One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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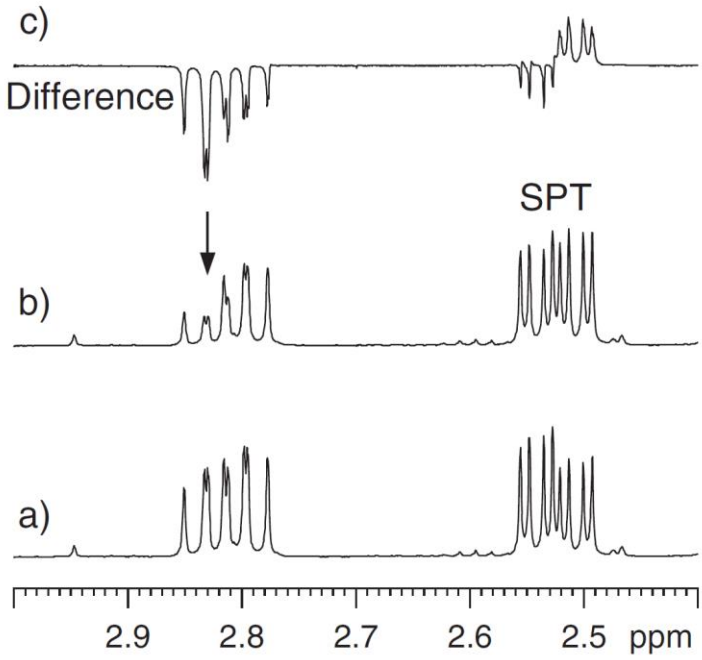
Lecture 54: Complications in NOE, Steady state NOE

Welcome all of you, in the last three classes we have been extensively discussing NOE phenomenon. In the last class specially we discussed about correlation time, how it is related to spectral density function, and how it affects NOE and we discussed lot about spectral density function, what happens when omega tau c is very much smaller than 1 extreme narrowing condition, when omega tau c is very much greater than 1, the diffusion limit, and we also saw that omega tau c is approximately equal to 1, that is a situation where there would not be any NOE. We saw various plots of omega tau c as a function of signal intensity, that is NOE enhancement, and we saw it is positive for small molecules and negative for bigger molecules. that is what we saw. And maximum gain in the signal intensity in extreme narrowing condition. We got the parameters equations for transition probabilities w1, w0 and w2; substituted them in the Solomon equation. Then we saw that in the extreme narrowing condition the gain in the NOE is half. Of course, depends upon the ratio of the gammas. For homonuclear case it was 0.5 that is what we observed. And for different heteronuclei the gamma factor comes into picture. And I showed the example of varieties of nuclei where gamma factor was there. And you know for carbon -13 it was 200 percent. For varieties of nuclei, up to even 1600 times we saw. That means if I record the carbon 13 spectrum with proton decoupling then I am going to get twice the enhancement in the intensity, 200 percent. Similarly we also saw that if I decouple carbon 13 and observe proton, the enhancement will be of the order of 12.5 percent, that is what we saw. So, all these things we understood. Of course, understanding NOE concept is bit difficult, lot more things are there to discuss. As I told you there are exclusively two books only an NOE itself. Imagine the vastness of the subject. So, everything cannot be discussed, but I gave you the basic ideas, some concepts to understand. And when you do an NOE experiment there are number of NOE experiments which I discuss in this class or the next class. And then we will understand how to do the experiments and everything. But there will be some complications while doing NOE, those complications we have to understand before interpreting the NOE properly. So, we will start with that today. So, starting with the complications of NOE, here; the selective saturation of the resonance is very very important, I am talking about steady state NOE. I tell you what is steady state NOE and everything. So, far we have not introduced to 2D NOE. For NOE I have been telling you, I hit a particular resonance and see the change in the intensity of the other peak. So, how do you selectively hit this a resonance. Selectively I have to saturate one particular resonance. The selectivity of the

