One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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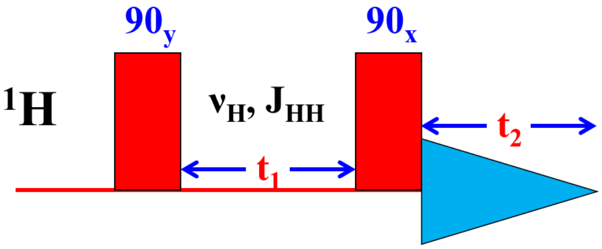
Lecture 39: 2D-COSY-I

Welcome all of you, since the last class we started discussing about two dimensional NMR. In the last week I introduced to you about what is 2D NMR, why do you require 2D NMR and I said clearly, if we have a pulse sequence designed with N time periods out of which N-1 are incremented and 1 is constant and we have N time periods, you do N dimensional Fourier transformation, you are going to get N dimensional frequency spectrum. So, we can have 1D, 2D, 3D, 4D etcetera and I also explained to you about the limitations of these experiments, time constraints and the size of the molecules. Both we discussed. And of course, for 1D and 2D spectrum you can get may be few minutes you can get these days in 25 to 30 minutes, or for 3D may be few days, for 4D may be few weeks, it goes like that. And you know it becomes very lengthy as the experiment becomes longer, when you go to higher and higher dimensions. And also it depends upon the molecular size. I also explained to you how depending upon the molecular size and type of information you want to derive, you can even decide what is the type of experiment you want. Whether you want 2D, 3D accordingly you can plan it. And I also explained that 2D can be broadly classified into 2 divisions, one is correlated type experiment, and the other is resolved type experiment. Both can be homonuclear and heteronuclear. And I explained withs lot of examples. I gave examples, about a homonuclear experiment like COSY, TOCSY etcetera, heteronuclear experiment like HSQC, HETECOR, HMBC etcetera, varieties of experiments are there. And any 2D experiment I also explained to you, as how do you interpret the 2D data, if you have a particular peak either on the diagonal or a cross peak. So, depending upon how the spins evolve in the t1 and t2 dimensions, and how the magnetization is getting transferred from one spin to another spin with which it is interacting, accordingly we will have the peaks in the t1 dimension and t2 dimensions. So, this what we discussed and we also saw simple examples of spectra of 1D, 2D, 3D etcetera. Varieties of spectra, the appearance of spectra, etc. So, with the basic introduction to 2D NMR, in general multidimensional NMR, from now onwards we will start going to specific experiments. Today I am going to discuss about what is called 2 dimensional correlated spectroscopy, also called 2D COSY. The COSY means correlated spectroscopy. And the requirement for this type of experiment is that the nuclear spins should be scalar coupled. There must be an interaction between the nuclear spins, J couplings must be there. So, let us see what is the 2D experiment, how it works, we can start discussing.



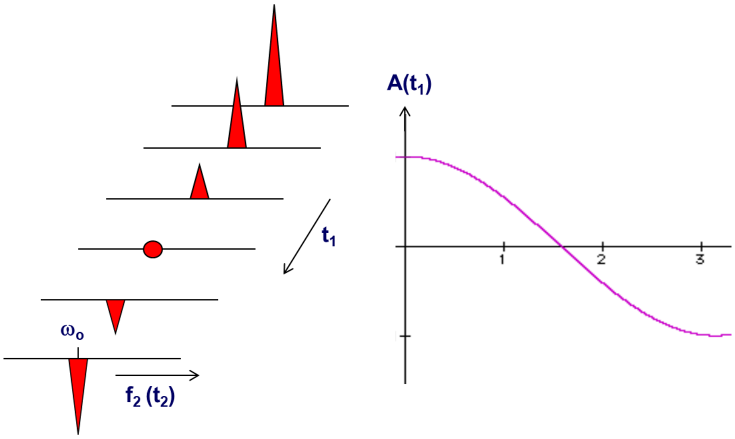
Of course, COSY experiment as I said is a very simple 2 pulse sequence. A I told you for any 2 dimensional experiment we have a preparation period, evolution period, mixing period and the detection period. And preparation period is the one where the nuclear spins are allowed to prepare, attain thermal equilibrium. Then you apply a radio frequency pulse, 90 degree pulse. During this period the nuclear spins evolve under chemical shifts and J couplings of spins. And afterwards you apply 90 degree pulse. That is a detection pulse. Start collecting the FID at a constant time period, that is fixed time. And both these pulses are 90 degree pulses. Of course, you can apply X or Y pulses depending upon your choice and this is a detection period, this is evolution period and this is a preparation period. Here of course, it is a very simple 2 pulse experiment, you may ask me where is the mixing period here. There is no mixing time, this itself you can consider as a mixing time. In fact the mixing time is absent here. So, in general there are 4 periods here, I told you already mixing period is optional we do not need to have a mixing period, we do not have it here. In principle some people will explain the second pulse itself as a mixing period, it does not matter. So, this is the two dimensional experiment simple pulse sequence. Remember COSY is a simple 2 pulse experiment.

