One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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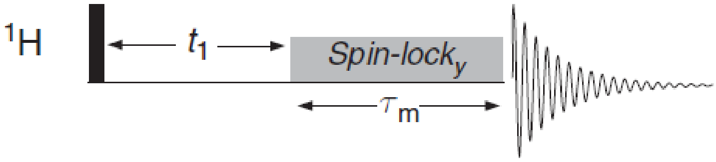
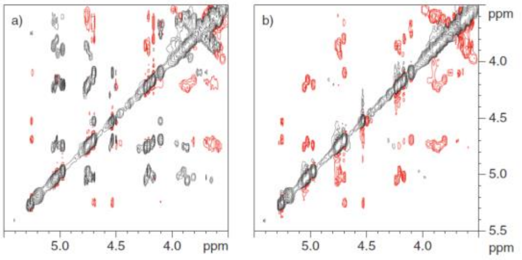
Lecture 55: ROESY and Tr NOE

Welcome all of you. So, we have been discussing about NOE concepts since last 2 or 3 classes. It is a vast subject and we have to discuss extensively. Varieties of things we discussed about NOE and then in the last class we discussed about types of NOE experiments starting with, steady state NOE, HOESY, ROESY, varieties of things we said. And then NOE experiment also has some complications. We saw that. What happens when you do the 1D NOE, steady state NOE. Even I wanted to show how we can do it. You selectively saturate a spin and then collect the signal. Go very much off resonance, identical RF power you apply, take it as a reference spectrum, take the difference, you see the signal. And then there will be some complications, you have to be extremely careful. So, the selectivity of the saturation is very important. If you do not selectively saturate a signal, and if you perturb the neighboring signal, there will be a leakage of RF. Then you will get wrong results, sometimes it could be confusing. Also if you have a multiplicity pattern you have to uniformly perturb them. Otherwise, what will happen? you will create a anti phase character, and when you take the area, it may get nullified finally. Thus you may not be seeing the NOE, although it is there. That also that I told you. And I told you about the 3 spin effect. If I have 3 spins, A, B, and C, if it is linear or angular, if you are irradiating A and see its effect on B, and B will be leaking through C. And then because of that leakage there may not be a correct NOE for that. If it is the angular thing, A to C if you want to see NOE it may be leaking through B, that is also possible. It may so happen instead of getting positive NOE, it may be leaking to B and may get nullified also. So, varieties of things we discussed. In the HOSEY I take the examples of fluorine and proton, and lithium and proton etcetera. It could be any of the heteronuclei, and other dilute spins, does not matter. So, when the HOSEY was seen I showed you that there is a cross peak which is very easy to interpret. In the HOSEY case it is not symmetric, spectrum is not symmetric. Whereas in the case of the NOESY it will be always symmetric. I have not discussed about the 2D NOESY. I will discuss in the next class or in this class itself. Then I took the example of ROESY, why is ROESY comes because omega tau C is approximately equal to 1 for certain molecules, then there is a situation at which NOE goes to 0. When NOE goes to 0, then what you do? So, we can alter the physical conditions, either go to higher magnetic field or change the temperature, change the solvent, etc. bring change in the tau C. or change the omega that is in your hands. Alternately an experiment has been designed where NOE can be done in the rotating frame, that is what I said, The rotating frame NOE is always positive, never goes to 0. We discussed varieties of advantages of ROESY. How it happens in NOESY, both the source spin which is irradiated and the other spin to which the magnetization transfer takes place are both along Z axis, plus and minus Z. But in the case of ROESY, you do it in the transverse plane. It is plus Y and minus Y, plus X or minus X, they are opposite each other, like anti phase character and then magnetization transfer takes place. And important thing I told you in the NOE case gamma B0 if you consider, B0 is the static field. In the ROE case B1 is the RF field, which is much smaller compared to B0. As a consequence, you can treat like an extremely narrowing condition. So, these are the things which you observed in the case of ROE also. And I showed you there is a 2D ROESY pulse sequence, I may not be able to show the 2D because the you know time getting short too many things to talk. But I showed you 2D ROESY sequence.