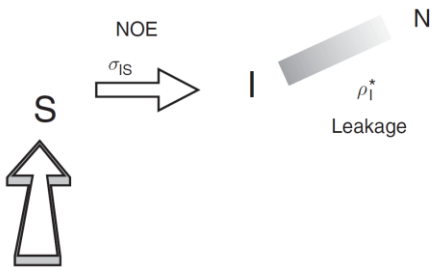
resonance is very very important. The frequency has to be properly set. Otherwise there will be little disturbance, and if you saturate a neighboring proton then the spillover of the RF can cause problems. So, that means the selectivity of the frequency is an important condition. And of course, if you have a multiplicity of the peak where do you want to hit? you have to perturb exactly at the center of this and make sure that multiplets are subjected to perturbation properly. If all the multiplets are not equally perturbed or not equally saturated at a time, then that results in selective population transfer. We discussed this long back. That can also cause problems. Antiphase terms of the j coupled multiplets are going to be seen, that is what we observed in population transfer experiments, when we discussed that. So, when you integrate the positive and negative intensity signals, the anti phase signals, the area of that integral is 0. So, that means if you do not selectively hit the multiplicity or saturate the multiplets of the peak properly, if they are not perturbed properly, the unequal perturbation can give us to anti phase multiplicity and if you take the total area, the area of that particular multiplets becomes 0. It will mask the genuine NOE. there may be NOE because of unequal perturbation whatever NOE is there will not be seen, it is masked. That is another thing selectivity and uniform perturbation of the multiplets are important things.



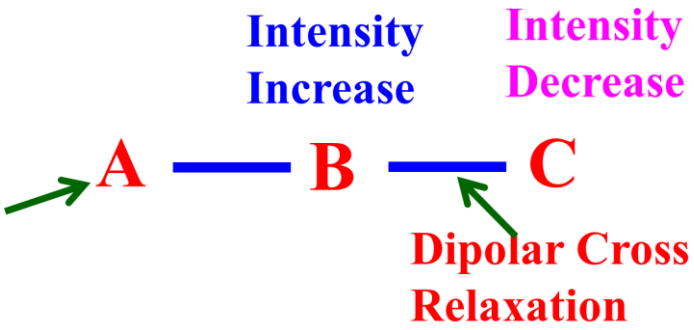
This is an example to show what happens in a spectrum like this. This is selectively saturated, but you see it is not uniformly saturated. It so happens and if the difference is taken see there are anti phase components. Now, if you take the area of this what will happen? it may become 0. Then you will not see NOE, if at all if there is any. So, these

are all problems of unequal perturbation of multiplets. When the multiplets are not properly perturbed you are going to get this type of problem.

There is another effect called 3 spin effect, we have to discuss that. For example, I have 3 spins. So, far you know all out understanding of NOE and everything was with 2 spins. We took into account in the understanding omega tau C, and you know dipolar relaxation everything, we took only example of 2 spins. It can so happen there can be many spins nearby. For example, we may have 3 spins. There is a famous thing what is called 3 spin effect.

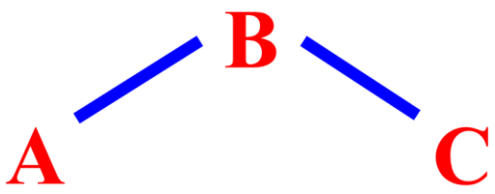


I am irradiating proton S, let us say, whatever it is, and it is giving NOE because of cross correlation with I spin, but there can be leakage of this I spin. Instead of gaining magnetization it may lose by give it another spin, next to it. This is called a leakage. This is a 3 spin effect, it can cause problems. for example, I will tell you how there is a there could be indirect effects, lot of indirect effects could be there. Take for example, a molecule which is linear.



I am saturating proton A, assuming there is a NOE I must see the intensity increase in B. That is true, there is NOE I will see that. And there will be dipolar cross relaxation between B and C. As a consequence, there is a intensity decrease here. And also it can so happen this also can decrease because it is giving its magnetization to C, it can happen. So, this is all indirect NOE effect. When a third spin is there, instead of seeing the intensity increase, it may come down or here also it may change in intensity, because population is getting transferred from B to C also due to dipolar cross relaxation between

B and C. These are all the important factors that one should know and this is a called a spin diffusion. The spin diffusion means, example we have three spins, A spin you are saturating, B should have enhancement, B can give it to C, this is a spin diffusion phenomena. That is the situation of linear molecule. Take a situation of a molecule which has some angle like this.

