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

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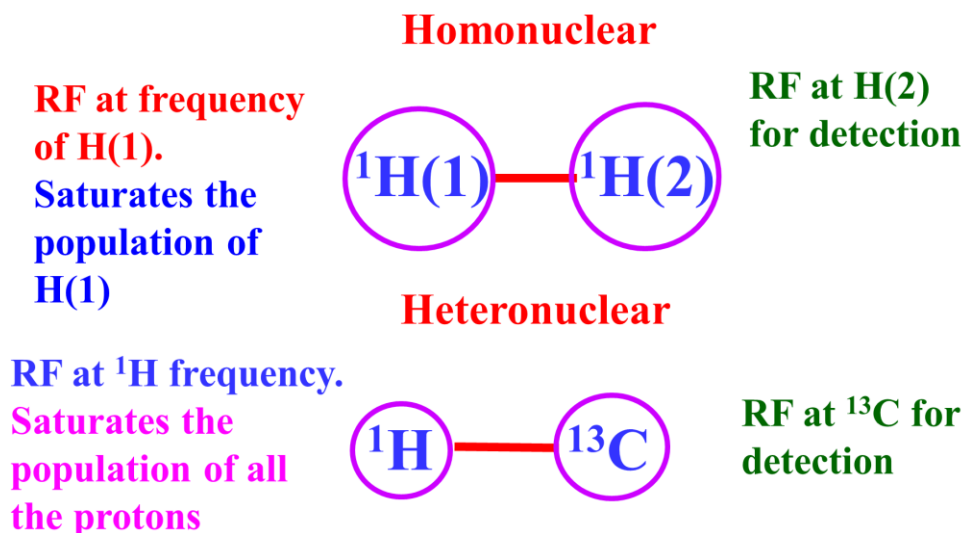**Indian Institute of Science – Bengaluru**

Lecture 51: Conceptual understanding of NOE

Welcome all of you. Since the last couple of classes, we have started discussing varieties of two-dimensional experiments apart from COSY and TOCSY which we discussed extensively. In the last class, we discussed two-dimensional INADEQUATE experiment, where we could get the correlation among the two dilute spins, especially carbon 13, carbon 13 correlation in their natural abundance. In fact, this helped us to identify and get the carbon skeleton of the molecules. We took lot of examples, we analyzed the pulse sequence, we understood how the pulse sequence work, how we can interpret the 2D INADEQUATE spectra, etcetera. Continuing with that, I also discussed a resolved spectroscopy technique, J resolved spectroscopy. I told you it is similar to the spin echo sequence, homonuclear spin echo or homonuclear J resolved experiment, where chemical shifts are refocused, but not the J couplings. And variation of the delay in the spin echo delay, can create intensity changes and then the intensity gets modulated as a function of the coupling strength and also the t1 delay. Double Fourier transformation of that gives the spectrum, where J coupling or the couplings will be in one dimension, indirect dimension and in the direct dimension, we will have both chemical shifts and the couplings. And the spectra will be tilted by 45 degree. If you tilt it by 45 degree, we are going to get spectrum where we have only chemical shifts along the F2 dimension and J couplings in the F1 dimension. The projection of the F 2 dimension spectrum gives you what is called completely proton broadband decoupled proton spectra. It is something very interesting, which removes all the couplings and you get individual peaks for each chemical shifts. That is one of the important things of this experiment. And also, we understood the heteronuclear version of that J resolved. How do we get heteronuclear J couplings resolved in one dimension. In other dimension, we have only chemical shifts. And there we have couple of experiments, the many pulse sequences are there. I introduced one where we can get only long range couplings, while suppressing the one bond couplings. And also, to speed up the data, the proton detected version of this heteronuclear J resolved also I discussed. While discussing, I gave lot of examples, lot of experimental data we saw and then we analyzed the spectra. I hope you people are comfortable with that. After that, today I am entering into another topic, which we discussed not extensively, but I introduced, I brought in those statements called nuclear Overhauser effect. I mentioned it at times when we were discussing the decoupling in the carbon-13 and various example like DEPT and etcetera. And I have discussed about polarization transfer and varieties of things. At that time, I also said we will discuss about

NOE, the nuclear Overhauser effect. This is another type of correlation experiment. It is all together a different one. It does not require J coupling. For this experiment the nuclear spins need not be J coupled. That is a different version.

