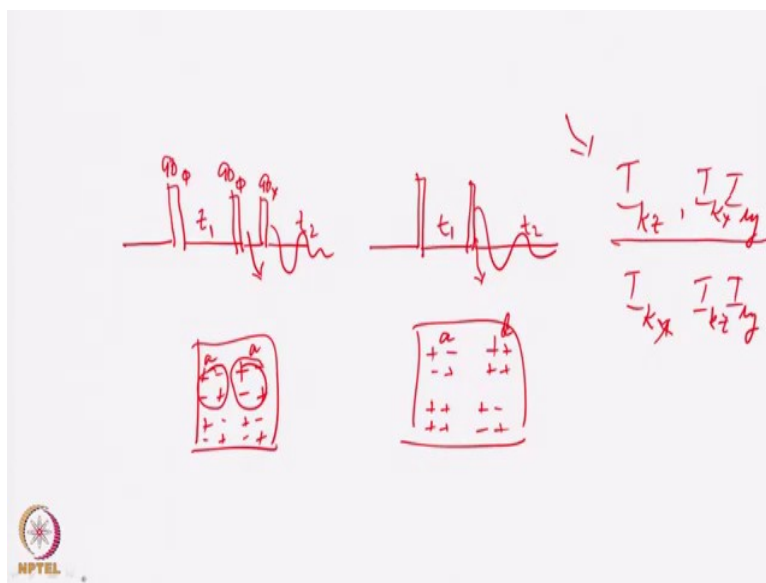**NMR Spectroscopy for Chemists and Biologists**
Professor Ramkrishna V Hosur
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Indian Institute of Technology Bombay
Lecture 49 - Two Dimensional Nuclear Overhauser
Effect Spectroscopy (2D – NOESY)

So, in the last class we discussed in detail an experiment called as double quantum filtered COSY.

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And that was a simply a pulse sequence which was like this and we had three 90 degree pulses. This is 90φ the phase of φ and this is $90x$ and the data was collected here. And, this is the t_1 period and this is the t_2 period. And, this is called as the double quantum filtered COSY. And, this is very similar to the COSY which we also discussed in the previous classes and that was just this, you have here t_1 period here and t_2 period here, just one pulse less.

And this is $90x$, but we could have other things as well. In doing so we actually demonstrated how double quantum filtered COSY produces some features which are very advantageous compared to the COSY, especially in the diagonal of the two dimensional spectrum, the double quantum filtered COSY had anti-phase character. So, you have ¿ ¿ ¿,¿. And, this was in comparison with the COSY where it was all ¿ here,¿, ¿, ¿ and these are dispersive line shapes here and these are absorptive line shapes.

Here we have all absorptive line shapes for both the diagonal as well as the cross peaks. And, therefore it had a big improvement in the resolution in the spectrum. So, that was particularly

advantageous from the point of improving the quality of the spectrum. But, notice also we demonstrated a new concept in that process and that is called as a selection of coherence transfer pathways.

In other words different components of the coherences were selected in two cases. For example, if I were to take a point like this here at this point or at this point, the density operated we have created had this sort of terms I_{kz} then I_{kx}, I_{ly} and I_{ky} and I_{kz} I_{ly}. So, in the COSY experiment these were ignored, these first two terms were ignored. This is I_{kx} and I_{kz} and I_{ly}, these terms were retained in the COSY experiment. And, in the double quantum filtered COSY experiment we retain this portion of the density operator by suitably adjusting the phases of these pulses 90φ, 90φ.

In fact this brings in a new concept, a totally a new thought with regard to manipulation of the magnetization transfers and the manipulation of the nature of the spectra. So, that is something which is quite important. And it becomes applicable in many other experimental sequences as well. So, I am going to demonstrate you one more such application where we choose a particular kind of magnetization namely I_{kz}, we will choose I_{kz} here. So, let us see how that can be done and that is what I am going to show you in today's class.

