One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
Prof. N. Suryaprakash

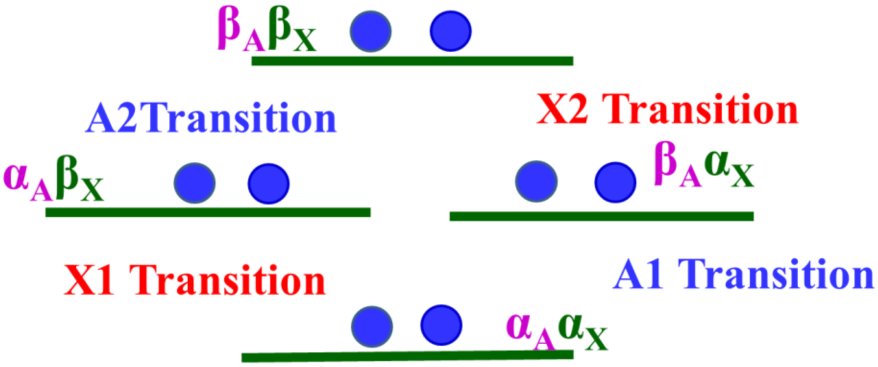
CSIR Emeritus Scientist, Solid State and Structural Chemistry Unit
Indian Institute of Science – Bengaluru

Lecture 52: Positive and negative NOE

Welcome all of you. In the last class we started discussing about nuclear Overhauser effect, an another interesting phenomena where you can get the correlation between two nuclear spins which are close in space. The requirement is only the spatial proximity and not any J coupling. There is no need of a covalent bond between two spins. And I discussed about various factors that affect NOE, viz., molecular motion and then Larmor frequency, pathways available for their relaxation, and varieties of things we discussed and also inter nuclear distance which is the dominant factor. And of course, we introduced the dipolar relaxation and allowed possible transitions. If I take two coupled spins there are single quantum, double quantum, zero quantum transitions. Zero quantum and double quantum areforbidden transitions in NMR and not detectable. Whereas single quantum are allowed as per the selection rules of NMR. But as far as the relaxation is concerned these double quantum and zero quantum are allowed. Remember only as far as relaxation is concerned, but not for detection.

That is why I said NOE not does not involve coherence. It involves only polarization, the population difference, that is what we said. And of course, I extended further. I wanted to show what is a dipolar relaxation. I showed you when by taking an example of two spins. Both the orientations of spins with respect to magnetic field remain same, but they can undergo tumbling in such a way, one spin creates a local field, dipolar field at the site of the other. And it is oscillating dipolar field. Of course, converse is true with other spin also. This is a dipolar relaxation phenomena when the local field created at that time will allow the spins to undergo flipping. This is a dipolar relaxation we discussed about dipolar relaxation, etcetera. And continuing further today we will discuss further about how do we get positive NOE, how do you get negative NOE, what are the relaxation pathways available, all those things we will discuss. Of course, as positive and negative NOE depends upon what is called a relaxation pathway, how the nuclear spins are undergoing relaxation.

To understand that let us take two uncoupled homonuclear spins A and X. Remember uncoupled I have highlighted with capitals because that is the important, and there is no need of a J coupling. And I have taken the two spin system A and X and I expect them to have a dipolar interaction in a magnetic field that is all the condition. Uncoupled homonuclear spins and having a dipolar interaction. As soon as I take the sample put it in a magnetic field, what is going to happen? This is what is called a relaxation. Outside the magnetic field the spin states are all degenerate, spins are randomly oriented. There is no separation of energy states, you all know. I did not discuss about longitudinal and transverse relaxation times T1 and T2 in this course because that was discussed in the previous course. And that is not the main aim of this course. And there are different relaxation phenomena like T1 and T2. As soon as you put the sample in a magnetic field within a short time, within that longitudinal relaxation time the spins start getting equally distributed among the energy levels. Please note, it is within a short time, less than are comparable to relaxation time, the spin get redistributed like this.