We will start understanding how this 2D works, two dimensional experiment. Before that, we should understand what is NOE. The concept of NOE is very important. So, I will introduce the concept of NOE for some time and then varieties of NOE experiments like steady state, 2D transfer NOE, HOESY, ROESY, everything we will try to discuss as much as possible. I will start with the conceptual understanding of NOE in this class.

Before going to NOE, I want to tell you about decoupling. What is the decoupling? Decoupling is nothing but the irradiation at one of the nucleus, so that you can break the coupling with other. It could be selective or broadband. And in carbon 13, when we were discussing, I told you we do broadband decoupling where we apply RF pulse, RF power at the proton frequency and ensure that all carbons are completely decoupled with all the protons to which they are coupled to. That is a broadband decoupling. So, what we do in decoupling? that was a heteronuclear, we can also do that in the homonuclear case. But homonuclear broadband decoupling is very very challenging task although it is possible to do in a different way. Generally, what homonuclear decoupling used to be done was selective, where you can selectively irradiate and hit at a particular frequency with the second RF and see the changes in other parts of the spectrum. Then you will know which spin is coupled to which spin. That is one of the ways by doing selective decoupling in the good olden days, we used to get information on which spin is coupled to which. That has now been replaced by two-dimensional COSY experiment where you can get correlation information, about which spin is coupled to which spin, which spin is sitting next to each other, all those things we discussed in COSY, TOCSY etcetera. The same thing in the homonuclear case in the good old days were done by what is called selective decoupling. So, broadband decoupling is little challenging, we will see how we can do that also if there is a possibility.

Homonuclear

RF at frequency of H(1).
Saturates the population of H(1)

^{1}**H(1)** —— ^{1}**H(2)**

RF at H(2) for detection

Heteronuclear

RF at ^{1}H frequency.
Saturates the population of all the protons

^{1}**H** —— ^{13}**C**

RF at ^{13}C for detection

In homonuclear what we do is we apply radio frequency pulse on one of the channels, what do you mean by that? I am saturating the spins, the population between two spin states I am making it equal and then of course, I apply a read pulse on another proton and start detecting the signal. This has to be done very very selectively, we cannot do broadband there. And of course, heteronuclear as I told you already, we irradiated one of the nuclei with a very strong RF, where the RF strength should be larger than the heteronuclear coupling constant, at least twice larger than that. And then we saturate all the populations of the protons and then detect the signal of carbon 13. In this process what happens? all the protons which are coupled to all the carbons are broken, the coupling is completely broken. And RF we apply at the carbon 13 frequency for detection. And while we are doing decoupling, one could come across several effects, one can see several effects while doing decoupling. What are these effects? For example, when I am irradiating at a chemical shift of I spin with a power let us say RF power larger than the coupling constant between I and S, let us say I and S spin are coupled. I am irradiating I spin and the RF power is strength of that is larger than the coupling strength. In which case what will happen? The multiplicity of the signal can disappear, instantaneously it will disappear. That means because we are breaking the coupling, I and S coupling, we are breaking because we are irradiating I spin. The S spin multiplicity disappears. We are going to get some reduced multiplicity depending upon whether we are decoupling broadband or selective. If it is broadband couplings will disappear, all the couplings will disappear and we get a single peak for S. And in continuing with the effects of decoupling that is a situation where decoupling with RF power is at least twice larger than the coupling strength. If the RF power is much smaller than the coupling interaction, also at the same time let us assume RF is not on resonance.

For example, I consider the two spin system like this. If I have to see the effect of this I had hit exactly at the center of the coupled multiplicity pattern and see the effect of it at the other region. On the other hand, instead of that if there is deviation from that, if it is not exactly on resonance, that is if it is slightly off resonance there could be different effects, some effects will be seen. In which case I and S coupling is not completely removed, rather it get reduced. It scales down a bit. If RF power is not enough and we are also not exactly on resonance. This can happen. That is one of the side effects of decoupling.

