NMR Spectroscopy for Chemist and Biologist

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Lecture 20

Polarization Transfer

So welcome to today's lecture, we will start new chapter today which is called polarization transfer. So, polarization transfer has profound effect in many aspects of NMR spectroscopy, polarization transfer is used to enhance the signal for a particular nuclei or it also used to measure the distance between two nuclei, because of this property actually it finds its wide application in chemistry and biology.

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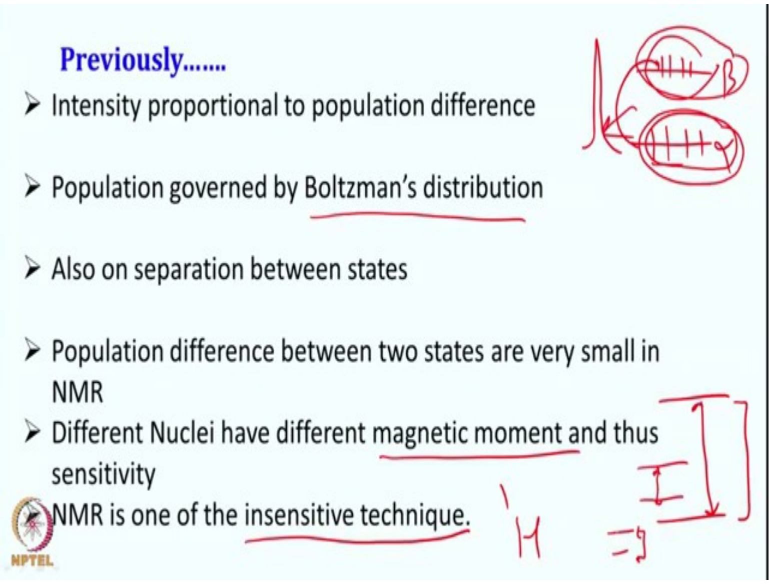

Previously…….

- Intensity proportional to population difference
- Population governed by Boltzman's distribution
- Also on separation between states
- Population difference between two states are very small in NMR
- Different Nuclei have different magnetic moment and thus sensitivity
- NMR is one of the insensitive technique.

So we will just go and revise some of the concept that we had seen earlier, previously like when we have started the course we have seen that intensity of the resonance is proportional to the population difference between two states, so suppose here I have a state, say α state and β state, how much is the population here and how much is the population here, so this difference actually gives you what will be the intensity of your signal.

Now as you have seen this population is governed by Boltzmann's distribution, now Boltzmann dictates how many spins are going to be in the lower state and how many spins are going to be in higher state, since the separation between this states is minimal so in NMR actually the difference between the ground state and the excited state is not much, therefore NMR is one of the insensitive technique, as we said that this difference between population here and population here is not much.

So, therefore intensity here is signal, so to just give you an idea how of how insensitive it is, if you record a fluorescence spectrum for a biomolecule, suppose we are probing a tryptophan which is a fluorophore and commonly used intrinsic fluorophore for fluorescence spectroscopy, so for that you need maximum 10 of 20 micromolar of sample, however for getting an descent NMR spectrum for a biomolecule you need few 100 micromolar say 500 micro molar to 1 mille molar for doing 1D spectrum, therefore one say that NMR is one of the insensitive technique that we had, but it has its own advantages and therefore it is popular like getting the sight specific resolution or information.

The other phenomenon this state difference also depends upon which type of nuclei is, so it actually dictates magnetic moment of the particular nuclei so carbon is 4 times less sensitive than the proton and therefore if difference in the proton is this much than carbon will be less compare to that and therefore carbon becomes inner less sensitive than the proton.

Nitrogen is further like its Υ is 10 times less so its magnetic moment is less and therefore this is the intensity that we obtain from the nitrogen will be even less. So this are the some of the phenomenon, so some are more sensitive nuclei like proton because of high magnetic moment so therefore separation between this two states will be higher, nuclei like carbon 13 or N15 will be less sensitive, simply because the separation between this two states will be less and the population difference between this two states will be also less, so this is one of the insensitive technique.

So just to summarize what we discuss the intensity for a particular signal depends upon the difference in the population of the ground state to the excited state like a typical electron spectra like fluorescence spectra or UV-visible spectra the difference between this two states, ground state and the excited state, population difference between this two states is quite huge therefore the signal intensity is remarkable, in case of NMR this difference in the population between this two states is smaller therefore it is one of the insensitive technique, this population is distributed by Boltzmann distribution and some of the nuclei like X nuclei nitrogen 15 or carbon 13 are even less sensitive.

So with this background we will move ahead for the polarization transfer and we try to understand the concept of polarization transfer, so all the time we have to think how to increase the sensitivity of some of the lower sensitive nuclei, even for many case proton is also not that sensitive effect compare with electronic spectra, however among another NMR active nuclei proton is the most sensitive.