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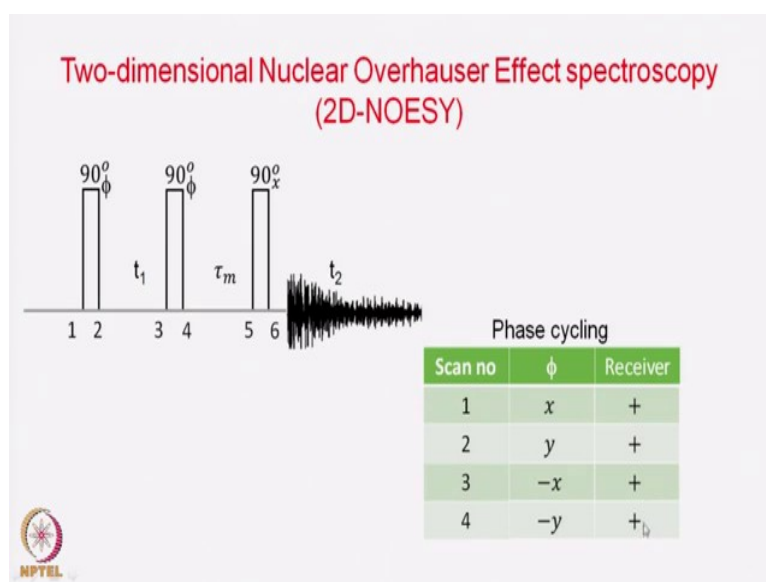
So, this an experiment called as two dimensional nuclear overhauser effect spectroscopy or 2D NOESY. This actually has been discussed previously by Professor Ashutosh Kumar during his lectures on polarization transfer. So, this is not coherence transfer in some sense that it is a polarization transfer. But, nevertheless it relies on choice of particular components

with the magnetization through the pulse sequence. This experiment has the following pulse sequence, you have this 90φ-90φ, you can actually have 90x, 90x here, it does not matter.

But, let us keep 90φ, 90φ to illustrate the point of selection of coherence transfer pathways. So, here we have 90φ, 90φ and 90x here and the phase cycling that is used for this experiment is given in this manner. You have changed the phase of the oxidization of pulses $x, y, -x, -y$ and ¿here.

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Selection of coherence transfer pathways

Comparison of COSY and DQF-COSY indicates a general strategy of selection of pathways for coherence transfer.
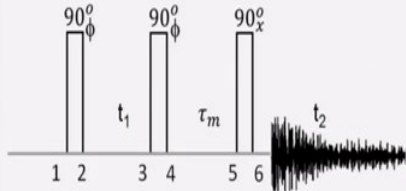
COSY: single quantum, k-spin magnetization is transferred directly to single quantum l-spin magnetization, and vice-versa, in the k, l-spin system.

DQF-COSY: initial single quantum, k-spin magnetization is converted to double quantum coherence between the spins, k and l and then transferred to single quantum l-spin magnetization, and vice-versa, in the k, l-spin system.

Such situations occur in other two-dimensional experiments as well, and the general strategy of selection of the pathway is achieved by so-called phase cycling of the pulses and the receiver.

Two-dimensional Nuclear Overhauser Effect spectroscopy (2D-NOESY)

Scan no	φ	Receiver
1	x	+
2	y	+
3	$-x$	+
4	$-y$	+

Phase cycling

Notice in the double quantum filtered COSY we have the phase cycles which initial single quantum k spin magnetization is converted into double quantum coherence between the spins. k and l then transferred to single quantum spin magnetization and vice versa in the kl spin systems. And, here the phase cycle was, this was ¿ for double quantum filtered COSY. But,

now we are going to use this remaining the same. We will make this ¿, how does that help us? So, this we can see through this product operator calculation once more.

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Product operator calculations

$\rho_4(\phi = x) = [-I_{kz}\cos\pi J_{kl}t_1 - 2I_{kx}I_{ly}\sin\pi J_{kl}t_1]\cos\omega_k t_1 +$
$[I_{kx}\cos\pi J_{kl}t_1 - 2I_{kz}I_{ly}\sin\pi J_{kl}t_1]\sin\omega_k t_1$ → (1)

$\rho_4(\phi = y) = [-I_{kz}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lx}\sin\pi J_{kl}t_1]\cos\omega_k t_1 +$
$[I_{ky}\cos\pi J_{kl}t_1 + 2I_{kz}I_{lx}\sin\pi J_{kl}t_1]\sin\omega_k t_1$ → (2)

$\rho_4(\phi = -x) = [-I_{kz}\cos\pi J_{kl}t_1 - 2I_{kx}I_{ly}\sin\pi J_{kl}t_1]\cos\omega_k t_1 +$
$[-I_{kx}\cos\pi J_{kl}t_1 + 2I_{kz}I_{ly}\sin\pi J_{kl}t_1]\sin\omega_k t_1$ →(3)