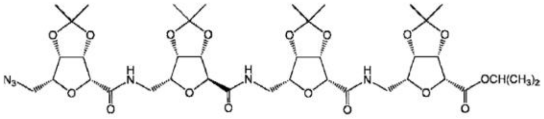


We will continue with that today. It is a 2D ROESY sequence here. And of course, there is a t1 period followed by spin lock. In the spin lock say what happens, I told you already. The chemical shifts get completely refocused, and mixing takes place between the spins, and then you collect the signal. This is a simple example of a 2D spectrum of a molecule like this, a 2D ROESY spectrum. This is called TOCSY, what is transferred there do not worry I did not discuss too much about those things. Why I am showing this is when you do a ROESY, what is the pulse sequence you used for TOCSY, it is identical. ROESY sequence and the TOCSY sequence are almost identical we saw that. A TOCSY experiment when we did, 1D TOCSY or 2D TOCSY I showed you there is RF pulse, mixing pulse and then we vary the t1 and apply the mixing pulse and collect the signal we saw that. There is no difference between the TOCSY sequence and the ROESY sequence, both have spin lock. But then as a consequence, what happens there are examples where in the ROESY you get TOCSY peaks.

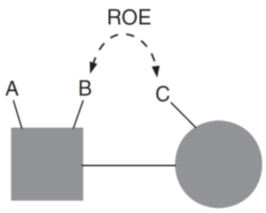
Dominated by TOCSY peaks



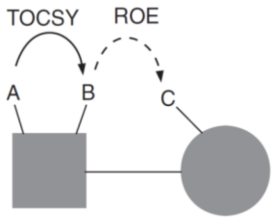
TOCSY peaks are largely suppressed



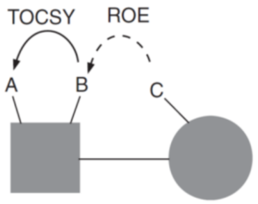
This is a simple classic example of a molecule where you can see, this is an experiment where ROESY is done in a different way, I will tell you what it is, slowly. Then you will see lots of peaks here which are present, which are missing here. These are all called TOCSY peaks. If you do a conventional experiment of a 2D you are going to get a ROESY spectrum along with TOCSY peaks which are dominant. In this case TOCSY peaks are removed, how we can remove that I will tell you now. These complications come in the ROESY, the TOCSY transfer takes place, that occurs during spin lock. COSY peaks also can arise from J coupling, there is signal attenuation from off resonance effects. There is a multi step transfer involving both TOCSY and ROE stages that can give rise to false ROE's. All these complications can come. Especially TOCSY peaks everything that can give you rise to complications. We need to somehow overcome that. How do we overcome? And these are the problems you come across.



Genuine
B-C ROE



False
A-C ROE



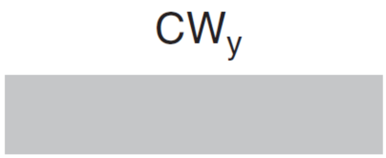
False
C-A ROE

For example, I have a hypothetical molecule like this, B to C are close in space, I am expecting ROE here, you may get it that is genuin ROE between B and C. But it can so happen there can be a TOCSY correlation you know. The TOCSY transfer of magnetization from A to B and then B to C, this is a false A to C ROE, you may get it may not exist, but you may see ROE. It can so happen there is a transfer of magnetization from C to B, and B to A there is a TOCSY transfer and then you will see as though C to A, ROE is there. Here you will see A to C ROE because of transfer of magnetization this way, but it will go other way then you will see there is a ROE, as if the between C and A. These are all complications in ROE. And then another few important things you should know is the signs of the cross peaks in ROE, because ROESY, and I will tell you later exchange spectroscopy EXSY, and then TOCSY, all those peaks will come into the picture here.

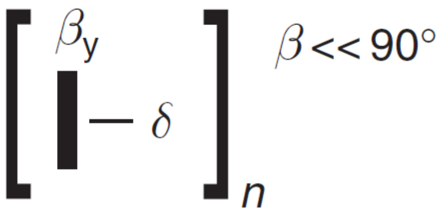
You see this is the signs of cross peaks in ROE experiment related to diagonal peak. By convention the diagonal peaks are always phased positive in ROESY. This is a ROESY cross peak, and I will say direct ROE if it is there, then the cross peak is negative with respect to diagonal. I am talking diagonal is positive and then indirect ROE 3 spin effect is there, we saw that A to B, B to C or C to B and B to A, in which case the cross peaks appear positive.