$$CH_3–CH_2–CO–CH_2–CH_2–CH_2–CH_3$$

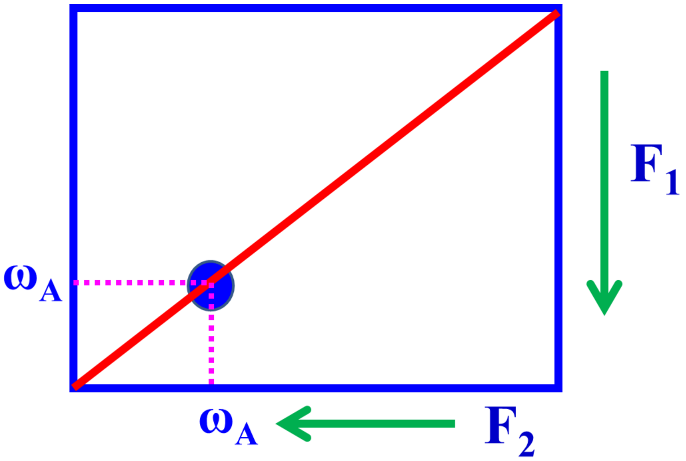
b f e d c a

What COSY gives? It is the connectivity among immediate coupled partners. Of course, it can also give long range interactions, but immediate coupled partner we can see. For example, you have a molecule like this a chain. The chain is broken somewhere here assuming that there is no interaction between this CH2 and CH2, then if I do a COSY experiment, COSY tells me this is correlated to this, you will get a correlated peak that tells me this CH3 peak is sitting next to CH2. And I get a correlation from this to this, telling this is sitting next to this. And also it gives correlation to this saying that this is sitting next to these two. Similarly this gives correlation to this and this, telling it is sitting between these two, this is sitting here, it correlates to this alone saying that this is isolated. Similarly, we can get the correlation between this and this very easy and interpretation of the 2D COSY spectrum is pretty simple. Please remember COSY gives you connectivity pattern among immediate coupled partners. Let us see how the COSY pulse sequence works. This is something about conceptual understanding that is needed.

