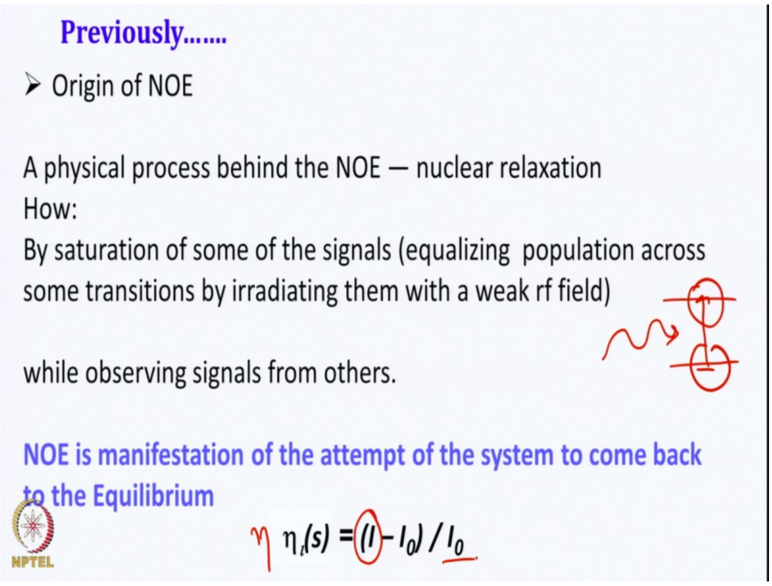
NMR Spectroscopy for Chemists and Biologists
Professor Dr. Ashutosh Kumar
Professor Ramkrishna Hosur
Department of Biosciences & Bioengineering
Indian Institute of Technology Bombay
Lecture No. 22
Steady-state and Transient NOE

Okay, so welcome back to today's lecture. We were discussing polarization transfer, where we started to discuss steady-state and transient NOE. So we continue from there.

(Refer Slide Time: 0:36)



Previously…….

- Origin of NOE

A physical process behind the NOE — nuclear relaxation
How:
By saturation of some of the signals (equalizing population across some transitions by irradiating them with a weak rf field)

while observing signals from others.

NOE is manifestation of the attempt of the system to come back to the Equilibrium

$\eta \quad \eta_I(s) = (I - I_0)/I_0$

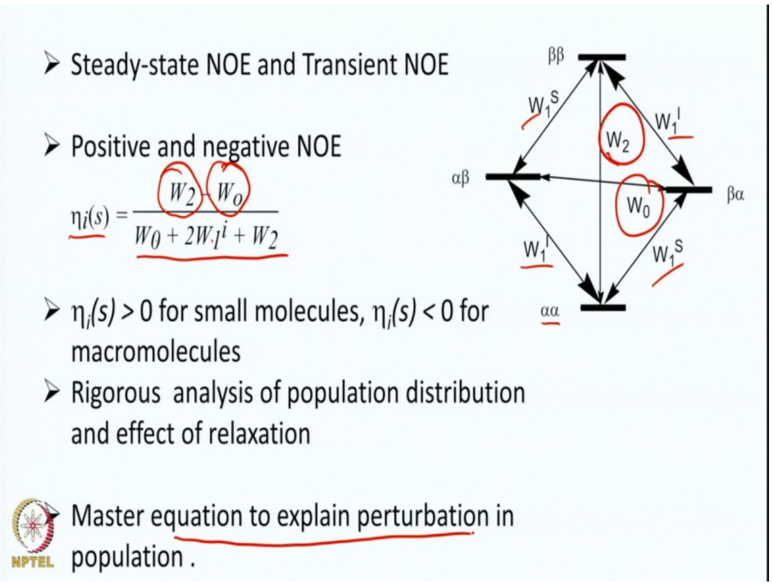
So now what previously we have looked? What is the origin of NOE? And what is the physical principle behind NOE that is nuclear relaxation, so how it is done? So what we are doing? We have a two signal, which are somehow coupled through a space and we saturate one signal, so by saturation what we mean by? We equalize the population of the transition by irradiating with weak field. So that means there are two levels here and we saturate, we apply an weak *RF* pulse here and now this is equalizing the population between this two states.

And then, while we absorbing what is happening to others signal. So that is what is NOE, two coupled system, one is perturbed and perturbed means equalize the population between these two states of this signal and then we look at the effect of that perturbation on other signal. So what we are observing? What is happening on other signal? So NOE is a manifestation of the attempt of the system to come back to the equilibrium.

So that means how it comes, come back to equilibrium. So because of perturbation the population equalizes and then what is happening, actually it is trying to come back by some mechanism called relaxation. So relaxation is trying to equalize that, sorry restored equilibrium and in attempt to store that there is a signal enhancement. That is given by η that is a NOE signal. So that is the signal because $\frac{I-I_0}{I_0}$. That is call NOE enhancement.

So essentially NOE is manifestation of the attempt of the system to come back to the equilibrium and that is given by these symbols, where I is the signal obtained because of this perturbation -I_0 is original signal. That is the ratio that is given as a NOE enhancement.

(Refer Slide Time: 2:50)



- Steady-state NOE and Transient NOE
- Positive and negative NOE

$$\eta_i(s) = \frac{W_2 - W_0}{W_0 + 2W_1^i + W_2}$$

- $\eta_i(s) > 0$ for small molecules, $\eta_i(s) < 0$ for macromolecules
- Rigorous analysis of population distribution and effect of relaxation
- Master equation to explain perturbation in population.

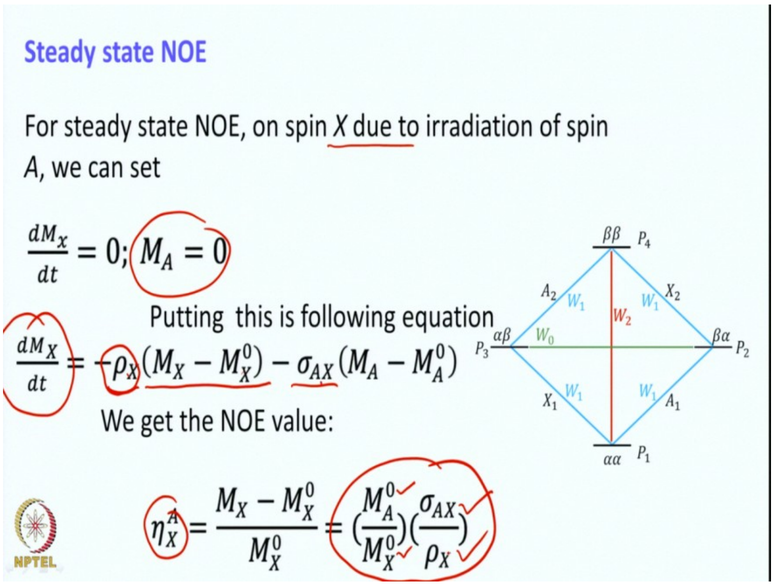
So next, what we look at the two kind of a NOE. One was steady-state NOE and another was transient NOE. We looked at what is the difference between steady-state and transient NOE and then will look at the positive NOE and negative NOE, in some cases you get positive enhancement, some case you get negative enhancement, so look at that.