$\rho_4(\phi = -y) = [-I_{kz}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lx}\sin\pi J_{kl}t_1]\cos\omega_k t_1 +$
$[-I_{ky}\cos\pi J_{kl}t_1 - 2I_{kz}I_{lx}\sin\pi J_{kl}t_1]\sin\omega_k t_1$ → (4)

I am putting the same four equations which were given earlier in the case of double quantum filtered COSY. The first experiment with phi is equal to *x* often we also refer to this *x* phase as 0 phase and *y* phase as 90 degree phase -*x* as 180 degrees and -*y* also as 270 degrees. So, sometimes you will find references as 0 degree phase 90 degree phase 180 phase and 270 degree phase. And, conventionally this are used in the coding on the spectrometers, you put it as 0, 90, 180 and 270.

So, when you have zero degree phase for the phi or the x phase then the density operator ρ_4 is the same as there was in the COSY. You have here

$$\rho_4(\phi = x) = [-I_{kz}\cos\pi J_{kl}t_1 - 2I_{kx}I_{ly}\sin\pi J_{kl}t_1]\cos\omega_k t_1 +$$
$$[I_{kx}\cos\pi J_{kl}t_1 - 2I_{kz}I_{ly}\sin\pi J_{kl}t_1]\sin\omega_k t_1 \qquad \text{→ (1)}$$

When φ was changed to 90 degree phase then of course you had these changes here, this remained the same.

$$\rho_4(\phi = y) = [-I_{kz}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lx}\sin\pi J_{kl}t_1]\cos\omega_k t_1 +$$
$$[I_{ky}\cos\pi J_{kl}t_1 + 2I_{kz}I_{lx}\sin\pi J_{kl}t_1]\sin\omega_k t_1 \qquad \text{→ (2)}$$

This changed to plus 2 I_{ky} I_{lx} and this changed to I_{ky} coefficient being the same and this changed to plus 2 I_{kz} I_{lx} sin π J_{kl} t_1. Notice here there is a change lx here and ly there. If you change the φ to -x, there is 180 degrees phase shift.

Then we got this remain the same

$$\rho_4(\phi = -x) = \left[-I_{kz}\cos\pi J_{kl}t_1 - 2I_{kx}I_{ly}\sin\pi J_{kl}t_1\right]\cos\omega_k t_1 + \left[-I_{kx}\cos\pi J_{kl}t_1 + 2I_{kz}I_{ly}\sin\pi J_{kl}t_1\right]\sin\omega_k t_1 \qquad \rightarrow(3)$$

So, this is changed that happened because of the change in the phase of the excitation here. Finally, when you had the phases 270 degrees or the -y then this first term remained the same, this changed the sign become plus 2 I_{ky} I_{lx}.

$$\rho_4(\phi = -y) = \left[-I_{kz}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lx}\sin\pi J_{kl}t_1\right]\cos\omega_k t_1 + \left[-I_{ky}\cos\pi J_{kl}t_1 - 2I_{kz}I_{lx}\sin\pi J_{kl}t_1\right]\sin\omega_k t_1 \qquad \rightarrow(4)$$

And, then you had -Iky you compare with this, this is the -I_{ky} and you compare with this, this is the -$2 I_{kz} I_{lx} \sin \pi J_{kl} \omega_k t_1$. I put all of them in the same page here for ease of understanding, how if you manipulate these additions and subtractions different terms will cancel out.

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We now add these four equations as indicated for the receiver phase in the table. This leads to the resultant density operator,

$$\rho_4 = \rho_4(\phi = x) + \rho_4(\phi = y) + \rho_4(\phi = -x) + \rho_4(\phi = -y)$$

$$= -\{4I_{kz}\cos\pi J_{kl}t_1 + 2(2I_{ky}I_{lx} - 2I_{kx}I_{ly})\sin\pi J_{kl}t_1\}\cos\omega_k t_1$$

z-magnetization   Pure zero-quantum coherence

During the mixing time (τ_m) which is in the order of few hundred milli-seconds,

- z-magnetization recovers towards the equilibrium value of the k-spin along the z-axis, dictated by the T_1 relaxation time.
- The zero-quantum coherence decays due to T_2 relaxation time
- The residual zero-quantum coherence acquires phase by evolution. So, by varying τ_m randomly or systematically in large number of steps, the zero-quantum coherence can be cancelled out.

For example, all of them I now add, instead of subtracting anywhere in the double quantum filtered COSY what I did? This was plus this was minus, this was plus and this was minus, now what I do, I add all of them ¿.