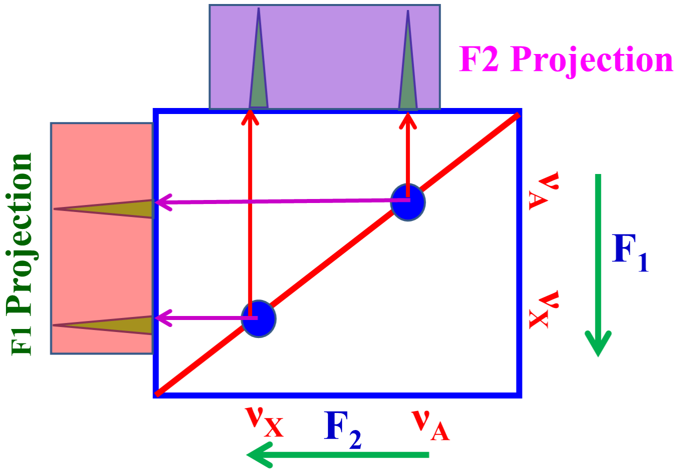
We will start with a 2D COSY for a uncoupled single spin, one proton which is uncoupled to anything else simple example is $CHCl_3$ molecule. We were looking at the proton we will not worry about the natural abundance carbon, which is one percent abundance, and assume that is not coupled to proton. So we are having a single proton one spin uncoupled to other spins. Of course, it would not couple to chlorine that is that is what I want to assume. So and we will start with the 2D COSY spectrum of a single spin. Let us see how this thing works. This is a usual pulse sequence which I already told you. First we are going to apply a 90 degree pulse. What does it do? we have been discussing. The 90 degree pulse tilts the magnetization to xy plane. Depending upon whether you are applying pulse along this axis or this axis, it will tilt it to other direction because all the three are orthogonal to each other. That is what we have been discussing. So first 90 degree pulse tilts the magnetization to xy plane. What will happen afterwards? The magnetization starts evolving during this time. This is a free precession time during which it evolves according to its chemical shift because there is no coupling here, it is single spin. So it has evolved according to its chemical shift. All right, after a time period t this proton would have moved to some distance. The vector has moved to some distance that we have already been discussing. It goes for $\pi*\upsilon*t1$. The t1 is the time you allow for the spins to precess in the xy plane, and how much it has moved is given by this expression. All right, now once you start moving like this, for example somewhere here you can resolve this into the two components along this, the x axis and y axis, and we have cosine component here and a sine component along x axis. So magnetization can be resolved at two components. This vector, the cosine component is along y and the sine component is along x axis, all right. What does the second pulse do ? What is happening to the vector during the period t1 after applying 90 pulse? the second pi/2 pulse acts only on the y component, that is what is happening. And the x component is not at all affected and it continues to precess the way. It is processing and it is not affected. Only y component of this is getting affected. Then what happens? we will wait for some time and collect the signal, do the Fourier transformation of this. It produces a spectrum with a single resonance line. Where is a single peak? because one proton is there no other coupling, $CHCl_3$ molecule I have taken, only one proton and it gives you one chemical shift, one peak. What is the intensity of this it depends upon how far it has rotated, how far it has precessed. We take the component of this along the detection axis, y axis, the cosine component will consider. And let us consider one peak, we will measure the intensity of this peak which varies as a function of $\cos\theta$. I told you it is the function of this time period, keeps on varying as given by this expression, the amplitude of the signal as a function of t1. How it varies is given by this expression. What we will do is take that peak and start measuring the intensity of this as a function of t1. I keep on varying the t1.



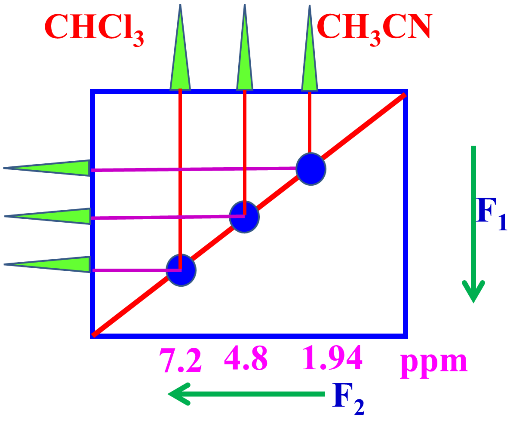
Initially the signal is let us say negative intensity, comes down becomes 0, goes positive and after some time it keeps coming down like this. So it starts like this and then becomes 0 and then goes up like this. It starts oscillating, it becomes a oscillatory function. Essentially what we did by doing this variation of the varying t1 you could build a pseudo FID. That is what I said in the general introduction when we were discussing about 2D NMR. We can build a pseudo FID in the F1 dimension. At longer times what will happen? the signal intensity completely diminishes, why? because it is also relaxing. It is oscillatory, at the same time decaying exponentially. At longer values of t1, you keep on waiting for a long time, the signal intensity becomes 0. That is what we saw in the FID, you keep collecting, and after a long time there won't be any signal at all. The maximum signal is during this period, keeps decreasing with time. So after a long time there won't be any signal, completely diminished now. We will do the Fourier transformation in the t2 domain. What you will get? we get a frequency data because we are collecting a signal for a fixed time period that is called a f2 period or also t2 period. There is also data in the other time domain. collect the data in the f2 domain or the t2 period, and also time domain data in the other t1 period. So both are there. What I am going to do is, I will do the Fourier transformation of the FID in the t1 domain. I can do individually the Fourier transformation in both the domains. For the FID collected during t1 if I do the Fourier transformation I get the frequency F1, this gives the frequency in two dimensions. When I do the Fourier transformation, in the f2 dimension I get omega 2. When I do the Fourier transformation in the t1 dimension I get omega 1. Now the 2 Fourier transformation in both the dimensions gives me spectrum in two dimensions, omega 1 comma omega 2. In both the dimension I get the frequency spectrum, true. I am collecting the FID both in t1 period and t2 periods, I am doing the Fourier transformation in both t1 and t2 periods, I will get a frequency spectrum like this. Where does the peak come after the double Fourier transformation. This gives a peak where the two signals intercept like this. It comes exactly on the diagonal, because this signal whatever was its frequency in the t1 period continues to remain the same in the t2 period also, because there is a single spin, and there is no way it can interact with any other spin. So it has not given its magnetization to any other spin, there is no transfer of magnetization. During the t1 period it has some frequency ωA, it remains same in the t2 dimension also, getting unaffected. As a consequence we get a peak on the diagonal. If from this peak sitting at the top come down vertically you get one frequency, go horizontally you get another frequency. This ω_A. The frequency is identical in both the dimensions, that is why it is coming on the diagonal. This is what happens. So if I take a small molecule, only single spin, a single proton not coupled to anything else and do a COSY you will get only one peak on the diagonal, that is what you should understand