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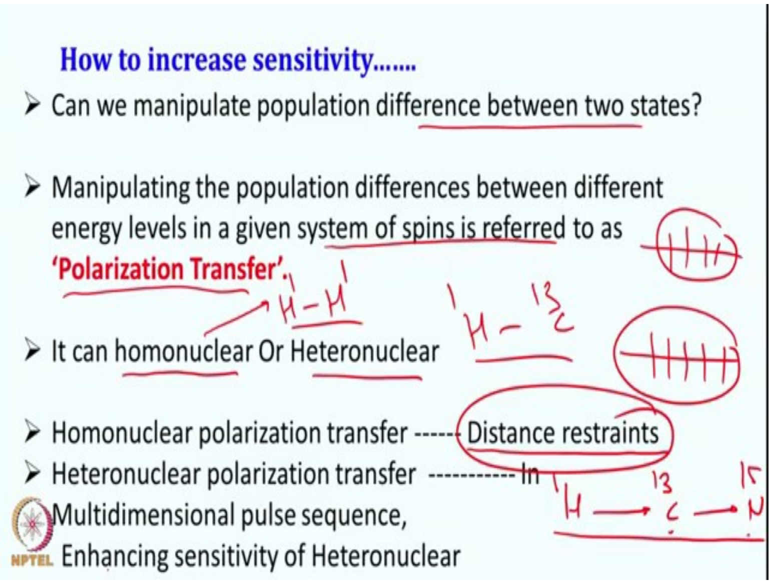

How to increase sensitivity…….

- Can we manipulate population difference between two states?
- Manipulating the population differences between different energy levels in a given system of spins is referred to as **'Polarization Transfer'.**
- It can homonuclear Or Heteronuclear
- Homonuclear polarization transfer ------ Distance restraints
- Heteronuclear polarization transfer ----------- In Multidimensional pulse sequence, Enhancing sensitivity of Heteronuclear

$^1H-^1H$

$^1H-^{13}C$

$^1H \rightarrow {}^{13}C \rightarrow {}^{15}N$

So can we manipulate somehow population difference between this two states if we manipulate the difference between this two states we can enhance the sensitivity because now difference will be more. Now manipulation of this population in two states actually energy level of the spins will be having different populations.

So here say we have a more population as expected because of the Boltzmann distribution and here we have less, can we manipulate so that here become more and more and here become less and less if we are probably able to do it we can increase the sensitivity of a particular resonance and the phenomenon where we do manipulation between the population of this two states is called polarization transfer.

Now this polarization transfer can be between two same type of nuclei which is called homonuclear polarization transfer or it can be between two different kind of nuclei that is called hetronuclear, so if we assume this population difference manipulation between proton and proton this homonuclear and carbon 13 and proton is called hetronuclear. Now this homonuclear polarization transfer gives you one of the important parameter which is used for measuring the distance between two nuclei so this gives you distance restrains.

Now in hetronuclear polarization transfer we will see further that actually it is used very much in the multidimensional spectrum where we transfer the polarization from proton to carbon 13 then further to nitrogen 15 and that is how we enhance the sensitivity of carbon 13 and nitrogen 15. So in multidimensional spectrum we do spin gymnastic or spin choreography where we transfer from one nuclei to anther nuclei and take it back and then we record the information in macromolecule, so you will see this concept in coming lectures and this essentially for enhancing the sensitivity of hetronuclear items such as N15, or 13C.

So lets us start with a homonuclear polarization transfer, the Overhauser was one of the pioneer who actually discover this and therefore this phenomenon is called nuclear overhauser effect or NOE, and actually this experiment actually opened the avenue of NMR in the structural biology because actually this gives direct measure of distance between two proton, so if you can measure the distance between two proton we measure the network of this distance and this distance is used for constructing the structure of the biomolecules like protein or DNA and even small molecules.

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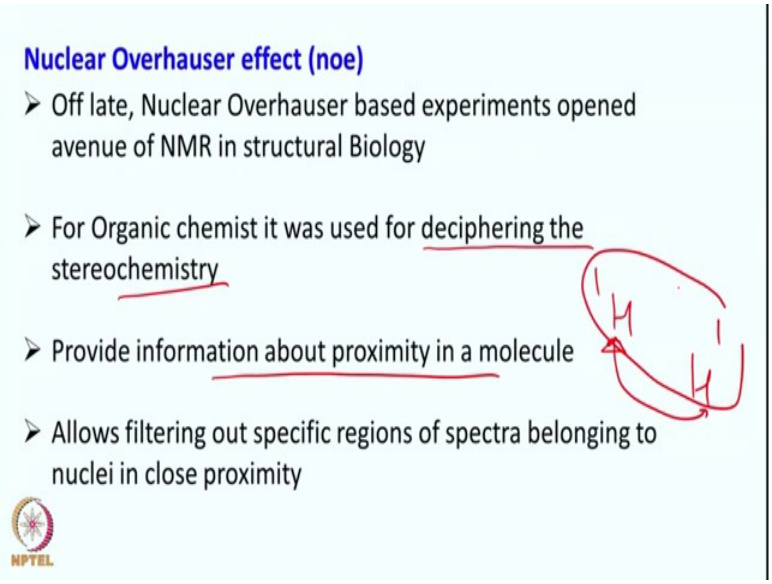

Nuclear Overhauser effect (noe)

- Off late, Nuclear Overhauser based experiments opened avenue of NMR in structural Biology
- For Organic chemist it was used for deciphering the stereochemistry
- Provide information about proximity in a molecule
- Allows filtering out specific regions of spectra belonging to nuclei in close proximity

Now so that nuclear overhauser best spectroscopy which is called NOESY and that we are going to see in coming lectures was one of the big through in structural biology for protein or nucleic acid structure determination. However organic chemist actually use also this concept for like deciphering the stereochemistry of a molecule so this again use this the more or less same concept, how this loop of spins are connected and the relative orientation of them can be measures and that fixes the stereochemistry of a small molecule.