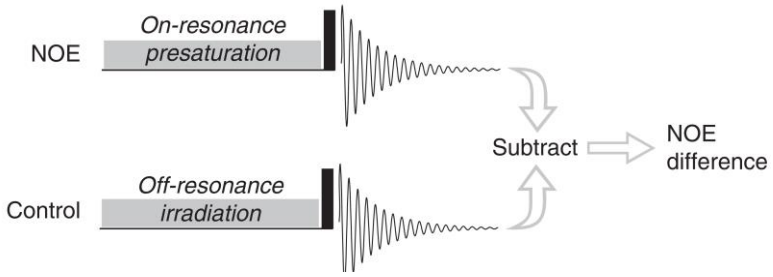


A, B and C are not linear. There is an angular structure. For the angular structure like this there can be direct NOE between A and C. In the previous case it is indirect NOE because A you were saturating, B was getting enhanced, B will give it to C, and you know you see NOE intensity on C. It is not actually real, because of the indirect effect from A to B, and B to C. In a molecule with angular structure, I am irradiating A, let us say a direct NOE effect can be there between A and C, that is positive. But what will happen because you know this can get nullified due to indirect effect between A and C. What will happen? There is NOE between A to B, and B to C. There can be direct NOE between A and C, that is positive we should expect. But because when you are saturating A, this also gets saturated. And then it this gives NOE to this. Then it can sometimes so happen, this can give back its magnetization to that. It can get diminished or get even nullified because of indirect effect between A and C. Although there is a direct NOE this can get suppressed. There are some of the problems, three spin effect on angular molecule, linear molecule, varieties of things we know. And frequency of selectivity, equal perturbation of the multiplet all are needed. So many complications are there in NOE, I just wanted to brief you. There could be many complications in NOE when you are doing the experiment.

How many types of NOE experiments we can do? there are different NOE experiments possible based on how the experiments are designed. For example, there can be what is called a steady state NOE, that is 1D difference NOE,conventionally what we used to do earlier. Of course, now also we do. There is another one called transient NOE, 2D NOE. There is also gradient version NOE. Usually steady state NOE we do in the 1D format, gradients version is also there. Usually 2D NOE is transient NOE. The rotating frame NOE called ROESY. There is also what is called transferred NOE, TRNOE, heteronuclear Overhauser effect. A couple of them I have listed. All these there are different types of NOE experiments. All are homonuclear and this is heteronuclear. We

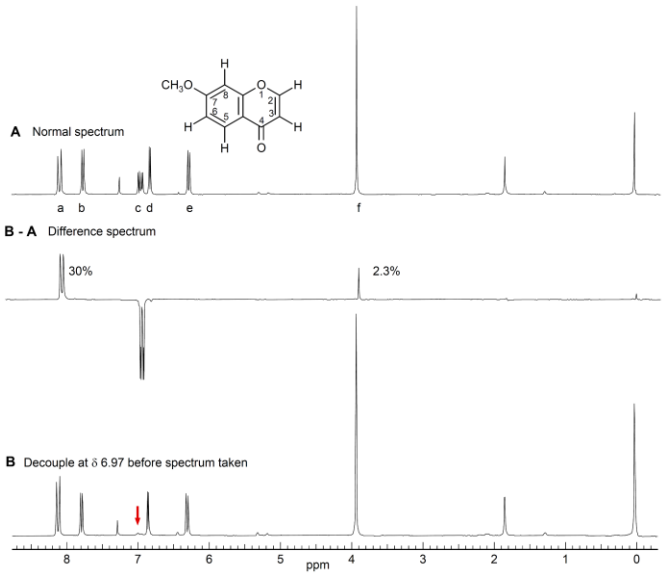
will see quickly couple of them, how it works, Idea of NOE so far we have discussed, the conceptual understanding of NOESY remains same, there is not much of a difference, but small experimental modifications will be there. And what will happen in each of these experiments, quickly we will see a couple of examples.

What is a steady state NOE? This is very simple experiment, where we are going to selectively saturate one of the spins for a long time with very low power, and then apply 90 degree pulse, start collecting the signal. During the long pre saturation time what happens spins coupled via NOE are able to reach a steady state, they are coupled to each other. You are saturating A spin, it may give energy to I spin slowly there will be NOE built up on I spin. And then it will reach a steady state, it will reach equilibrium. This occurs on the T1 scale, the relaxation time scale. So, this is the simple experiment, called a steady state noe experiment.



Saturate one of the spins and NOE coupled spins, the spins coupled via NOE, after a long time reach a steady state. That is what it is. Then how do you carry out this 1D difference NOE experiment, very easy. All that you have to do is, you have to selectively saturate a proton or a particular spin or signal whereby a very low power RF is applied. I showed in the previous slide pulse sequence, apply very low RF, pre-saturate, apply a 90 degree pulse and collect the signal. That is what we do that is a simple one-dimensional experiment. Only thing is pre saturation is done before applying a detection pulse. Before applying the detection 90 degree, pre saturate the selected signal. Apply a similar RF power at a very far off distance so that none of the intensity of the signal are disturbed, they should not be perturbed. Why? we have to maintain the ideal conditions, so that I want to see the difference of the two. What we do is first collect the spectrum by saturating a proton, and then identical similar RF power you to apply far away from the spectrum of your interest So, that means if there is a disturbance because of the RF it is uniform throughout for both the spectrum. Collect the spectrum. This one which is you are not saturating any signal, where power is applied at a far off place is called a reference spectrum. Take this reference spectrum and then take a difference of this reference spectrum with the spectrum you have chosen, or you have collected after selectively saturating a particular signal. Take the difference like this. This is an experiment, do a control experiment where you can irradiate the signal, and another one

