One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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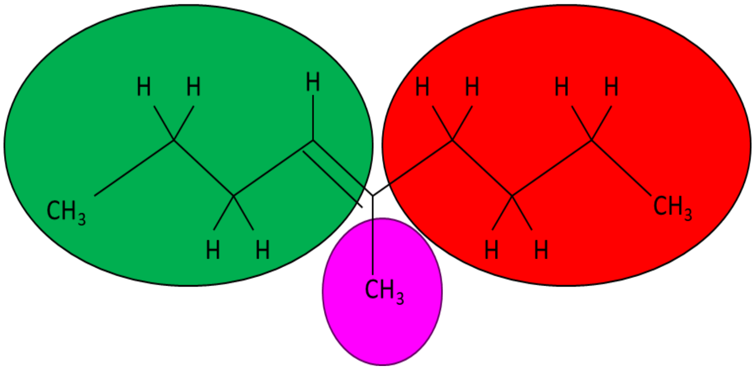
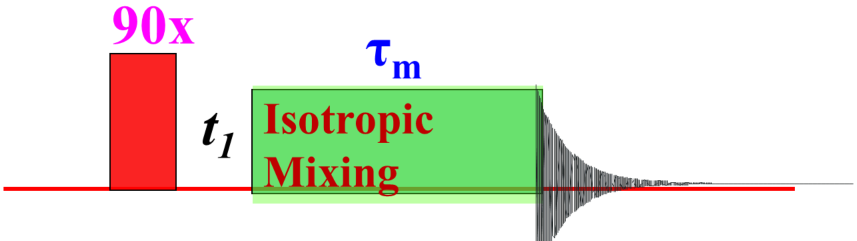
Lecture 42: TOCSY

Welcome all of you. In fact, last class or last two or three classes, we started discussing extensively on two dimensional NMR. After giving basic introduction to the concepts of 2D and the basic pulse sequence and how we interpret the 2D data, and I took examples of 2D COSY spectra. We started interpreting the 2D COSY spectra also. Many examples we took and we wanted to know how we can utilize the 2D COSY to get the connectivity information right from small molecules to big molecules, even sugars, glucose where alpha and beta isomers were there. We started analyzing the spectra just using the concept of correlation of two coupled protons. See, basic thing I have told you is that COSY requires that spins should be scalar coupled. Then there is a transfer of magnetization from one spin to another spin and then this correlation information is reflected as cross peaks in the 2D COSY spectra. So, we knew how to analyze the 2D COSY spectra. Start with one of the peak where you are confident, sit on the diagonal, come vertically down, go horizontally and then go in a ladder manner and then you will be able to almost interpret all the peaks of the spectra. So, that way we can assign all the peaks of a given molecule. After understanding quite a bit with that and also I told you about the DQF-COSY, certain variants of the COSY, and the way we represent the 2D data, magnitude mode, double absorption mode, etc. I said the double absorption mode is preferred for better resolution. DQF-COSY is much better than COSY, I told you. You will get a sharp peaks and also peaks near the diagonal are not masked. We also saw some examples of small flip angle COSY, long range COSY, etc. How we can get the correlation of the long range couplings or we get the relative signs of the couplings based on the direction of tilt of the cross peaks. So, lot of things we discussed. We will go further today with another topic called total correlation spectroscopy called TOCSY. In simple term, TOCSY establishes connectivity among all the coupled partners of a given spin system. This is a very simple statement. In the COSY also we did the same thing. Remember, I told you COSY establishes the correlation among the coupled partners,