Population difference for spins A and X

$$\Delta p\ (X1) = 0 \text{ and } \Delta p\ (X2) = 0$$
$$\Delta p\ (A1) = 0 \text{ and } \Delta p\ (A2) = 0$$

I have taken the example of two spins, four energy states; beta beta, alpha beta, beta alpha and alpha alpha. Already we discussed. This is A transition, this is A transition. This is X transition and this is X transition. Each of these two transitions have numbered A1, A2, X1 and X2. And I told you already since there is no j coupling, the frequencies are identical. A will give two transitions, X will give two transitions. I have, for the sake of understanding the intensity of the peaks, just to get the intensity of the peaks I have written certain populations here. I have written some number. Why did I write that number because I just wanted to ensure that the population difference gives me the intensity and in a weakly coupled two spin system which are not even coupled, there are only four possible transitions. Two of them are equal, for each spin two of the transitions are equal. So, I want to ensure that the transition should have identical intensities that is why I have written. These are the the possible distribution of populations, immediate after putting the sample in the field. Immediately after putting. I am not even waiting for the T1 period. If you wait for the T1 period there is a redistribution here. This is what happens if you understand NMR. You put the sample immediately, let us say your sample has a very long relaxation time, you start collecting the signal you will not get. That is because you have to wait for the spins to align and in the sense population redistribution should take place. Now I am not allowing the population redistribution to take place. Immediately after putting the sample I looked at the population distribution. It is like this. Now take the population difference and find out the intensity of the peaks. A2 transition 2 minus 2 equal to 0. A1 transition, 2 minus 2 equal to 0. Similarly all the four transition two A and two X which are equivalent has 0 intensity. This is exactly what happens as soon as I put the sample in a magnetic field,a nd if you do not allow the spins to completely attain thermal equilibrium. Then you will not get any signal. This is what happens. No signal for A or X now. What we will do is, we will wait for some time that time is relaxation time, that time is called T1. T1 is called longitudinal relaxation time. The time required for the spins to attain thermal equilibrium, that is the growth of magnetization along the z axis. The population gets redistributed with time because of the relaxation forces you. Now see the difference immediately after putting the sample is one phenomena, now I have waited for some time. Let us see what is the population difference. Some redistribution has taken place now. There are four spins here two here two here and no spins her. But never be under the impression there are no spins in this state here, beta beta state, okay. There are spins, but for the sake of understanding the intensity of the peaks I have taken some number. That does not mean there are no spins in the higher energy state beta beta state. There are spins. So for calculation, this is 4, this is 2, this is 2 and 0 is taken. After waiting for sufficiently long time that is T1 period. Now let us calculate the intensity of both the peaks for A and X. The A2 transition 2 minus 0 intensity is 2, here A1 transition 4 minus 2 equal 2, The X2 transition 2 minus 0 is 2. The to X1 transiton, 4 minus 2 is 2. Now each X transitions, 2 transitions of X, 2 transitions of A both of them are equal in intensity. That is why we get two singlets, two peaks of same frequency overlaps and both of them have equal intensity which is 2, 2, okay. X transition intensity is 2, A is 2 after thermal equilibrium. After the time T1 when you put the sample in a magnetic field. Let us see now after some time. We know that the A and X transition have equal intensities, and I told you spins in the beta beta state are there only for calculation purposes taken 0 never been that is the under the impression there are no spins in the beta beta state. What we will do is, continuing further I will saturate transitions A1 and A2. I will equalize the populations. What do you mean by saturation? making the population of both the states equal. I will saturate transitions A1 and A2. What I will do this was the equilibrium population, right 4, 2, 2 and 0. When I equate when I make sure they are saturated, now these two become equal, and these two become equal. This is what happens. I have saturated both A transitions. I am NOT touching X transition at all. The X spin is remaining same. We will calculate the intensity now. A transition has 0 intensity obviously because spins are saturated, no signal will be there.

For both A transitions, 3 minus 3, 1 minus 1, 0 population. So no signal for A. What about X? calculate the X intensity, 3 minus 1 is 2, 3 minus 1 is 2. So what it means here is, you have saturated one of these spins, and still got 2, 2 intensities. It means saturation will not alone give any enhancement in the intensity.

Let us wait for some time after saturating what is going to happen? You will not be continuously saturating you are saturating for some time and then take out the RF. The system has to attain equilibrium. It will come back to thermal equilibrium. That means the saturated spin when attains thermal equilibrium, there is a population redistribution. And you can again calculate the intensities. Now when it comes back you have equal intensity again you have 2, 2, 2 and 2. What is this situation that happened now. In the sense we saturated one of the spins, both the transitions, we saw the change in the intensity is 2. After some time allow the spins to come back to thermal equilibrium, still it is 2 only. There is no enhancement, there is no change in the intensity of the signal at all. that means there is no Overhauser enhancement. What does it imply for you? the saturation alone does not cause any change in the intensity or will not give rise to Overhauser enhancement. Then what we have to do to get the NOE. Let us do one more trick now, we will saturate at the same time we will introduce what is called cross relaxation.