Let us take the example of two peaks instead of one. We will take the example of two peaks that is A and X, and now I am putting a condition that they are uncoupled spins. What do you mean by uncoupled JAX is 0, coupling is 0. I assume there is no coupling but the chemical shift of A and the chemical shift of X both they are different, the chemical offset frequencies are different. Let us see what happens to this type of molecule, the two uncoupled spins.



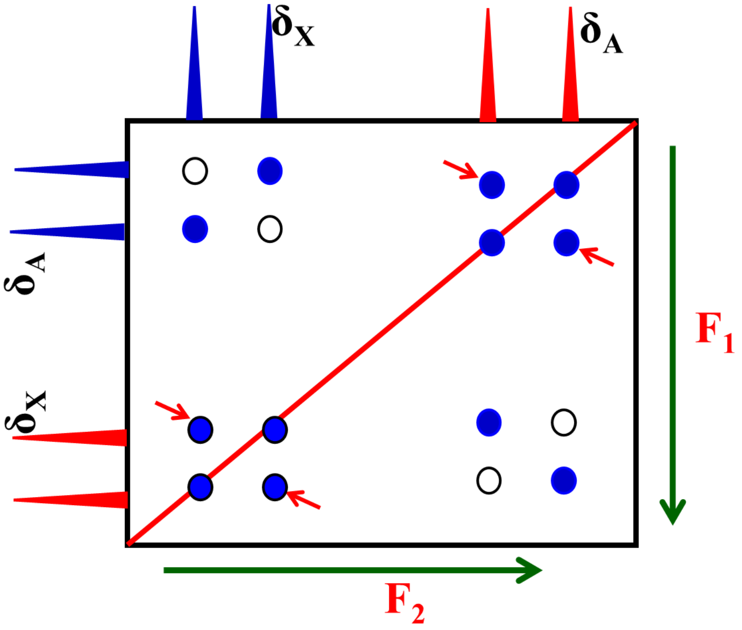
In the COSY pulse sequence when I do the Fourier transformation of these two uncoupled spins, if I take the frequency A, it remains same in this axis and also in this axis. Take the peak here go horizontally I get a peak go vertically you get a peak and this frequency and this frequency is same. So if I say this is υA correspond to frequency A or chemical shift correspond to A. This also the chemical shift correspond to A. It is a diagonal peak. Now come to this peak, again from the peak sitting at the peak go horizontally you get a peak, go vertically up you get a peak. And this is frequency X that means this also X this also X that means when I have a diagonal peak its frequency remains the same both in the t1 period and in the t2 periods. Its frequency is identical whether you measure in the f1 dimension or in the f2 dimension. That is what the peaks which are coming on the diagonal in a two uncoupled spins COSY spectrum. We have two peaks coming on the diagonal and vertically go to along omega 2 axis or along omega 1 axis you get the identical frequency for both of them, and this is what it is. And if I can take a projection of this spectrum you get two peaks like this. I can take the projection on f1 also. Then also I get two peaks that means whatever you take, a one-dimensional spectrum of these two uncoupled spins, two molecules let us say water and CHCl3, which are not coupled at all, they are two different molecules. Take the NMR spectrum, the proton NMR spectrum, we get two peaks one for CHCl3 one for H2O. So that they will be on the diagonal, take the projection along this axis you get two peaks one for CHCl3 one for H2O here. The same here also same peak CHCl3 for this if you take horizontally if you draw a line this also CHCl3 peak and this is water peak. If you assume this is CHCl3 and this is water this also water peak, this is also water peak this also CHCl3, this also CHCl3. That means if I take the projection I can take it along f1 axis or f2 axis does not matter, when I took uncoupled spins we get the spectrum, on either dimension you take the projection, it is nothing but a simple one-dimensional spectrum. And what do you understand from this for uncoupled spins the peaks always come on the diagonal. And if you take the projection of these uncoupled spins if you take the projection along f1 or f2, it correspond to simple one-dimensional spectrum, where you get single peaks.