So, therefore the NOE or nuclear overhauser effect actually provides the information about the proximity of the molecule, so if this proton in space if they are connected this will be shown in this experiment called nuclear overhauser effect, this is actually through a space through distance correlation through space correlation it is not through bond, as we have seen many experiments works through bonds and here it is through correlation. Now this also allows a filtering specific region of the spectra that belongs to nuclei in close proximity in space.

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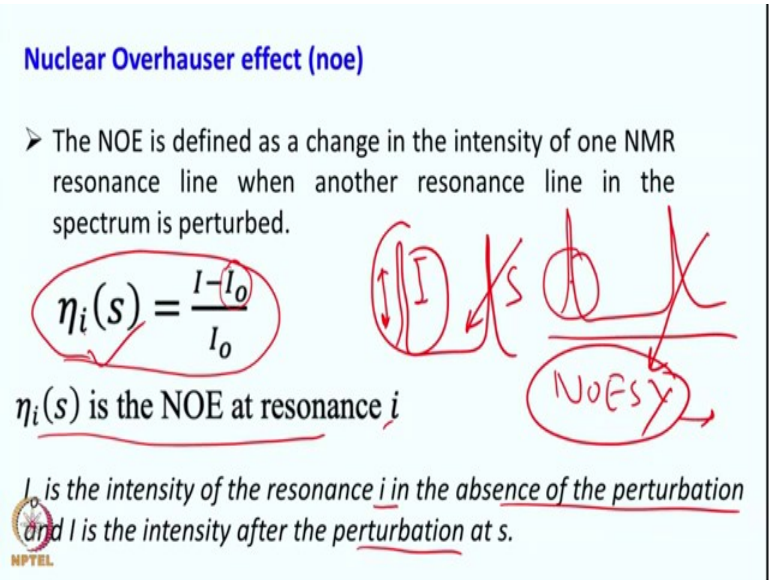

Nuclear Overhauser effect (noe)

- The NOE is defined as a change in the intensity of one NMR resonance line when another resonance line in the spectrum is perturbed.

$$\eta_i(s) = \frac{I - I_0}{I_0}$$

$\eta_i(s)$ is the NOE at resonance i

I_0 is the intensity of the resonance i in the absence of the perturbation and I is the intensity after the perturbation at s.

So these are the some of the useful point for NOE. So what actually is NOE is, NOE can be defined as the change in the intensity of one of the NMR resonance line when another resonance line in the same spectrum is perturbed. So let me explain you very well, so suppose here are this two resonance and they are connected to each other via space, now if I perturb this resonance the effect of this perturbation of this will be seen on this resonance, this effect is called like nuclear overhauser effect and the spectroscopy that was developed based on this each called nuclear overhauser effect spectroscopy or NOESY which is going to come in future lecture.

So, what happens because of this perturbation there is a enhancement of signal or actually decreased in the signal of this particular nuclei, because we are perturbing the resonance of this nuclei and that is given by this formula,

$$\eta_i(s) = \frac{I - I_0}{I_0}$$

is actually is NOE of any resonance I.

And here so we are perturbing this signal for *S*, so now I_0 is the intensity of the resonance *I* in the absence of any perturbation and *I* is when we are perturbing it. So, that is what here we had *S* was being perturbed and we are looking how the intensity of I is changing whether increasing or decreasing or altered so that is given by this η factor.

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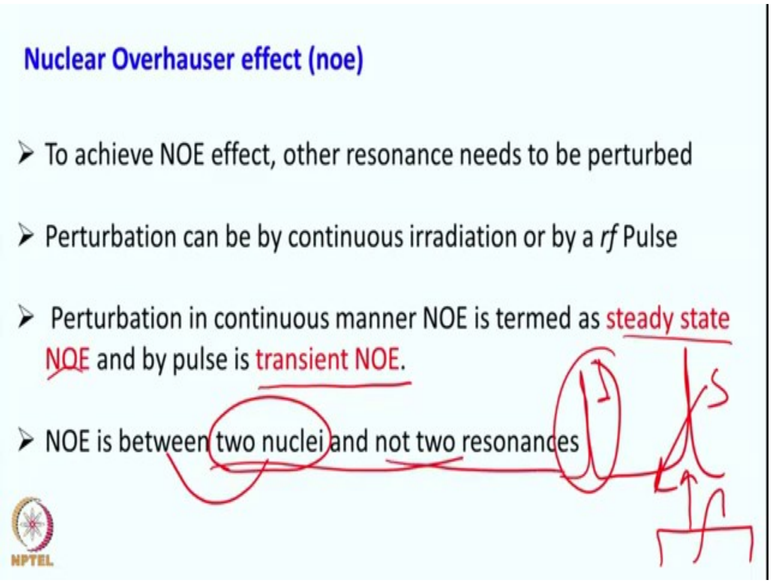

