One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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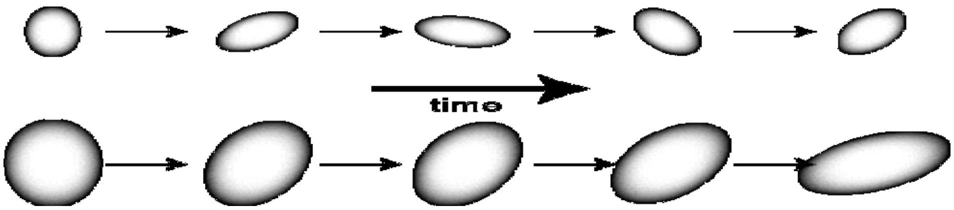
Lecture 53: NOE and correlation times

Welcome all of you. In the last two classes we discussed about nuclear Overhauser effect, the basic concepts. I told you what is an NOE. You will see the enhancement in the intensity of the signal or of course reduction in the intensity, both are possible, when one of the spins is irradiated with a low RF. And ofcourse, the spins need not be scalar coupled. I told you basic requirement is they should be spatially close to each other. Spatial proximity is an important condition to observe NOE and we discussed various parameters which are responsible for the NOE. I said NOE depends upon the internuclear distance, it depends upon the magnetic field at which we are going to study, it depends upon the correlation time. In addition to that it depends on the pathways available for the spins to relax. Then we discussed about double quantum, zero quantum and single quantum relaxation pathways and what will happen to the signal intensity when the spins adopts double quantum relaxation pathway or the cross correlation is because of zero quantum pathway. In double quantum relaxation pathway, there is enhancement in the intensity, in the zero quantum relaxation pathway there is a reduction in the intensity by 50%, we saw that. And of course the single quantum pathway of relaxation will not give rise to any NOE. And of course this dipolar relaxation is the main reason for observing NOE. Then the question is where are the fluctuating fields coming from for the spins to relax. The spins will relax through dipolar interactions and there must be fluctuating fields for that. This also we discussed about the dipolar interaction. I said if we have two spins close to each other, molecule may be undergoing tumbling. The orientation of the spins with respect to the magnetic field do not change. However, diagrammatically I showed you, when the spins are undergoing motion, that is rotation or changing the position, one spin will introduce or create oscillating magnetic field sat the site of the other spin. Of course vice versa. And these oscillating fields are responsible for the spins to relax, that is what I said. Then these are the origin of the spins to relax and we discussed about the fluctuating magnetic fields. So these fluctuating magnetic fields releases energy. And of course when the molecules are fluctuating I mean undergoing motion to create a fluctuating magnetic field there is a term called rotational correlation time, which I said yesterday. And of course we discussed about the spectral density function also. Spectral density function depends on upon the Larmor frequency and the correlation time, I said.

I will start from today the correlation time. It tells about the motion of the molecules, and the correlation time is you know defined as τ_C. It correlates the orientation of molecule at two different times and depends upon several factors. In principle it is the time required for the molecule to undergo one rotation by one radian that is the definition. But anyway basically this is the important thing, you understand. And of course it depends upon the molecular weight. Bbviously when the molecules are undergoing tumbling it depends upon the size or weight of the molecule. And this is the reason I will say the correlation time τ_c defines the sluggishness of the molecule. A short correlation time means fast random motion. A long correlation time means sluggish motion of the molecule. I am going to stop here, because I need a pen, okay, I got the pen, alright. A long correlation time means sluggish motion of the molecule. Let us see small molecules tumble more quickly here, I can see that these are the small molecules, these are large molecules they tumble more slowly, these molecules tumble very quickly.