So here we look at there are four states of two spins, so here there are two spin, say spin I and spin S and we can notify this as α,α state, α, β state, β α state and β, β state. So two transitions belongs to I this and this and two transition belongs to S this and this. Now that is what transition we have written. So actually if we perturb one of the spin, either S or I because of perturbation, the population equalizes and then we are looking at how by different relaxation mechanism it is coming back and what is the effect of that relaxation on the other spin.

So we look at positive NOE and negative NOE and we have seen that actually this double transition and this zero transition probability, actually that plays important role in giving the NOE enhancement. So the ratio of this with the ω_0, ω_1 and ω_2 is given as a NOE enhancement and then depending upon what kind of NOE signal we get that will be enhancement for that.

So we have seen for generally for small molecule, it is positive NOE and for large molecule, it is negative NOE and we have also looked what is the reason, so for positive NOE its ω_2, which majorly contributes and for negative NOE ω_0 that majorly contributes. So we have done rigorous analyses of population distribution and we have looked at the effect of the relaxation, when it equalizes the population and we have developed an master equation to explain the perturbation in the population. So up to here we have seen in the previous class.

(Refer Slide Time: 5:10)



Steady state NOE

For steady state NOE, on spin X due to irradiation of spin A, we can set

$$\frac{dM_X}{dt} = 0; M_A = 0$$

Putting this is following equation

$$\frac{dM_X}{dt} = -\rho_X(M_X - M_X^0) - \sigma_{AX}(M_A - M_A^0)$$

We get the NOE value:

$$\eta_X^A = \frac{M_X - M_X^0}{M_X^0} = \left(\frac{M_A^0}{M_X^0}\right)\left(\frac{\sigma_{AX}}{\rho_X}\right)$$

Now, we go back and do little more rigorous analysis to explain this steady-state on NOE. So let us see for steady-state NOE what we are doing? We are looking at the spin *X*, on spin and *X* what is the effect to irradiation of spring *A*? So we are irradiating say spin *A* and looking the effect of that irradiation on spin *X*. So like M_A can be 0 and we are looking at how M_X is changing with time to 0.

So, we put that in master equation and what we can get?

$$\frac{dMx}{dt} = -\rho_x\left(M_X - M_X^0\right) - \sigma_{AX}\left(M_A - M_A^0\right)$$

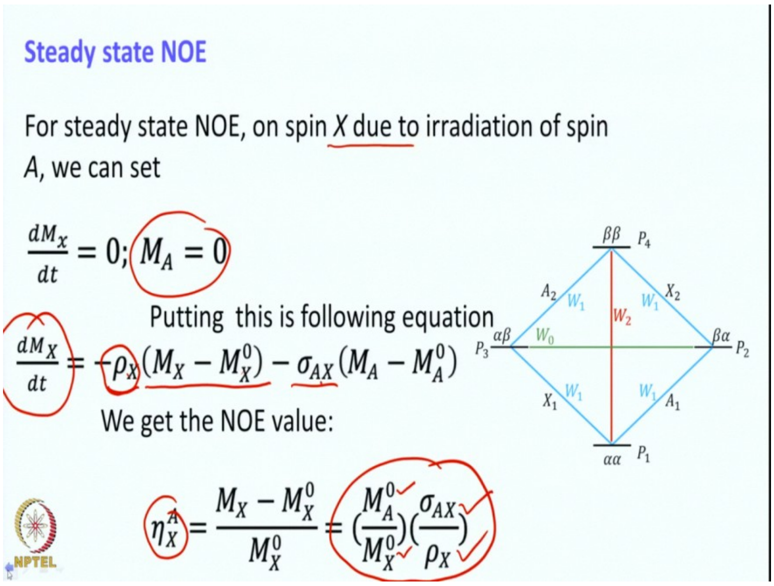
So this is one rate and there is another rate here. So this is called auto relaxation rate and this is called cross relaxation rate. So cross relaxation rate is happening because A and X spin are correlated.

So in that case if you do the analysis, we get an enhancement of NOE of X that will be given

$$\eta_X^A = \frac{M_X - M_X^0}{M_X^0} = \left(\frac{M_A^0}{M_X^0}\right)\left(\frac{\sigma_{AX}}{\rho_X}\right)$$

So that is a kind of steady-state enhancement we are getting and that we have seen earlier.

(Refer Slide Time: 7:05)



Steady state NOE

For steady state NOE, on spin X due to irradiation of spin A, we can set

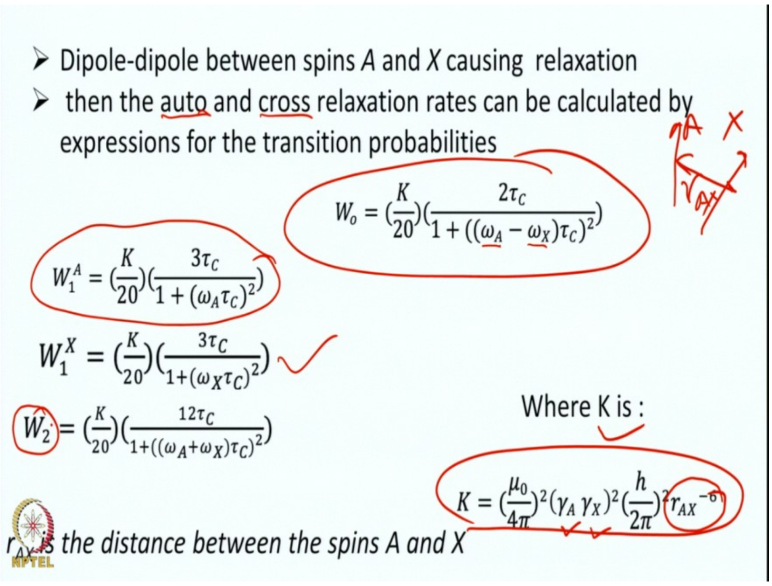
$$\frac{dM_X}{dt} = 0; \; M_A = 0$$

Putting this is following equation

$$\frac{dM_X}{dt} = -\rho_X (M_X - M_X^0) - \sigma_{AX}(M_A - M_A^0)$$

We get the NOE value:

$$\eta_X^A = \frac{M_X - M_X^0}{M_X^0} = \left(\frac{M_A^0}{M_X^0}\right)\left(\frac{\sigma_{AX}}{\rho_X}\right)$$