Let us take the example of an hypothetical molecule, two spins A and X coupled between themselves. This is a coupling strength this is the chemical shift of A and chemical shift of X. I am saturating A spin that means I am making the population between two spin states equal, then this peak is not seen at all. We are not going to see since that population difference is 0. But then you see the effect of it because this is

coupled to this through scalar coupling, through covalent bond. This doublet collapses into a singlet and intensity become double.
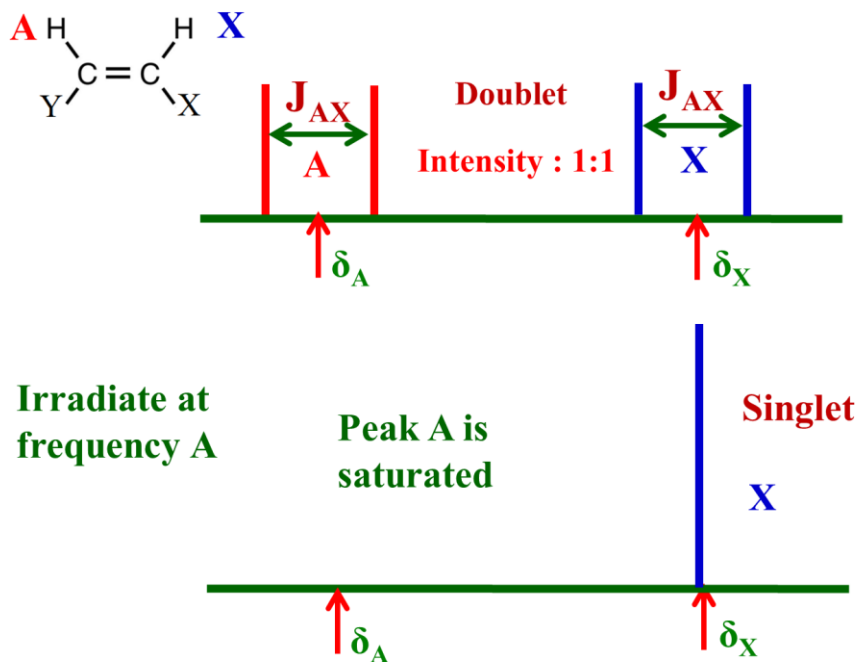
Look what happened? I irradiated this proton A and this became a singlet. Of course this type of decoupling phenomena, its effects everything, how we do, what are the all the details have been discussed in one of the previous courses. I am just giving you the idea what happens when we are doing the decoupling exactly on resonance with a power sufficiently larger than the coupling constant. And the change in the intensity what you are going to observe is due to change in the population distribution, that is true, because of the population distribution.

Now it can so happen when I am trying to do the decoupling the intensity can increase and intensity can decrease also. There is another type of another effect of decoupling. When it is going to happen we will see that. And that, is a sort of I would not say it is a decoupling, it is a sort of irradiation at another frequency. And you will see the change in the intensity of the peak similar to your decoupling. And what is going to happen if I hit a particular peak at a particular RF frequency. Then what will happen is that the non irradiated peak intensity will change. In this case as I told you these two nuclear spins need not be j coupled. What is the requirement is the spatial proximity. As long as both of them are close in space, then if I hit one of them there is a change in the intensity of the other. And for this thing as I told you spins need not be j coupled, only spatial proximity is important. And if you go far and far away between them, if they are far away

separated, then the NOE effec becomes very small or sometimes you may not see. So, spatial proximity of the two spins is important, and they need not be j coupled.

Then what will happen? This phenomena when a two spins are close in space when I hit one of them, the change in the intensity of other is called nuclear Overhauser effect. This NOE is positive, if the intensity of the non irradiated signal increases. I have two signals here I am irradiating signal here and this intensity can go up, that is positive. Or the intensity can come down from what it was earlier, that is called a negative intensity, negative NOE. So, the intensity increasing and decreasing corresponds to whether the gain in the signal intensity or loss in signal intensity. This is nothing but positive intensity a positive NOE or negative NOE. So, if the two spins are close in space I irradiated one of them if there is a change in the intensity which is increasing then we say there is a positive NOE between these two. Otherwise, if the intensity comes down, it is a negative NOE. Of course, you can come across the situation there may not be any change at all there is no NOE at all for that matter in which case this intensity may not even change at all. That is a situation where there is no NOE.

I am considering a situation when there is NOE, NOE can be positive or NOE can be negative depending upon whether the intensity of the non irradiated peak is increasing or decreasing. All right, before going to the conceptual understanding of what an NOE is let us understand some salient points of NOE. At least I will read out couple of sentences, bullet points, like I did for the J coupling which are very important. And each of these can be discussed, at stretch.