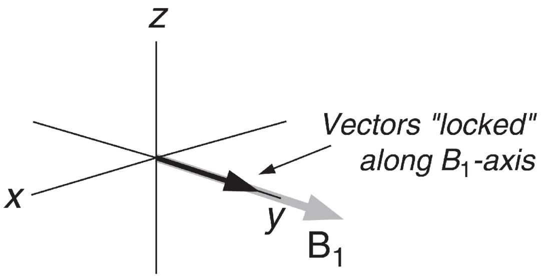


immediate coupled partners. In a given molecule, we know one is coupled to next one, next one, next one, it goes like that. Systematically you have to go in a ladder manner. Whereas, in the TOCSY, it establishes connectivity among all the coupled partners of a given spin system. Of course, spin system we discussed long back while introducing even in the concepts of NMR itself. Of course, in the context of TOCSY, I am telling you what is a spin system again. A spin system is nothing but a set of protons in a chain of unbroken J coupling interactions. That means, I have spin system, one may be coupled, A may be coupled to B, B may be coupled to C like that. It can go systematically. All the spins should form a part of a coupled spin system. One of the couplings may be 0. That is if I take 4 spins, 1, 2, 3 and 4, 1 may be coupled to 2, 2 may be coupled to 3, 3 may be coupled to 4, 1, 4 coupling may be 0, does not matter, but it is part of the coupled spin system. And if there is a break in the chain, then it forms a different spin system. This also we discussed long back when we discussed the spin system. Just to give an example like this, take a hypothetical molecule like this.

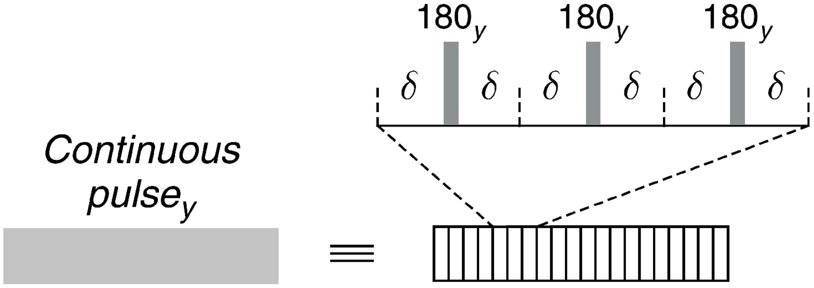
And this forms a spin system because you see, CH3 is coupled to CH2 which is coupled to this CH2 and this is coupled to this CH. Let us assume this CH3 is not coupled to any of these things. So, this forms a spin system and similarly, this side, this CH3 is coupled to this, coupled to this and coupled to this, that forms another coupled spin system and I assume the long range coupling with CH3 to any of these protons is not there. There is no coupling at all. That means, CH3 forms an isolated spin system by itself. So, in this given molecule, there are three coupled spin systems and TOCSY gives cross spins among all the spins of a coupled partner in a given spin system. For example, when I do a TOCSY experiment, I can get at a time cross spin from this to this, this to this, this to this, everything will be observed in one experiment. Remember, when you are doing COSY, you are going from here to here, here to here, here to here, step by step, you are trying to go to, understand the correlation information to identify the coupled partners. But TOCSY does everything in a single experiment, that is the advantage. What is a TOCSY pulse sequence?



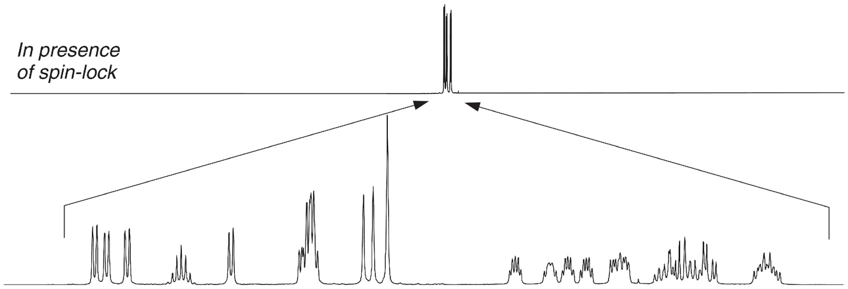
Of course, it does the polarization transfer. Basically, it is similar to COSY sequence. Only thing is, instead of a second pulse, we have what is called isotropic mixing. What is an isotropic mixing? I will explain to you soon. So, we do not have a hard pulse. We have two hard pulses in the COSY, 90-t1-90-t2, that is a COSY sequence, but here 90-t1 and isotropic mixing for a short duration; we apply a soft pulse for a long duration and that is called a mixing pulse. And during this mixing pulse, something happens, that is what is called isotropic mixing and this is the pulse sequence we use for TOCSY, very simple pulse sequence. But the question is, how does this simple pulse sequence works? Remember, when we do this thing, mixing pulse, is applied along this axis, then we say the spin systems are locked along this axis. If the spins are brought from z-axis to let us say y-axis, and apply RF power here, RF pulse with the B1 power along this axis, then we say all the spin vectors are locked along the RF axis, along the B1 axis. This is what the mixing pulse does. It locks the spin system. And what is the duration for which you are going to apply the RF pulse? This spin lock pulse, is a soft pulse, very low power pulse with a constant phase for a long duration, you see, it is a long pulse. For example, hard pulse, told you, when you want to observe proton NMR, 90 degree pulse would be 5 to 8 microseconds, I said. Carbon 13 may be little more. Whereas here, the long soft pulse is of the order of milliseconds, 10 milliseconds, 20 milliseconds, 50 milliseconds, 80 milliseconds, like that. It is of the order of several milliseconds. That is the soft pulse we use. That is a spin lock pulse. Now, what happens in the spin lock? We apply a spin lock pulse.