- Dipole-dipole between spins A and X causing relaxation
- then the auto and cross relaxation rates can be calculated by expressions for the transition probabilities

$$W_0 = \left(\frac{K}{20}\right)\left(\frac{2\tau_C}{1 + ((\omega_A - \omega_X)\tau_C)^2}\right)$$

$$W_1^A = \left(\frac{K}{20}\right)\left(\frac{3\tau_C}{1 + (\omega_A \tau_C)^2}\right)$$

$$W_1^X = \left(\frac{K}{20}\right)\left(\frac{3\tau_C}{1 + (\omega_X \tau_C)^2}\right)$$

$$W_2 = \left(\frac{K}{20}\right)\left(\frac{12\tau_C}{1 + ((\omega_A + \omega_X)\tau_C)^2}\right)$$

Where K is :

$$K = \left(\frac{\mu_0}{4\pi}\right)^2 (\gamma_A \gamma_X)^2 \left(\frac{h}{2\pi}\right)^2 r_{AX}^{-6}$$

r_{AX} is the distance between the spins A and X

So, why this is happening? Because both spins are actually dipole and there is a dipole, dipole interaction, because though both spins are connected by the dipolar interaction in space, so suppose this is *A* spin and this is *X* spin, they are both are dipole and connected by dipolar interaction. So that is causing relaxation, so when it cause relaxation there is auto relaxation rate and there is a cross relaxation rate and that can be calculated by measuring the transition probability.

That transition probability we have looked in the previous slides. So that is ω_0, ω_1 and ω_2 if you can calculate this, one can find it out what is the ω_0 for this correlation. So that will be given by

$$\omega_0=\left(\frac{K}{20}\right)\left(\frac{2\tau_C}{1+\left(\left(\omega_A-\omega_X\right)\tau_C\right)^2}\right)$$

τ_C is the correlation time of this molecule. So we can give for ω_0.

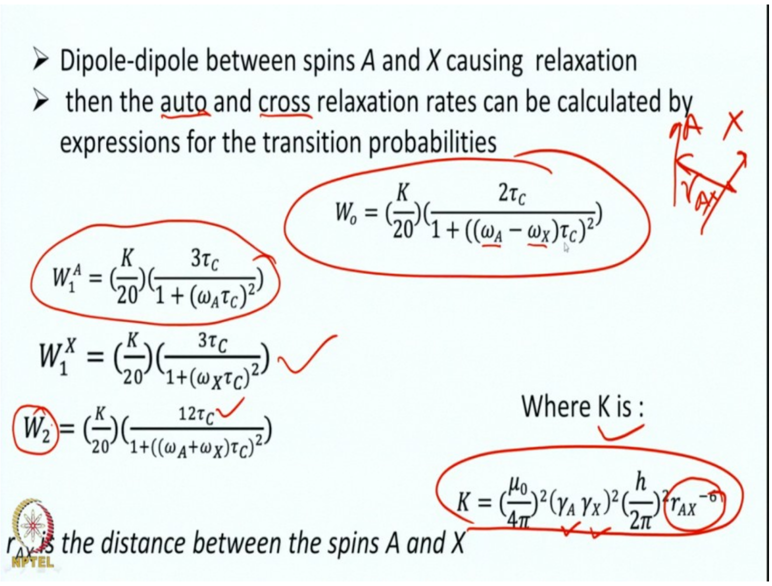
Similarly, we can have for ω_1^A, ω_1^X and that we can get the correlation by doing simple algebraic equation. So we can get ω_A, ω_X and ω_2 that is transition probability for double quantum transition. So we can get and the *K* here can be defined as this relation,

$$K=\left(\frac{\mu_0}{4\pi}\right)^2\left(\gamma_A\gamma_X\right)^2\left(\frac{h}{2\pi}\right)^2\left(r_{AX}\right)^{-\sigma}$$

which takes care of the gyromagnetic ratio of A and X and also distance. If you look at here distance dependent.

So this interaction transition probability has important term, the separation between these two spin. So this is r_{AX}. Okay r_{AX} is the distance between two spin. So if you put it, you can get the transition probability of zero quantum transition, single quantum transition and double quantum transition and there is a term correlation time. So if you put everything together.

(Refer Slide Time: 9:07)



- Dipole-dipole between spins *A* and *X* causing relaxation
- then the auto and cross relaxation rates can be calculated by expressions for the transition probabilities

γA X

r_{AX}

$$W_o = \left(\frac{K}{20}\right)\left(\frac{2\tau_C}{1+((\omega_A-\omega_X)\tau_C)^2}\right)$$

$$W_1^A = \left(\frac{K}{20}\right)\left(\frac{3\tau_C}{1+(\omega_A\tau_C)^2}\right)$$

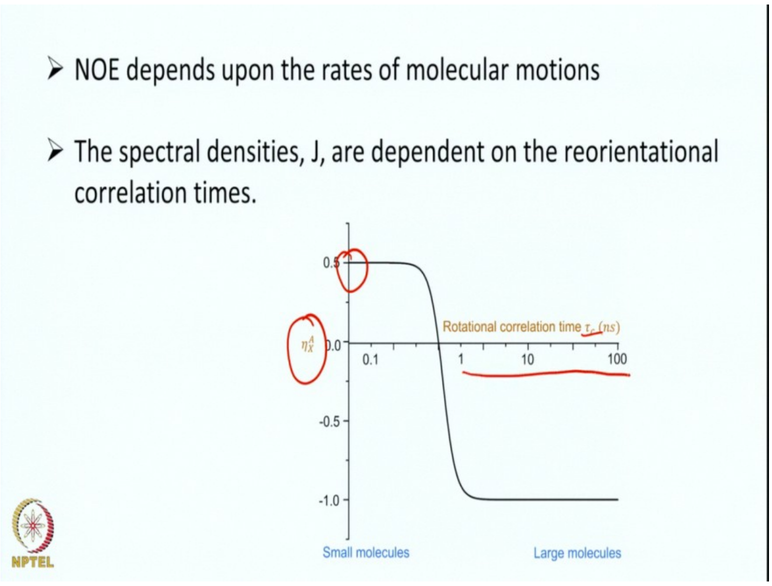
$$W_1^X = \left(\frac{K}{20}\right)\left(\frac{3\tau_C}{1+(\omega_X\tau_C)^2}\right)$$

$$W_2 = \left(\frac{K}{20}\right)\left(\frac{12\tau_C}{1+((\omega_A+\omega_X)\tau_C)^2}\right)$$