First of all I tell you NOE depends upon dipole-dipole relaxation. There is a dipole-dipole interaction that is through space interaction, it is not through covalent bond. This is an important phenomena important mechanism of relaxation. Even in a conventional case if you want to see if the spins are undergoing relaxation the dominant relaxation phenomena for spin half nuclei are dipole-dipole interaction. I am telling you for spin half nuclei the dipole-dipole relaxation is a dominant phenomena. So, NOE depends on this dipole-dipole relaxation. It is one of the important concepts and it is due to through space interaction, it is not through covalent bond.

For example in a given molecule the two nuclei can be separated with 10 bonds or 20 bonds away, does not matter. Let us say you take a protein, there is one spin here it undergoes folding and everything in three dimensional space and other one comes close by here. And these two spins maybe they are separated by several bonds more than 10 or 15 bonds, it does not matter so long as they come close in space between them. Each of them is a individual dipole nuclei, a magnet, there can be interaction between the magnets

through space, that is possible. So, this is a through space interaction the spatial distance is what matters. How close they are in space matters, not how many bonds they are separated. That is another important point of NOE. As I already discussed, I told you NOE can be either positive or negative. That is one of the important things, depending upon the change in the intensity of the non-irradiated peak, whether increases or decreases. Most important parameter is r_{ij}. this is where NOE is used to get the structure of the molecule. If you get the magnitude of NOE, how much is the percentage of increase in the NOE, we can correlate it to the distance between the two interacting spins r_{ij}. We can get the information about internuclear distance and also about the molecular motions. The magnitude of NOE depends on this r_{ij} and the molecular motions. So, most important if I find out what is the strength of NOE, how much is the NOE, I can correlate it to the distance. I can get geometry information, the conformational information. These are all important points of NOE.

Another thing we can say about NOE, before we go further for discussion. There are several factors which are responsible for the NOE. I would not say affecting, or these are the factors that are responsible for NOE. One is various pathways that are available for relaxation. NOE as I said is due to dipole-dipole relaxation. How the dipole-dipole relaxation takes place? what is the pathway available for the spins to relax that is a dominant phenomena. That tells about whether the intensity is positive or negative or no no intensity change, the zero NOE. Positive intensity in a positive NOE, negative NOE or 0 NOE, that is defined by the pathways available for relaxation.

And another thing it depends upon the magnetic field strength or in other word indirectly I would say the Larmor frequency. The NOE what you do at the lower field and that phenomena for the higher field are different. Of course the concept is same, but it depends strongly on the Larmor frequency. For example, as we go further I will tell you it depends upon a term called omega tau c, where tau c is a correlation time, omega is the Larmor frequency. And then depending upon this we can get NOE positive negative or 0 it depends strongly on the Larmor frequency. Or in other words, the spectrometer at which you are doing NOE experiment. It depends strongly on that.

And also I told you it depends upon just now I introduced the word called correlation time or which depends upon mobility of the molecule. I will tell you as we go ahead further what is a correlation time it depends upon the mobility of the molecule. How fast molecules are undergoing tumbling motion, that also affects NOE intensities.

As I already told you it depends upon $1/r_{ij}^6$. Remember 1 over Rij to the power of 6, that is in the denominator, that shows slightly deviation in this value can affect the NOE drastically. So, it is depends on the spatial proximity, that is why the spatial proximity information can be derived because it strongly depends upon 1 over Rij to the power of 6.

I told you about dipole-dipole relaxation, but in this course I never discussed about dipole-dipole interaction at all. In one of the previous courses, in the advanced course when I was introducing solid state NMR I discussed a lot about dipolar interaction, quadrupolar interaction, etcetera. Here for the benefit of the audience, I will spend one or two minutes about dipolar interaction. If you consider two interacting spins separated by the distance r we can treat them as like a vector and it can be making certain angle with respect to magnetic field.
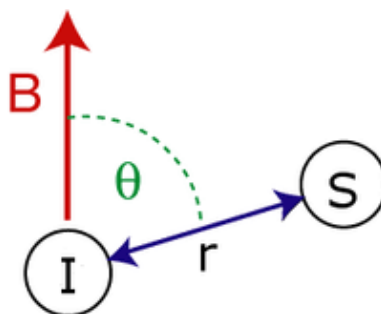

