NMR spectroscopy for Structural Biology NS
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Lecture: 19
2-D NMR or 2-D Co-relation Spectroscopy: General Concept 3

So, we have seen the complications arising because of the active and passive coupling splitting's in the fine structures and we have seen that the plus minus nature is actually sometimes a disadvantage because there can be cancellations of the peak intensities. The component intensity is in a cross peak you may lose a cross peak. Therefore a strategy has to be designed that was designed in a particular experiment called as J scale COSY.

Where you increase the coupling constants along the F_1 dimension by using a suitable pulse sequence by designing a suitable pulse sequence and that is what is called as the J scaled COSY. So, along the F_1 dimension the J is scaled up.

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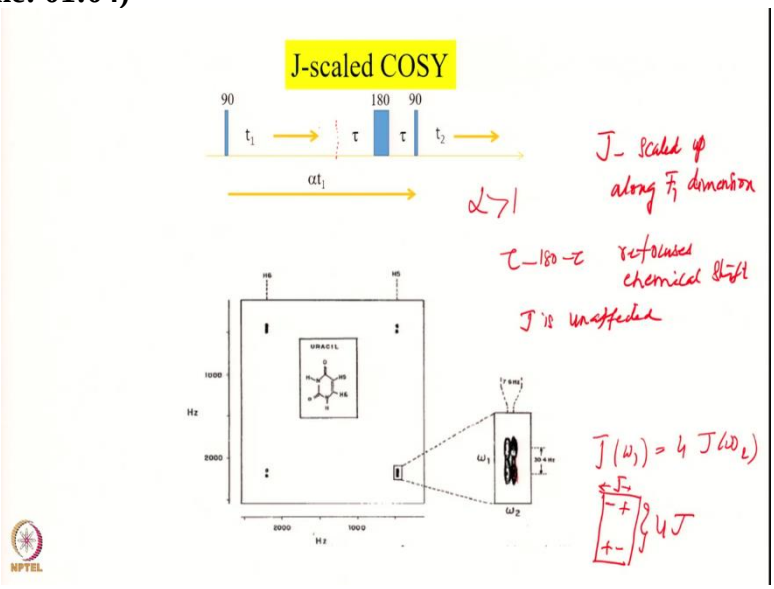

So, here the idea is scale up the J they scaled up along F_1 dimension. So, I will just you show you the spectrum first initially look at the spectrum of a simple 2 spin system this is uracil here uracil is a 2-spin system which has 2 protons H5 and H6. So, there is a one diagonal peak and one cross peak. So, I notice here we have the fine structures in each one of these the blow up of this peak is shown here in a particular peak that is the cross peak shown here along the ω_2 or the F_2 whatever that is.

So, F_1 or F_2 what we call it F_2 dimension the splitting is 7.6 hertz that is a normal coupling constant coupling constant between H5 and H6 is 7.6 hertz now along the F_1 dimension or the omega one dimension the separation between the components is 30.4. So, 30.4 means this is 7.6 into 4, 4 times it is scaled up right. So, therefore here J along $\omega_1 = 4J$ along ω_2 this is scaled up right.

So, 4 times 4 into that is $4J$ ω_2. So, this is achieved by design of a pulse sequence you manipulate your experimental sequence in such a way that the coupling constant appears scaled up along the F_1 dimension and that pulse sequence is shown on the top there. So, use the normal

thing is you have a COSY experiment where you have 2 pulses 90 t_1 90. But now what you do is after the 90 you wait for a certain time t_1 let us say until up till here.

This is your t_1 period you introduce additional delays additional a sequence tau 180 τ sequence is like a span echo sequence like the spin echo sequence you introduce in such a way but the total time between the two 90 degree pulses is alpha times t1 and here $\alpha > 1$. So, the coupling constant is evolving during the t_1 period for the period alpha times t_1 whereas the chemical shift is evolving only for the t_1 period because the τ 180 τ refocuses chemical shift.

So, what it does τ 180 τ refocuses chemical shifts whereas the J is unaffected J is unaffected therefore J continues to evolve for the period alpha times t 1 you can choose whatever is the Y value of alpha. You can choose α is equal to 2 3 4 5 whatever depending upon in certain situations recently I have noticed that people are using alpha of 30 and that is if you have extremely small coupling you want to blow it up it in such a large manner that you see the fine structure and the cross peak structure there.