Then the question comes, how does the magnetization transfer takes place among all the coupled spins? And I said previously, this is a RF pulse, which is a continuous pulse like this, soft pulse, continuous pulse. Then I can imagine this RF pulse as if we have a sequence like this. You know what is the sequence called? It is a spin-echo sequence, a delay, 180 pulse and a delay. It is a spin-echo sequence. Can you not imagine this mixing pulse, a continuous RF pulse as if it is broken into a lot of, innumerable number of spin lock pulses, large number of spin lock pulses, several 90 degree pulses with a minute delay between them, very small delay. Can I not think of that? Then It is nothing but a closely spaced 180 degree pulses with a delay between them. And N such pulses, N such spin-echo sequences. This is the way you can imagine. In which case, I could say each of these delta 180 delta pulse sequence or that particular group block, I will call it as a spin-echo block. So, I can consider continuous RF pulse which we apply for mixing as nothing but a chain of a huge number of spin-echo sequences together.

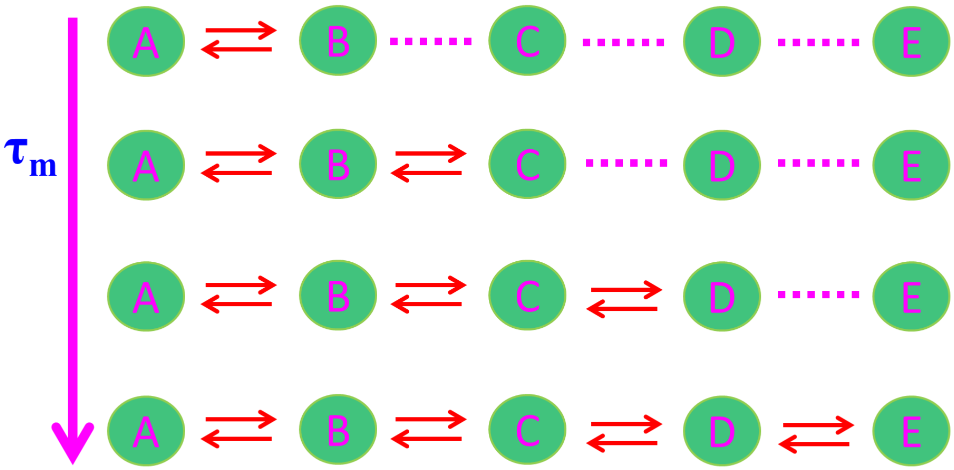


That is what you can imagine. Of course, you know with a homonuclear pulse, what happens when I apply a spin-echo? We have discussed this in the spin-echo. Homonuclear spin-echo refocuses chemical shifts. It does not refocus J-couplings. That is what I told you. I discussed that. It refocuses chemical shifts, but not the J-couplings. So, there is no chemical shift evolution in the mixing time, because after each spin-echo sequence, there is no chemical shift evolution. There are infinite or N number of such