Depending upon what is the angle it is making, the dipolar interaction changes. And this dipolar interaction, when there are multiple spins are present, there will be pair wise interaction among all these spins. And now I would say this spin is interacting with this spin when it is in a magnetic field. And this is the angle theta I can even find out what is the dipolar interaction strength, since it depends upon orientation. Of course, we are dealing with solution state NMR, in this course. We are not discussing anything about solid state NMR. In the solution state there is a isotropic motion, that is, random motion is going on, the molecules are undergoing thermal agitation all along. I told you NOE is a orientation dependent phenomena. At any given instant of time if you consider one internuclear vector in this direction, you can find out equal and opposite internuclear vector. That is the vector with the same magnitude but in the opposite direction. So, when you take the ensemble average of all these, the dipolar interaction goes to 0. So, in the isotropic medium the dipolar interaction is averaged out to 0. You will not be able to see it, because it is a orientation dependent and isotropic motion all possible orientations are present and then the ensemble average goes to 0.

Continuing with the dipolar interaction it drops off very fast with the distance between two spins rij. Of course, dipolar coupling is always given by $1/r_{ij}^3$, but NOE is given as $1/r_{ij}^6$ because NOE involves two dipoles. Each dipole interaction depends upon internuclear distance $1/r_{ij}^3$ that is the thing which matters. So, NOE can occur with a distance of less than 5 to 6 angstroms, less than that, 2 angstroms, 3 angstroms. As it comes closer and closer the possibility of seeing stronger and stronger NOE is there. As the distance between two spins become longer and larger, let us say, distance increases

beyond 5 to 6 angstroms or even more, you may not see NOE or it may be very weak. This clearly tells you we can get distance information. Up to let us say less than 5 or 6 angstroms, usually we get very strong NOEs, especially proton-proton NOE that I am discussing now.

With this let us try to understand NOE in two spins case before going into the complications. In a given molecule there could be N number of protons. In a molecule many protons could be present, but to understand the concept, let us start the discussion with only two spins. Now, NOE does not involve coherences. Remember like any other NMR experiments, we detect the oherence you measure that, Bring the magnetization to x and y axis that is a coherence, we collect the FID. But here no coherences are involved. It is just a polarization, the difference in population between the alpha and beta spin states. If I consider spin half nucleus there are two alpha and beta spin states. The population difference between these two spin states is what is defined. So, it is a polarization which is important not the coherences. NOE does not involve coherences, that is the important point you should remember. NOE is merely a polarization. And before I want to go further I want to introduce certain type of transitions in the coupled spin system.

For example, you remember in the second or third class itself we discussed about selection rule in NMR. What did we say selection rule is where the change in the magnetic quantum number between any two spin states or energy states should be either plus 1 or minus 1, that is what we said, yes it is true. This means at any given instant of time only there is change in the one quantum of energy. When the spins are undergoing flipping between any two states there is only one quantum of energy is getting changed. The change from alpha to beta spin state is what we are going to detect. The change is only one quantum of energy and that is what the selection rules allows. But can there be higher quantum and lower quantum lower than 1, it is 0. Or higher than 1, it could be 2, 3, and 4 quantum NMR? Can we detect higher quantum NMR? selection rule does not allow, I told you they are forbidden transitions. But how many such type of transitions are possible, in just two coupled spin system? For 3 spins it is more, for 4 coupled spins it is even more. But we are taking the example of only two coupled spin system.
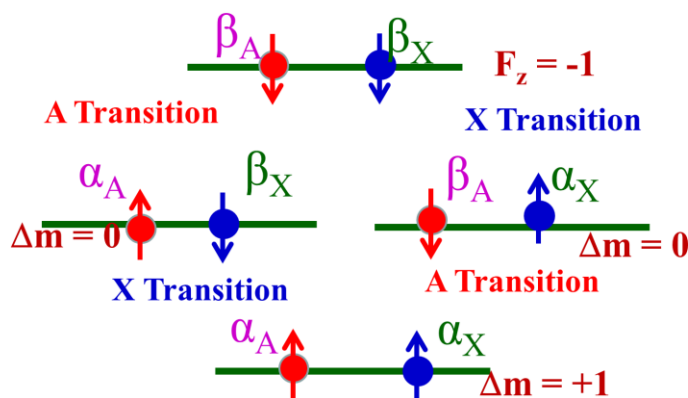
I will discuss about single quantum double quantum zero quantum transitions in such a spin system. Of course, energy states already I we have discussed n number of times. It is a beta beta state, alpha beta, beta alpha, and alpha alpha. The magnetic quantum number of each states is Fz. It is nothing but the sum of each individual magnetic moments of each individual spin. So, minus half minus half is minus 1, 0, 0m plus 1 that all we know. Now, I told you about single quantum transition which is change in the delta m by plus or minus 1, this and this is allowed single quantum. I told you that is a transition because A spin is undergoing flipping here. This is also A transition. All the red spins which are undergoing these transitions are for A. And again alpha to beta, beta to alpha that is allowed. This blue I have made it for x spin, alpha to beta, and beta to alpha. So, these are all X transitions. Any other transition single quantum is possible here? No, there is no way you can get any other transitions here. So, the maximum possible single quantum are four. I told you when n spins are coupled, especially two spins here, we have four energy states and four single quantum transitions we are seeing. If you go to higher number of coupled spin system, it will become different we can calculate in a different way. I have told you already $^{2N}C_{N-1}$ transitions are possible, all right.