What is cross relaxation? it is the relaxation that occurs between two spins via dipole-dipole interaction. When we discussed about the relaxation I have discussed this at a stretch, a lot of thing about it, in one of the previous courses. The relaxation that occurs between two spins via dipole-dipole interaction. We will introduce the cross relaxation between two spins now. In the presence of irradiation of a spin the transition probabilities have varieties of effects. One W_A^1 transition, the single quantum, then there is no effect in the A transition that is being irradiated. W_1^S has no effect, since the transition is already saturated at equilibrium. In the presence of irradiation of A spin, the transition probability have different effects. When I irradiate A spin, the transition that is being irradiated has no effect. But no effects on S spin transition that already at equilibrium. That is okay, something interesting. We will allow the system to relax in the presence of irradiating field using different pathways. What are the different pathways? You know when we the nuclear spin attained thermal equilibrium, it will start coming back like this. This is called single quantum transition, the spins are relaxing through single quantum pathway. That is what I said here. The single quantum pathway only we are seeing. There is no effect. But what we will do is we will allow the spins to relax through different pathways. That is, double quantum pathway between alpha alpha and beta beta states, or zero quantum pathway between beta alpha and alpha beta states. Why not the spins undergo transition between these states. This is a double quantum relaxation, this is a zero quantum relaxation. What will happen, if you allow the spins to relax like that. In the previous example in the previous slide I showed, the spins were relaxing through single quantum pathway only to attain thermal equilibrium. Now we will attain thermal equilibrium with a different pathway. What we will do is, now let us say we have saturated A spin, both A transitions are saturated. This is the situation. The states are saturated, the population difference is zero. We will allow some the spins to come through not the single quantum pathway, but directly through double quantum pathway like this. What happens? I have not allowed the spins to come through single quantum, then it would have been a different thing. This would have come here, this would have come here and we would have attained thermal equilibrium that is a single quantum relaxation pathway. But I have chosen double quantum relaxation pathway. Now let us calculate the intensities of all both A and X spins. Now A transition 1 minus 0 is 1, 4 minus 3 is 1. A transition has 1 intensity, OK. See the X transitions now. The X transition is 4 minus 1 is 3, 3 minus 0 is 3. A transition is 1 intensity, whereas X transition is 3 intensity. This is what happens, when I allow this spins to come through double quantum relaxation pathway. If I recalculate the intensities of A and X spins, A intensity is 1, X is 3. There is a 50% gain in the intensity for one of the peaks, ok. What happened here, we saturated one spin, there is change in intensity of the non irradiated spin. This is what is NOE. I told you in the previous class the definition of NOE. I am irradiated A spin there must be change in intensity of the X spin. And that is what happens here. I irradiated A spin, the X spin has increased the intensity by 50%. There is a gain in the intensity, this is a situation called positive NOE case. If the spins undergo relaxation through double quantum pathway, there is a positive enhancement in the signal intensity by nearly 50%. And you are going to get positive NOE. Ok. Anyway this is not important thing we are not discussing, this is a case of positive NOE. Let us go to other one ,I continue to irradiate with the low power wait for the spins to attain equilibrium. What will happen? it will all come back to original state where we started with, 4, 2, 2, 0 intensities. Here again both A and X spins have equal intensity, 2, 2, that is fine. So what we did here we irradiated A spin and allow it to come through double quantum pathway we saw gain in the intensity for the X spin. Continue low power irradiation allow the system to attain thermal equilibrium then this is the spin population redistribution, ok that is fine.

I will consider another situation again I have saturated A spin I have made populations equal between the states. I will do one more thing now. Instead of allowing the spins to relax through double quantum pathway, why you cannot allow it to relax through zero quantum pathway? like this. If the spins are allowed to relax through zero quantum pathway there is a different phenomenon. Let us now calculate the intensity of A spin and X spin. The X spin intensity is 2 minus 1, and 3 minus 2, both are intensity 1. In the earlier case X was 2 intensity, now it became 1. Of course this is a irradiated spin we will not worry. What happened to X spin? I irradiated A spin allowed the spin to relax through zero quantum pathway the intensity came down by 50%. There is a loss in the intensity. This is a case of negative NOE. Remember, when the spins relax through double quantum pathway there is a 50% gain in the intensity. This is the difference. Ok.