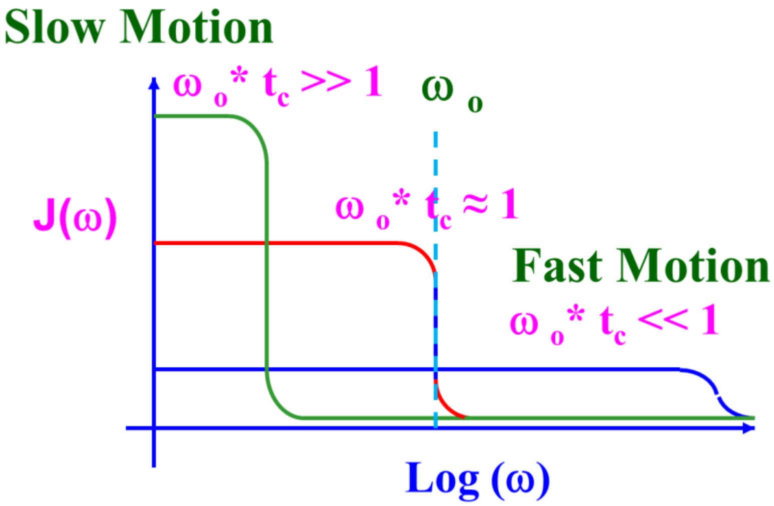
And we can make a rough estimate of the correlation type for a given mass, depending upon the size of the molecule or its molecular weight. If the molecular weight is given by MR and this is the rough estimate to tell you what should be the correlation time.

$$\tau_c \, (\mathrm{ns}) = M_r * 10^{-12} \, \mathrm{s}$$

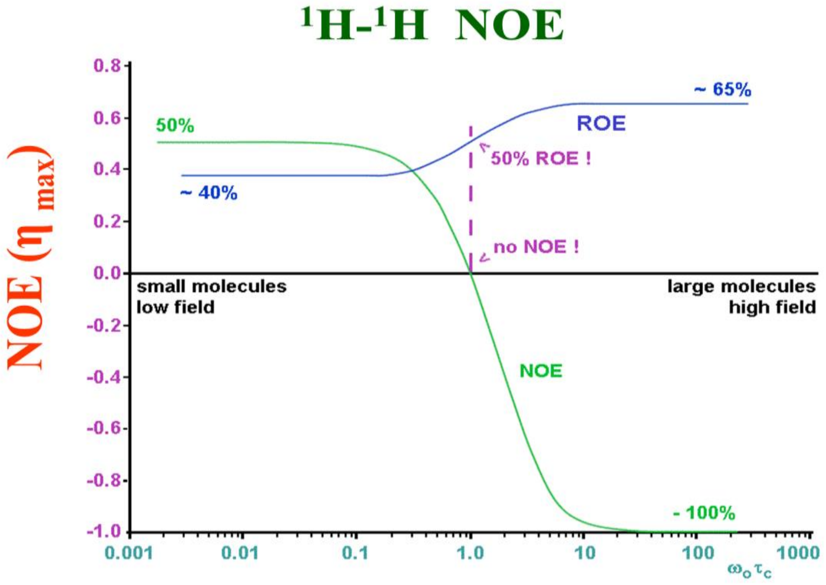
Of course it can be different but it is an estimate. If I know the molecular weight this has to be multiplied by 10 to the power of minus 12 seconds, then I know what is the correlation time in nanoseconds. So the molecular motions are within nanosecond scale. I can make a rough estimate based on that. And just for an example if I take a 3.3 kilo Dalton size of the molecule, it is a small protein or whatever it is, then the correlation time I can calculate as 2 nanoseconds. That means you can find out what is the resonating frequency. Not the correlation time, just frequency, because I know the correlation time and so once I know 2 nanosecond 1 over inverse of 2 to the power of minus 9. We can find out, that turns out to be 500 MHz. So that is the frequency of correlation time for a particular molecular weight. Motions with higher frequency will be generally absent for a perfectly rigid molecule. For a perfectly rigid molecule if you take very higher frequencies are generally absent. These are just information I am giving you. The spectral density at twice the Larmor frequency 2 omega is very small for proteins and other molecules. Usually twice the Larmor frequency is very high frequency, such molecules have to rotate very fast. Usually proteins and others are very big molecules you know that, they are all macromolecules. As a consequence, they cannot undergo rotation faster the correlation time will be not that much. So it takes more time to undergo rotation. As a consequence spectral density function at twice the Larmor frequency is very small. So this is especially for macromolecules. And the zero frequency also is there, I mean the zero frequency component of the spectral density function. It increases with increasing the molecular weight because the rotational frequency becomes lower and lower. As a consequence of that the zero frequency component of the spectral density function increase. That means if I draw a spectral density function I can find out how it is changing as a function of molecular weight. We will see that now. The spectral density function depends upon two factors now. One is the correlation time, and the other is the resonating frequency. These are the two factors that defines spectral density function.