So, we get two transitions for A and two transitions for X, but here I am taking the example of two spins which are not j coupled. See here there is there is no shifting of alpha alpha, beta alpha, alpha beta and beta beta states. There is no stabilization or destabilization I told you. Remember when I was discussing about the sign of the j coupling such situation was there, it is not here. So, no coupling. So, as a consequence frequencies for both A transitions are identical. The frequencies of both X transitions are identical. They overla,p as a consequence you get one peak for A and one peak for A, that is what it is. We will go further.

So, so far we understood delta m equal to plus 1 and minus 1. Now can we think of a situation where delta m equal to plus 2 or minus 2. It is possible, where is that situation here? This state has total magnetic quantum number minus 1, this is plus 1. Let us take the difference of these two. Whether this transition is allowed? here minus 1 to plus 1 or plus 1 to minus 1 that is SQ. This change in delta m is equal to plus or minus 2. This is a double quantum transition. Remember this is not allowed for detection. What about this transition? can you think of spins 0 to 0 alpha beta, becoming beta alpham the same energy state 0 to 0. That is not allowed, because we cannot detect it. So, this is called zero quantum transition. But remember double quantum and zero quantum transitions are forbidden in NMR, as far as the detection is concerned. But are allowed in the context of relaxation through dipole-dipole interactions. That is why I did not take j coupling, I considered two spins which are closed in space, I was considering dipole-dipole interaction. vIn the context of dipole-dipole interaction or relaxation through dipole-dipole this is allowed. The double quantum and zero quantum relaxation pathways are

allowed,vbut detection is possible. That is why I told you remember NOE is not a coherence it is only population difference, you understood why I am telling that? We cannot see the transition. The spins can come from alpha alpha to beta beta, it can go from beta beta to alpha alpha, etc. There is a change in the population, that is possible. That is allowed as far as NOE is concerned through dipole-dipole relaxation. But you cannot detect the signal directly. This higher quantum detection is done in a different way I discussed this in one of my previous advanced courses. So, we will not worry about that. So, please remember one thing that 0 quantum and double quantum are forbidden transitions in NMR, as far as the detection is concerned, but in the context of relaxation they are not forbidden, they are allowed. OK.

With this we will understand now what is a dipole-dipole relaxation. Let us consider two spins which are not J coupled. Remember, but closed in space, that is what I want. There should be dipole-dipole interaction, but there is no need of a covalent bond for this. But only thing is the spatial proximity is important, I bring them close in space. These two neighboring nuclei in a molecule I can treat them as two dipoles, true. Each nuclear spin I told you is like a dipole. That will be tumbling in solution like this.
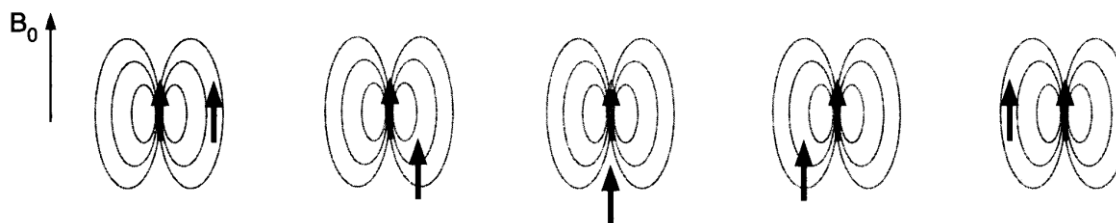
Let us say one dipole is here, and the other dipole is here. It will influence this, it will create a local field at the state of this nuclear spin. And what is happening is this nuclear spin is not sitting in the same place. It keeps changing its position. From here it comes here, from here, it goes here, like that it keeps changing its position. It is tumbling in solution. But remember one thing the direction of orientation between these two nuclear spins is same. The orientation of each nucleus with respect to each other and with respect to the static magnetic field do not change. With respect to static magnetic field both are parallel here that orientation with respect to this remains same. That is not changing, but one spin is undergoing tumbling while introducing the local field, the dipolar field, at the site of the other nucleus that is possible.