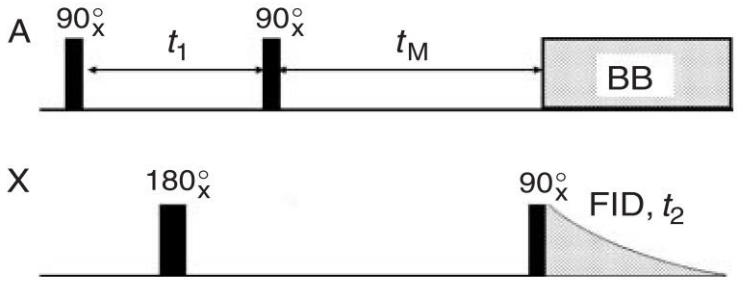
irradiate very much far away, that is called off-resonance, collect the signal. Do on resonance selectively on a particular signal continuously pre saturating, keep identical power in both the cases. Again collect the signal, take the difference between the two, and then you are going to see NOE. If there is any difference, if there is any enhancement of the signal, if there is no enhancement on any signal, if you take the difference the identical spectrum will be there then there will not be any signal. You will get 0. But if there is NOE on some of the signals when selectively saturating one of the signals, then that signal will show in that, it will not get nullified when you take the difference. This is what happens. The difference of the two spectra shows NOE enhancement if there is any.



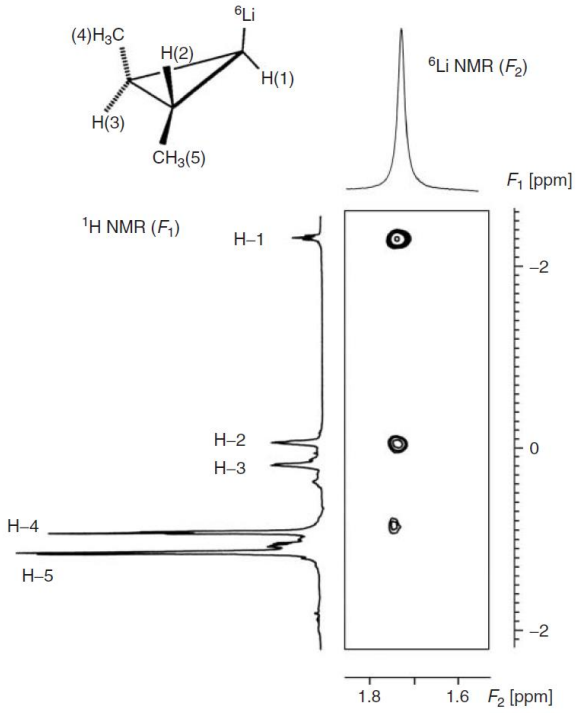
This is an example to show. See we are saturating one of them. This is a conventional spectrum and this is what is saturated. And then you see the difference. This is normal spectrum, this peak is saturated here, you see the difference. I will tell again this is a normal spectrum and this particular peak is saturated here, there is no signal because of saturation. And the difference of this and this is taken. Of course, this is negative because it is saturated and this is full signal is here, and there is no signal here, that is saturated. And you see this, there is enhancement here and here All these things get completely canceled out whereas, there is enhancement here and here. That tells you this proton and this proton are having NOE, or getting NOE because of this proton you are saturating. That will establish these two are in closed in space, close to each other. There is a spatial proximity between these two, that is what it tells you. If the saturation recovery is not set to few t1, we have to set for a few t1, selective saturation is not enough to create full NOE, so of the order of several t1 we have to put. Normally what we have to do is to keep 5 times T1, otherwise spins would not have completely relaxec back, the saturation signal would not have completely attained equilibrium. Only after it attains equilibrium you have to apply a pre saturation pulse otherwise there will be error in the NOE. So, you

have to wait for certain time normally 5 timesT1 you have to do. But in most of the cases in the routine experiment that is not necessary we give only 1 or 2 times T1 or maximum 3 times T1 and then do the NOE experiment, that is a steady state NOE.