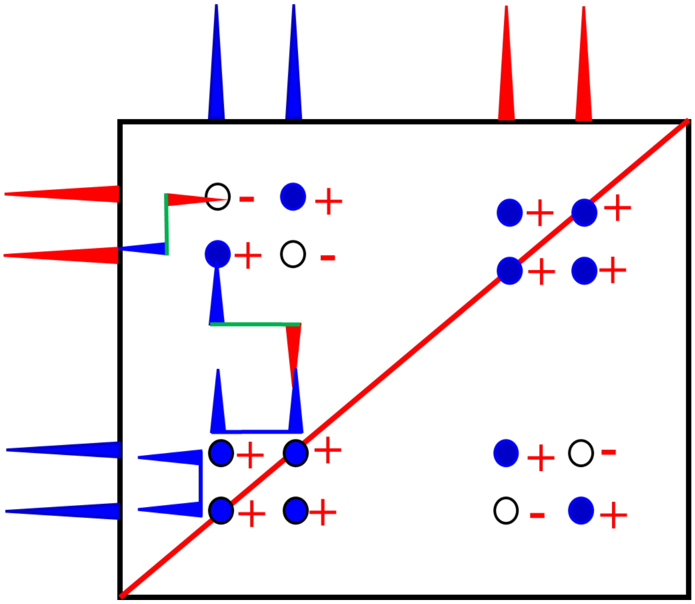
Now let us see what happens in a realistic example. I take three solvents CH3CN, H2O and CHCl3. All the three I take the COSY spectrum. None of them are interacting, they are all three different molecules. The CHCl3 comes here, H2O comes here, CH3CN comes here. One is close to 2 ppm, one is at 4.8 and other is 7.8 ppm. I get three peaks so three peaks will be there on the diagonal. You take any peak on the diagonal go vertically up go horizontally you hit a peak and that correspond to peak which is CH3CN coming at 1.94 ppm. Go to the next one, go vertically go horizontally you hit the same peak and in both the dimensions that correspond to what will be coming at 4.8 ppm. Identically you can talk about CHCl3 also. That is how it is. So if you take the spectrum of three solvents which are non interacting, then you get three peaks on the diagonal that is what it is. You simply understand the logic, if I take the two dimensional COSY spectrum of uncoupled spins, they always appear on the diagonal. So take the projection of that frequency in f1 axis or f2 axis, it remains same. It pertains to simple peak corresponding to what you get in the one dimensional spectrum.