$$J(\omega) = \frac{2\tau_c}{1 + \omega_0^2\tau_c^2}$$

So basically there is an expression for spectral density function which is given like this. It is simply j omega is twice the correlation time, divided by 1 plus omega squared tau c squared. This is the expression which has been derived. I am not going to the details of the mathematics because it will take enormous time that is not the important thing, but you should know what is the spectral density function depends on. It depends upon correlation time and spectrometer frequency, the Larmor frequency and what does it mean at ω_0 equal to 0, I put this as 0 then what will happen maximum will be there at ω_0 is zero, then this denominator is only 1. So it implies at ω_0 equal to 0 the spectral density is maximum, correct. The spectral density for this, you can find out what it is. The spectral density when it be put this as 0 you are going to get maximum, that is fine. But then as you keep changing the omega, as you start going away then you have higher and higher frequencies. As a consequence, the spectral density function drops off very fast. So that means how fast it drops off when this changes, also depends upon tau c. So these are the two factors which play together. How fast the spectral density function drops off is controlled by τ_c. And this is an expression for that. Whatever I said in words is shown in figure here. See this is spectral density function, it is maximum here and you see it starts dropping off and I have written here three different types of graphs. This is a situation when omega tau c is very much greater than 1. omega naught is a Larmor frequency and the tau c is the correlation type. When this parameter is very much larger than 1, you get a spectral density function like this. It has a large value here and then drops off very fast. And if you go to a small molecule where there is a fast motion omega tau c that means is very much smaller than 1 in which case what is going to happen see there is a spread like this and then dropping off is very slow.



And in the intermediate situation, when omega tau c is approximately equal to 1. This is the graph you are going to get. This is a graph of spectral density function plotted as a function of omega. And when you take omega into tau c together and this is the graph you are going to get. And this is called a slow motion region, and this is a fast motion region for the molecules. So, these curves predicts how the relaxation rates vary with the correlation type. This will define also the relaxation rates. How it varies as the correlation time changes. This is the graph to understand that and one thing I want to tell you the total amount of the oscillating field is always a constant. For example, go in this graph the total amount of the fluctuating field you are going to get is an area of this and this whatever you take, it is a constant. The total amount of the oscillating field is constant, but the upper limit of the frequency varies with correlation type. The upper limit here is larger, here smaller here, and even smaller here. So, but the area of the curve, that is the total amount of the oscillating field, that is constant. Now spectral density that means it represent the probability of finding a fluctuating magnetic component at any given frequency as a result of motion. What it means? look at this what I am trying to say there is a fluctuating magnetic component of this field. It has a frequency and as a function of the motion how it is changing we will come to know here. See as omega t is changing spectral density is here for a different frequency and it will tell you how the magnetic component is there, what is the probability of finding a fluctuating magnetic field at any given frequency because of molecular motion, because tau c is embedded into that equation. Basically if you draw a graph of homonuclear NOE between protons how it is going to change as a function of omega tau c; always omega tau c comes as a factor, the multiplier. As a function of omega tau c if a plot the NOE, this is NOE and the curve appears like this. This is from this journal. Now you can see forget about this part this I have not discussed.