sequences echoes. As a consequence, during the entire mixing period, there is no evolution of chemical shifts. All the spin vectors then remain locked along y-axis because there is no evolution. They do not move. They do not precess. So, when there is no chemical shift offsets, they do not start precessing. It gets locked along the y-axis or on a particular axis in which you are applying the RF pulse, mixing pulse. For the entire mixing period, that means there is no net evolution of the magnetization, for the entire mixing time. So, all the protons then experience the same effective field because there is no evolution of the chemical shift. That means chemical shift differences are zero. It is as good as telling there are no chemical shifts. Remember, when I was explaining spin-echo, I took the example of two spins A and X and they were two different spins. One is on resonance, other is off resonance, but finally I showed both the chemical shifts get refocused and there would not be any difference in chemical shifts. Exactly here, there is no chemical shift evolution and chemical shift difference is practically zero and we are doing this in the spin lock period. That means in the rotating frame. I did not discuss about the rotating frame in this course, but in one of the previous courses, extensively I have discussed the rotating frame. Imagine when I bring the magnetization to x-axis, I am already in the rotating frame and I am applying a spin lock pulse along the axis and then spin vectors get locked. So, there is no chemical shift evolution. Difference of chemical shifts are zero. That is fine. What about the J couplings? Remember in the homonuclear spin-echo, I discussed chemical shifts are refocused, but not J couplings. J couplings always continue to evolve after the 180 pulse. So, this is what happens. So, throughout the spin lock period, you have created a situation, such that the chemical shift evolution zero, but J coupling continuously evolve. It is similar to a free precision. During the spin lock period, like a free precision, there is only J coupling evolution and no chemical shift difference. That is what happens. What do you call a situation? We discussed about strong coupling and weak coupling. When I said strong coupling, the chemical shift difference between the coupled protons becomes smaller and smaller. It is almost comparable or less than the J coupling. But in this situation, what is happening?

All the coupled spins if you consider, there is no chemical shift difference at all. Whereas the J coupling exists, that means delta delta is zero, J coupling is present. What do you call the situation? This is the situation of what is called strong coupling. In the case of the strong coupling, there is no chemical shift here, only J couplings evolve. This is what the spin lock pulse creates. It creates a situation of strong coupling. Then in which case, since there is no chemical shift, all the coupled spins lose their identity. You do not know which chemical shift belongs to which spin. If the spins are not locked, in that case, you can identify and say this is chemical shift of proton 1, 2, 3 like that. Here you cannot distinguish. The spins lose their identity. They are indistinguishable because chemical shifts are zero. And this strong coupling is induced on the entire spin system. This is what happens. So, spin lock pulse in summary, remember, creates strong coupling effect on all the coupled spins. And pictorially, you can imagine like this. Let us say this is the one-dimensional NMR spectrum. So many chemical shifts are there, couplings are there, different groups are there. You can find out what are the chemical shifts. And the multiplicity pattern tells you J couplings. But when I do spin lock, what happens? All these chemical shift differences become zero, no chemical shift. Whereas, J couplings are still present. And this is the situation reated in the spin lock sequence. No chemical shift differences, but there exists J coupling. And this is imposed on all the spins. The strong coupling phenomena, what it does? It creates an oscillatory exchange of magnetization among the coupled spins. During the spin lock time, what happens? There is a strong couplingphenomena, spins do not know themselves what they are, they are indistinguishable. But there is a coupling. So, what will happen? There is the exchange of magnetization among them. Different spins give the magnetization to different spins, but only to coupled spins. because there should be scalar couplings. That is a necessary condition. Only among the coupled spins there is exchange of magnetization. If there are different groups of coupled spins, for one coupled spin system, among them there is a exchange of energy. For the other coupled spin, there is exchange of magnetization. But between these two spins, there is no exchange of coherence. That does not take place. So, the strong coupling creates a oscillatory exchange of coherence. Now, how it works? I will show you here picture really for an AX spin system. Consider the AX spin system, two coupled spin system. See, the magnetization get completely transferred from A to X during the process, during spin lock. When does it give? Complete transfer takes place after half the J coupling. The J coupling between A and X spin, if it is JAX, half of that period is enough for the complete transfer of magnetization from A to X. Of course, I am taking A to X, but it could be other way also. But after sometime what will happen? It will start coming back. Initially A will give to X, then from after half the coupling, half JAX, it start coming back from X to A. This is what happens. So, this is a polarization exchange in a two spin case, two spin case. But what happen? In a bigger spin system, large number of protons, not only two, but when many spins are coupled. Then there is a continuous propagation of magnetization among the spins. One will give to other one, other one give next to it, this will give to next one, like that. And this you can understand the propagation of magnetization pictorially like this.