All right, with these we will go further. We will try to understand the COSY spectrum of two coupled spins. What happens? In the same example, earlier in the couple AX system, I set JAX as 0. But now I make it nonzero. Let us see what happens. If it is nonzero exactly A and X are coupled with a coupling constant J_{AX}, and I have the chemical shifts, nu A and nu X. Fine we will understand what is happening with the pulse sequence. First what the 90 degree pulse makes the magnetization associated with the spin A? I will talk about only one spin right now, for one spin A. What happens to spin X, it should be identical because the interactions in NMR are mutual. We will understand what is happening to spin A.The 90 degree pulse makes the magnetization associated with spin A to precess during t1 at its chemical shift nu A, because it has a chemical shift nu A. This is the free precision period. After the application of pulse you are not doing anything. The spins are free to precess also. It has coupling that one thing but what will happen when it is precessing at this frequency and this frequency. It produces a frequency peak at this frequencywhich is same in both the dimensions nu A. I am considering only nu A like assumed if it is uncoupled, there is only one chemical shift it gives a peak which is at nu A, this is what we observed in the previous slide for uncoupled spin. The frequency remains same in both mega 1 and omega 2 dimensions. So it gives a peak. What do you call this peak? this is a diagonal peak. That is what we observed. so nu A because it is precessing at its own frequency in both the dimensions, in both t1 and t2 period it gives a diagonal peak, which correspond to identical frequency in both t1 and t2 periods. Further what happens? the second 90 degree pulse transfers part of this magnetization to the coupled X-spin. During the process here spins are interacting it will transfer part of its magnetization to coupled X-spin. That is a polarization transfer or what is called magnetization transfer. That takes place and what will happen to this transfer magnetization? it gives it to which spin? it gives the X-spin. A spin gives part of its magnetization to X-spin, why? because they are J coupled, there is a scalar coupling between these two. So it will transfer part of its magnetization to X-spin. Then what will happen to that? This X-spin not only precess at its own frequency, also it precess at the frequency of A so that now X-spin will precess at both the frequency of A and also the frequency of X. That is what happens, so then it will give me a peak corresponding to two different chemical shifts in two dimensions. That means it gives peak at (nu A, nu X). That means I get a peak, remember we understood how to interpret the 2D spectrum. If you get the peak go along omega 1 dimension you get a frequency corresponding chemical shift of A, go along the other dimension you get the chemical shift of X. Both will be there, it will get you will get the peak which correspond to two different chemical shift in two dimensions. All right, what do you call this? This is called a cross peak the chemical shifts are identical (nu A, nu A) it is diagonal. But nu A the spin A also gives a cross peak in addition to diagonal at frequency (nu A, nu X) which correspond to cross peak. Okay for example it is like this. It gives a peak here nu A and also gives a peak here let us say this is here and this is X so nu A gives a peak at its own frequency on the diagonal and also gives a frequency at X, understand. It gives two different chemical shifts in two different dimensions. And this peak is called a cross peak. But the one sitting on the diagonal is a diagonal peak. So nu A gives a cross peak at the frequency (nu A, nu X), meaning along the chemical shift of A, go horizontally you get a peak here, come vertically down you get a peak here. This corresponds to chemical shift of X, this corresponds to chemical shift of A. That is what it means. So it gives you a peak cross peak, where in one dimension you get the chemical shift frequency of one, in other dimension you get the chemical shift frequency of the other, called the cross peak. So the precession of magnetization during t1 and t2 period for spin A are not identical. They are no longer the same frequency. See this Spin A in the t1 period is at frequency A, in the t2 period it has frequency (nu A, nu X). So we have two frequencies for this peak, one comes on the diagonal which comes at the identical frequency of its own in both the dimensions. And also you get a cross peak which comes at the frequency of its own and also a peak at the corresponding frequency of the X spin frequency. That means you get a diagonal peak which correspond to chemical shift of A in both the dimensions and also get a cross peak which correspond to chemical shift in one dimension of A in one dimension pertains to chemical shift of X in the other dimension. This how you are going to get peaks for the A, Okay then I will ask a question. What is going to happen for X spin? we are discussing about A spin. Please remember I was telling you interactions in NMR are always mutual. If A is interacting with the X and giving its magnetization to X, same thing happens. The X also gives to A. That means the X spin behave identically, it evolves at its own frequency in t1 dimension and also at its own frequency in the t2 dimension. That means it is a peak at (nu X , nu X) which is a diagonal peak. And also nu