Where K is :

$$K = \left(\frac{\mu_0}{4\pi}\right)^2(\gamma_A\gamma_X)^2\left(\frac{h}{2\pi}\right)^2 r_{AX}^{-6}$$

r_{AX} is *the distance between the spins A and X*

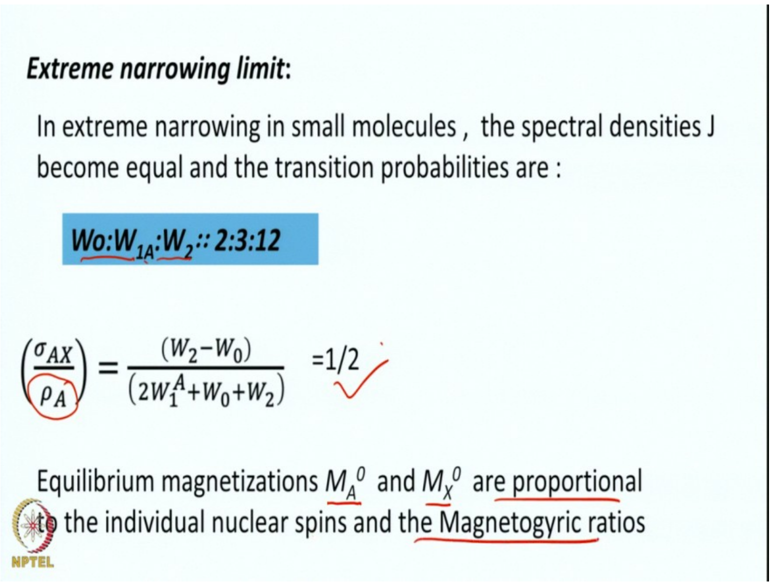


- NOE depends upon the rates of molecular motions
- The spectral densities, J, are dependent on the reorientational correlation times.

Now we know that NOE depends upon rate of molecular motion because the tumbling time τ_C is important factor, here τ_C. So that is a molecular motion, so now spectral density are actually dependent upon what is the reorientation times. So if we perturb how much time it takes to reorient itself, that is orientation correlation time τ_C that is a relation.

So here is the enhancement factor of NOE η_X^A and as you can see for small molecule we have positive NOE and for large molecule as we go, we have a negative NOE all the way up to -1 and the correlation time is increasing. So that means for shorter molecule, which has a faster correlation time you have a positive NOE that is 0.5 and for smaller molecule you have a negative NOE. So that is a dependence of correlation time with the NOE enhancement factor and for small molecule and larger molecule that is the relation.

(Refer Slide Time: 10:11)



Extreme narrowing limit:

In extreme narrowing in small molecules , the spectral densities J become equal and the transition probabilities are :

$W_o:W_{1A}:W_2 :: 2:3:12$

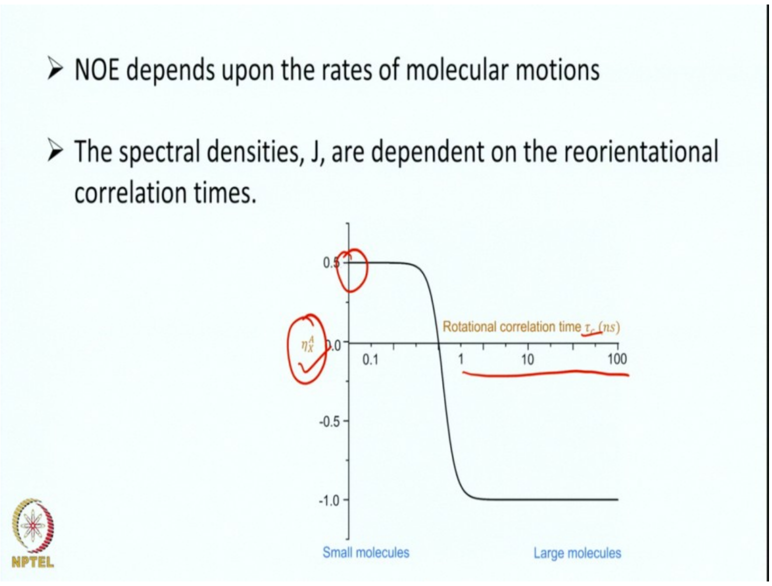
$$\left(\frac{\sigma_{AX}}{\rho_A}\right) = \frac{(W_2-W_0)}{(2W_1^A+W_0+W_2)} \quad =1/2 \checkmark$$

Equilibrium magnetizations M_A^0 and M_X^0 are proportional to the individual nuclear spins and the Magnetogyric ratios

So, what happens? In extreme narrowing condition for small molecule, this is the case when extreme narrowing condition comes because they can tumble very fast. So the spectral density becomes equal to the transition probability. So we can write it, ω_0, ω_A that is zero quantum transition, single quantum transition probability of A spin and the double quantum transition probability is in ratio of 2:3:12 and that if you get it, do the algebra.

So ratio of this cross relaxation rate and then auto relaxation rate, one can get it half. And that is what we were saying. So equilibrium magnetization of A spin M_A^0 and X spin are proportional to individual nuclear spins and there gyromagnetic ratio. So if we put that all the equation.

(Refer Slide Time: 11:06)

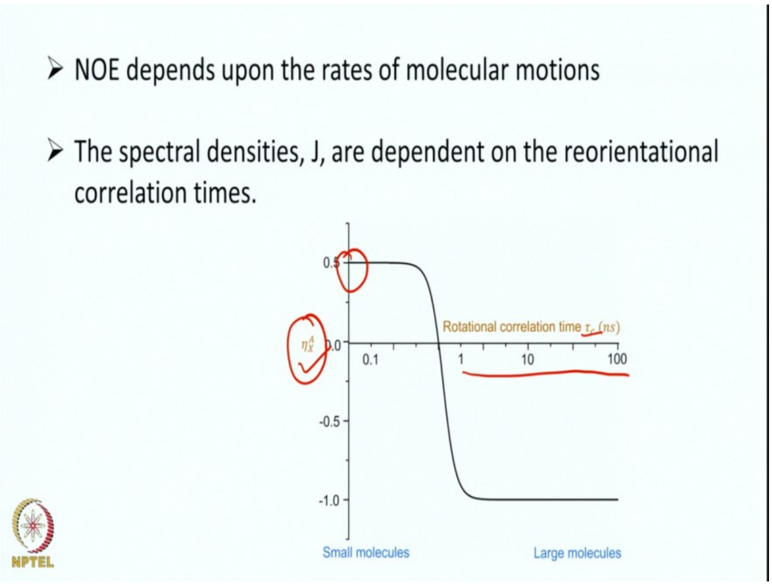


- NOE depends upon the rates of molecular motions
- The spectral densities, J, are dependent on the reorientational correlation times.