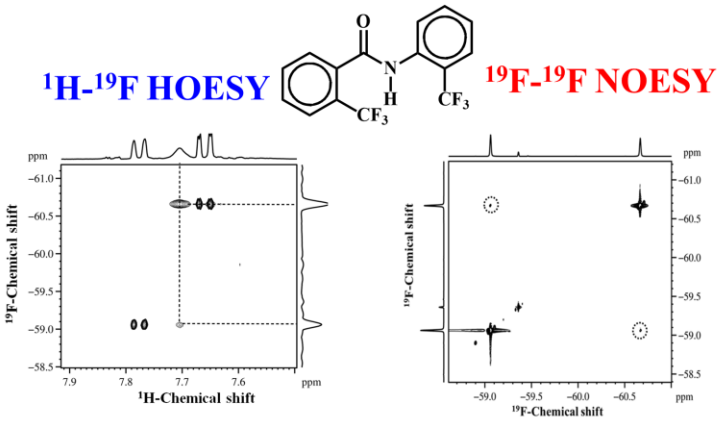
After conceptual understanding of all these techniques we will take several examples of 1D NOE steady state NOE everything and then interpret the spectrum really with many examples. Now, I will introduce HOESY, HOESY is called heteronuclear Overhauser spectroscopy. Here the pulse sequence is very simple, you have 90 tau 90 and then we have 180 pulse at the center of one of them, obviously that causes decoupling. This is what happens, here the magnetization evolves during the t1 period I am applying 90 pulse.



At the proton frequency the magnetization is evolving, and then we apply a 180 pulse here that causes decoupling between two spins, and then apply a 90 degree pulse again on the proton channel, and then mixing time here, mixing pulse, give a time for the mixing. The spins will start mixing, talking to each other. After some time apply a pulse on the detection channel that is on X, we are detecting X nuclei, while decoupling proton completely. If it is carbon 13 you are doing, this is carbon-13, this is proton, apply a proton pulse, the spins evolve here at the proton frequencies and decouple carbon proton coupling here, and then in the t1 dimension apply 90 degree pulse, allow the spins to mix, there is exchange of energy between the spins based on the spatial proximity between two heteronuclear spins, then apply carbon-13 90 degree pulse, and collect the FID while doing proton decoupling. This is a simple pulse sequence for HOESY, alright. This is an experiment to show how we can do a HOESY on varieties of nuclei.



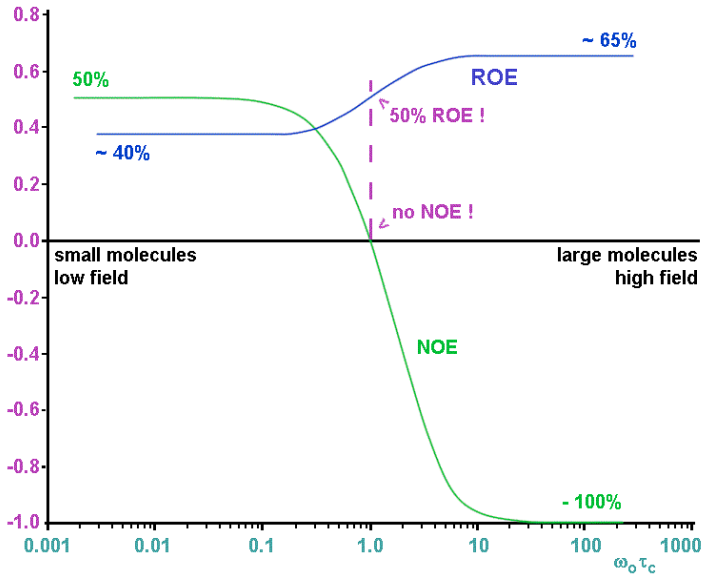
This is a HOESY spectrum of proton and lithium. See this is the thing, proton here and lithium is giving correlation to this and this. Based on the correlation you know how close they are in space. This is what it is.



And is a this is a classic example from our work. This is the molecule we are looking at. Here there is CF3, there is CF3 here and this is HOESY spectrum. Here there is a fluorine and proton, our idea was to find out how close they are because we are working on the hydrogen bonding. We wanted to see the spatial proximity. It is important for hydrogen bonding, we wanted to see how close they are in space. So we did fluorine 19-1H HOESY, if they are close in space, if there is a spatial proximity between these two, we must get the cross peaks. We did that, and we are seeing the cross peaks here, we

observed that. This is a proton axis, this is fluorine axis. There are two fluorines here, each of them giving rise to certain cross peaks. This is the next one, to compare that we also did the NOESY. Of course we have not discussed about the 2D NOESY, I will discuss that when I come to detailed discussion of NOESY, lot of examples we have to take we will discuss that. This is 19F-19F NOESY. Here it establish a spatial proximity between proximity between this CF3 and this CF3, and that is what it is. This is CF3, this is a diagonal peak, gives the chemical shift and this is a cross peak and this is a cross peak showing they are close in space. These are all NOE cross peaks. Here of course is the HOESY, this cannot be symmetric like this because they are heteronuclei. I told in a like in HSQC they are not symmetric like in COSY, similarly NOESY not symmetric, but not HOESY.