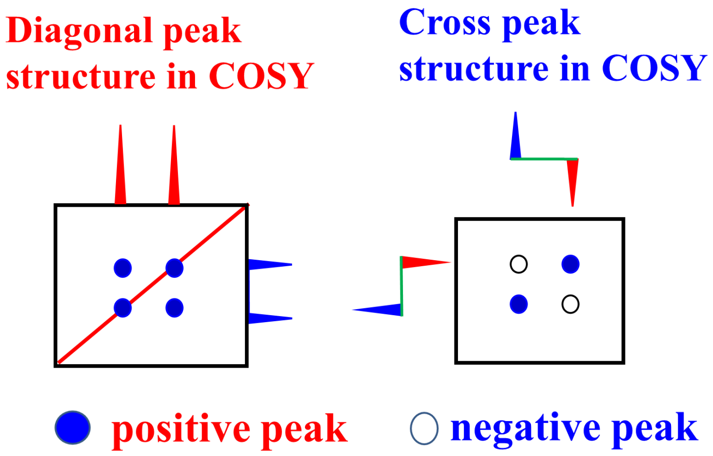
X in the t1 dimension, it gives a peak at its own chemical shift, plus in the t2 dimension it gives a frequency at chemical shift of A.



You understand, very interesting thing A give a peak at its own frequency and a the frequency of X. Similarly X will give a peak at its own frequency and also at the frequency of A. What does it mean? It means A will give rise to diagonal peak at its own frequency, X will give diagonal peak at its own frequency. A will give a cross peak at X, and X will give you the cross peak at A. So this is what it is. We have diagonal peaks pertaining to both the spins and this is the pattern we get in the COSY. What is that pattern we will see. The diagonal peaks will have four peaks in the square pattern. Why it comes? If I take this one diagonal peak for let us say this is a chemical shift of A, it will have four peaks in the square pattern. Why four peaks? because A is split into a doublet due to X. Similarly X will have four peaks in the square pattern. The diagonal peaks have fine structure of four peaks. And this separation correspond to J_{AX} and this also correspond to J_{AX}. Identically for this also. So the cross peaks also will have the fine structure similar to this. Now A will give its own frequency as diagonal, it also gives cross peak at the frequency of X. This is also four peaks, these cross peaks. What does this will give? Again this peak separation will give J coupling. You understand now, what happens for X? The X also gives a cross peak to with A, not only at its own frequency, but also gives cross peak. So we will get four peaks for cross peaks, similar to square pattern. Basically what is that you are going to get is, you will get for diagonal four, four peaks, cross peaks four, four peaks. So totally you are going to get 16 peaks for both the spins put together. For both peaks one and two, the diagonal peaks are in-phase doublets, okay. We get four peaks, that is fine. But further I am going to tell you the diagonal peaks are in-phase doublets in both f1 and f2 dimensions. The center gives the frequency nu 1 and nu 2 and that the separation is JAX. What do you mean by in-phase doublet? that we have to understand. If I have a doublet like this, A is coupled to X it is a doublet, similarly X is a doublet. On both the frequencies like these are in phase. Let us say both are positive like this, this separation gives you a coupling constant J_{AX} and this is called in phase doublet. They are in-phase so both the dimensions I have four peaks. The diagonal peaks are in phase and if I take the projection both are in phase. If I take this projection both are in phase both are positive so both the dimensions they are in-phase doublets. What about the cross peaks? The cross peak always arises in COSY because of transfer of anti-phase coherence to anti-phase coherence. What is an anti-phase coherence we were discussing this during spin lock discussion and other the spin echo, and also INEPT. We discussed a lot about J modulation. At various places we discussed what is anti-phase. The anti-phase is like this.