And then you can measure the coupling constant very precisely. So, this is the demonstration that you have with this sound of a pulse sequence you can get ah higher resolution along the F_1 dimension. It was particularly important to do it along the F_1 dimension or the ω_1 dimension because that is where we have do not have enough resolution we are not able to collect that many data points along the t_1 dimension as you collect along the t_2 dimension.

T_2 dimension you can easily create 2048 or 4096 data points but the same is not possible along the t_1 dimension because it will increase your experimental time. So, much that it becomes quite impractical. Therefore here you artificially change the coupling constant by designing a pulse sequence. So, that a scaled up coupling constant appears along this one. Therefore in this one what will happen is you have you will have plus minus here but the minus plus appears at a much larger distance.

So, therefore this separation is 4 times J and this is just J this separation is only J therefore the cancellation here is much reduced and you will see a good cross peak intensity in your experimental spectrum. So, this is one strategy to get over this difficulty of cancellation of cross peak intensities. Now let us see some other experiment where you want to avoid the difficulties in crowded spectra.

What is the crowding that is happening here is here is an experiment I am showing which is called as omega 1 decoupled COSY.

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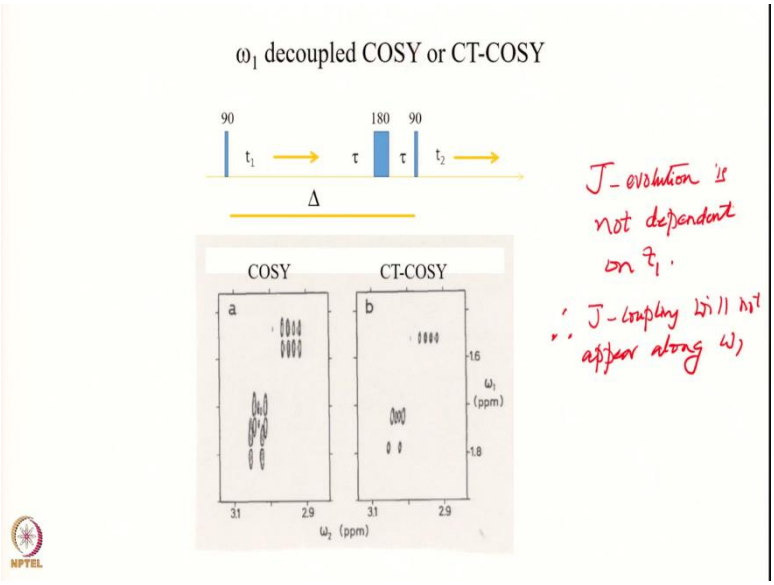

So, notice the pulse sequence here looks very similar to that in the case of J scale COSY except that this period which was earlier alpha times t_1 it is now a constant time period delta is a constant time period. So, from every F or every FID this period is kept constant δ which means as t_1 increases this starts decreasing the tau starts decreasing. So, in the previous case as t_1 increases tau also increases.

So, that you have the αt_1, $\alpha > 1$ therefore αt_1this keeps on increasing. The separation between the two pulses continuously increases because you have this whole period was alpha times t_1 here this period is kept constant this total period from here to here is kept constant. So, as t_1 increases this tau decreases here. So, that this period is kept constant what is the consequence the consequence is J evolution that happens all the way from here to here is not dependent on t_1 at all.

So, J evolution is not dependent on t_1 therefore what is the consequence J coupling will not appear along ω_1 axis. So, this is what we said whatever was the information during the evolution period will appear along the ω_1 axis or the F_1 axis whatever information is present along the t_2 period will appear along the ω_2 axis. Now you see if the J coupling does not appear along on 1 what it means it is equivalent to doing decoupling along the ω_1 axis.

It does not show this is an experimental spectrum to demonstrate that here. So, here is the normal COSY of a particular molecule does not matter what molecule it is you can see this one here. Here is a fine structure you are seeing a fine structure of a particular cross peak. Here also there is a fine structure but notice here there are two cross peaks here there are two cross peaks overlapping on top of each other quite close although they are different we can see that they are different there is 1 4 here there is another 4 here.