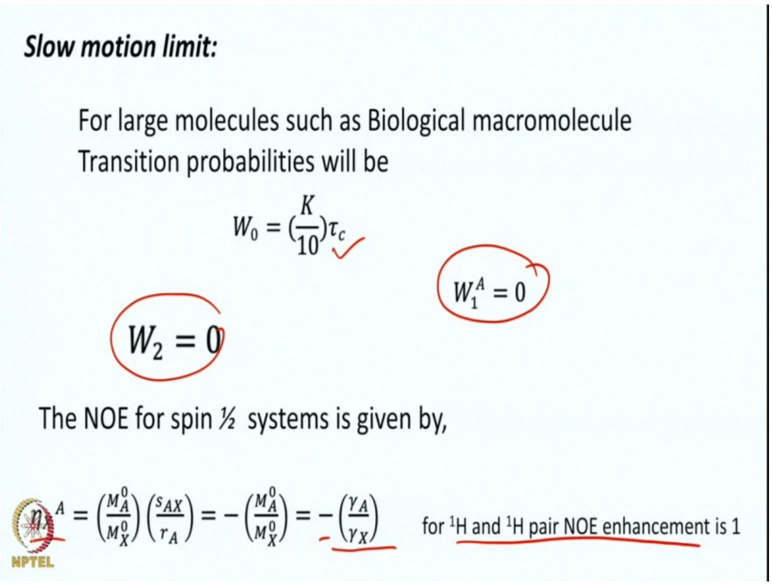
So the magnetization of A is proportional to the Υ_A which is gyromagnetic ratio. Similarly, this X spin magnetization proportional to gamma X. So for a half spin system, suppose it is a proton, proton or proton carbon that is a ½ spin system. What we have is NOE enhancement will be $\frac{1}{2}\frac{\gamma_A}{\gamma_X}$. Now if you take this, so for a carbon proton NOE pair the enhancement is 2. Why? Because Υ of proton is 4 times more than the carbon. So this will be 4 divided by 2 that is 2.

For a nitrogen this will be half 10 by 1 that is 5 and for proton proton because both spins are now proton. So 1 divided by 1 and multiplied with half that is 0.5. So if you look at clearly here for proton proton like a small molecules where we are looking, the NOE enhancement between two protons. We have only 50 percent enhancement. If it is carbon we have two times enhancement, if we have nitrogen we have 5 times enhancement. Okay.

(Refer Slide Time: 12:22)



- NOE depends upon the rates of molecular motions
- The spectral densities, J, are dependent on the reorientational correlation times.



Slow motion limit:

For large molecules such as Biological macromolecule Transition probabilities will be

$$W_0 = \left(\frac{K}{10}\right)\tau_c$$

$$W_1^A = 0$$

$$W_2 = 0$$

The NOE for spin ½ systems is given by,

$$\eta_X^A = \left(\frac{M_A^0}{M_X^0}\right)\left(\frac{s_{AX}}{r_A}\right) = -\left(\frac{M_A^0}{M_X^0}\right) = -\left(\frac{\gamma_A}{\gamma_X}\right)$$

for ^{1}H and ^{1}H pair NOE enhancement is 1

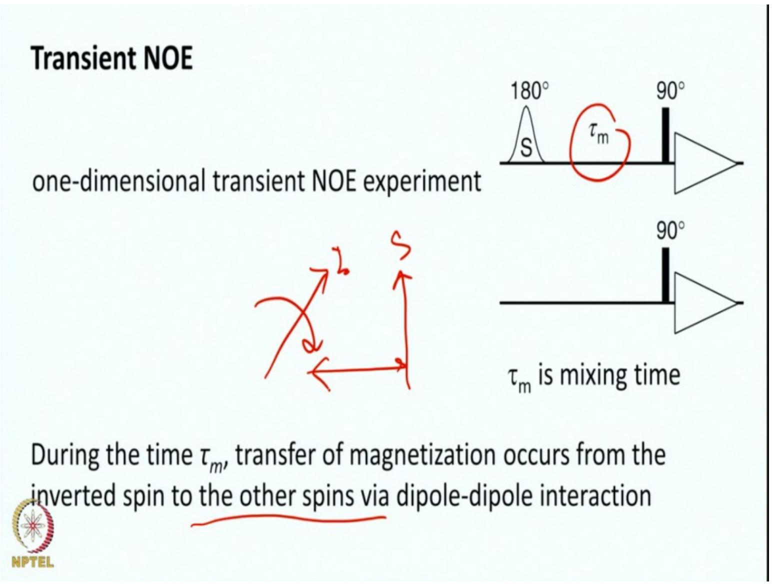
So now this is for fast tumbling molecules, small molecules what happens in the slow motion, so for a large molecules. Suppose biological molecule protein the transition probability mostly will be governed by the zero quantum transition and that will be given by

$$\omega_0 = \left(\frac{K}{10}\right)\tau_C$$

So here and the first quantum transition is 0, zero quantum transition is also 0. So NOE for such system will be given by this relation, so ultimately it comes $\frac{\gamma_A}{\gamma_X}$.

So now, if we are taking the for large molecule two proton is pairs. So this will be 1 by 1, so that is one enhancement and that is negative, and that is what exactly we saw for the large molecule. For large molecule the NOE enhancement in fairly broad correlation time 1 to 100 and enhancement is minus 1. So that means 100 percent enhancement. But it will be negative enhancement, so for large molecule. It is a negative NOE up to 1. So that is what is for slow motion limit. So for that is and enhancement we get for biological molecule so that is steady-state NOE.