Origin of ROESY Cross Peak	**Sign of Cross peak**
Direct ROE	**Negative**
Indirect ROE (3 spin effect)	**Positive**
TOCSY	**Positive**
TOCSY-ROE	**Negative (False ROE)**
ROE-TOCSY	**Negative (False ROE)**
Chemical Exchange	**Positive**
COSY TYPE	**Anti Phase / Mixed Phase**

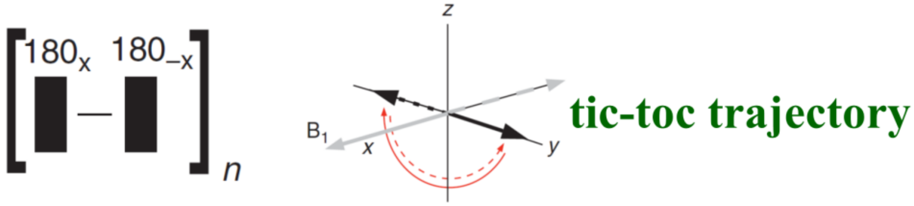
There can be a TOCSY correlated peaks in which case if the diagonal is positive, the cross peak is also positive. Through the TOCSY there can be an ROE in which case the cross peak is negative, this is a false ROE. There can be ROE and TOCSY we saw that in the example, A to B, B to C or C to B, B to A both cases were discussed. In both the cases ROE is negative, I mean the sign of the cross peak is negative. So, as a consequence you have a false ROE. There can also be chemical exchange and the sign of the cross peak is positive. There can be COSY type peak, we have mixed phase, anti phase and in-phase, all these things can happen. And then to overcome that there are certain methods. Firstly, you can apply very low power RF for spin locking, very very low power, comparable to maximum resonance frequency offset. That means we do not need to use the higher power, that is one way.



Alternately you can use pulse spin lock. Instead of continuous RF, you can apply pulse with a delay like this, and repeat n times. And this pulse this can be angle less than 90 degree, n number of times you apply for the same time period, mixing time period. You apply n number of such series of pulses, that is a spin lock you are doing in a pulse manner, not a CW manner. That can also help.



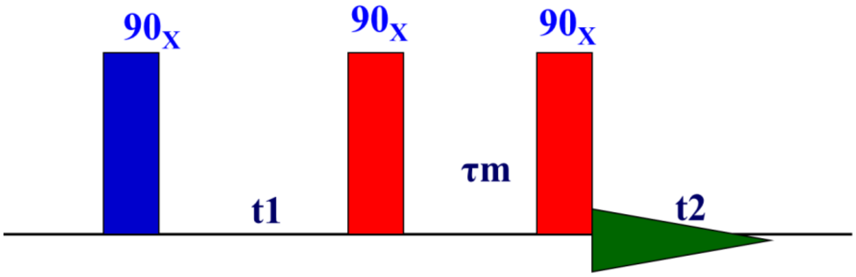
Alternately, you can apply what is called alternating phase spin lock like this.



180 plus x 180 minus X like that. Repeat it n times like here, then what will happen? this called a transverse ROE. What will happen? once the magnetization is here, goes here, and see this will come here, this will come here. These magnetization which are opposite to each other. The source spin is here and the other spin is here. They are all opposite to each other, in plus y and minus y axis. They keep changing their positions. Once this comes here, this goes here, everytime when you apply 180 plus pulse and 180 minus x pulse, this trajectory keeps changing. This is called tick track trajectory. You can do tick track trajectory, make this spins to follow this, then you are going to get what is called this is called a transverse NOE. This is another easy way to overcome some of these problems, because where the TOCSY peaks coming can be overcome, Many of these problems can be overcome by modifying this thing. With this I will be almost completing the concepts of NOE, but I will show you here the spectrum which I wanted to show here earlier.