Another one I want to introduce, this is what is called a ROESY, the rotating frame Overhauser effect. Why do we do that? what is mean by rotating frame? see a lot about rotating frame everything we discussed I have discussed in one of myvery first or second courses. If anybody is interested they can see some of the lectures there where I discussed what is the rotating frame and everything.



Now look at this graph. We saw this graph. I told you, this is a situation where omega tau c is equal to approximately 1 where you will not see any NOE. This happens for small and mid-sized molecules. Now you I want to use the NOE for various purposes and it can be used to establish some assignments, and regiospecificity of the molecules, finding out spatial proximity, conformation ,etcetera. But then if NOE is not there, how can I use it. This is a problem now at omega tau C equal to 0, there is no NOE. How do you overcome this situation. Of course one thing is change the spectrometer frequency because it depends upon omega tau C, change the spectrometer frequency instead of 100 MHz go to 800 MHz, or whatever the frequency, or change tau C. The tau C change

means molecular tumbling, how do you change the molecular tumbling? you vary the temperature, increase the temperature tumbling becomes faster. If it is fast, lower the temperature, the tumbling becomes slower. Or change the solvent of different viscosity. If the viscosity is very very small, the molecules tumble faster. In a highly viscous solvent they tumble slowly. So various experimental tricks we can adapt if you want to overcome the situation there is no NOE situation. yYou can do any of these three things, change the spectrometer frequency, change the rate of molecular tumbling by changing temperature of the solvent. All right, instead of altering the solution conditions, why can't we do a different experiment? that is a instead of physically altering all these conditions we can do that. Then we can do NOE in the rotating frame, that is an advantage. In that situation what is the advantage you may ask me why should I do in the rotating frame. Remember in the rotating frame there is no negative NOE, no zero NOE, always positive NOE you will get for any size of the molecules. So why can't we do that? So that is what we do, but only thing is it has a limitation. It is less effective for molecules with longer T1 due to application of longer mixing times. In the rotating frame we are going to apply a longer mixing time. As a consequence then if you have spins with longer T1 then what will happen? it will be less effective. Don't worry about that. that's some condition, some limitation which I told you to be careful while doing the experiment. Let us see about the cross relaxation rate for homonuclear spins during NOE and doing ROE, both. This is what we observed the cross relaxation time during NOE. Not the gain the signal intensity, you don't get confused, with rho IS, that is not that. This is sigma IS, cross relaxation. This is a cross relaxation term for NOE.

$$\sigma_{IS} \propto \gamma^4 \left\{ \frac{6}{1+4\,\omega_0^2\,\tau_c^2} - 1 \right\} \frac{\tau_c}{r_{IS}^6}$$

The same cross relaxation time you can calculate for ROE. This turns out to be this.

$$\sigma_{IS} \propto \gamma^4 \left\{ \frac{3}{1+\omega_0^2\tau_c^2} + 2 \right\} \frac{\tau_c}{r_{IS}^6}$$

Now consider a situation, varieties of substitution. Here I want to take the example. Supposing omega tau c is very very small. Consider a situation, omega tauc is approximately equal to 1, in a situation omega 2c equal to 1, then what will happen? see this is 2, then still it is a positive term. This is what I wanted to show you, this one, this one I am going to see, still it is a positive term. Here of course things are different, I am not discussing that. Varieties of things will happen. In the ROE when a omega tau C is

approximately equal to 1, then you will see that 3 by 2 plus 2 into this one, this cross relaxation is there that gives rise to positive NOE. The ROE is always positive for all values of omega tau c, because cross relaxation is there. For small molecules ROE matches with that of the NOE, approximately 40 percent matching is there iin NOE, approximately 40 percent. For large molecules ROE goes up to 68 percent. For small molecule there is no issue, it can match with the transient type of NOE, this is a steady state NOE, we were talking ROE, we can also do in the 1D way and 2D way. I will tell all those later. For a small molecule ROE matches with that of the transient NOE, up to 40 percent. For large molecule this goes up by 68 percent. Under no circumstances ROE becomes 0, under no circumstances. However, NOE can become 0 when omega tau c is approximately equal to 1, but ROE never becomes 0. That is important thing you should remember.

The NOE and ROE growth rates are identical for small molecules. If I look at the growth rate how the NOE is building up, in both the situations it is identical. But for large molecule they differ a lot that is what we see growth rate is up to 68 percent for large molecules. All right we will consider the situation when omega tau c is very much smaller than 1.