Nuclear Overhauser effect (noe)

- To achieve NOE effect, other resonance needs to be perturbed
- Perturbation can be by continuous irradiation or by a *rf* Pulse
- Perturbation in continuous manner NOE is termed as steady state NOE and by pulse is transient NOE.
- NOE is between two nuclei and not two resonances

Now to achieve this NOE effect as we discussed the other resonance has to be perturbed and this perturbation can be by any means, here is my resonance for *S*, here is my resonance for *I* we are perturbing this, now perturbing means you have to eradiate this resonance and that can be eradiation can be done by something called continuous waves, so what we are doing we are applying a constant *RF* at this particular frequency that is called continuous eradiation and the perturbation in the continuous manner NOE is termed as steady state NOE.

We can do also if we apply a tangent pulse here for a radio frequency pulse at the *S* resonance and that is called transient NOE, so NOE effect is between two nuclei not two resonance. So if nuclei one gives the resonance of *I*, if the nuclei gives the resonance of *S* the NOE has to be between this two different kind of nuclei not this two difference, so just understand difference in this so resonance is generally between the nuclei not between the resonance.

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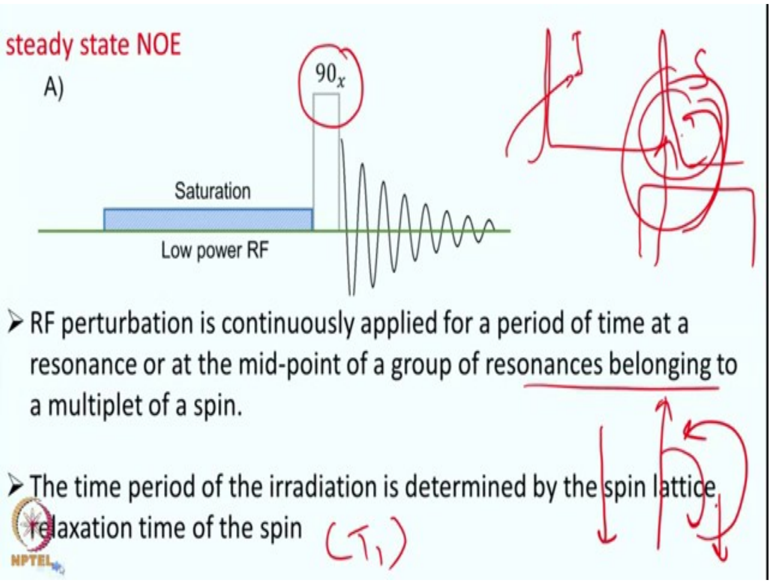

steady state NOE

- RF perturbation is continuously applied for a period of time at a resonance or at the mid-point of a group of resonances belonging to a multiplet of a spin.
- The time period of the irradiation is determined by the spin lattice relaxation time of the spin (T_1)

So that means for getting NOE we have to perturb a particular nuclei which gives a resonance, so, how we are going tom perturbed by continuous waves continuous eradiation. So here is the schematic of that experiment that we are going to do some as we have discussed earlier that we two resonance, one for I and another for S, here is my S, here is my I, we are saying continuously we are eradiating this resonance which is shown here and this continuous eradiation is done by a say low power *RF*.

We are just want to eradiate in the continuous manner then after continuous eradiation we apply a 90° pulse which is a hard pulse, means this all nuclei can be excited and one the excitation is done so this is applied 90_x pulse which effect actually all of the nuclei and then we record the spectrum.

So, this RF this continuous *RF* perturbation right down on S resonance at the midpoint here and this is by week *RF* pulse, so what happens not his S is eradiate, and it can have a multiplet or singlet or whatever but whole this spin system is eradiated and the time period for the eradiation how much we have to eradiate that depends upon what is this spin lattice relaxation.

So if you remember this spin lattice relaxation is the like a time it takes to come back in the equilibrium state when we put it in the $-Z$ direction. So suppose spin wise here and we put it in $-Z$ direction how much time it takes to come back to the equilibrium that is called spin lattice relaxation T_1 relaxation. So that T_1 determines that how long we have to eradiate, because we want this guys to completely eradiated therefore we need to know how much time it takes to come back to equilibrium.

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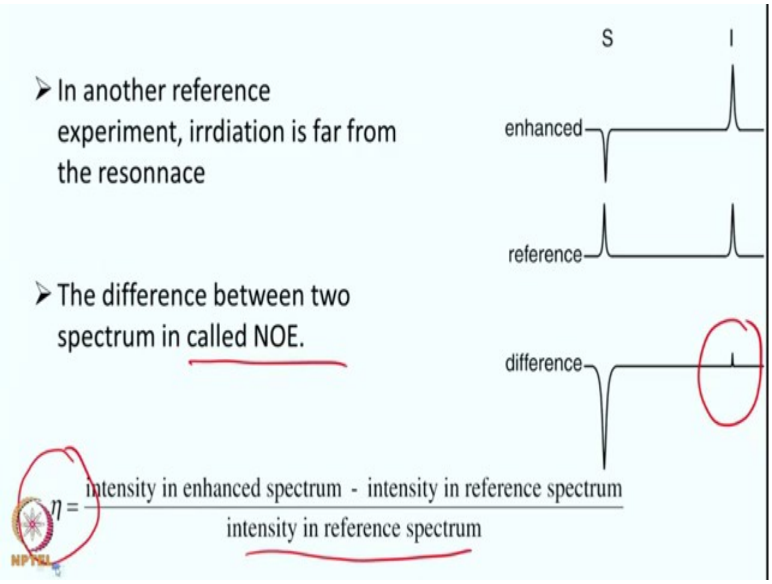