I will tell you about it, this is called ROE not NOE. that is a rotating frame Overhauser effect. I will come to that at the end of this talk or maybe in the next lecture. But at the moment we will concentrate only on the NOE curve. The NOE curve you can see this is positive, that is 50% positive intensity. when it is there, it is 50%; it goes through 0 comes down and becomes minus 100. That is and the possibility of getting this enhancement in the intensity is + 50 to -100. That is it can be half or minus 1. That is a possibility of getting the NOE. If you draw as a function of omega τc if you plot this as a function of omega tau c this is NOE. What does it tell you? For the small molecules here, in this region, this is a region pertaining to small molecules, in that region we say NOE is positive. For a large molecule here in this region you see large molecules and also omega tau c is larger here, that means at higher and higher magnetic field then what is going to happen NOE is negative. For small molecules at lower magnetic field NOE is always positive. For bigger molecule in this region it is negative you see it started coming here different values. But you can find out a situation where it is this curve is crossing 0 point that correspond to omega tau c is approximately equal to 1.106 or 1.116 some number is there. Approximately consider 1, this is a point at which omega tau c equal to 1 there is a switch over point NOE is 0 here, no NOE at all, totally 0. So that means depending upon the omega tau c you can have a positive NOE or negative NOE. That depends upon whether it is a small molecule or large molecule, whether you are doing at high field or at low field. Various parameters are coming into picture. Of course omega tau c means omega when I am taking it account the factor of magnetic field is embedded into it. This is important point you should remember. Based on the omega tau c the NOE can be either positive or negative. It is positive for small molecules and negative for large molecules, alright. Going more into the details of that when omega tau c is less very much less than 1 the molecules are tumbling very fast that is what I said and that gives a positive NOE. This condition is called extreme narrowing condition. That is the omega tau c very much smaller than 1 is called extreme narrowing condition. This you can experience only for small molecules, that too in non-viscous solvents. Why non-viscous solvents because mobility has to be more. In a viscous solvent there is rigidity for the molecules to move, it cannot rotate tumble very fast. So it has to be for a small molecule a non-viscous solvent, where the omega tau c is very much smaller than 1. This is called extreme narrowing condition.

We can go to another situation omega tau c is very much greater than 1. The molecule tumbles very slowly, and there is negative enhancement for this. Here it is a positive NOE it is a negative NOE. And this condition is called diffusion limit, It diffuses very slowly and this can be seen. Earlier I said it is for small molecules, here this omega tau c is very much greater than 1 in the diffusion limit. Generally experienced for proteins or macromolecules, big molecules especially in viscous solvents. Also in both in proteins and big molecules and in viscous solvents when omega tau c will be greater than 1 you will experience this one. The molecules tumble very slow, so extreme narrowing condition for omega tau c is very much small smaller than 1, and positive NOE. And for small molecules in non-viscou solvents when omega tau c is greater than 1 negative enhancement, the diffusion limit applicable for bigger molecules, macromolecules proteins, etcetera, and mostly in viscous solvents.

And we go to the next situation this is omega tau c approximately equal to 1. In this limit the NOE goes to 0. When can it happen? It will happen for certain medium size molecules and at certain Larmor frequency, because this factor is coming into the picture omega naught into tau c. So, either of them can be different, either this can be larger or this can be larger, doesn't matter, but the product should be approximately equal to 1. So it can happen depending upon certain medium size molecules and at certain Larmor frequency this factor becomes 1. And NOE will not be seen in such situations, okay.

Remember, we discussed three conditions, omega tau c very much smaller than 1, omega tau c very much greater than 1, and omega tau c equal to 1. And when omega tau c is exactly equal to 1 there is no NOE. One is a extreme narrowing limit and the other is the diffusion limit. All these we discussed. In the extreme narrowing limit, for small molecules, positive NOE. And in diffusion limit, for big molecules and in non-viscous solvents, it is negative NOE. That is all what we discussed. Alright, the zero quantum transitions are thus favored by small molecules, why ? we saw that. Generally zero quantum transition are the favorable because of this one. In zero quantum process energy is small as it is the difference in the Larmor frequencies. Only when there is small molecule generally there will be zero quantum transition probabilities more. For double quantum transitions the energy is the sum of the Larmor frequency is there omega i plus omega s. These transitions are stimulated by rapid tumbling of the molecules. The molecules undergo rapid tumbling at omega 2, which is equal to sum of the Larmor frequencies, which is if I told you already in the previous class, for the resonating frequency of 500 MHz W2 is almost 1 GHZ, close to that. And we can derive all these expressions for, omega 1, omega 0, and omega 2.