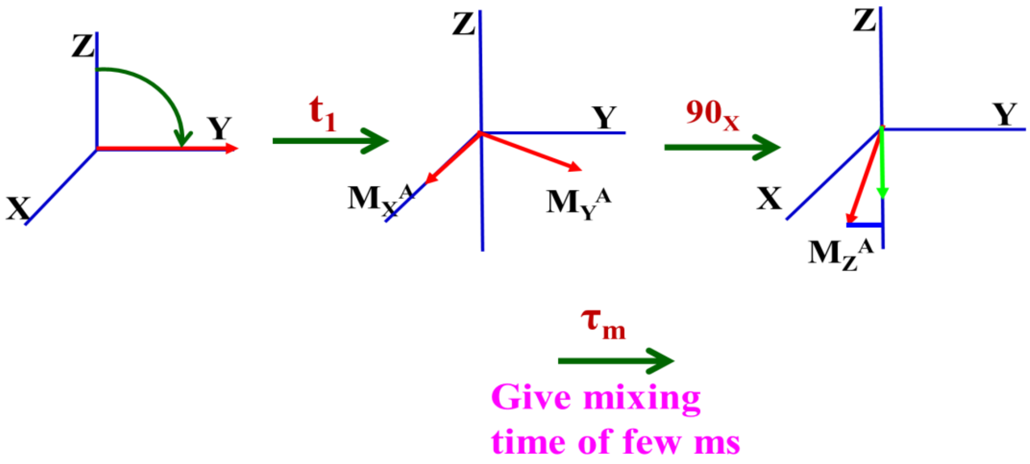
This what I told you, This is the TOCSY dominated ROE peaks. This is what is transverse ROE. What is transverse ROE, where the spin lock pulse is 180 plus x and 180 minus x, continuously n number of times you apply, tick track trajectory is followed. Then this TOCSY peaks are largely suppressed. This is a transverse ROE. So, these are all some of the tricks which you have to follow. So, basically I have given you lot of concepts about NOE, HOESY, ROE, steady state NOE, everything, lot of things we discussed. Hopefully you have got a point about what we have to do. What we have to do with type of experiment, how do you overcome some of these complications etcetera. And with this we will really jump into some new experiment. So, far I was telling you, of course, only this example of 2D ROESY, and HOESY I showed. The homonuclear 2D NOESY have not shown at all you, will really see the 2D experiment. That is called transient NOE. I discussed the steady state NOE, ROESY, HOESY, but the transient NOE I did not discuss. The transient NOE can also be done in 1D way by using gradients, I am not worried about that I am not discussing that. But I will discuss 2D NOE, that is called NOESY, that is another important thing. So, with this we will go to a different topic now all together and you will see what is NOESY and everything. We will go into the NOESY experiment. So, we will start now discussing about what is called 2-dimensional NOESY which is also called transient NOE, TRNOE. We already discussed steady state, we discussed HOESY, we discussed ROESY. Now, we will discuss NOESY a 2-dimensional experiment, not 1-dimensional experiment that is steady state NOE.

What is the difference here? In the case of NOESY, NOESY is a non-selective experiment in nature. In the case of steady state NOE you remember I told you, selectively you have to excite or saturate one of the spins and see the effect of polarization transfer to the neighboring spin. Selective, you have to do, one by one. This is non-selective in nature. All NOEs to all the protons can be mapped in a single experiment, that is an advantage. So, it is a non-selective experiment. Here NOE is built up during a short mixing time. With the spins relaxing along z axis. And the spins will be relaxing, going back to z axis, because it is z z magnetization transfer, I told you. The magnetization transfer takes placefor NOESY along z axis. This provides accurate distance information if you use the built up curve for the measurement. You can do several NOEs with a different T1, how it builds with the mixing you can monitor. Then you get a very accurate distance information by using this type of experiment. And most effective at determining rough estimate of relative proximity of protons up to 5 to 6 angstroms, you can almost get the rough estimate of the distance. That very effectively you can do. Of course if you want to do precisely you have to take the build up curve. Here the recycle delay between the scans can be very short, unlike in the steady state where we have to give a long delay, you know at least 3 times of the spin which is taking long time to relax. And the artifacts are much smaller here than in steady state experiment. And that is the advantage. Mot many artifacts will be there. But in the steady state I showed you, you know, depending upon selectivity of excitation and all those things we have lot of problems there. All those things can be overcome, they will still be there, but very minimum artifacts. What is the two dimensional experiment in NOESY? It consists of a 3 pulse sequence like this very simple. What it does?