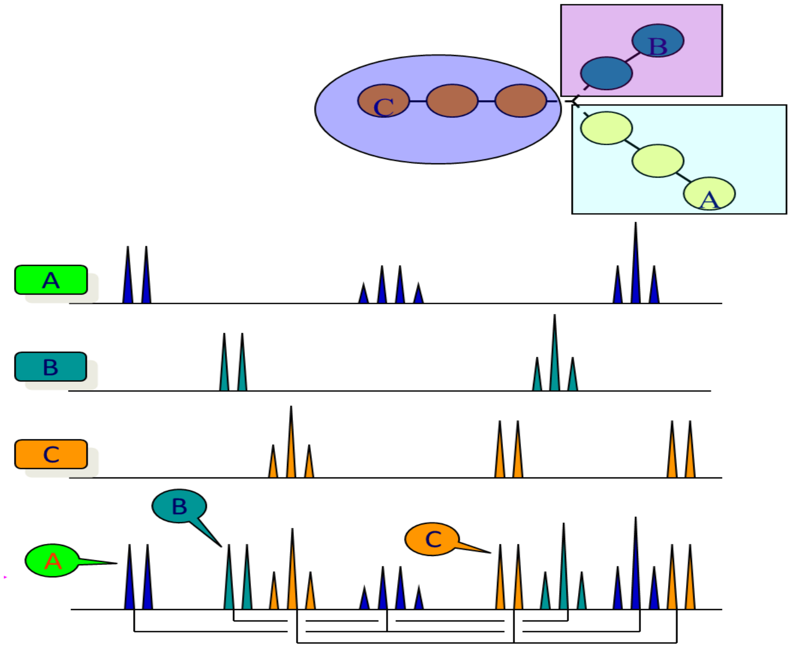
Let us say, this is tau m I am calling as a mixing time. I keep changing the mixing time. I keep on increasing the mixing time from some value, minimum value to maximum value as I go down. First, I will consider five spins are coupled among themselves. For a short mixing time here, there is exchange of energy between A and B. I will start with A and then it will give energy to B. But simultaneously after sometime, it will also start coming back from B. So, between A and B there is an exchange of energy for certain mixing time. But what I do is, I increase the mixing time to some other value, little bit more. Let us say from 5 millisecond, I go to 10 milliseconds or even more. Then what happen? Not only from A to B, then B also gives to C further, to the next coupled partner it will give. B will give it energy to, C will give it back to B again, and again B will give to A. There is a cyclic phenomena, A to B, B to C. Then again C to B, B to A. This keeps on going like this. A cyclic process is there. And again increase it for some more time, from C, it goes to D. So, A to B, B to C, C to D it goes on. And then finally, ro all the coupled spins, the last coupled spin also will get the magnetization. What is happening here? Depending upon the mixing time, the propagation of the magnetization takes place. It keeps transferring the magnetization from one spin to other one depending upon its coupling strength and also the duration, because it also depends upon the coupling strength. If it is larger, there is a greater transfer of magnetization. Let us say this is 10 Hz, magnetization transfer may be more. Let us say this is only 1 hertz, then it is weak coupling. As a consequence, transfer of magnetization may be less. So, the extent of magnetization transfer depends upon the mixing time, time for which you allow the spin system to exchange energy and also strength of the couplings. Both are important. So, this is how the magnetization gets propagated during the spin lock among all the coupled spins, coupled spins of a given spin system. This is what we should always remember, magnetization may travel in either direction in a spin chain, not always unidirectional. It goes, comes back, goes comes back like that, it is happening. So, there is a cyclic exchange of energy among them. So, what does it mean? If A goes to B and come back to A, it goes to C and come back to B and then to A. That means, if I take a TOCSY spectrum, it is symmetric. Magnetization from A is given to B means, B is also given to A. Similarly, A gives to B, B gives to C, again it comes back to A. So, that means, whenever you see the correlation, it is always symmetrical about the diagonal, similar to COSY.