(Refer Slide Time: 13:35)



Transient NOE

one-dimensional transient NOE experiment

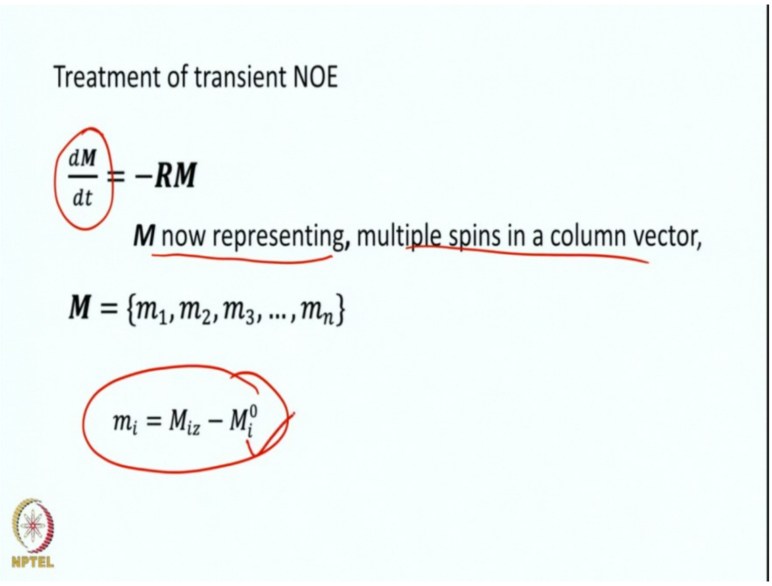
τ_m is mixing time

During the time τ_m, transfer of magnetization occurs from the inverted spin to the other spins via dipole-dipole interaction

Now coming back to transient NOE, just to refresh your memory what is transit NOE? That we have two spins like previously *I* spin and *S* spin. We are applying one selective pulse on a spin here which is inverting the population and then we are waiting for some time, which is called mixing time. Then we apply a 90˚ pulse and we detect what is happening after this, so that is one-dimensional experiment.

And as we discussed this tm is the mixing time, during the spin mixes. So transfer of magnetization occurs during this mixing time, the inverted spin to other spin by a dipolar coupling. So that is what is happening, here we have two spins, spin *S*, spin *I*, we are applying 180˚ pulse and then we are waiting and during this time by dipolar coupling, the spin, spin mixing is happening.

(Refer Slide Time: 14:38)



Treatment of transient NOE

$$\frac{dM}{dt} = -\boldsymbol{RM}$$

M now representing, multiple spins in a column vector,

$$\boldsymbol{M} = \{m_1, m_2, m_3, \dots, m_n\}$$

$$m_i = M_{iz} - M_i^0$$

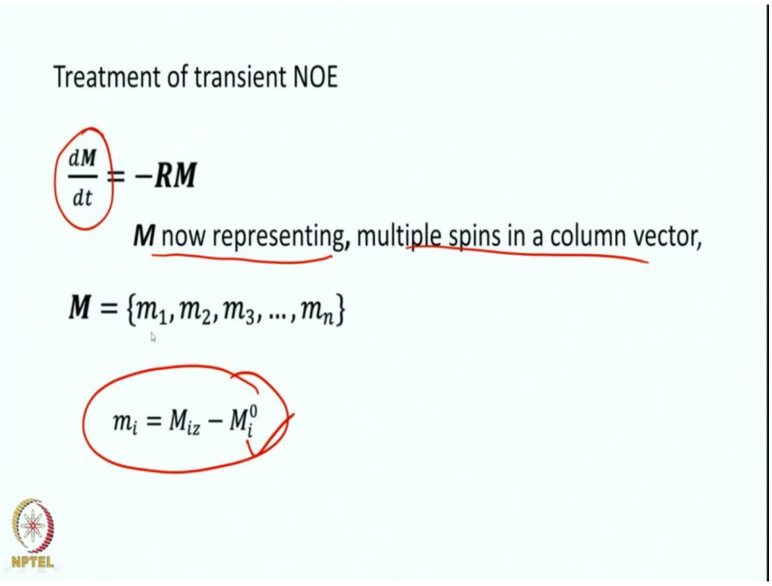
So we can do the same analysis of change of magnetization of spin with respect to time and that will be given by this master equation. So

$M = \{m_1, m_2, m_3 \dots . m_n$

And

$m_i = M_{iz} - M_i^0$

(Refer Slide Time: 15:07)



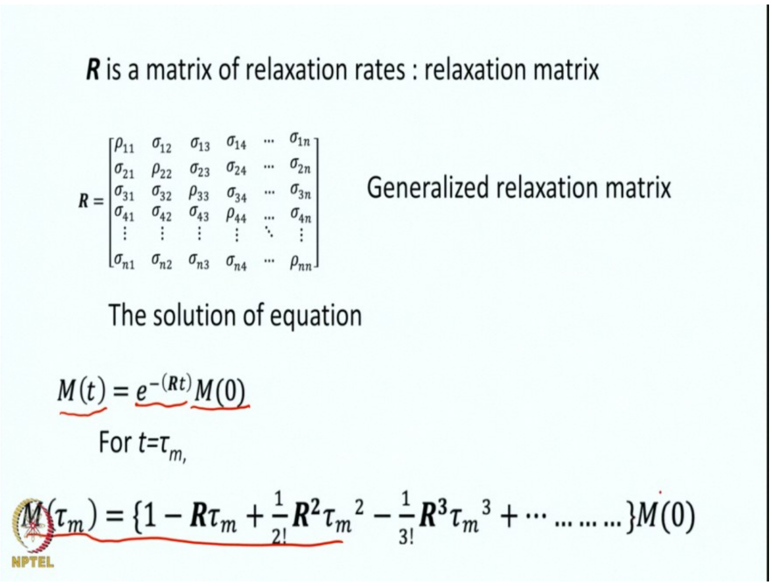
Treatment of transient NOE

$$\frac{dM}{dt} = -\boldsymbol{RM}$$

M now representing, multiple spins in a column vector,

$$\boldsymbol{M} = \{m_1, m_2, m_3, \dots, m_n\}$$

$$m_i = M_{iz} - M_i^0$$



R is a matrix of relaxation rates : relaxation matrix

$$R = \begin{bmatrix} \rho_{11} & \sigma_{12} & \sigma_{13} & \sigma_{14} & \cdots & \sigma_{1n} \\ \sigma_{21} & \rho_{22} & \sigma_{23} & \sigma_{24} & \cdots & \sigma_{2n} \\ \sigma_{31} & \sigma_{32} & \rho_{33} & \sigma_{34} & \cdots & \sigma_{3n} \\ \sigma_{41} & \sigma_{42} & \sigma_{43} & \rho_{44} & \cdots & \sigma_{4n} \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ \sigma_{n1} & \sigma_{n2} & \sigma_{n3} & \sigma_{n4} & \cdots & \rho_{nn} \end{bmatrix}$$

Generalized relaxation matrix

The solution of equation

$$\underline{M(t)} = \underline{e^{-(\boldsymbol{R}t)}}\,\underline{M(0)}$$

For $t=\tau_m$,

$$\underline{M(\tau_m) = \{1 - \boldsymbol{R}\tau_m + \frac{1}{2!}\boldsymbol{R}^2{\tau_m}^2} - \frac{1}{3!}\boldsymbol{R}^3{\tau_m}^3 + \cdots \ldots\ldots\ldots\}M(0)$$