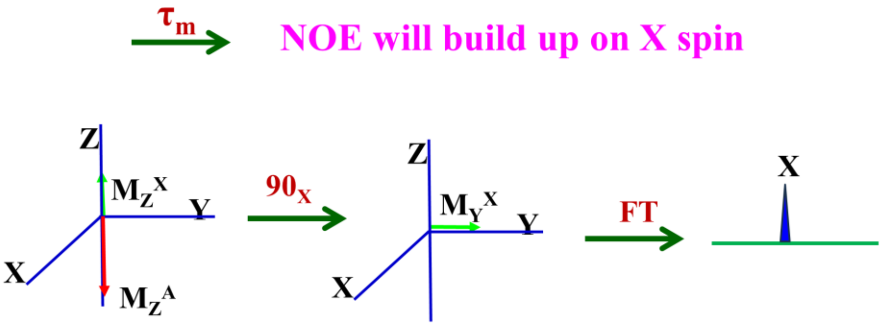


You apply 90 degree pulse and then bring the magnetization to x-axis or y-axis. Apply another 90 degree pulse and then allow the spins to mix here, in the mixing time, then apply a detection pulse, and start collecting the signal. This what happens. During the t1 period the transverse magnetization, let us I am doing proton proton noe, the protons evolves in the transverse plane in the t1 period, the magnetization protons, after applying 90 degree pulse the magnetization is brought here, spins start evolving in the xy plane.

Apply 90 degree pulse it creates longitudinal magnetization, because the magnetization what was there in the x-axis or y-axis, will be taken back to z-axis, because it is a180 pulse, 90 plus 90 it will be with a delay of course. As a consequence, the spin vectors would have moved by some distance in t1, and then allow them to mix up during the mixing period. The magnetization transfer via cross relaxation, chemical exchange whatever it is, can take place. And apply a last pulse which creates transverse magnetization and FID is collected. This is a simple way. And the magnetization transfer for two spins A and X that are close in space, how it happens is like this.

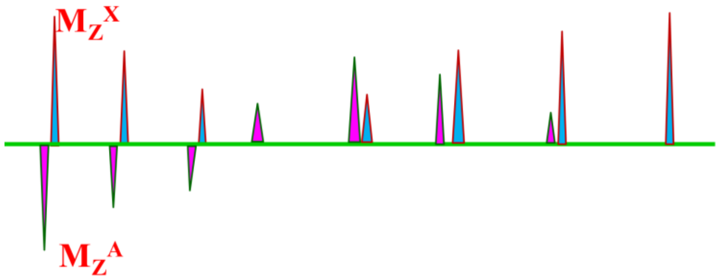


Bring the magnetization from z-axis to y-axis, allow for the time t1 for the spins to start moving, they start processing and they move by certain degrees based on the t1, how much time you have waited. Now let us say x and y component the spin A is there, the vectors have started moving with t1 like this. Apply another 90 degree pulse. Then this component will come to this axis, move would like this. And I am considering only the component along Z axis. The other spin, let us say, I am not considering, only one of them, I am considering A spin. I am not worried about the x spin, remains same, just for the sake of understanding. And this component of the magnetization only I am considering, one which comes here. This one would have moved far away. I am not worried about that. Only one of the component is taken and give some time for mixing, for a few milliseconds. What happens in the mixing time? The spins with their macroscopic magnetization along minus z-axis, here minus z-axis, that means are in the excited state, moved from from alpha to beta state, that means they have higher energy they have to go back to a ground state or go to the equilibrium state by giving away the excess magnetization to somebody. Spins have to get rid of excess energy. That means the spins which are along minus z-axis in the excited state will come back to plus z-axis by giving magnetization. That is getting rid of the excess energy. Where does it give? it will give to the neighboring spins. That is what happened. The magnetization is transferred to neighboring spins that are close in space. How it can happen? because of dipolar relaxation. The two spins close in space experience dipolar relaxation. Remember I discussed about the dipolar relaxation, how one of the spins keeps moving all across keeping their orientation of two spins same. It creates a local dipolar field at the site of the other nucleus which is responsible for relaxation through that fluctuating dipolar field I discussed. Now because of that what will happen, through the dipolar relaxation the magnetization is transferred from one spin to other. This what happens during the mixing time.