- In another reference experiment, irrdiation is far from the resonnace
- The difference between two spectrum in called NOE.

$$\eta = \frac{\text{intensity in enhanced spectrum} - \text{intensity in reference spectrum}}{\text{intensity in reference spectrum}}$$

So, what accessories for this experiment we have to do we have to record two experiment, one for we eradiate the S resonance, one where we do not eradiate then we take the difference of this two and the difference will tell the NOE enhancement, so because of eradiation it is enhancement in the *I* spin.

So intensity in the enhance spectrum minus intensity in the reference spectrum divided by the intensity in the reference spectrum this is celled actually NOE between the two spectrum, now this can only happen if this two spins are connected through space, so if they are not connected they will not happen and that is what we do in the reference spectrum. So, in reference spectrum we can eradiate far away from the resonance of the spin in the actual spectrum we are eradiating at the S spin, in the reference spectrum we are eradiating far away from the resonance and then we take a difference and we get a NOE.

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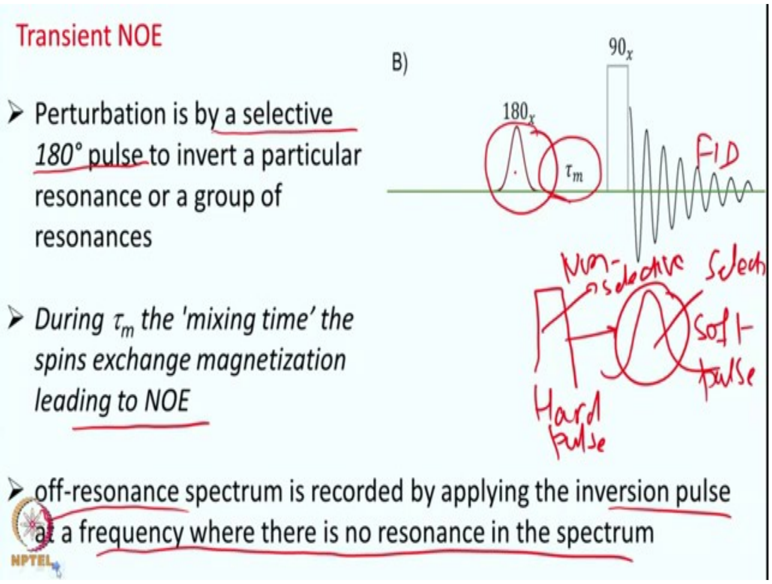

Transient NOE

- Perturbation is by a selective 180° pulse to invert a particular resonance or a group of resonances
- *During τ_m the 'mixing time' the spins exchange magnetization leading to NOE*
- off-resonance spectrum is recorded by applying the inversion pulse at a frequency where there is no resonance in the spectrum

Now in another experiment which is called Transient NOE, so as we have discuss transient NOE we are not going to give continuous pulse but small *RF* pulse of 180˚. Now this small RF pulse on 180˚ essentially will invert the resonance of this particular nuclei, so perturbation is done by this selective *I* pulse, if you see sign like this, this is for selective *RF* pulse the sign like square is for hard pulse means this non selective everything is excited and this kind of the Gaussian or various shape this is called soft pulse, so this is soft pulse, this is hard pulse, soft pulse is selective, selectively exciting particular resonance this is hard pulse is non selective.

Now this selective pulse is inverting the particular resonance or a group of a resonance say S spin then after that we have to do something called mixing, so mixing time what happens during mixing time actually spins exchanges their magnetization and that actually leads to NOE, so here this τ_m is important so now missing between this two spins has happened then we apply a hard pulse 90˚ hard pulse means that excite all the nuclei and after that we record is response this is FID.

So you record response as you discussed we have to do two experiment, one where we eradiated and another is a reference spectrum which we do off resonance spectrum is recorded by applying the inversion pulse at a frequency where there is no resonance in the spectrum and then we take a difference between two like on resonance spectrum and off resonance spectrum and that difference gives you how much will be enhancement.