Now you understand what are the relaxation pathways which are responsible for both positive and negative NOE. A double quantum pathway is a dominant relaxation pathway in a spin system you have a positive NOE. If the zero quantum pathway that is dominant then you will have negative NOE. So, the dipolar relaxation is one of the root causes for NOE. And the pathway that is available for relaxation defines whether the intensity is positive or negative or whether it is a positive NOE or negative NOE. Continue low power decoupling again attain thermal equilibrium, that is all what happened here come back to equilibrium. So, this is a situation. What we do in an NOE we radiate one of the spins and see the effect on the other spin. What happens within the system we do not know, the spins can relax through zero quantum or double quantum path way. There is also single quantum relaxation, it is also possible. We saw there was no effect on the intensity you know after irradiating and allowing the spins to come to equilibrium, the intensity remained same. So, double quantum and zero quantum are the only important phenomena, but W1 single quantum relaxation pathway do not create NOE in a molecule or any spin system of your interest. If this relaxation pathway is a dominant phenomenon, that is a dominant relaxation pathway then you will never see NOE. So, in any given molecule or any given spin system of your interest if you want to see NOE the spins must relax through double quantum pathway or through zero quantum pathway. That is an important condition.

When will it happen? When is W2 dominant? When is W0 dominant? If W2 is the efficient pathway for relaxation, it can happen in the case of small molecules where there is a fast motion and the frequency of tumbling is larger. The large frequency is there. So, this W2 relaxation pathway is dominant for small molecules which is undergoing fast motion. That is where you get positive NOE. All right, that yields positive NOE. So, small molecules generally, and of course depends upon various phenomena like Larmor frequency etcetera, you will have a W2 relaxation pathway and gives positive NOE. What happens if W0 is the efficient pathway this is only for the large molecules like big proteins etcetera. These molecules cannot tumble very fast like small molecules, they are little sluggish. As a consequence, they undergo slow motion and small frequency will be generated. As a consequence you will get negative NOE. Say W0 if dominant you get negative NOE. It can happen only for big molecules, large molecules. In small molecules W2 is generally dominant and you get positive NOE. Please remember these are the important sources of relaxation pathways that is for a positive and negative NOE. But in reality in a given molecule you do not know what is happening inside the nucleus. For the purpose of calculation I took it individually, what happens like that. But in reality they are not separated, they are not exclusive processes. Both phenomena are simultaneously happening. One may be dominant other may be less dominant or if both may not be dominant, both may not be existing. We do not know. both may be dominant. So, there is a process which is going on. There can be a competition between both the processes, not only between these two, there can be competition along with single quantum relaxation pathway. So, all the three W1, W2, W0 are not exclusive processes as there can be competition among them, they are competitive processes. So, that means please remember the statement positive and negative NOE is dictated by the dominant relaxation pathway, which is the relaxation pathway. So, you know, if this is the dominant, this is positive. If this is dominant negative NOE. That is all you have to understand. Alright, in summary please remember we are comparing only W2 and W0 W2 is very very large dominant large compared to W0 then NOE is positive. When W0 is large that is dominant then NOE is negative. When W2 is equal to W0 there is no NOE, you will not get NOE at all. The dominant is W1. We can even calculate NOE. There is a way we can calculate NOE also how do you calculate, etcetera. There is a process for this. How do we get the NOE, percentage of NOE we have to calculate. I irradiate A and look at the S spin, usually we always say I and S spins, this terminology came because when the NOE was discovered first it was electron, you know electron that was irradiated to see the enhancement in the signal intensity. But here we are doing only with two nuclear spins. But still term I and S remains same, does not matter. The gain in the signal intensity due to NOE is given by a famous equation called Soloman equation. There is a qualitative treatment for the phenomena of steady state NOE. A very big description is there and this is itself takes enormous amount of time to discuss and to arrive at the equations and everything. There are two books on NOE itself by Noggle and Mike Williamson, two books exclusive on NOE itself. Imagine the vastness of this topic. So, do not want to go to mathematical description to arrive at everything. But this is the final expression called Soloman equation. What this will do? this one, eta is always referred to as a symbol for gain in the NOE factor. Bracket S means I am irradiating that spin I am irradiating the S spin and I want to see the gain in the intensity for I spin. What happens if I irradiate S see the gain in I spin that is given by this expression. W2 and W0 we already said the relaxation pathways W1 that is the transition probabilities, the relaxation possibilities. And this in short form is written like this.