TOCSY spectrum is also symmetrical about the diagonal. That is what you should understand. Next question is, when such a situation is created, when do the spins experience identical local fields? In the sense, they all behave, you know, as if chemical shifts are 0 and everything behave as if experiencing a particular local field. When does situation come? That will come under a condition called Hartmann-Hahn match. That Hartmann-Hahn match is given by this equation. Gamma of one spin with RF power of that multiplied by that RF power into gamma of the other one into multiplied by the RF power of the other spin.

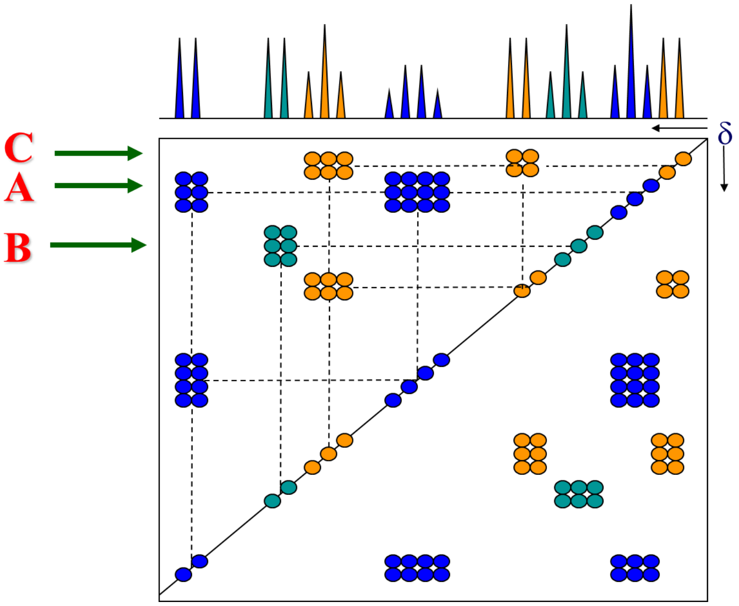
$$\gamma_A B_{1A} = \gamma_X B_{1X}$$

If this condition is matched, this is called Hartmann-Hahn match, then there is local field experience at A and B, both the nuclear spins are identical, then you are not distinguishing these two spins. Then only there is a transfer of magnetization. And for homonuclear case, this is called Hartmann-Hahn match, homonuclear Hartmann-Hahn. This experiment is called HOHAHA, homonuclear Hartmann-Hahn. Same if you consider the heteronuclear spin, identical experiment can be done. It is called heteronuclear Hartmann-Hahn called HEHAHA. These are the two experiments. This is nothing but the TOCSY experiment for homonuclear spins and TOCSY experiment for the heteronuclear coupled spins. And both will happen, magnetization transfer takes place only when there is a Hartmann-Hahn matching. Now, always you should remember, I have been telling you, transfer of magnetization take place only among the coupled spins. But no transfer of magnetization among different spin systems. Please understand, if there is a group of spins coupled here, several spins, there is only exchange of energy among them. And if there is another spin system, there is exchange of energy among them like that. But between these, there is no exchange of energy. That is not possible. So, the TOCSY does not allow that. So, how do you imagine that?

Imagine it is a 400 x 100 meter relay going on. What is happening? Each country, you can consider the spin system. For example, you look at this one, this is Great Britain. This is another one, it is Japan. Then you have Jamaica like that. What is happening? See this Netherlands fellows, they form one spin system, Jamaica one spin system, Japan one and Great Britain one spin system. They are all running. But remember, they are carrying a baton. What is baton? In NMR spin system, I call it as energy. Baton is nothing but energy. So, this spin can give energy to this fellow, can give baton to him. He has to give it to him to complete the race. So, he will give baton to him. Similarly, Japan fellow will give baton to him. But he cannot give his baton to this fellow. That is not allowed. Inter spin exchange is not allowed. So, this is exactly the comparison for transfer of magnetization transferamong the coupled spins in a TOCSY experiment. Each of them, if you consider as one spin system, there is a transfer of baton among their teammates like among all the spins of the same coupled spin system. But there is no transfer between them. This is how TOCSY works. And the spectra of three groups of different spin systems, I can show, as an example. I will consider yellow is one spin system, red one system and green one spin system. There are three different spin systems. Let us say this A group