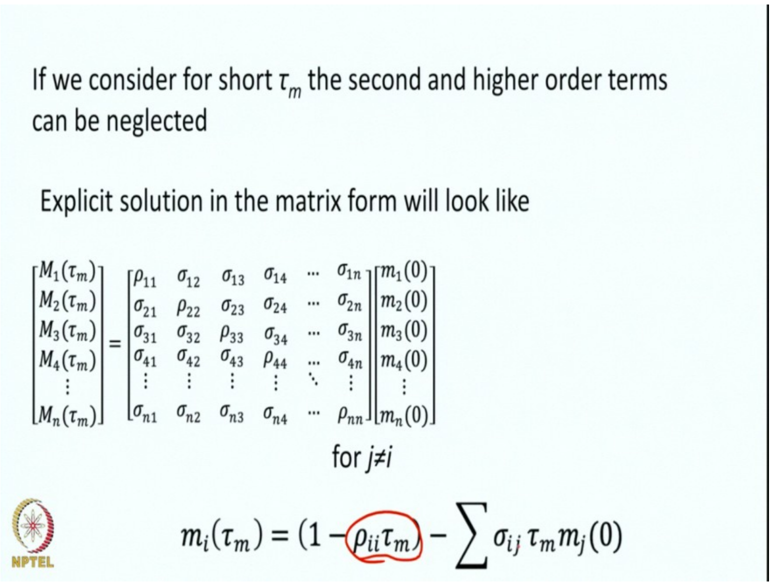
So we can write the R is various rates. The auto relaxation and cross relaxation rate, so if you look at the diagonal element is correlating with self that is auto relaxation rate and then you have cross relaxation rate. So that is the R matrix and then we have a column vector given by M. So if you take this.

So solution for any spin magnetization at any time t will be given by

$$M(t) = e^{-(Rt)}M(0)$$

So for any time τ_m, because we are mixing for time τ_m. The magnetization will be given by this series multiplied with equilibrium magnetization.

(Refer Slide Time: 15:52)



If we consider for short τ_m the second and higher order terms can be neglected

Explicit solution in the matrix form will look like

$$\begin{bmatrix} M_1(\tau_m) \\ M_2(\tau_m) \\ M_3(\tau_m) \\ M_4(\tau_m) \\ \vdots \\ M_n(\tau_m) \end{bmatrix} = \begin{bmatrix} \rho_{11} & \sigma_{12} & \sigma_{13} & \sigma_{14} & \cdots & \sigma_{1n} \\ \sigma_{21} & \rho_{22} & \sigma_{23} & \sigma_{24} & \cdots & \sigma_{2n} \\ \sigma_{31} & \sigma_{32} & \rho_{33} & \sigma_{34} & \cdots & \sigma_{3n} \\ \sigma_{41} & \sigma_{42} & \sigma_{43} & \rho_{44} & \cdots & \sigma_{4n} \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ \sigma_{n1} & \sigma_{n2} & \sigma_{n3} & \sigma_{n4} & \cdots & \rho_{nn} \end{bmatrix} \begin{bmatrix} m_1(0) \\ m_2(0) \\ m_3(0) \\ m_4(0) \\ \vdots \\ m_n(0) \end{bmatrix}$$

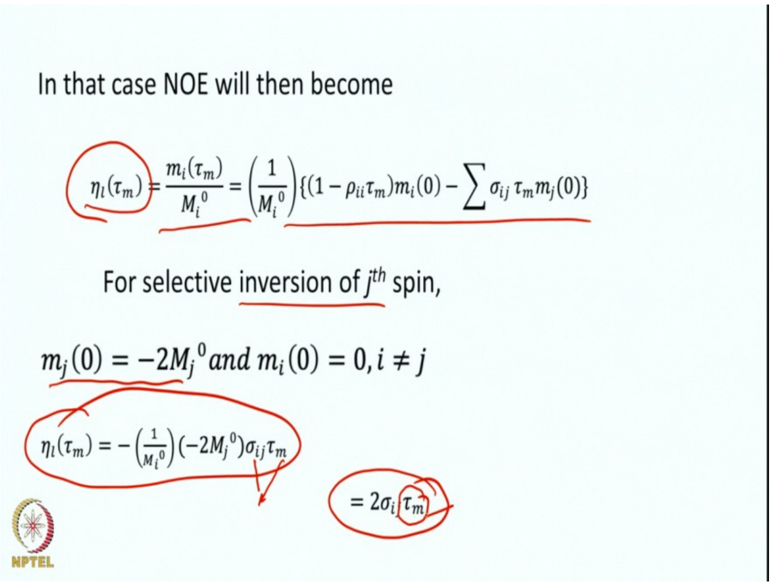
for $j \neq i$

$$m_i(\tau_m) = (1 - \boxed{\rho_{ii}\tau_m}) - \sum \sigma_{ij}\,\tau_m m_j(0)$$

So if you do that, we consider the short tm. So then second and higher-order term can be neglected. So we can find explicit solution which will look like magnetization of any spin at time *t* will be given by this and then will be equilibrium magnetization for any spin $j \neq i$. So one can give it a simple formula of m_i, magnetization of an spin at time *t*, will be given by

$$\eta_i(\tau_m)=\frac{m_i(\tau_m)}{M_i^0}=\left(\frac{1}{M_i^0}\right)\{(1-\rho_{ij}\tau_m)m_i(0)-\sum\sigma_{ij}\tau_m m_j(0)\}$$

(Refer Slide Time: 16:33)



In that case NOE will then become

$$\eta_i(\tau_m)=\frac{m_i(\tau_m)}{M_i^{\,0}}=\left(\frac{1}{M_i^{\,0}}\right)\{(1-\rho_{ii}\tau_m)m_i(0)-\sum\sigma_{ij}\,\tau_m m_j(0)\}$$

For selective inversion of j^{th} spin,

$$m_j(0)=-2M_j^{\,0}\,and\;m_i(0)=0,i\neq j$$

$$\eta_i(\tau_m)=-\left(\frac{1}{M_i^{\,0}}\right)(-2M_j^{\,0})\sigma_{ij}\tau_m$$