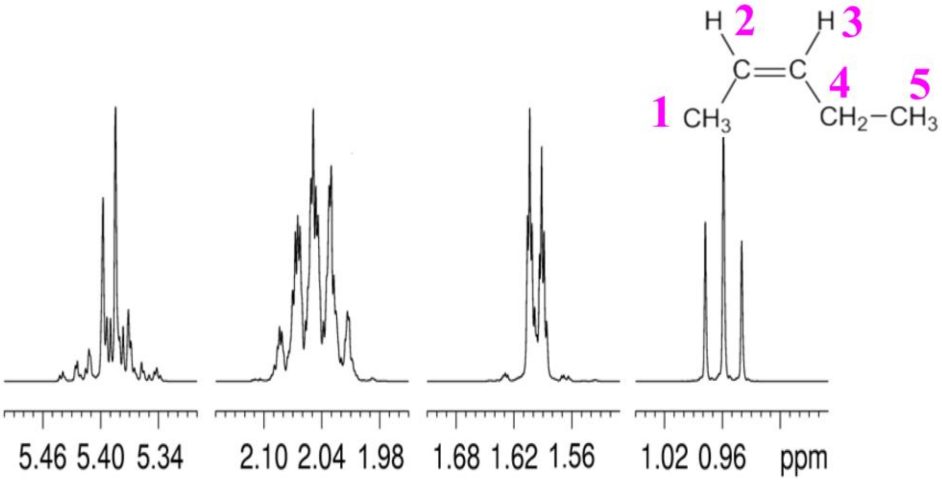
During the mixing time magnetization along minus z come back to +z-axis and gives magnetization to spins which are already along z-axis through dipolar relaxation. And give some time NOE will start building up slowly like this. Slowly z magnetization builds up and give its magnetization to X spin, and now apply 90 degree pulse, the X spin magnetization along z comes to transverse plane and start doing the Fourier transformation you will get that signal. This is what happens. You understand the concept bring the magnetization to minus z axis by applying 90 pulse, t1, another 90 pulse, worry about the component of the magnetization along minus z axis, it is in an excited state, it has to give its energy, get rid of its excess magnetization by giving to some other spin which is along z axis, which is close in space through dipolar relaxation, that spin is also along z axis and then slowly there is a build up of magnetization of the other spin. If you apply the third 90 degree pulse and do the Fourier transformation you get a signal for that other spin. I inverted A spin but I got magnetization, the signal for X. This is what happened during mixing time. There is a transfer of magnetization from proton A to proton X. And pictorially how the enhancement comes? A crude diagram I am giving you. As a function of increasing t1

Intensity of X spin with increasing t_1

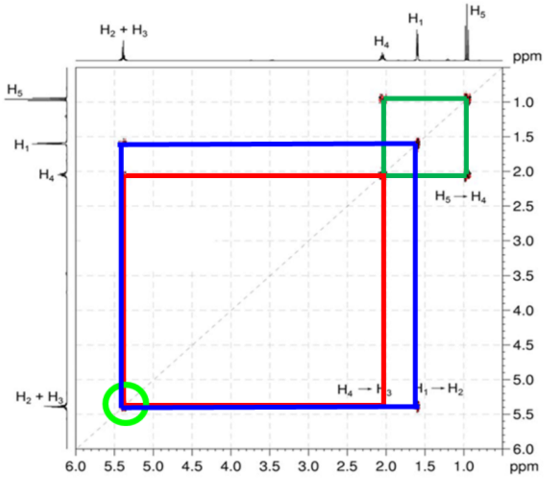


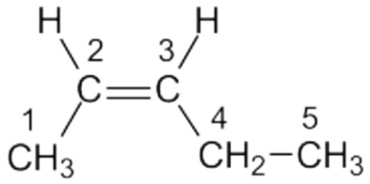
Let us say you have inverted the magnetization Z, I have written X spin here. It has some magnetization. Then it starts giving to this, this comes down gives magnetization, then there is some dipolar relaxation is also going on, it will take magnetization, it comes down, comes down, then this goes up, this goes up like this. Finally from the minus z, completely it goes to z axis. And this gives all its magnetization to X spin. The A spin red spin will give to blue spin. It follows a trajectory, oscillates, The x spin enhancement oscillates at the frequency of A. A spin magnetization comes down, goes up, comes down, goes up like this. And then this magnetization energy keeps coming down. It doesn't go to negative, it oscillates. This oscillates at the frequency of S spin. Now, NOESY cross peaks. Where do you get the NOESY cross peaks. In 2D NOESY the cross are between two chemical shifts, between two nuclei that are close in space. As I told you there is no need of a j coupling between them only close spatial proximity is very important. The COSY peaks appear as artifacts in 2D NOESY spectra. The artifacts are removed by phase cycling of the first two pulses. There are COSY artifacts that appear in the NOESY spectrum, but you can remove that by doing phase cycling. There are several ways of removing this thing, okay. There are a lot of improvements in the pulse sequence.