$$W_{1\mathrm{I}} \propto \gamma_{\mathrm{I}}^2 \gamma_{\mathrm{S}}^2 \left[\frac{3\tau_{\mathrm{c}}}{r^6\left(1+\varpi_{\mathrm{I}}^2 \tau_{\mathrm{c}}^2\right)} \right] \qquad W_{1\mathrm{I}} \propto \gamma_{\mathrm{I}}^2 \gamma_{\mathrm{S}}^2 \frac{3\tau_{\mathrm{c}}}{r^6}$$

$$W_0 \propto \gamma_{\mathrm{I}}^2 \gamma_{\mathrm{S}}^2 \left[\frac{2\tau_{\mathrm{c}}}{r^6\left(1+(\varpi_{\mathrm{I}}-\varpi_{\mathrm{S}})^2 \tau_{\mathrm{c}}^2\right)} \right] \qquad W_0 \propto \gamma_{\mathrm{I}}^2 \gamma_{\mathrm{S}}^2 \frac{2\tau_{\mathrm{c}}}{r^6}$$

$$W_2 \propto \gamma_{\mathrm{I}}^2 \gamma_{\mathrm{S}}^2 \left[\frac{12\tau_{\mathrm{c}}}{r^6\left(1+(\varpi_{\mathrm{I}}+\varpi_{\mathrm{S}})^2 \tau_{\mathrm{c}}^2\right)} \right] \qquad W_2 \propto \gamma_{\mathrm{I}}^2 \gamma_{\mathrm{S}}^2 \frac{12\tau_{\mathrm{c}}}{r^6}$$

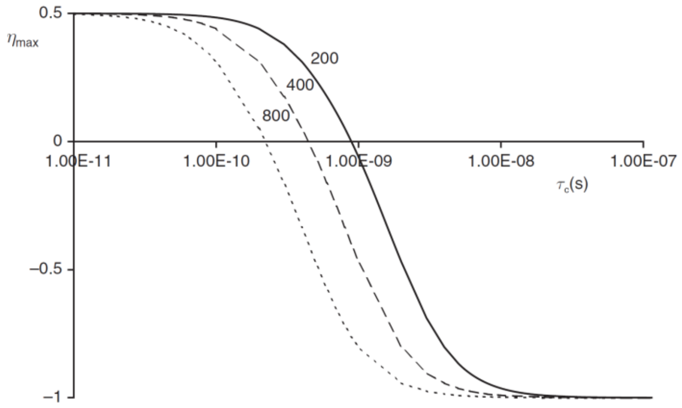
All the three we can arrive at, okay. An extensive theoretical calculation one can do, but you should remember one more thing in these three equations all of them of course we have taken into account the gamma. The gamma also takes into account the population distribution I told you. So if it is homonuclear situation both gamma are equal, when we have heteronuclei, the gamma i and gamma s are there.