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Product operator calculations

$$\rho_4(\phi = x) = \left[-I_{kz}\cos\pi J_{kl}t_1 - 2I_{kx}I_{ly}\sin\pi J_{kl}t_1\right]\cos\omega_k t_1 +$$
$$\left[I_{kx}\cos\pi J_{kl}t_1 - 2I_{kz}I_{ly}\sin\pi J_{kl}t_1\right]\sin\omega_k t_1 \qquad \rightarrow (1)$$

$$\rho_4(\phi = y) = \left[-I_{kz}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lx}\sin\pi J_{kl}t_1\right]\cos\omega_k t_1 +$$
$$\left[I_{ky}\cos\pi J_{kl}t_1 + 2I_{kz}I_{lx}\sin\pi J_{kl}t_1\right]\sin\omega_k t_1 \qquad \rightarrow (2)$$

$$\rho_4(\phi = -x) = \left[-I_{kz}\cos\pi J_{kl}t_1 - 2I_{kx}I_{ly}\sin\pi J_{kl}t_1\right]\cos\omega_k t_1 +$$
$$\left[-I_{kx}\cos\pi J_{kl}t_1 + 2I_{kz}I_{ly}\sin\pi J_{kl}t_1\right]\sin\omega_k t_1 \qquad \rightarrow (3)$$

$$\rho_4(\phi = -y) = \left[-I_{kz}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lx}\sin\pi J_{kl}t_1\right]\cos\omega_k t_1 +$$
$$\left[-I_{ky}\cos\pi J_{kl}t_1 - 2I_{kz}I_{lx}\sin\pi J_{kl}t_1\right]\sin\omega_k t_1 \qquad \rightarrow (4)$$

So, now we can go back and see which are the terms which will survive. This term has remained in the same throughout, so this will remain. Now you look at this term, minus 2 I_{kx} I_{ly} and where does that appear again? It appears again here as minus 2 $_{Ikx\ Ily}$. But, appears with the same sign and therefore this does not go away. And, similarly this 2 I_{ky} I_{lx} appears here and it also appears here with the same sign, therefore if we add all of this, this also will remain.

This will remain as these two terms into I_{ky} I_{lx} and this is -2 I_{kx} I_{ly} this will remain as with the same coefficients. Now, what happens here? Now this I_{ky} cancels with this -I_{ky} and this I_{kx} cancels with this minus I_{kx}. And, what happens to this? This 2 I_{kz} I_{ly} cancels with this and this 2 I_{kz} I_{lx} cancels with this. Therefore I will have only limited number terms from the addition of all of this.

We now add these four equations as indicated for the receiver phase in the table. This leads to the resultant density operator,

$$\rho_4 = \rho_4(\phi = x) + \rho_4(\phi = y) + \rho_4(\phi = -x) + \rho_4(\phi = -y)$$
$$= -\{4I_{kz}\cos\pi J_{kl}t_1 + 2(2I_{ky}I_{lx} - 2I_{kx}I_{ly})\sin\pi J_{kl}t_1\}\cos\omega_k t_1$$

z-magnetization   Pure zero-quantum coherence

During the mixing time (τ_m) which is in the order of few hundred milli-seconds,

- z-magnetization recovers towards the equilibrium value of the k-spin along the z-axis, dictated by the T_1 relaxation time.
- The zero-quantum coherence decays due to T_2 relaxation time
- The residual zero-quantum coherence acquires phase by evolution. So, by varying τ_m randomly or systematically in large number of steps, the zero-quantum coherence can be cancelled out.

And that is indicated here, I will have

$$\rho_4 = \rho_4(\phi = x) + \rho_4(\phi = y) + \rho_4(\phi = -x) + \rho_4(\phi = -y)$$
$$= -\{4I_{kz}\cos\pi J_{kl}t_1 + 2(2I_{ky}I_{lx} - 2I_{kx}I_{ly})\sin\pi J_{kl}t_1\}\cos\omega_k t_1$$

z-magnetization   Pure zero-quantum coherence

And, the whole thing is multiplied by cosine omega k t1. Now, look at what these terms are, this is clearly *z* magnetization only, this is the *z* magnetization of the *k* spin. Now, if you go back and look at the natures of the product operators, this term here 2 *Iky Ilx* - 2 $I_{kx} I_{ly}$ is pure zero quantum coherence. So, which means by this operation of the phase cycling we have retained the *z* magnetization and zero quantum coherences.