$$\eta_{\mathrm{I}}\{\mathrm{S}\} = \frac{\gamma_{\mathrm{S}}}{\gamma_{\mathrm{I}}}\boxed{\left[\frac{W_2 - W_0}{W_0 + 2W_1{}^{\mathrm{I}} + W_2}\right]} \equiv \frac{\gamma_{\mathrm{S}}}{\gamma_{\mathrm{I}}}\left[\frac{\sigma_{\mathrm{IS}}}{\rho_{\mathrm{IS}}}\right]$$

NOE term

Gamma S divided by gamma I into this one. This is called cross relaxation. This is an NOE term. First of all we will say this is NOE term. This whatever the entire term given in the bracket is a NOE term. And W2 minus W0 is called the cross relaxation rate constant. This is difference in double and zero quantum transition probabilities. The transition probability W2 and W0 if we find out the difference, that is called cross relaxation rate constant. In the bottom, the denominator, what we saw is the sum all the three terms. This is the longitudinal dipolar relaxation rate constant, rho. This is sigma. These are the two factors which contribute to NOE. This is the denominator this is the cross relaxation term in the numerator. This is summarized and put like this as a factor. Like this gamma S over gamma I into sigma S over rho IS. This is the factor. Why we have to introduce gamma? what was the need to introduce gamma? remember if it is a homonuclear spin you do not need to worry, gamma is identical. But we can also do NOE between the heteronuclear spins. We can get NOE between heteronuclear spins, that is the reason. In which case the populations are different. For example carbon 13 is 4 times less in population, it is 4 times less than that of protons, in a given magnetic field. So gamma factor has to be taken into account to take into account the equilibrium population of different nuclear spins, heteronuclear spins. That is why gamma is taken into account. When I say I take protons, we do not need worry about it. All we have to deal with is sigma IS over rho IS, that is all important thing. Cross relaxation over the longitudinal relaxation rate constant. So, W2 minus W0 define what is the sign of NOE. That is important thing. What are these the rates of W2, W1 and W0 processes? if somebody can ask the question what is W2, okay it is the transition probability. But what are these rates? how fast they were undergo transition? what is the rate of W2, W1 and W0? That depends upon another function called spectral density function given by J(ω). It depends upon spectral density function. And what is that for the case of W1. This spectral density function if you look at J(ω), it is identical to Larmor frequency of the spin that is undergoing flipping. That is what is our Larmor frequency? let us I am recording the spectrum in 400 MHz spectrometer, then W1 is 400 MHz. That is all. If both the spins are the same, the homonuclear spins, then the Larmor frequency is around 400 MHz, and for both them it is 400 MHz. That is the W1 transition probability. The frequency of W1 is nothing but the Larmor frequency. What is W0? W0 is energy difference, the difference between Wi minus Ws. What is Wi and Ws resonating frequency of two homonuclear spins if I take, I am dealing homonuclear spin now, when WI and Ws same. Let us a protons, they are two different chemical shifts. The resonating frequency is just difference in the chemical shifts. What is that chemical shift value? It is of the order of few Hz or few kHz. Remember W1 is in MHz, W0 is few kHz or few Hz. Then what is W2? then W2 is the sum of the resonating frequencies of WI and WS. What is the resonating frequency of two spins? both are in MHz. This is 400 MHz, this is 400 MHz, then what is W2? W2 become 800 MHz. That is the difference. See W0, W1 and W2 depend upon the spectral density function, which in turn depends upon various factors.

W1 depends on the Larmor frequency W0 is the difference in the chemical shifts, the difference between Larmor frequencies, and W2 is the sum of the Larmor frequencies. So these are the three factors. W1 is Larmor frequency, W0 is difference in the Larmor frequency and W2 is sum of the Larmor frequency. These are the three factors which you have to consider. All right, what is the spectral density function in numbers for proton at 400 MHz. Just to give you the numbers, of course, W1 is 400 MHz, W2 is double of that, sum of the Larmor frequencies, 800 MHz and W0 is a difference in the chemical shift few kHz. That is all. The spectral density functions the frequencies of are of this order. Only these three we have to consider where I am considering two homonuclear spin systems. Then what is the spectral density? you may ask me a question. We said spectral density function everything, fine. What is the spectral density means? Of course it is a distribution of the frequencies of oscillating dipolar interaction. I told you about dipolar interaction between two spins. Both of them have the same orientationwith respect to magnetic field, that orientation will not change. But then when they undergo a tumbling motion, the local field at the site of the other nuclear spin starts changing. That is what we saw. One nuclear spin induces dipolar field at the site of the other nuclear spin. That keeps changing. what are the frequencies we are going to get.