One interesting thing you should observe here is, there is tau c at the top; and of course there are three factors here coming into the picture. And all of them are inversely proportional r to the power of 6, r to the power of 6 is coming in the denominator. All these three equations are arrived at for three situations omega 1, omega 0, and omega 2. All the three. So far we discussed about the spectral density function, correlation time, varieties of things, diffusion limit, extreme narrowing limit, etc. Now we will go to a situation. Let us say I have done the experiment, I want to get the distance, what is the use of NOE? that is what I told you, it will give you spatial proximity information. We have to use the enhancement in the NOE to arrive at the distance information. How they are dependent on the distance? of course previously I told you 1 over r_{ij} to the power of 6. Let us see that. We know the expression for w0, w1 and w2. They all contain r_{ij} information, I showed you in the previous slide. They are all in the denominator, r_{ij} to the power of 6, that is there. Why r_{ij} to the power of 6, you may ask me a question. Of course logically we can understand this. One can also derive it mathematically, but logically consider a magnet, the strength of the dipole dipole interaction depends upon 1 over r_{ij} to the power of 3. 1 over r_{ij}^3; it is in the denominator. Whatever it is, to the power of minus 3. But if I have to talk about two magnets dipole, to have a dipolar interaction dipole dipole interaction for NOE, then we need two dipoles and NOE depends upon two dipoles because of that, these Ws will depend upon r_{ij} to the power of 6. The inverse of r_{ij} to the power of 6. That is what is the dependence of NOE. It depends upon 1 over r_{ij} to the power of 6. Here I have taken heteronuclei, that is why r_{IS} is written. r_{ij} is what normally we talk. r_{ih} to the power of 6 inversely proportional to that so distance NOE depends upon the distance. So if you know the NOE we can get distance information. And for omega τc very much smaller than 1, the extreme narrowing condition, I am considering a situation omega τc is very much smaller than 1, then all the terms containing omega in the equations for omega w0 , w1 and w2 are negligible. Why? we can see here. Look at this one. This is very much smaller than 1. If you put it, practically makes it 0. Then what is going to happen? this term can be eliminated . then 3 we have τc over r to the power of 6. That is what it is. This is a situation when omega τc is very much smaller than 1. When it is very much greater than 1, we cannot ignore this term. That denominator contributes. This is a situation for all the 3 w0 and w1 and w2, we calculate for this extreme narrowing condition. Then here also the term, this one goes to 0. We can ignore that iin both the both the situations because omega omega τc is very small, both of them can ignore. And again we are going to get this 2 τc over r power of 6; this term goes to 0. No go to w2, again 12 tc, this term goes to 0. 12 tc over r to the power of 6. So we know the transition probabilities, that is w0, w1 and w2. All of them are inversely proportional to r to the power of 6. And of course multiplied by certain τc. Now assuming this molecule exists and in the extreme narrowing condition, I substitute these parameters, what we arrived at for all the three. We can substitute in this equation this is the equation, remember I told you about enhancement in the signal intensity. This term is called η, it is enhancement term. If I saturate this spin, S how much is the intensity gain I am going to get, gain or not is given by η. The η tells you about the NOE factor. When I am irradiating the spin S and this was equation, I told you earlier okay. So this is the situation we will substitute that for all the W's what we arrived at, for extreme narrowing condition.

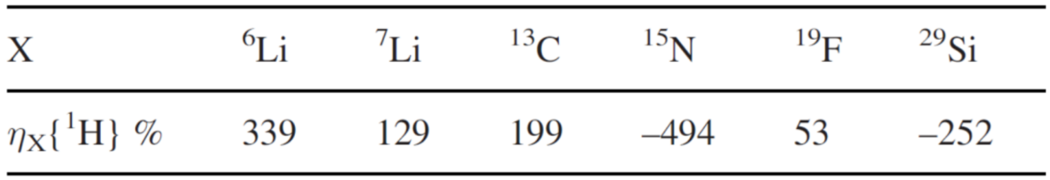
$$\eta_I\{S\} = \frac{\gamma_S}{\gamma_I}\left[\frac{W_2 - W_0}{W_0 + 2W_1{}^I + W_2}\right] \equiv \frac{\gamma_S}{\gamma_I}\left[\frac{\sigma_{IS}}{\rho_{IS}}\right]$$

$$\eta_I\{S\} = \left[\frac{\left(\frac{12\tau_c}{r^{-6}}\right) - \left(\frac{2\tau_c}{r^{-6}}\right)}{\left(\frac{2\tau_c}{r^{-6}}\right) + 2\left(\frac{3\tau_c}{r^{-6}}\right) + \left(\frac{12\tau_c}{r^{-6}}\right)}\right] = \left[\frac{12 - 2}{2 + 6 + 12}\right] = \frac{1}{2}$$

