfer from $l \rightarrow m$ as well. So transfer can occur from $k \rightarrow l$ and $l \rightarrow m$ and if there are more it can go further $m \rightarrow p \wedge p \rightarrow q$ and things like that. So it can relay through the entire magnetization the coupling network and that is the strength of the TOCSY experiment that is why it is called as total correlation spectroscopy.

Look at the example here, here is an experimental example this is the COSY spectrum of a particular molecule whose one dimensional spectrum looks like this because it is put on both

sides in the same manner. And this is the corresponding TOCSY spectrum of the same molecule. So you notice here that in the COSY spectrum you got a correlation from this diagonal peak to this a produces the cross peak here that is to this diagonal peak and it produces a cross peak here to this diagonal peak here.

So correlations that established between these 3 spins in the COSY spectrum, so this proton is therefore coupled to this proton and also to this proton. Now you see here in the TOCSY spectrum you have those two same peaks present here, these two are the two COSY peaks which were present here and here. But you get additional peaks here, this peak here and this peak here these have two additional peaks which have coming.

So how did they come? They came because of the relay of the magnetization through the network of the coupled spins. So because these ones are further coupled to other spins and that is how you generated those two. See for example here, this diagonal peak is here okay so this diagonal peak is coupled to this you can see this cross peak here.

But this diagonal peak here is also showing a cross correlation here and therefore that is the same which is the relay here. So you have this relay coming here through the relay. So therefore you have this kind of correlations appearing in the TOCSY spectrum through the relay mechanism. So therefore it is called two-dimensional total correlation spectroscopy or the TOCSY. Similarly here you start with this here you had only one cross peak showing one correlation, one coupling from here to here.

But this diagonal peak here, this proton may be coupled to several others, this one is coupled to several others. And that is shown by these 3 peaks which are additionally appearing here. This one is appearing additional and these ones are appearing additional which means this irrespective diagonal peaks are connected to more spins and magnetization is relay to those spins in the spin lock period. And they will evolve during the t_2 time period with the respective frequencies and therefore they will appear here in the 2D spectrum as cross peaks and the respective chemical shifts of this spin which had evolved during the t1 period.

So therefore this generally contains more number of peaks but it is extremely useful and it has more information. So complete spin networks can be identified from the TOCSY spectrum. So with that we will stop here so we completed the two dimensional correlation experiments homo-nuclear correlation experiments with this.