Now, both these are not observable. So now what happens? To make them observable we need another pulse. And that of course is another 90 degree pulse which is required. So, that is there in the pulse sequence. The last 90 degree pulse converts them into observable magnetization for us to measure what happens before that.
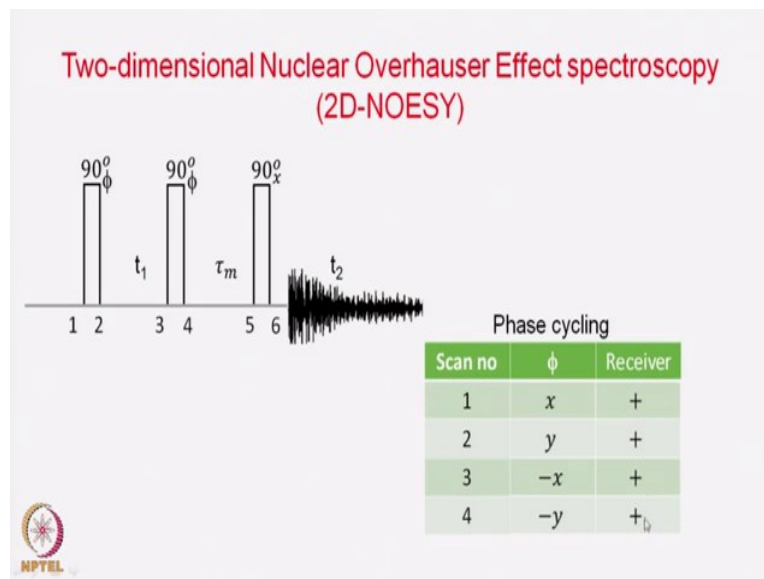
Now but during the next period in the pulse sequence during this period τ, we now have a period called τ_m which is the mixing time. We looked at the density operator at this point in time and we found that operation of this type we had only the z magnetization here and the zero quantum coherence. And this τ_m is of the order of few hundred milliseconds. Now, what happens during this period?

The following things happens: The z magnetization recovers towards the equilibrium value of the k spin. Because, it is the k spin magnetization it has to come back to its equilibrium magnetization along the Z axis. And, this will be dictated by t_1 relaxation time of the k spin. Now, the zero quantum coherence is a coherence, therefore it is a phase coherence. This will

actually decay due to t_2 relaxation time. During the few hundred milliseconds it will decay due to t_2 relaxation time.

Now, what else we can do? So now what we are interested in the NOESY experiment is the z magnetization. We do not want the zero quantum coherence here, we want to keep only the z magnetization, that means we want to eliminate this. Partially it will go away because of t_2 relaxation time, during the long mixing time we might have. But, then we can also do a trick to see that this gets cancelled out. What is the trick? We do we can systematically vary this τ_m the mixing time over a large number of steps.

So, suppose you were recording experiments at 400 milliseconds or 200 milliseconds you record experiments for 200. If you are doing record with 190 milliseconds 195 milliseconds 200 milliseconds, 205 milliseconds, 210 milliseconds the average till remains 200 milliseconds. But, just around that number you vary the mixing time to a certain degree. So, what happens then is so because that time is variant the zero quantum will acquire different phases. So, which means acquire different phases meaning now if you take an average a sum of all of these ones it is possible that the residual zero quantum coherence will cancel out because these phases were systematically varying.

So they can pass over the 360 degrees. And, if you have sufficient number of steps there they can be cancelled. Or, if you do randomly vary this over a larger number of scans and then of course this also will cancel out. If you do a large number of increments scans because you collect the data for number of scans, you collect 4 scans, 16 scans, 24 scans or 32 scans or whatever so that you have a good single to noise ratio. And, for every scan which occurs in of course in multiples of 4 as indicated in a phase cycle you keep changing the tau m value. Then, when it is randomly varied the phases will also be random. And, when you add all of those the zero quantum coherence scan get cancelled out.

So, this way you remove the zero quantum coherence and you retain only the z magnetization. So this is an illustration of how phase cycles can be used for selecting particular magnetization pathways. Coherence transfer pathways or magnetization pathways through the experimental pulse sequence.

This demonstrates a different choice of coherence pathway selection. Different combinations of phase-cycles are indeed possible.

During the mixing time, the recovery of the z-magnetization of k-spin causes transfer of magnetization to a dipolarly coupled spin, say l.

$$I_{kz} \overset{\tau_m}{\to} \lambda I_{kz} + (1 - \lambda)I_{lz}$$

λ represents the residual magnetization on the k-spin and $(1 - \lambda)$ represents the transfer to the l-spin.