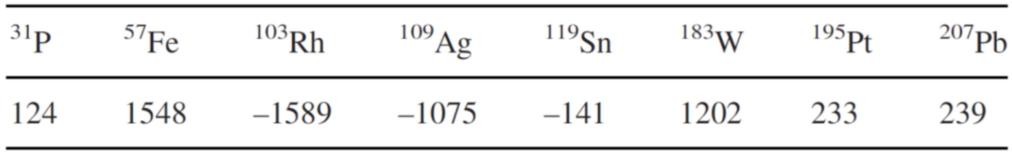
You substitute for this equation, we got in the previous two slides back I showed you what is w1 w0 as well, when we eliminate omega omega τc parameter, this is what the terms we got for w2 , w0 and this is for w1. All we got same thing. We substitute for this equation now we can calculate very easily r to the power of 6. Here if you take it out here also r to the power of 6, you take it out. this cancels out and τc also gets cancels out as a common factor. Then what is left here 12 minus 2 , divided by 2 plus 6 plus 12, equal to ½. This is the equation I am going to get. What this equation tells me η, the gain in the intensity when I irradiate a spin S, at the site of I spin, the maximum gain is half, when? In the extreme narrowing condition. I told you an extreme narrowing condition the maximum gain in the intensity is half, that is what it is. This can be shown graphically like this.



What will happen when I change the resonating frequency, how it changes. For two homonuclear spins as a function of tumbling rates, these are the tumbling rates, but at different Larmor frequencies, at 200, 400 and 600 MHz. But by and large it follows same same pattern, almost remains same. But the only thing is omega τc is shifted a bit, depending upon what is the tau C, tau C anyway varies. For a given molecule tau C is kept constant, this one varies. As a consequence this omega tau C, this position is 0 crossing over shifting with different Larmor frequencies. This is what is expected because the factor omega tau C is a coming as a multiplying factor. So omega tau C matters. When tau C is changing like this, because this is also getting changed. All right. The enhancement in homonuclear case we will find out. For homonuclear case, for example protons, both gamma is same, in this expression, okay, So gamma A is equal to gamma X. I am taking an AX spin system. What is the maximum NOE you are going to get? 50%, half. What happened in the case of heteronuclear spins? It goes by the ratio of the gamma, because in this heteronuclear case gamma is not same, so gamma ratio comes into picture. See this is what the factor $\eta_I\{S\}$ is equal to this. Remember this thing, this I showed in the last two last two classes also. So if this is equal to this, now this is equal to this, half. So in the homonuclear case this is identical, gamma S equal to gamma I so this is maximum is half. But in the heteronuclear case this we have to take into account, ratios of the gammas are important, and this what happens. So in the heteronuclear case it goes with the ratio of the gammas, okay. How much is the enhancement of intensity we are going to get. The enhancement in the NOE, if proton is irradiated. My irradiation spin is proton. That is gamma eta I into S, in this flower bracket is S. S is my proton. What is enhancement in the intensity for I? that is for different nuclei when I irradiate proton how much gain I am going to get. This is what it is. See the irradiated spin is proton and enhancement is seen on the X nuclei. What is the X nuclei there are varieties of nuclei is given here. How much NOE maximum we can get if I irradiate proton?



X	^{6}Li	^{7}Li	^{13}C	^{15}N	^{19}F	^{29}Si
$\eta_X\{^1H\}$ %	339	129	199	–494	53	–252



^{31}P	^{57}Fe	^{103}Rh	^{109}Ag	^{119}Sn	^{183}W	^{195}Pt	^{207}Pb
124	1548	–1589	–1075	–141	1202	233	239