The cross peaks are anti-phase that is one is positive other is negative like this you can get. This is called anti-phase doublet. And the cross peaks are always anti-phase in both the dimensions. For example these four cross peaks square pattern. If one is positive and there is negative in this dimension. Again here this is positive and then you go this is negative here so in this dimension it is positive and negative here positive and negative. This is how it goes. What it means? the cross peaks are anti-phase doublets in both the dimensions both in t1 dimension and t2 dimension or omega 1 omega 2 dimensions. Similarly diagonal peaks are in-phase doublets in omega 1 dimension and omega 2 dimensions. So this is the pattern and doublets are always out of phase by 90 degree that is what is called anti-phase here. So in homonuclear 2D COSY always the cross peak appear as pairs. In homonuclear it is always a pair and it is symmetric with respect to diagonal. Obviously, also logically it is true. When I have a peak like this in homonuclear case chemical shift in both the dimensions are identical because same nucleus we are considering in both the dimensions. And this is the diagonal. If this peak comes here and this is the diagonal peak, and if the cross peak comes here, cross peak comes here because interaction is mutual. So with respect to diagonal they are always symmetric. The cross peaks are always symmetric with respect to diagonal, and they appear as pairs. This is the important property of 2D homonuclear COSY. Remember it is homonuclear. For heteronuclear. it won't be like this, that is different. So COSY pattern of two coupled spins how do they look, is like this. If I have two weakly coupled spin system, I already discussed this in a proper nomenclature A and X spin what is the pattern you are going to get? you get doublet for A and doublet for X. This is the chemical shift of A, this is chemical shift of A, similar this is A and X in the same order. This is diagonal, this is f1 dimension this is always f2 dimension. I get four peaks here exactly like this. Four peaks of A appears here at its own frequency they are called autocorrelation peaks. A will correlate within itself because there are two doublets. This will correlate to this. This will give cross peak to this, this will give cross peak to this. So as a consequence you get cross peaks. And this gives cross peak between themselves they are called autocorrelated peaks. And similarly X also gives four peaks in the square pattern that means this is also autocorrelated peaks, this and this are correlating giving rise to cross peaks between themselves. Both A and X spins give autocorrelated peaks. Finally what is going to happen is A frequency will give rise to cross peaks at its coupled partner X. Okay, this like this. X also gets modulated because of frequency of A. It gives its energy or magnetization to A, X will give to A and A will give to X. They will give rise to cross peak like this.



As I told you they are in-phase doublets. All are positive here, anti phase doublet in this dimension. If this is positive, this is negative in this dimension. If this is negative, this is positive. That is what it means. So this is the COSY pattern you are going to get for two spins which are weakly coupled. so signs of diagonal and cross peaks are like this. If I say closed circle, it is positive, open circle means negative. That means here negative positive, positive negative, positive positive, positive positive. Both the dimension positive here, one dimension positive and negative in other dimension here. Also positive negative two peaks so they're anti phase character like this, plus plus minus minus like that. So if you want to plot the spectrum this will be like this. Both are in-phase in both the dimensions. Here one is positive or negative, both are anti-phase or cross peak in both the dimension. In this dimension also anti-phase, in this dimension also anti-phase. So, diagonal and cross peak structure can be understood like this. If it is a diagonal peak it is like this, if it is a cross peak it is like this. if I take the projection this is the cross peakd projection in both the dimension. This is the projection of the diagonal peaks in both the dimensions. So, please remember COSY diagonal peaks are in-phase in both dimensions, cross peaks are anti-phase in both the dimensions and this correspond to positive peak, this correspond to negative peak. The time is getting over, we will continue with the further discussion on the COSY in the next class. But so far what I discussed explained to you COSY is a simple 2-pulse sequence, 90 degree t1, 90 degree t2 that is all, a 2-pulse sequence. Preparation period is there, evolution period is there and detection period is there, mixing period is absent. I took the example of how the spins evolve. After 90 degree pulse the magnetization comes to x-axis, in the x-y plane it start precessing. It will have x component, y component, x component is one thing which goes keeps precessing it does not get altered, y component is the one which you are going to detect. It will have projection along x-axis and y-axis, the cosine and sine components. The cosine component is the one what we are anyway going to detect it and depending upon how much it has precessed, its intensity changes. I took the example of one spin which is not coupled at all, an isolated spin. I told you it comes on the diagonal whose frequency remains same in both the dimensions. Took two spins example, an example of two spins uncoupled gives two diagonal peaks, frequency remains same in both the dimensions for that. Then I took the example of coupled spin, then it I said for coupled spins we get diagonal four peaks of square pattern for both the A and X spins. Cross peaks also give four peaks in the square pattern, but I said diagonal peaks are in phase in both the dimensions all are positive signal. Whereas, the cross peaks are anti-phase in both the dimensions, one is positive other is negative in both the dimensions. And this is what we understood the structure of cross peaks and diagonal peaks in a two simple weakly coupled spin system. And we will continue further on how we can use COSY. In the next class we will discuss this. Thank you.