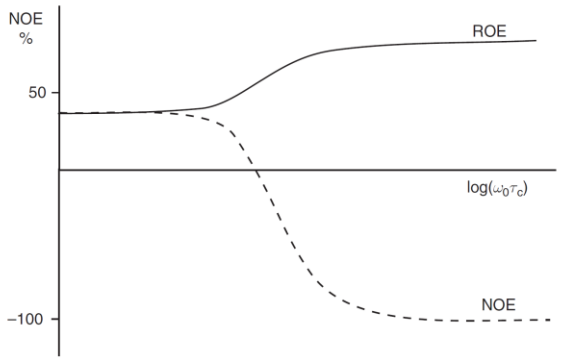
$$\sigma_{IS} \propto \frac{5\gamma^4\tau_c}{r_{IS}^6}$$

The cross relaxation rate for both transient NOE, that is NOE and ROE can be given like this. For omega tau c greater than 1, this is what it is.

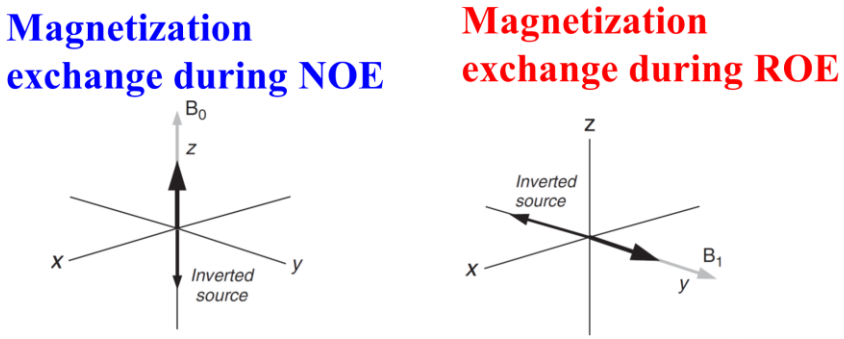
$$\sigma_{IS}^{NOE} \propto \frac{-\gamma^4\tau_c}{r_{IS}^6} \qquad \sigma_{IS}^{ROE} \propto \frac{2\gamma^4\tau_c}{r_{IS}^6}$$

In situation omega tau c smaller than 1 this equation applies to both NOE and ROE. When omega tau c is larger than 1, these two equations are different, For ROE it is different see here for ROE and NOE both are different. One has negative sign other is positive.

So, the dependence of ROE and NOE on omega 2c for isolated two homonuclear experience if I consider, this how it is.

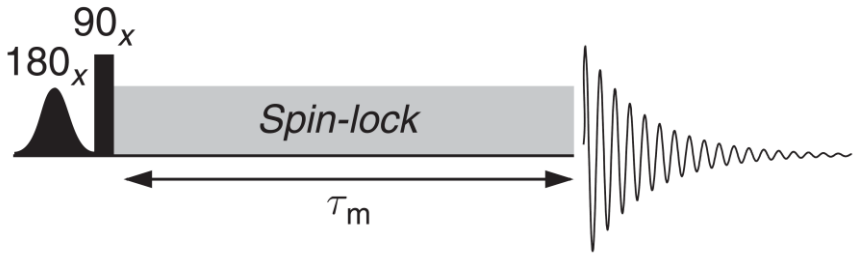


ROE starts here, goes here from 40 percent up to 68 percent. It is always positive. Whereas NOE starts at 50 percent, comes down, it goes to 0, goes negative. This is a simple one which graphically illustrates how the ROE and NOE will change. As omega tau c keeps increasing or changing here, on a logarithmic scale ROE keeps building up. Whereas, NOE from positive becomes negative. All the time, you should see here, ROE has never become negative. Starting with positive value, and it goes only positive. For large molecule ROE grows twice as fast as NOE and on an opposite side here, you see. The ROE, conceptually the magnetization develops in the tranverse plane, whereas NOE is developed along z axis. This is very interesting thing. Of course when we discussed about the NOE I told you there the magnetization transfer takes place only along z axis in NOE. Whereas ROE will take place in the x-y plane. the transverse plane. Remember two things NOE development is there along z axis, along the longitudinal axis. Whereas, ROE development is along transverse plane, in the x axis or y axis. So, the magnetization exchange during NOE and ROE if you want to see pictorially, it is like this.



In the ROE we have a anti-phase magnetization vectors one for the inverted spins, and the other one which is close in space where the magnetization transfer takes place. This is

inverted spin, this is other spin. And in the ROE same thing happens. This is the inverted spin and the other spin is here. Both are anti-phase in the x-y plane, along y axis in the transverse plane. The effect of spin lock is to retain the spins. I will show in the next one pulse sequence, that there is spin lock pulse in ROE. The effect of spin lock is to retain the spin vector. So, that it would not get dispersed off, so that ROE develops only during this time due to cross relaxation. If you do not spin lock, then they vectors may start moving away. So, this is a simple one pulse sequence for 1D ROESY.