They are slightly shifted along the ω_2 axis this one is to the left compared to this one here but there is an overlap here. This overlap can actually cause cancellations of the intensities of the peaks which indeed that just happened how do we know this well. Now we do a decoupling experiment as I said that you remove the J coupling along the F_1 dimension along the ω_1 dimension this you are seeing 2 sets of peaks because of the coupling along the F_1 dimension or the ω_1 dimension if you remove this what happens.

So, you get only one peak one set of peaks which is at the middle of this which is appearing at the middle. So, therefore you have no coupling information along this axis but the full coupling information is retained along the ω_2 axis here. A greater benefit is seen here you see that overlap which was seen here is removed you can clearly see that there is one multiplet here there is another multiplet there.

Internally some cancellations have happened some internally within this multiplied in the second one some cancellations have happened and this is because of the plus minus character along the F_2 dimension and they are close by of course there is a cancellation there. Whereas in this case there is no cancellation some cancellation is there but still you are able to see the peaks in the middle and here the peaks in the middle are gone.

So, this is the reflection on the magnitude the relative magnitudes of the coupling constants which I was mentioning to you earlier relative magnitudes of the coupling constants changes the peak patterns and the peak intensities and you can clearly identify that there are 2 spins here. There are 2 protons fine structure 2 cross peaks here and there is one cross peak here and that cross peak find structure is looking like this.

Therefore this experiment is called as constant time COSY constant time COSY because this period from here to here is constant is kept constant and the because it is also causing a decoupling along the ω_1 axis it is also called as ω_1 decoupled COSY ω_1 decoupled COSY or constant time cross this is the advantage and you can create great benefits of this in a very crowded spectrum.

Now the next experiment which I want to explain to you is called as the total correlation spectroscopy. Now this is actually quite a significant advance because this will completely eliminate the plus minus character in the cross peaks this is the COSY here.

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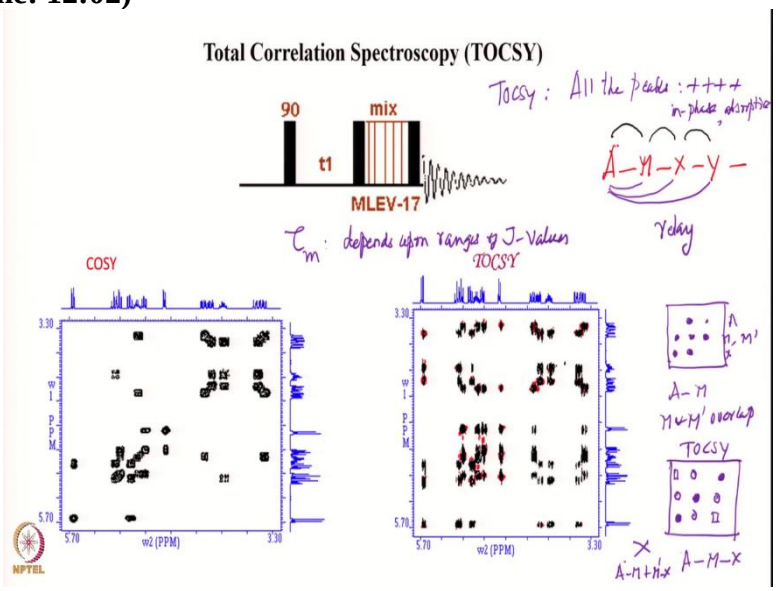

Now the TOCSY spec let us explain the. So, pulse sequence a little bit here. So, it goes in the semi you have the 90 degree pulse you have the t_1 evolution period. And the mixing now is not one pulse but is a series of pulses there are several we will not go into the details of those ones but this is the series of pulses which are nicknamed as MLEV 17. There are 17 pulses here we will not go into the details of those.

This is a particular sequence of pulses all of them 180 degree pulses the result of this is will produce a fine structure in the cross peaks which does not have the plus minus character it will only have plus plus plus plus character both in the cross peaks as well as in the diagonal peak and you will see many more cross peaks here because of the elimination of the cancellation effects and you will also have a relay.

What are the differences here? Now I am showing here comparison of the COSY spectrum and the TOCSY spectrum here this is called as total correlation spectroscopy because it shows many more correlations in a given spin system. Now here is the COSY which is the one dimensional spectrum of a particular molecule it does not matter what I do not know do not even know what this molecule is but some molecule which has a one dimensional spectrum looking like this and the same is present here as well.