The NOESY cross peaks are symmetric with respect to diagonal. Similar to your COSY and TOCY, NOESY cross peaks are also symmetric with respect to diagonal. The intensity of the cross peaks give qualitative information about the spatial proximity. Higher the intensity, indicates they are very close in space. If the spins are far away separated in space, the intensity comes down drastically. And if they are very far away there may not be be any intensity at all, there may not be any NOE at all. And qualitatively you can say just by looking at the intensity of the cross peaks, you can find out about about that spatial proximity, whether they are close or not, approximately you can get qualitative information. Of course how do you get quantitatively? That is a different thing. You should know that if you want to get it, you have to monitor the build-up curve everything. That is what I told you while discussing the concepts. Assuming that you know how to do NOE, okay you you understood NOE, 2D NOE, the transient NOE everything. But how do you use it? okay this much concept is understood, you are doing transient NOE, all the peaks you can get in the NOE at a time, it is not a selective thing, it is non-selective, all the NOEs whichever is there in the molecule can be simultaneously obtained.   We are using a three pulse sequence, I am not discussing much about it. There are varieties of modifications. I am discussing how do we interpret the NOESY spectrum, simple NOESY spectrum. We will take couple of examples. I am taking NOESY spectrum of a simple molecule cis pentene. How do we interpret that molecule how do you interpret the NOESY spectrum. Of course from the NOESY we are getting the conformation information. We will see that this is 1H spectrum of cis 2 pentene. This is structure of the molecule. All the protons have been numbered.

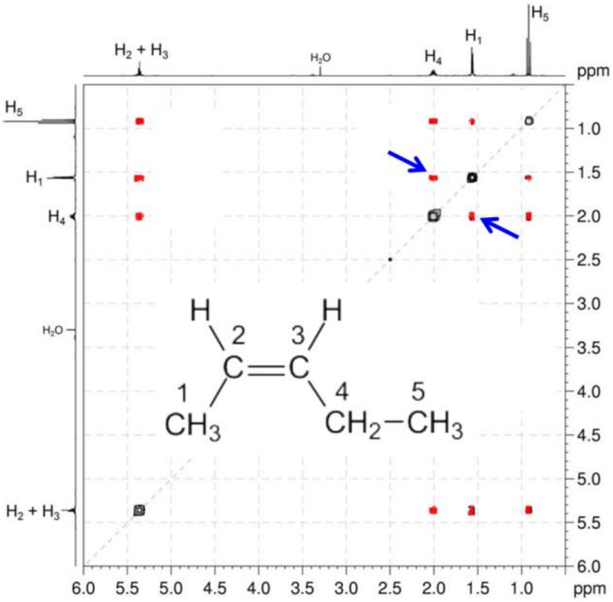


Well you can interpret it interpret the spectrum without any difficulty because we know that this is proton 5 and the high field region is a triplet because of CH2, this CH is a quartet because of this, and each another quartet is coupled because of this geminal coupling, it can also have a trans coupling. I don't know whether it has a cis coupling they are far away 1 2 3 4 5 bonds away. I don't know whether it is there, but anyway complications are there. What I am trying to say is interpretation is not that easy, I mean not that easy although molecule is simple. Of course qualitatively by using some idea we can say which peak is which, but multiplicity pattern everything we can understand definitely. First of all what is important is identifying protons, making assignment of the different protons. Let us start assigning the peaks. Here first of all there are 5 protons you see there are 5 protons here, 2 are overlapped, 5 groups of protons are there.





The COSY we have discussed and we know how to make the assignment. Start with this, one of the peaks on the diagonal, come vertically down, go horizontally, go vertically completed the square. I know this proton and this proton are coupled. This is proton 5, this is proton 4. This is proton 4 and the next one is third one, this proton 4 is here, that is why I said it is so complex. It has coupling from various things Ok that is also done. We have understood, 5 and 4 assignments we have made. What next? if you carefully see this 4 has a cross peak here, go across, complete the square. Then that has to be 3, but you can see that 2 and 3 peaks which are overlapped, not resolved. There is not enough of resolution for us to say, but they are there. But then that one of the peaks gives a correlation to other one, and complete that square. I would say this is proton 1. So, if I go systematically I know 3 to 2, then 5 to 4 then we understood 4, 3, 2 and 1. The COSY spectrum you have already analyze n number of spectra, in this course earlier. So, we have understood how to interpret this. That is fine. what next? we will take the NOESY spectrum of the same molecule. It is very interesting.