We apply one180 pulse on the selective spin, followed by immediately a 90 pulse, apply a spin lock, it is called a mixing time. that is where ROE takes place, collect the signal. That is what it is. ROE develops during mixing time. This is simple one dimensional ROESY pulse sequence, ROE. And thi is what is called the rotating frame, where you are doing the spin locking, your spins are in the rotating frame, and that is where ROE is going to develop. So, for ROE the targeted peak is always first inverted by a selective 180 pulse, and then followed by a non-selective pulse. Then what will happen the effect is like a 270 pulse. When you have a 270 pulse, the signal gets inverted, related to others. The peak is negative with intensity, because you have a receiver here and bring the magnetization to along this axis. This is positive here, the signal is on the other direction after bringing it by 270 degree, it is the negative signal.

So, there is a negative signal. So, when the magnetization spin locked in the transverse plane, what happens during spin lock? Of course the chemical shifts are refocused, that is what we discussed in the TOCSY. During the spin lock, the spins lose their identity. There would not be any chemical shifts at all. They are all refocused and spins will relax only in the rotating frame. That relaxation time rotating frame is called T1ρ. Spins relax in the rotating frame, even though they are spin locked along the particular axis in the transverse plane. The spins can still relax, that is called relaxation in the rotating frame, denoted as T1ρ. So, in NOE the effective field is B0, but in ROE the effective field is B1. Why? I showed you in the picture, in NOE both its inverted peak is along z axis ,negative z and other spin along z axis. But in the ROE it is like this. One is along y axis other is minus y axis, that is a rotating frame. In the rotating frame the magnetic field is B1, RF field which you apply. Whereas in NOE the magnetic field is the static magnetic

field, which is huge, very large. This is what makes the NOE and ROE dynamics completely different in both the cases because magnetic fields are different.

Let us understand what it does. If you take gamma B0, the resonating frequency is in MHz. The B0 is huge of the order of several Tesla, whereas gamma B1 is very small of the order of few kilohertz, because Ba is small. So, gamma B1 is very much smaller than gamma B0. Similarly, omega 1 is very much smaller than omega 0, because they are all related to each other. Omega is equal to gamma B0 over 2 pi is the resonating frequency, that is what we discussed. Thus for omega tau c smaller than 1, for all values of tau c you have everything positive. Omega tau c for all smaller than 1, the molecules behave as if they are in the extreme narrowing limits, hence ROE is always positive. It is like a small magnetic field, it is an extreme narrowing limit. Hence ROE is always positive. And of course we can extend this to a 2D way to do the experiment, everything I will come to that, I may not go to 2D, but same experiment if you do, here you vary this as a function of t1 and collect the signal, and we apply spin lock for a mixing, this only vary, collect the signal here. This is t2 and then do the 2D Fourier transformation you get a signal like this. I will come to that later and there are also certain complications in ROESYand everything. Since the time is getting up I am going to stop here. Today what we discussed a lot of things about NOESY, NOE experiments, varieties of things we discussed like several experiments what is ROESY, steady state NOE, what is HOESY, how we get the signal? what are the pulse sequences, what is the pulse sequence for HOESY, and what happens in HOESY during spin lock. When the omega tau is approximately equal to 1 there is no NOE. In such a situation instead of changing the physical parameters, we can go to an experiment called ROESY, where you can do the NOE in the rotating frame. In that case NOE is always positive, never goes to negative, never goes to 0. That is what we saw, and we saw that for small molecules, NOE is up to 40% matches with the ROE, matches with NOE. For bigger molecule it goes up to 68%, always positive compared to NOE which is the opposite in sign. And the growth rate is faster at for ROE. For bigger molecules it goes up to twice the speed. We observed all those things, how NOE grows in both NOE, ROE, everything, how signal gain will be there in both NOE, ROE etcetera, we discussed a lot today. So, I am going to stop here, bit more about complications for ROE etcetera I will tell in the next class. We are almost coming to the end of the understanding of the concepts of NOE. I took almost 2 to 3 classes or even more may be 3 classes, just to tell you about NOE and its concepts because it is a huge topic to understand. The lot of complications are there, but gives a very, very useful information about the structure of the molecules. So, there are a number of experiments I said in NOE itself we can do. I mentioned only couple of them and gave one or two examples But we will see the utility of them in the subsequent classes, one or two of them. So, I am going to stop here. Thank you very much.