And you have the diagonal which is reflecting this one dimensional spectrum and you have this cross peaks. So, what this cross peaks are telling you well this peak is coupled to this one here there is a cross peak here. Now here this one is this proton is coupled to 2 different protons one cross peak here another cross peak there therefore you are seeing this and this cross peak is this here again you see there is a cross peak between these two.

There is a cross peak between these 2 this is like the COSY pattern and this proton here has coupling to this proton here that is a diagonal is here of this one it is also coupled to this proton whose diagonal is here. Now we see this is also coupled to this one which is here. So, therefore these two protons they were the diagonals area they are coupled between themselves therefore there is a network of coupling which is indicated by cross peaks.

Now what we saw here that this proton is coupled to this one there but this proton is also coupled to this one here you see and this fellow has another one here. Therefore this diagonal peak is coupled to this proton and also to this proton. So, you see how we can identify and then you go from here to here you can draw and this proton is now coupled to not only to this but also to this. So, the network of couplings you can establish.

How the various protons are coupled in the COSY spectrum the nearby neighbours coupled in this in information you get here. And there is one single doublet here and that is this fellow this is coupled to this proton and that you are seeing this cross peak here and this one does not have anything else it has only one. Now this one however is coupled to something else which are these two here.

So, this is how we analyze the COSY spectrum analysis of the COSY spectrum in a complex system. So, you could not identify this thing from the one-dimensional spectrum this one-dimensional spectrum would not allow you to identify all these correlations which proton is coupled to which proton both are these are proton spectra all on both axis you have the proton frequencies. So, from the one dimensional spectrum you cannot figure out which one is coupled to what.

But in this COSY spectrum you can figure out the entire network of couplings by monitoring where the cross peaks are. Now in this case of course the resolutions are pretty good although the intensity patterns are different in different peaks and that is because of the magnitudes of the coupling constants which results in partial cancellations of the intensities of this peaks. Now what is the particular advantage here further. Now we notice here if we take the same proton this is the TOCSY you not only see these 2.

As we saw here those 2 you also see some others peaks there you also see some others there and why does that happen why does that happen? And that happens because of the following let me consider a system which is like this AMXY and. So, on linear system in the COSY spectrum I see this coupling separate cross peaks and I also see this coupling in the TOCSY spectrum what I see is a different colour in the TOCSY spectrum I see this I see this and I also see this all the three.

So, from A I will see a cross peak to M then to X and also to Y although there is no coupling between A to X there is no coupling between A to Y, I still see a cross peak from A to M and A to X and A to Y this is because of what we call it as the relay. Total correlation that is why it is called as total correlation spectroscopy the entire network of couplings will show up in the TOCSY spectrum you can identify by analyzing the TOCSY spectrum.

So, which are the spins which are in which are J coupled in the entire coupling network obviously you will have more peaks in the TOSCY spectrum than in the COSY spectrum. So, now what will be the particular advantage here one particular situation I will illustrate this to you what will be the situation. Suppose I consider a spin system which is like this and this is MX and I see a cross peak here cross peak there and I see a cross peak here and a cross peak there in the COSY.

If I see it like this what it could mean is that there is an AM cross peak there is an AM coupling this will tell me that there is a am coupling but there is also a cross peak from the M but however suppose there are 2 protons M and M' overlapping. Let us say there is M and M' here they are at the same chemical shift. Now if a AM coupling but if there is a M' X coupling but there is no a MX coupling there is no MX coupling but there is an M' X coupling.

So, I see a cross peak from M to X but this can as well be an M prime X coupling M prime X cross peak. So, how do I figure that out. So, COSY will not be able to tell me that information. Now if I do a TOCSY of the same I have the diagonal here this diagonal this diagonal same as before. Now if I have a peak here which will be there in the COSY I will also have a peak here as in the COSY but if it is AMX and not am plus M prime X if it is an AMX I will see a cross peak here in the TOCSY.

But if it were am plus M prime X then I will not see this will be AMX this will be AMX and not am plus M prime X. So, this is not this is not true okay. So, therefore this I can eliminate I can eliminate this in the TOSCY spectrum this is a TOCSY. So, in the TOSCY spectrum I will see this additional peak if there is AMX system and not am plus M prime X. So, therefore that is how this experiment is useful to figure out if there are overlapping peaks and you make a mistake in your connectivities in the analysis of the COSY spectra.