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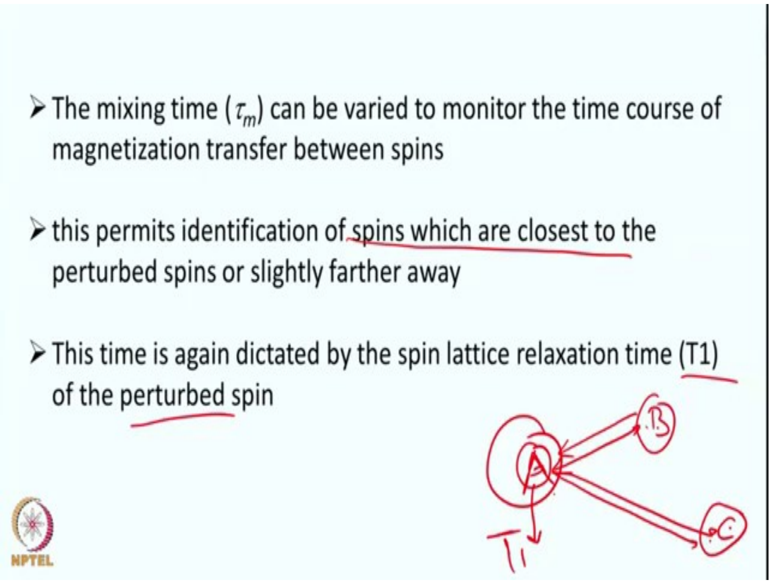

- The mixing time (τ_m) can be varied to monitor the time course of magnetization transfer between spins
- this permits identification of spins which are closest to the perturbed spins or slightly farther away
- This time is again dictated by the spin lattice relaxation time (T1) of the perturbed spin

Now one of the parameter we have discussed in the transient NOE is the mixing time τ_m. So τ_m can be varied to monitor the time course of magnetization transfer as we discussed during this time two spins talk to each other, so if you put it for a longer time they will talk more and magnetization transfer will be more, if we put it for a shorter time then the magnetization transfer will be less so this means this permits the identification of the spins which are closer to the perturbed spin or which are slightly farther from the perturbed spin, if you vary this τ_m actually this can give you precised distance information from the perturbed spin.

So if say just say just for an example A spin is here and we are perturbing this *A* spin, *B* spin is here and *C* spin is here, so effect will be more seen here then the effect will be seen here, so that means that the intensity can change if it is closer or it is far, now looking at the intensity we know that *A* is closer to *B* or *A* is closer to *C* so in this case *C* will be like intensity difference we will tell the distance and that actually gives you distance information, this whole experiment we will dictated by what is spin lattice relaxation of the perturbed spin.

So, we have to determine now how much is the T_1 time lattice spin relaxation for *A*, this is important parameter because this gives the time when it goes to equilibrium state, so then perturbation we have to tune accordingly looking at this spin lattice relaxation time for this perturbed spin.

So to summarize what we are doing the how we are going to get NOE in brief by two way, one is called steady state NOE one is called transient NOE, in steady state NOE we are eradiating a particular resonance or a group of resonance with particular spin by a continuous wave, one parameter to look after is the T_1 of the perturbed nuclei, that we have to look at in transient NOE we are doing same thing more or less but here we are perturbing by a selective pulse and then again we are mixing the spin states so that the magnetization of that perturbation effect can be transfer to another spin and that give you distance information.

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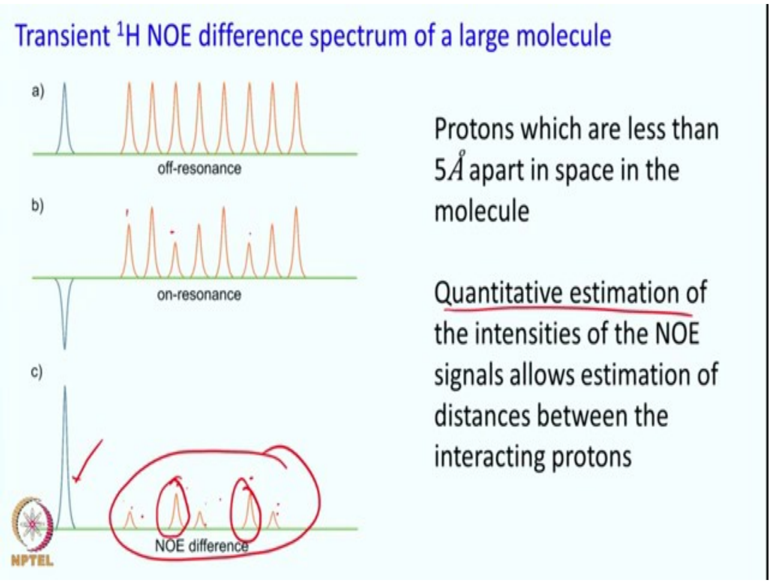

Transient ^{1}H NOE difference spectrum of a large molecule

Protons which are less than 5$\mathring{A}$ apart in space in the molecule

Quantitative estimation of the intensities of the NOE signals allows estimation of distances between the interacting protons

So here is transient NOE spectrum for a large molecule, so here is the off resonance spectrum we are actually perturbation based on quite a far and one on resonance spectrum here the perturbation based on this spin and then you see the effect at the some of the resonances their intensity changing.

So if you take the difference between this two you can find that all these resonances which shows the some signal where actually enclosed proximity of the perturbed spin like here, so now that give the distance information and distance information is not too long it is approximately about 5 Armstrong, but we can find it out that one thing you can see it that intensity of this resonance and this is more than this, this and this.