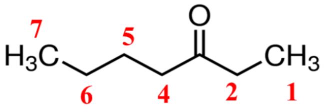


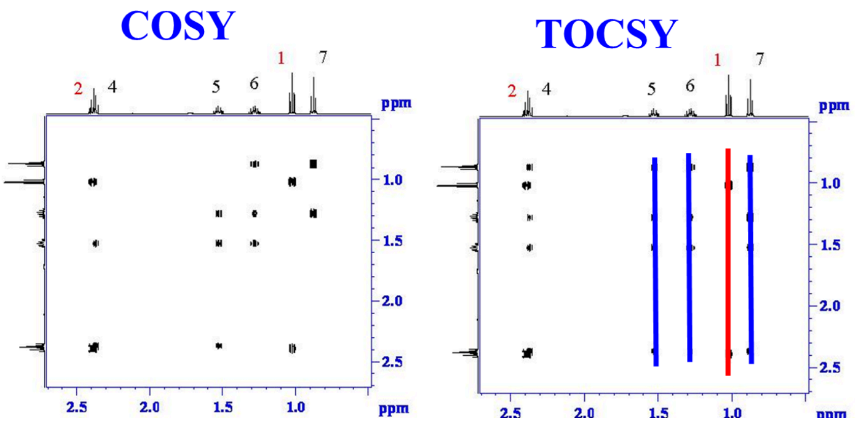
I do not know. We will not worry about the interpretation. Hypothetical spectrum, let us not worry about valence bond matching, whether reality of the existence of the molecule, all those things. This is a hypothetical spectrum for spin system A. There are three protons, there is coupling among them and this is the pattern I get. Now, we will go to B. The B has two protons and two groups of protons are there. That is for B. Go to C. There are three groups of protons and three chemical shifts and three groups of peaks. But in reality, how, what do you get when you record NMR spectrum of this molecule? You do not get this, all of them individually. All of them are superposed and you get a spectrum. This is a conventional spectrum you get for this molecule. If you take the NMR spectrum, this is a conventional spectrum. So, what we will do is, I will take this molecule and run a TOCSY.



What it does? TOCSY also has a diagonal exactly like COSY. Here all the peaks on this diagonal matches with the one-dimensional spectrum, similar to COSY. Remember, I told you in COSY, the diagonal spectrum, diagonal peaks correspond to one-dimensional spectrum. Here also, the diagonal correspond to one-dimensional spectrum and that entire TOCSY spectrum is superposed here. Whether you take projection this way or this way, it does not matter. Both are same because it is symmetric. So, now I have taken the projection here. What we will do is, we will carefully look at it, how to interpret it. Let us say there are three different colors for three different spin system. I will start with this orange color or yellow color, whatever that color is. Now, there are two peaks here on the diagonal. Come down, you will see four peaks here, coupled to something and then come down. There are three groups of peaks to which it is coupled to. From here, come down, go here horizontally and you see a peak here. And you can complete one square. Continue further, come here, come here, go here and complete one square and this is also another one. So, this, this and this, we can say are coupled among themselves, because all of them form a coupled spin system. See, you can find out this is one spin system, this is another coupling and entire spin system is coupled. Fantastic. All these spins which are coupled among themselves in a spin system are giving rise to cross peaks here. All the three of them, see starting with this, go continuously and you can say this, this and three are coupled. If you want to identify, go here horizontally and this one coupled, go horizontally this one. So, these three see similar color, they form a spin system. Go to the next one, blue. Blue, there are three groups here, one, two and three and it comes here, it is coupled to this and come down here and here and go here and is coupled to this. So, this, this and this are coupled. What is the left over? Green, start with this and then go here, it is coupled to this, does not matter. There are only two things, it is coupled to that. So, very easily you can identify. See, green, this to this and this to this. Only two spins are there. Similar to COSY, you can see the coupling pattern. Exactly, this is the way yoy have to interpret a TOCSY spectrum. So, what do you understand? Just go vertically like this or horizontally like this, that is all. You can identify all the coupled partners in one experiment. What COSY does? Where you have to go in systematic manner? Instead of that, simply draw a vertical line, you will identify all the coupled partners or go horizontally, that also tells you. You can identify all the coupled partners in one simple TOCSY experiment. So, that is what happens and you can identify everything easily. So, the corresponding to spin systems A, B and C have been identified. This is like unraveling the overlap.