The second important point here is all the peaks will have in phase character another important factor which I should mention that all the peaks are plus plus plus plus there is no plus minus plus minus at all. So, therefore there is all in phase this is called as all in phase all in phase character in the TOCSY. In the TOCSY all the peaks will have in phase character that is plus plus plus plus or minus minus minus minus whatever you want to call it because that is but there is no plus minus plus minus.

Therefore there will be no cancellation of intensities and that is an important factor which is extremely useful because there is no loss of intensity it is not. So, much dependent on the

magnitudes of the coupling constant although at some extent it does because this what you call as the mixing time here the mixing time depends on what is the coupling constant you should use. So, how much is the mixing time and that is this is called as a mixing time suppose I say tau M is a mixing time this depends on the ranges of the coupling constants.

So, what are the important messages to take here in the TOCSY spectrum there is a relay of information through the coupled network all the cross peaks have in phase character and all of them are absorptive in nature and all of them in phase absorptive therefore there is no dispersive character at all. So, the resolution here is much better than what you have in the cause. So, is notice here this is true for both the diagonal as well as the cross peak.

Therefore the diagonal is also pretty clear the peaks which are very close to the diagonal can also be resolved can also be resolved in this TOCSY spectrum. Therefore these days whenever you have a molecule you straight away record A TOCSY spectrum but only certain situations you want to remove certain number of peaks and be more specific with regard to the near neighbour interactions then you will use the TOCSY spectrum and then you lose the cause or the double quantum filtered COSY.

So, this sort of experimental techniques one can use to obtain the relevant information in your space system. So, we will not go into the details of this mixing sequences here these are quite complex and this involves a series of pulses several pulses and all the other characters with regard to the data collection the resolution etc will be the same as in the case of normal 2D spectrum other 2D spectrum.

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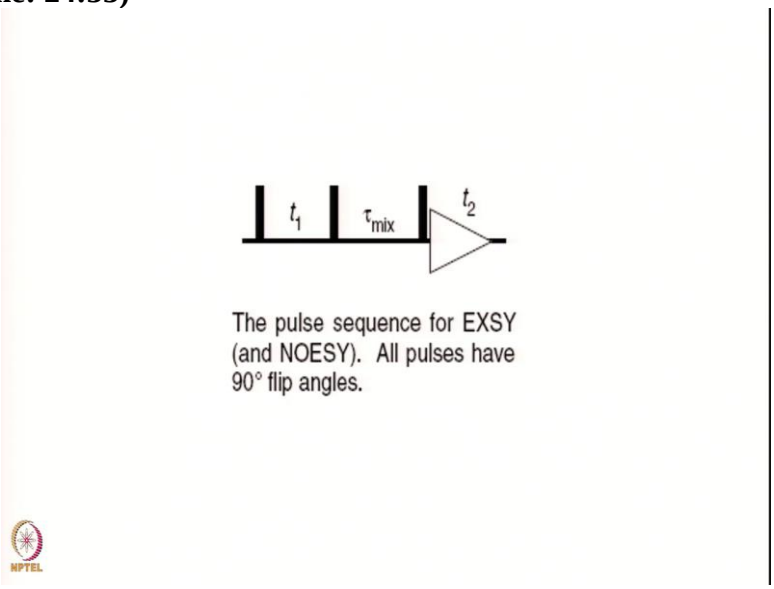

The pulse sequence for EXSY (and NOESY). All pulses have 90° flip angles.

Now this brings us to another important experiment and that is called as the NOESY. So, we will only make a small introduction to this we will take up this in the in greater detail in the next class. This is again an extremely important pulse sequence and this is extremely useful for determination of the structures of the molecules macromolecules the proteins nucleic acids this is this experiment is also called as EXCY this is also called that means exchange spectroscopy or it is also called NOESY.

This NOE correlated spectroscopy and here you have completely different principles of magnetization transfer in the previous cases we had actually used on the basis of the J coupling the J coupling was the driving factor for all of these ones. Now in the in the there are other

mechanisms of the transfer of information and that happens in the case of the NOSCY or the EXCY spectra and this we will take up in the next class. I think we can stop here.