Now, during the mixing time the recovery of the z magnetization of the k spin causes transfer of magnetization to a dipolarly coupled spin say. How does the relaxation occur? The relaxation occurs due to interaction between two or more spins. So, if the k spin is dipolarly coupled to some other spin let us say l spin then of course there will be a transfer of this magnetization. Because, it is in a non-equilibrium state the system will have to return to equilibrium. When it has to return to equilibrium, it will pass on this disturbance so the non-equilibrium to some other spin which is in interacting with it through dipolar interaction.

So, some portion of this z magnetization will get transferred to a real spin. Therefore, we can schematically say it this way: That I_{kz} during the period τ_m becomes λI_{kz} of course λ will be less than 1. And, remain residual of that will be on the l spin. If lambda remains on k spin 1 minus lambda appears on l spin. Of course here we consider two spins only but there can be more spins.

If there are more spins some portion will stay here and the other will get distributed to more one spins depending upon how many spins are dipolarly coupled to the k spin. So, this is the network of coupled spins and magnetization gets distributed through this network coupled spins.

So, finally at the end of the mixing time, the density operator will be,

$$\rho_5 = -4\left\{\lambda I_{kz} + (1-\lambda)I_{lz}\right\}\cos\pi J_{kl}t_1 \cos\omega_k t_1$$

The final 90° x-pulse converts the z-magnetization into observable transverse magnetization.

$$\rho_6 = 4\left\{\lambda I_{ky} + (1-\lambda)I_{ly}\right\}\cos\pi J_{kl}t_1 \cos\omega_k t_1$$

Diagonal-peak   Cross-peak

Both the diagonal and cross-peaks have the same fine-structure with in-phase components and peak-shapes (absorptive).

So, now the density operator at ρ_5, I mean we have now looked at the end of the mixing time. So, I have written here the density operator which consists of the z magnetization of the k spin plus the z magnetization of the l spin. This actually has come from the k spin. Notice therefore that it also has the modulation in the t_1 as per the presentation of the k spin. So, this is although this is lz here this is modulated by $\cos\omega_k t_1$ and $\cos\pi J_{kl}t_1$. So, this along the F_1 dimension it will appear at the frequency of ω_k. And, therefore this is the k magnetization z spin.

Now, the last 90 degree pulse. Both these are not observable, z magnetization is not observable. The final 90 degree x pulse converts this z magnetization into observable transfers magnetization. So, the kz goes to -ky so therefore you take away the minus sign put it plus here, λ_{ky}. And, similarly this will be 1 - λ I_{ly} and this remains the same. Now, clearly this is k and this k therefore after t_2 evolution and things like that this will produce the diagonal peak and this will produce a cross peak.

So, during the F_1 dimension it will be k magnetization and F_2 dimension this will be l magnetization. Whereas this term is k magnetization along both F_1 and F_2 dimensions. So, the both diagonal and cross peaks have the same fine structure, that is an importance fact. You look here both are y, so this is the ky and ly, so therefore they have the same phase, you have the same phase. And, both are multiplied by the same coefficient as indicated by this, therefore they will both have the same shape both peak shapes on the F_1 dimension.

And, here it will be absorptive line shape because we have this $\cos\omega_k t_1 \cos\pi J_{kl} t_1$. This produces 2 peaks at $\omega + \pi J_{kl} t_1$ and $\omega - \pi J_{kl} t_1$. So, 2 frequencies and but this will be cosine and therefore they will be absorptive line shapes.

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In case of multiple-spin systems with many dipolar coupling the magnetization gets distributed through the network of spins.

Explicit calculations of polarization transfer during the mixing time through cross-relaxation (or chemical-exchange) have been discussed in the previous classes, and here we provide the final result of the intensities of the diagonal and cross-peaks.