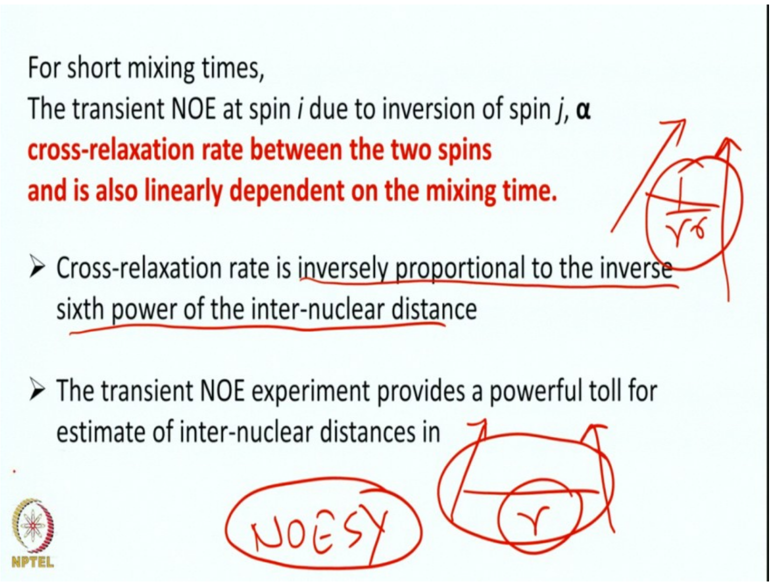
$$=2\sigma_i\tau_m$$

So if you do that one can get the contribution of the NOE enhancement and that NOE enhancement at any time tm can be given by this formula, so we can sum over the time that is there and for selective inversion of say we are doing on j^{th} spin, one can find it out what is the NOE enhancement? So that will be given by say for

$$\eta_i(\tau_m)=-\left(\frac{1}{M_i^0}\right)(-2M_j^0)\sigma_{ij}\tau_m$$

So if you look at we can simplify this and one can get essentially $2\sigma_{ij}\tau_m$. So it depends upon the tm. NOE enhancement at the end we can conclude that it depends upon tm. So if we have short mixing time the enhancement can be 1 and if we have long mixing time enhancement can be other. So that means if you keep increasing your NOE can maybe probably increase.

(Refer Slide Time: 17:36)



For short mixing times,
The transient NOE at spin *i* due to inversion of spin *j*, **α**
cross-relaxation rate between the two spins
and is also linearly dependent on the mixing time.

- Cross-relaxation rate is inversely proportional to the inverse sixth power of the inter-nuclear distance
- The transient NOE experiment provides a powerful toll for estimate of inter-nuclear distances in

$\frac{1}{r^6}$

NOESY

r

So that is how for short mixing time, the transient NOE at spin *i* is due to inversion of spin *j* and cross correlation rate between two spins is also linearly dependent upon mixing time. That is what I was saying. So relaxation rate between two spins is linearly dependent upon mixing time, so if you increase mixing time the relaxation rate will vary.

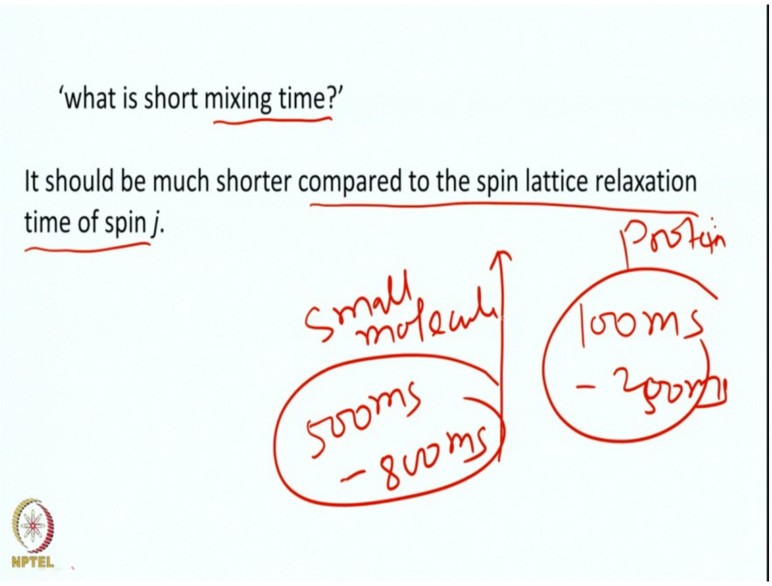
So cross relaxation rate is inversely proportional to the inverse power of the inter-nuclear distance. So what it says? Here we have two spins and r^6 that we have seen. So relaxation rate depends upon $\frac{1}{r^6}$, so that is actually very important factor. So the NOE ability between two spins is distance dependent and that depends upon the dipolar interaction between this two spins and that dependency is $\frac{1}{r^6}$. So if you keep increasing the distance the effect is going to be minimized.

So this transit NOE experiment actually provides the powerful toll for estimate the inter-nuclear distance in coupled spins. So here suppose these two spins is there, how much NOE enhancement that depends upon *r*? Shorter you have more enhancement, longer you have less enhancement and therefore, looking at the cross peaks coming because of cross relaxation rate if you can measure the intensity of that cross peak we can get an estimate of the distance between this two spins.

So actually this is very important and this actually opens and new avenue for measuring the inter nuclear distance between these two spins and that is what essentially was used to develop an experiment, which is called nuclear overhauser effect spectroscopy NOESY and this actually open a new avenue in structural biology. Because you can precisely or maybe up to a great accuracy you can measure the distance between two coupled spin.

So then, what a short mixing time? I was saying that here we mention for a short mixing time. The transient NOE of spin *i* due to inversion of spin *j* depends upon cross relaxation rate. So how do we know that is what a short and what is long? It is all relative term.

(Refer Slide Time: 20:12)



'what is short mixing time?'

It should be much shorter compared to the spin lattice relaxation time of spin *j*.

small molecule

500ms - 800ms

Protein

100ms - 200ms

So, short mixing time is essentially. It should be much shorter compared to the spin lattice relaxation time of spin *j*. So you know spin lattice relaxation time. It is an inversion recovery. If you invert the spin *j* how much time it takes to come back to equilibrium that is called t_1 time or spin lattice relaxation time. So that the short mixing time has to be less compare to the spin lattice relaxation time and then this will hold true.

Typically what we have is like a for protein, suppose relaxation time is, spin lattice relaxation time is say 1 second. So your short mixing time will be few millisecond that will be compared to less. So therefore, for say small molecules you can have a short mixing time up to a few hundred milliseconds, like 500 milliseconds or 800 milliseconds. However, protein has relatively short spin lattice relaxation time.

Therefore, mixing time which is used in case of protein is generally 100 milliseconds to 200 milliseconds or 250 milliseconds for small organic molecule, you can go all the way up to 500 milliseconds to 800 milliseconds. So that is a short mixing time. That is relative for this for protein by a big biomolecule. This is for small organic molecule. These concepts are used in NOESY.

So, what I am going to doing next is class? I will take you this concept of magnetization transfer of polarization transfer. How this is going to use as a measurement of a distance by few experiment called NOSEY and ROSEY briefly, I am introduce you and then we go to the heteronuclear polarization transfer by an concept called inept transfer. So we will continue with this. So now, if you have a any questions please write it to us or ask us will try to solve it. Thank you very much.