Then you ask me a question what happened to this spin. I am showing for one spin. The same phenomena is applicable for other spin also. This can also create a local field at the site of this like the way it is doing. That is also possible. So, what is happening? The nuclear spins are undergoing tumbling motion in the solution. The orientation of each of these nuclear spin with respect to magnetic field remains same. But at the same time

when they are undergoing reorientation, changing the orientation with time, it is creating the local field at the site of the other nucleus. One creates the local field at the site of the other nucleus like this, and their relative orientation in space will change, but not with respect to the magnetic field. Then the local field experienced at one nucleus as a result of the other nuclei keeps changing, keeps fluctuating. I is called a fluctuating magnetic field, local fluctuating magnetic field, dipolar field. It is not constant and the molecule undergoes tumbling. This nuclear spin keeps changing its orientation, creating local field at the site of other spin, and that keeps on affecting that the local field experience by one nucleus. As a result the other keeps on fluctuating because of molecular fluctuation. And this will cause what is called dipole-dipole interaction. There will be a dipole-dipole interaction as the molecules are undergoing tumbling. but it is there. This tumbling molecule it creates variation in the magnetic field at the site of the nucleus. That is what I told you, as it keeps changing the local field created at the site of the nucleus keeps on changing. And this oscillatory field causes the spins flipping of the adjacent nucleus this is the interesting concept.

Remember this nuclear spin here, I saw this was undergoing tumbling motion with respect to this. And then it is creating the local field at the site of other spin. You can see that this local field is the one which is responsible for that other coupled spin, the neighboring spin to undergo flipping from alpha to beta state or beta to alpha state, whatever that state is it causes flipping of the neighboring nuclear spin. This is only oscillatory field not a constant field. If the oscillatory field is the constant field means it can match with only one frequency but not other things. So, oscillatory field keeps on changing in frequency. You know it gets oscillatory and causes the flipping of the spins of the adjacent nucleus. This is what is called dipole-dipole relaxation and it is a dominant dipole relaxation mechanism for many spins of nuclei. And this is the source of nuclear Overhauser effect, please remember. There are several mechanisms but dominant mechanism for NOE nuclear Overhauser effect is the dipole-dipole relaxation. Now, we will see the time is getting up, we have to discuss more about the relaxation, negative and positive NOE etcetera. We will come to that later, but right now today what I was trying to tell you is you are going into a new topic called NOE which does not require scalar coupling, does not require covalent bond between the spins. All it requires in a close spatial proximity and in which case it depends upon the distance between the interacting spins or closely spaced nuclear spins.

It depends upon the magnetic field, it depends upon the tumbling motion of the molecule. I showed you molecules are undergoing tumbling. And the tumbling motion depends upon the size of the molecule and it depends upon the magnetic field, I said Larmor frequency. All those factors affect the NOE and the salient feature of the NOE is it depends upon the internuclear distance and the through space interaction and no need

of a covalent bond. And then we also introduced about what is called a single quantum, double quantum zero quantum transitions. The single quantum transitions are always allowed. The double quantum and zero quantum transitions are forbidden in NMR, the selections rules do not allow. However, in the context of relaxation they are allowed. Nuclear spin can undergo dipole-dipole relaxation through this double quantum pathway or zero quantum pathway. That is allowed because NOE does not involve coherence it involves only population difference, that is what I told you. It involves only polarization not the coherence. So, we introduce this and then we will discuss further about NOE, what we have to do, how the positive NOE negative NOE comes, how the dipole relaxation occurs, everything in the next class. Thank you.