For a symmetrical two-site exchange $A \leftrightarrow B$, with equal populations at the two sites, equal spin-lattice relaxation rates, and equal transverse relaxation rates, the intensities of the diagonal (a_{AA}, a_{BB}) and cross-peaks (a_{AB}, a_{BA}) are given by the following equations,

Of course, in case of multiple spin systems with many dipolar couplings as I mentioned to you before, the magnetization gets distributed through the network of spins. And, we have already done these explicit calculations of polarization transfer during the mixing time through cross relaxation or chemical exchange in the previous classes, so this we will not repeat that here. Because, you remember this Solomon equations, relaxation matrix that appears which describes the transfer of magnetization from 1 spin to another spin through the process of cross relaxation or chemical exchange.
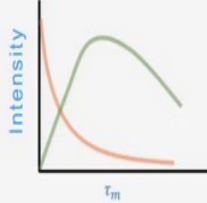
And, therefore we are not going to repeat that expression here and we simply take the final result. There we provide the final result of the intensities of the diagonal and cross peaks. Now, considering a symmetrical two-side exchange to *A, B*. Of course this exchange it can be chemical exchange or it can also be cross relaxation with equal populations at the two sides. If you consider a two site exchange process, equal populations at the two sites, the equal spin that is relaxation rates.

And, equal transfers relaxation rates of the two spins of the two sites. The intensities of the diagonal which we label here as a *AA* and this a *BB* and this is a *AB* and a *BA* are given by the following equations:

So, these are for the diagonal and this will obviously depend upon the mixing time. So, what we get here, this is the final result as I mentioned to you. This is the diagonal peak intensity will be given by

$$a_{AA}(\tau_m) = a_{BB}(\tau_m) = \frac{1}{2}[1 + e^{-2k\tau_m}]e^{-\tau_m/T_1}$$

And, this is of course dictated by the t_l relaxation time that is z magnetization is recovering along the longitudinal axis. And this is the exchange rate and in case of relaxation, there it will be cross relaxation rate coming up here as well.

So, where k is the exchange rate and t_l is the spin lattice relaxation time. The equilibrium magnetization at the two sites is assumed to be the same. Now, you can see here when tau m is 0, so this term is 1 and this term is 1, so what you get here? 1. So, therefore the intensity of the diagonal peak is maximum and that is 1, it is essentially normalized. And what about the intensity of the cross peak? If $\tau_m = 0$, say this one is 1, therefore 1 minus 1 goes to zero and this is of course 1.

So, intensity of the cross peak is 0. So, if now plot these two functions as a function of τ_m, along this axis you have the intensity of the diagonal or the cross peak. You see the diagonal peak intensity decreases like this as per this equation. And, the cross peak intensity goes up like this and transfer and then of course after some time this also decreases. This is because of what the process called as spin diffusion which has been discussed earlier, that is the relay of magnetization from one spin through the network of dipolarly coupled spins.

Now, it will initially increase to the nearest neighbor exchange spin. And then if there is spin diffusion occurring two multiples spins then of course it can decrease. This is in the case of cross relaxation and this is dependent on the inter proton distances and how many protons are close by in space that determines how this diffusion transfer is happening, what is the efficacy of this transfer from one spin to another spin. But, of course in the initial times when the mixing time is short, then of course it is linearly dependent on the mixing time and the transfer will be restricted to the 1 spin only. Whichever one is the closed it will go there only and that will have a specific application.

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The ratio of diagonal to cross-peak intensities will be,

$$\frac{a_{AA}}{a_{AB}} = \frac{1 + e^{-2k\tau_m}}{1 - e^{-2k\tau_m}}$$

For short mixing times (τ_m), compare to T_1,

$$\frac{a_{AA}}{a_{AB}} = \frac{1 - k\tau_m}{k\tau_m}$$

Thus, by monitoring the intensity ratios as a function of τ_m, the exchange rates can be calculated.

Now, if you took the ratio of the diagonal to the cross peak intensities and this will give you

$$\frac{a_{AA}}{a_{AB}} = \frac{1 + e^{-2k\tau_m}}{1 - e^{-2k\tau_m}}$$

Now, for short mixing times tau m compared to t_1 this will get reduced because you can simply expand this and you will get here this will be $1 - 2k\tau_m$. 1, 1 will cancel. Then, you will have only $k\tau_m$ below and here you will have

$$\frac{a_{AA}}{a_{AB}} = \frac{1 - k\tau_m}{k\tau_m}$$

So, we can see that this is directly related to tau m and by doing experiments at different τ_m values you will able to measure the exchange rates. Thus by monitoring the intensity ratios as the function of tau m the exchange rates can be calculated.