I will show you the comparison of the TOCSY spectrum of a molecule 3 heptanone with COSY. What is the advantage of TOCSY with COSY? You will identify. This is a TOCSY spectrum. What is the TOCSY? I told you there are two spin systems here. Remember, we analyzed the 1D NMR of this, also COSY of this one. And CH3 is coupled to CH2 and there is in between, another group and this and this are not coupled.





So, there is a breakage in the coupling. I told you, the continuous, all the spins, the group of spins coupled among themselves without any breakage in the chain. A group of unbroken chain with a coupled spin system, all the spins form a coupled spin system. Here, there is a breakage in the chain. So, this is one spin system and these four form another spin system. So, there are how many spin systems here? In this molecule, there are only two spin systems. So, if I do a TOCSY from here, here, here, here, first this gives energy to this, this to this and this to this. The magnetization transfer takes place among all these four groups of protons and again for these two protons, there is a magnetization transfer taking place. So, all you have to do is sit on a diagonal, draw a vertical line and find out all the coupled protons, start like this. Now, this is a group, only there are two peaks here. Start here, come here, go here and then go here, completing this square. How many, there are only two things which are coupled here, whether you go vertically or horizontally, you will see, see one and two, only two things are coupled. Of course, you can also go horizontally and find out, only two are coupled, because \spectrum is symmetric, whether you go vertically or horizontally, it does not matter. You can still identify all the coupled spins in one experiment. So, you identify one group of coupled spin system. What next? Start with the other one. See, it starts from here, draw a line, it comes through four cross peaks, go horizontal again like this, like this, like this. There are four such things. So, there are four vertical lines you can draw. Each one, does not matter whether any one of them will do to serve your job. But I am showing you, each of them can identify all the four coupled protons of the same spin system like we saw here in 2D case. Here also same thing. So, all the coupled protons have been identified. The simple TOCSY experiment identified two spin systems. Is it not a better thing? Compare this with the COSY spectrum. What was the advantage here? In the COSY, what did we do? TOCSY identified both the spin systems like this, whereas in the case of COSY, this was easy, you couldnot identify in one shot. For the other thing, what you have to do? Start with this, there is another peak here, start with this, go up, again go up, go up, go up. You have to go through like a ladder. Here, there is no such phenomena, just one vertical line or one horizontal line identify all the coupled spin system. This is the beauty of TOCSY. You see the difference? This is called a relay transfer. COSY directly transfer the magnetization between immediately coupled neighbors, whereas TOCSY does this like a relay, that is why I gave the example of the baton passing in a running race. It is a relay. So, one spin gives its energy to other one, that gives to the next, next, like that and a relay goes like this. See, this is a 500 MHz COSY spectrum, where you can see this forms one spin system and then this is coupled to this, this is coupled to this, this is coupled to this and like this. You have to go like that. Same thing if I do a TOCSY, this is what happens. Very easily, in one shot, I can identify all these things. Here, I have written a number here, 2, 3, 4, 5, 6. All of them are coupled, 2 is coupled to 3, 4, 5 and 6, everything is coupled here. Same thing you can identify here, but you have to go in a stepwise manner. It is like a step function, we could keep going. Whereas TOCSY identified everything in one shot. This is the advantage of TOCSY. I gave you several examples. And now the time is getting up, what I am going to do is, I will stop here. We will continue with the TOCSY bit more in the next class and then we go to heteronuclear correlation, another experiment where we can correlate different nuclei. Here, so far COSY and TOCSY, we have been doing only for homonuclear spins. TOCSY also I showed, only in homonuclear case. Both are comparable, both have diagonal, both are symmetric with respect to diagonal, cross peaks are symmetric. In both the cases, diagonal will correspond to 1D spectra, everything is identical. Only thing is in the case of COSY, you have to go in a stepwise manner to identify all the coupled partners. Whereas in the case of TOCSY, one row, you just draw a vertical line, one row or one column, whatever you do, you will identify all the coupled partners. And we took one or two examples. I will stop here, we will continue with this further. Thank you very much.