So what is says that these two resonances seems to be closer to the perturbed spin and that is how one can get the distance information in a macromolecule, so if you do quantitative estimation of this intensity of this NOE signal we know the distance between all this spins, so we need to measure a standard distance and the NOE effect and with compare to all those intensity can be relatively calculated how much will be distance between this two nuclei or the group of resonances. So now we understood the importance of the NOE.

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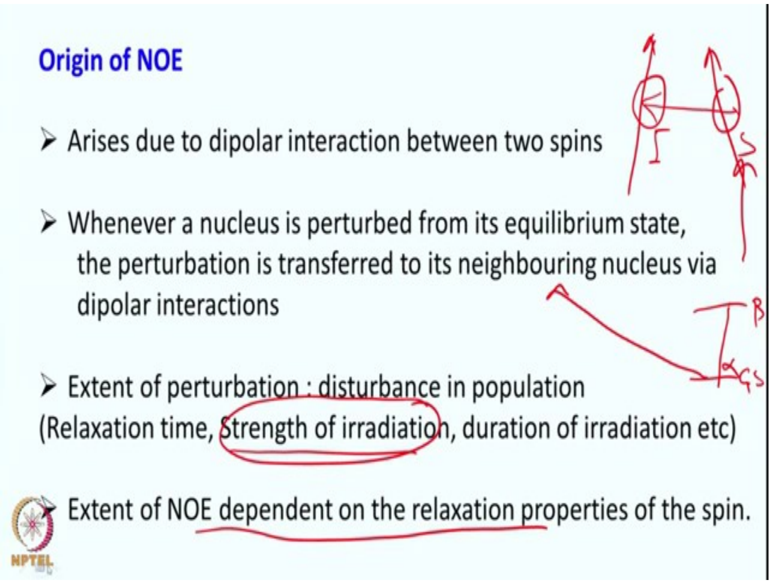

Origin of NOE

- Arises due to dipolar interaction between two spins
- Whenever a nucleus is perturbed from its equilibrium state, the perturbation is transferred to its neighbouring nucleus via dipolar interactions
- Extent of perturbation : disturbance in population (Relaxation time, Strength of irradiation, duration of irradiation etc)
- Extent of NOE dependent on the relaxation properties of the spin.

Now next we want to understand what is the origin of this NOE, so NOE arises because of the dipolar interaction between two spins, now as you know each spin is a dipole and between this dipole there is an interaction so that means this interaction if you remember from your undergraduate physics, dipolar interaction is the distance dependent that is why we said that up to 5 Armstrong the NOE effect can be seen. So NOE arises because of the dipolar interaction, the also it depends upon if you look at their angular aspects to that so how spins are oriented with respect to each other, so this actually gives you lots of information about the orientation of the spins the distance of the spins and many of those stuff.

So whenever a nuclei is perturbed from its equilibrium states, equilibrium state what we mean where the lower state has more population then he higher state so that is equilibrium state, the perturbation is transferred to its neighboring nuclei by a dipolar interaction so suppose this two spins, spin I and spin S are connected by dipolar interaction we are perturbing spin S, now perturbing means we are changing the population in this S spin, the population between this two states the ground state and the excited state or the α state and β state, so if you are changing the effect of that will be transfer to this spin and that is because they are interacting by dipolar interaction.

So this extent of perturbation how much we are creating depends upon how they are relaxing, so relaxation rate of this and how strongly we eradiate this what is the strength of the that radiation pulse, what is the duration of that radiation, so all those plays effect and therefore this effect is quite quantitative because we can change the eradiation strength and duration of the eradiation and we can look at how far the eradiation effect has reached, so extent of NOE depends upon various relaxation properties you will look at more qualitative and quantitative manner how the NOE is dependent upon relaxation property of the particular spin.

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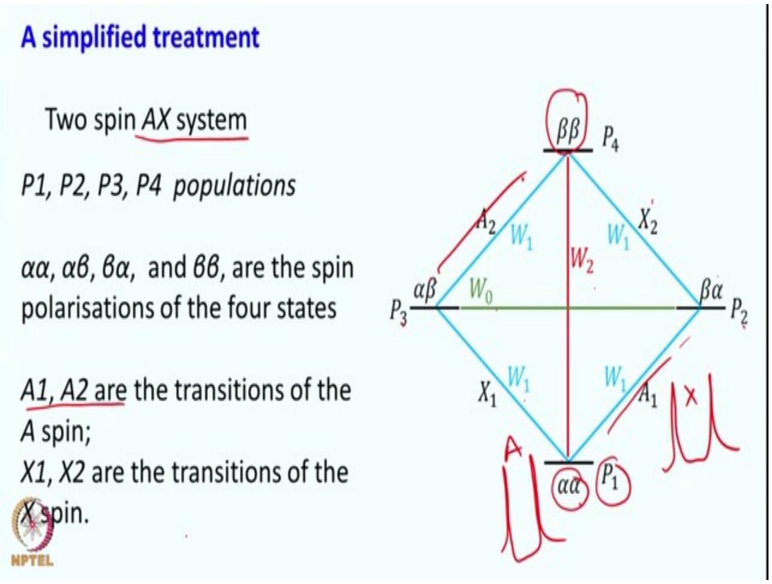

A simplified treatment

Two spin *AX* system

P1, P2, P3, P4 populations

αα, αβ, βα, and *ββ*, are the spin polarisations of the four states

A1, A2 are the transitions of the *A* spin;
X1, X2 are the transitions of the *X* spin.