If I take the NOESY spectrum of the molecule, this is Cis-Pentene, we have to see what are the cross peaks we are seeing here. There is one cross peak here, remember this was not seen in the COSY. There is one cross peak here between 1 and 4, this cross peak. In COSY between 1 and 4 there was no cross peak. As a consequence, this is a NOESY peak. There is a cross peak between 1 and 4. H4 and H1 correlation is seen in the NOESY spectrum. What does it tell me? these two are close in space proximity, that is why it is giving cross peak. There is a cross peak between 1 and 4, because they are on the same side of the double bond. If this CH2 were to be here and this proton were to be here, of course my multiplicity pattern here would have been different, that is a different question. CH3 would have been double of a triplet. But nevertheless we do not know whether this one is on this side or other side, we do not know, that is a trans. But when these two are close to each other there is a spatial proximity, so close there is a correlation between these two.

So, CH3 and CH2 is going to give cross peak here. That confirms for me it is a cis geometry, because these two are on the same side of the double bond. Then these two protons should be on the other side of the double bond. They are on the same side, this establishes cis geometry for the molecule. So, you now understood how we can use the NOESY spectrum to get the conformation structure of the molecule. Now it can I can confidently by looking at the NOESY peaks, this these two protons are one side of the double bond and these two protons are on one side of the double bond, establishes cis geometry for the molecule. And also you can see H2 and H3 correlations. There is another conformation. Look at this here, H2 and H3 correlation is there in the NOESY. But unfortunately they are not resolved, because the chemical shifts are so much overlapped. There is a cross peak between these two. You can see two and three are also correlated through space. There is a confirmation that two and three are also on the same side of the double bond. These two together establishes cis geometry for this molecule.

I will take one simple example and before we complete the time will be getting over. I will take this last example. May be it I think this time is up I am going to stop. I will take this example in the next class, tomorrow or so. I am going to stop here, But today what we did? we started with the complications we get in the NOESY, NOESY cross peaks and varieties of problems we can think of, it comes because of the TOCSY peaks could be there everything. How do you overcome that? we saw that. If there are TOCSY peaks, instead of CW we can have a pulse mixing time, or tic-tac sequence. 180 plus X-180 minus X for the entire mixing period, repeated n times for the duration of the mixing, then TOCSY peaks are removed, partially you will eliminate them. And we saw very clean spectrum for the ROESY, where TOCSY peaks are removed in one of the examples we saw. Then subsequently, we thought after that we should go into real applications, real 2D NMR. NOESY, there are varieties of NOE experiments I told you, steady state NOE, HOESY, ROESY which we discussed in this class. We started discussing NOESY, I told you NOESY is a 3 pulse sequence, 90, t1, 90 mixing time 90 start collecting the signal. And we saw in the t1 period, the magnetization start evolving, then again after a 90 degree pulse, one of the component magnetization vector go to minus z axis, we look at the component of it and then because it is an excited state at minus z axis it wants to get rid of its magnetization by giving it to other spin, which is close in space. That we saw pictorially how it happens, it gives magnetization to the neighboring spin only through dipolar interaction, the dipolar relaxation is there. It relaxes by giving its magnetization to spin which is close in space, we saw that one. That is how it works actually. Then finally if you do the experiment two-dimensional Fourier transformation, you are going to get a NOESY spectrum which is symmetric with respect to diagonal like our COSY and TOCSY spectra. That is what we saw. And of course the interpretation of that is very easy, I showed you. The TOCSY peaks can also come, I told you several complications. It gives rise to COSY peaks, and many things will be there. But then we have to use a judicious way to discuss and then identify the cross peaks. I did not go into more details of that, how to do and everything. But remember there will be complications but those can be minimized by some methods and you are going to get only the cross peaks. We took the example of Cis-Pentene and we saw the cross peak between two CH2 groups which established that they are close in space, because they were giving NOE cross peak. In the same molecule other two protons on other side they were unresolved, and they also give rise to cross peak. This established the spatial proximity of these three groups and establish a cis-geometry for the molecule, that is what we understood. So you can utilize the NOESY in getting the information about the geometry of the molecule, confirmation of the molecule. So that is how we can use the NOESY. I give you few more examples and specific examples in the next class. I am going to stop here, thank you very much.