For lithium nearly 340 times enhancement in the signal, that is really large, a huge enhancement you are going to get. And go to the case of other carbon-13, 199 that is almost 200 percent, the intensity gets doubled here. Here it gets nearly three and a half times here, Nitrogen -15, it is five times. See this is the ratio of minus 494 times enhancement for silicon. Of course negative sign is because there are nuclei with negative magnetic moment. Look at this one, the rhodium almost 1600 times enhancement, so also iron. So depending upon the nuclei in which you are seeing the enhancement, this I am talking about heteronuclei, I am irradiating proton, I want to see what is enhancement in the other heteronuclei. In which case there is a significant advantage, if you do NOE. The gain in the signal intensity is enormous, you will see that. That gives a lot of information. NOE gives lot of information here, okay. And another important thing if I when I was discussing carbon 13 I told you, if you remember. It is an important factor for improving the sensitivity of the low gamma nuclei. For example when I was doing broadband decoupling of carbon 13, remember I said several ways of decoupling continuous wave broadband decoupling, gated decoupling, decoupling without this NOE, all those factors we discussed. The broadband decoupling when it is done when we look at the carbon 13 there is enhancement. There is NOE enhancement, that is what I told you at that time, there is enhancement due to NOE, which I said I will discuss later. This is the reason we do decouple proton absorb carbon -13. There is enhancement in the intensity by nearly two times, that is 200%. And you can see that here gamma I and over gamma 2X. I saturate I spin and observe the gain in the X spin. X is the observing spin. The total intensity is given by this equation I_0 is the original intensity. 1 plus eta is the factor of the gain, the total intensity. When I am decoupling proton, just substitute here it turns out to be 2. That means I am observing carbon 13, I am decoupling proton and this is the enhancement in the intensity. It is nearly 200%. That means twice the signal enhancement will be there. Alright instead of that if I see proton what will happen? We will see that. Of course enhancement is given on the proton, when 13C is decoupled. Remember now it is reversed. In the previous example I observed carbon 13and decoupled proton. But here I am doing the reverse, I am decoupling carbon-13 and observing proton. So in our expression whatever the nuclei which is there in the flower bracket that gets changed here gets changed. Here this is carbon 13, this is proton. Now this is what is the intensity you are going to get, about 12.5%. So remember even in the case of detecting proton there is a gain in the intensity of the proton signal when we decouple carbon 13. When we observe carbon 13 and decoupled proton then 200% is the gain in the intensity. But here it is only 12.5% when I decouple carbon 13, that is because of the gamma. And that factor is there that's what I discussed. Okay. In the NOE so far we discussed a lot, how we get the gain in intensity everything we understood, various factors that affects NOE that also we discussed. In this class we discussed a lot about Omega tau C, correlation time, when the Omega tau C very much less than 1 there extreme narrowing limit,, when Omega tau C very much greater than 1, it is a diffusion limit, Omega tau C approximately eqial to 1, there is no NOE. Varieties of things we discussed. And what is tau C, what it defines about the tumbling motion of the molecule. and tau C depends upon molecular weight and the sluggishness of the molecule, everything we understood. And of course I also showed a couple of equations for the transition probabilities of W0, W1 and W2. When we substituted that in the Soloman equation for the enhancement in the intensity, where you know W2 minus W0, W2 plus 2W1 all those the expressions are there. When we substitute these parameters W1 W2 W0 in the solomon equations, for homonuclear case we showed that maximum gain is half in the extreme narrowing limit. When there are different heteronuclear present it goes with the ratios of the gammas. And we saw several examples, when we radiate proton how much gain is there in different nuclei. Basically what is important is when we are looking at the carbon 13 NMR, we detect carbon 13 while decoupling proton, that is where NOE factor comes into picture. When that is done I showed you there is 200 percent gain in the intensity. The enhancement intensities is there, almost each peak will be doubled. Whereas if I decouple carbon 13 and observe proton, there is 12.5 percent enhancement of the intensity. So these are all some of the important things about NOE we discussed. The NOE is a very very important factor to get the information about spatial proximity. I told you NOE depends upon r_{ij}, that means if you know the intensity if you know the NOE factor, we can correlate to the distance. So we can get distance information. So far that is what we discussed. The time is getting over. I am going to stop here. But telling this is easy, but there will lot of complications involved in doing NOE. We will discuss that in the next class. Thank you