The distribution of the frequencies of the dipolar field, the oscillating dipolar field that is created at the site of the other nucleus because of the tumbling motion is nothing but J(w) called spectral density function. So, it is nothing but the distribution of frequencies of the oscillating dipolar interaction term. Okay, now to induce spin transitions molecules must tumble at the appropriate frequency to provide the fluctuating field. Remember this is a very important point. Let us say a transition has to undergo between two energy states, This has some energy. Of course when we discuss about the relaxation everything in the one of the previous courses extensively I discussed this. To induce the spin transition the molecule must tumble at appropriate frequency. Then only the spins will undergo transition. Then what is the tumbling frequency? it should match with this. When the tumbling frequency matches with this energy separation then spins will undergo transitions. So, this is important thing, the dipolar fluctuating field should match with this energy separation. And the next question is where do you get this fluctuating field? of course a lot of factors are interconnected. First the spectral density function that has some frequency which is related to Larmor frequency, and where do the spectral density function come? it is because of the fluctuating dipole? the magnetic field is created because of the tumbling motion of the nucleus. What is the reason for fluctuating magnetic field? where do you get fluctuating field, electromagnetic fields to generate this dipolar field at the site of other nucleus? That is because origin of the fluctuating magnetic field in solution state is the motion, the rotational motion of the molecules. The molecules are not staying steady, the spins are aligned with the magnetic field, but this in turn generates magnetic field because they are undergoing fluctuation. That is what we saw in the figure, when the two dipoles I took the example, one dipole is stationary let us say I took the example of this just for calculation purpose. And other one is undergoing tumbling motion while creating magnetic field, you know all through that other spin, keeping both the orientation with magnetic field same. Spins stay aligned with the external magnetic field but in turn while undergoing motion, tumbling motion generates the fluctuating magnetic fields. That is the frequency of rotation of the molecule, when they are undergoing rotation. So, this is the origin of the fluctuating field and this is given by this thing. The molecules can undergo tumbling, fluctuation, rotation. These are all the important factors. So if the molecules are steady, let us say, you take a perfectly rigid solid, no motion at all neither translation or rotational motion, in which case spins take enormous amount of time to relax. It might take ages to relax. These fluctuating fields are dipoles that is generated at the site of one spin because of the motion of the other spin gives rise to this type of spectral density function, which is responsible for relaxation. And these fluctuating fields are the pathways for the system to release energy and that causes relaxation. You understood what causes relaxation? the spectral density function which has different frequencies. Where do these fluctuating frequencies come from? How do fluctuating magnetic field comes? it is because of the molecules undergoing motion, rotational motion, tumbling motion like that. And these tumbling motional frequencies matches with this rotational frequency of the molecule, and that causes flipping of the spins. And this rotational correlation time tells us about the motion of the molecules. How fast the molecules are undergoing motion? how fast? are they going faster or slower. That is defined by tau c called correlation time. I will discuss more about this later, since the time is getting up. I will discuss more about the correlation time everything in the next class. But right now you understand today we discussed a lot about various relaxation pathways available for spins to relax, and we discussed about the spectral density function. I said the spectral density function has different frequencies, w1 w2 w0. The spectral density functions for these things are nothing but w1, the Larmor frequency, w2 the sum of the Larmor frequency of the two spins, and w0 the difference of the Larmor frequency. In a given spectrometer frequency let us say 500 MHz is w1 500 MHz, w2 is 1 GHz, whereas w0 is the difference of the chemical shifts. And these fluctuating fields come because of the rotation of the molecules, which in terms generate the fluctuating fields at the site of the other spin, which causes the spins to undergo flipping. This causes dipolar relaxation. Let us say in a given molecule if the fluctuating field and the frequency generated is twice the Larmor frequency, then w2 is dominant. if it is of the order of the Larmor frequency then w1 is dominant, if the frequency of the fluctuating field is difference of the chemical shifts then w0 then is dominant. This is how you have to understand what happens to dipolar relaxation, how they contribute for the NOE. okay with this I am going to stop today. We will continue with these relaxation time, correlation time etc in the next class. Thank you