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In case of cross-relaxation mediated transfer, the cross-peak intensity is proportional to the cross-relaxation rate (σ_{AB}) at short mixing times (linear regime)

$$a_{AB} \propto \sigma_{AB}\tau_m$$

$$\sigma_{AB} \propto \frac{1}{r^6}$$

Where, r is the distance between spins, A and B. This allows estimation of inter-proton distances in complex molecules from NOESY spectra recorded with short mixing times.
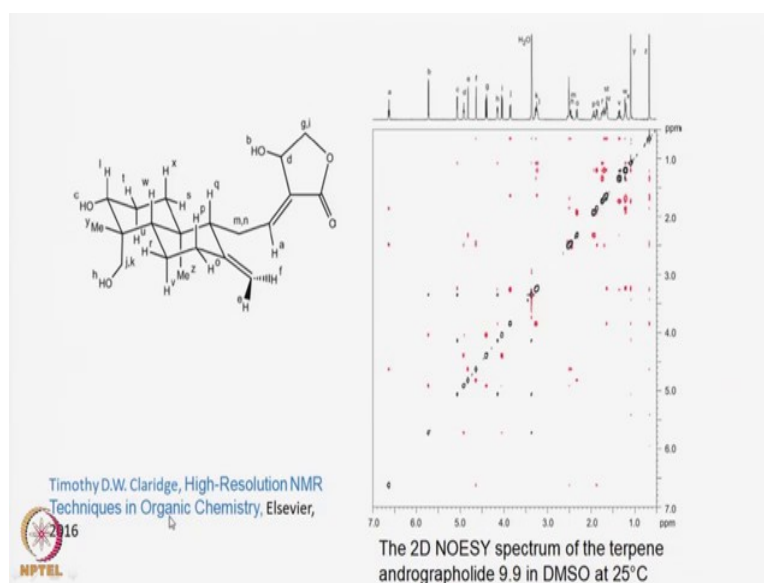
Now, in case of cross relaxation mediated transfer the cross peak intensity is proportional to the cross relaxation rate. As discussed in the previous classes and here will be write that the a *AB* the cross peak intensity is proportional to

$$a_{AB} \propto \sigma_{AB}\tau_m$$

Where σ_{AB} is the cross relaxation rate and we have also seen the cross relaxation rate is inversely proportional to the inter proton distance. If you are talking about proton proton NOE, it is rated to the inter proton distance and to the inverse 6 power of the distance.

Therefore, if you perform experiments at different values of tau m then you can actually estimate the cross relaxation rate here. What you do, you measure this cross peak intensities as a function of tau m and then if you fitted to a linear equation you get the σ_{AB}. Once you get σ_{AB} you can calculate the inter proton distance *r*. So, this allows estimation of inter proton distance in complex molecules from NOESY spectra recorded with the short mixing times.

The 2D NOESY spectrum of the terpene andrographolide 9.9 in DMSO at 25°C

Timothy D.W. Claridge, High-Resolution NMR Techniques in Organic Chemistry, Elsevier, 2016

So, this is the very important observation and it has found extreme value in majority of applications. In fact the large amount of biological NMR is now based on this kind of a concept. Structural determination of large molecules has become possible because of this kind of a strategy. So, here is an experimental example here, this is a molecule which has large number of protons here. Say so many protons are there are labelled here with A B C D E F G H and so on so forth. And, this is the two dimensional NOESY spectrum.

This is the diagonal, you can see the diagonal here the black and all your cross peaks here are in red. So, this is the one dimensional spectrum and it monitors the intensities of all of this. And, you can actually establish correlations firstly with regards to which protons are in close proximity. And they also have different intensities because there are different inter proton distances. By looking at the intensities of the peaks here, you can actually the various inter proton distances.

So, you have so much information here and all of these peaks which are present here are the carriers of structural information. You quantify all of these peaks here then you will be able to establish the network of inter proton distances here. And, it will allow you to determine the structure of the molecule. And this is an extremely important application for chemistry and biology. This picture has been taken form this book here, the Timothy Claridge, High-Resolution NMR Techniques in Organic Chemistry.

So, therefore we have discussed here the important application of the NOESY with regard to the structure determination of molecules. And, while this is an important thing from the application point of view we also demonstrated how pathways can be selected by

appropriately choosing the phases of your pulses and the phases of the receivers. Adjust them so that you select what you want in your spectrum. If you chose coherences then you get J correlated spectra. If you choose z magnetization, then you get dipolarly correlated spectra which protons are correlated through dipolar interactions and therefore they carry distance information.

In the other case the J coupling information is obtainable. So, this 2D NMR therefore provides the variety of possibilities for obtaining the desired kind of information in your spectrum. And that has the therefore been the biggest revolution in NMR spectroscopy. So, we will stop here and continue with other pulse sequences in the next class.