So for just simplified treatment we will just look at two spin system like weekly coupled system that we had discussed earlier, *AX* system, now *AX* system will have four states, say *αα* state, *αβ* state, *βα* state and *ββ* states, so these are the four states for the *AX* system, now the population in actually this four states let us write *it P1, P2, P3* and *P4* so as we have seen this two transitions here *A1* and *A2* is for *A* spin suppose write and this two are for *X* spin, so this transition will be one line and that transition will be on another line, and similarly here we have two transition giving one line and another line, so that is the four states and the population *P1, P2, P3* and *P4* of this states.

Now as we have seen earlier this transition be define as ω_1, ω_1, ω_1 and ω_1, this is called single quantum transition, what is happening here, there is only one flip so α goes to β state here in this case first spin α goes to β state. So change in the quantum here is 1, again here, here and here so therefore this are called single quantum transition.

Now here if you look at $\alpha\alpha$ goes to $\beta\beta$ states so that is called double quantum transition and in this case there is a spin flip flop, so α goes to β and β goes to α so this is called zero quantum transition, so we have three kind of transition probability in the spin system ω_1, ω_2 and ω_0. So we looked at the four this populations and the polarization of this four states so $A1$ and $A2$ are transitions for A spin and $X1$ and $X2$ are the transitions for the X spin.

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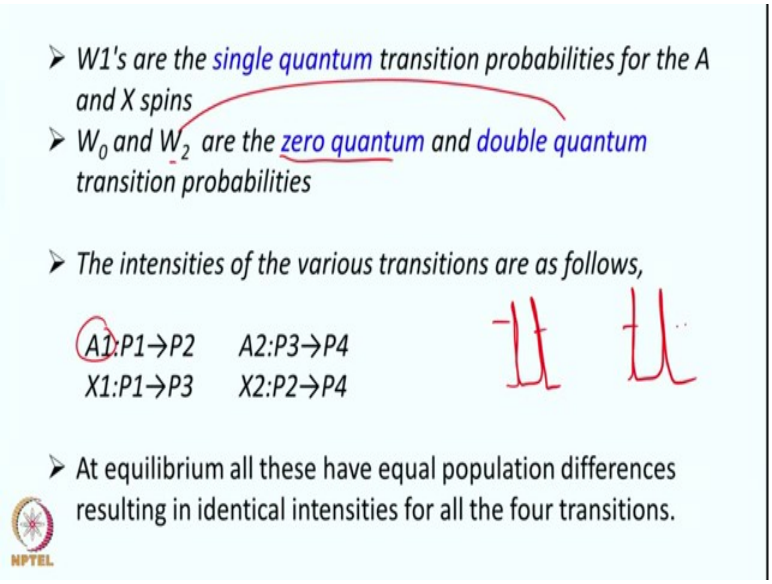
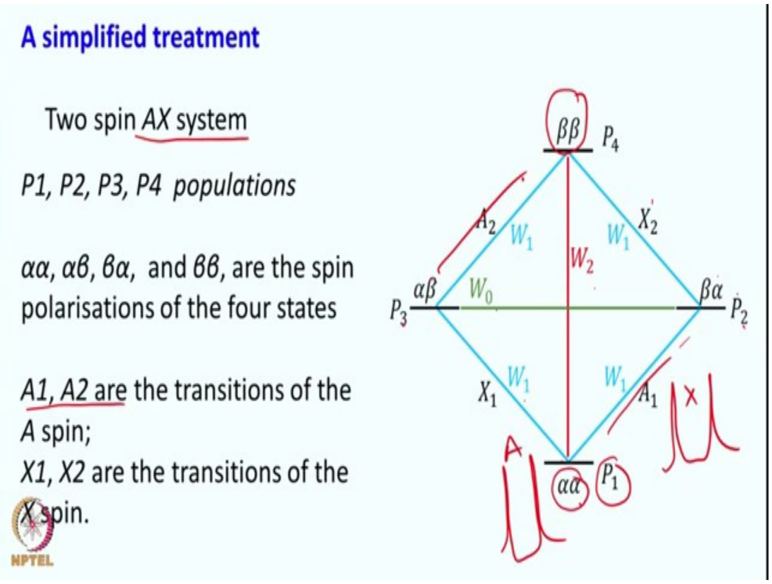


- W1's are the single quantum transition probabilities for the A and X spins
- W_0 and W_2 are the zero quantum and double quantum transition probabilities
- The intensities of the various transitions are as follows,

A1:P1→P2 A2:P3→P4
X1:P1→P3 X2:P2→P4

- At equilibrium all these have equal population differences resulting in identical intensities for all the four transitions.



A simplified treatment

Two spin AX system

P1, P2, P3, P4 populations

αα, αβ, βα, and ββ, are the spin polarisations of the four states

A1, A2 are the transitions of the A spin;
X1, X2 are the transitions of the X spin.

So, that is what we looked that the ω_1 is transition called single quantum transition and ω_0 is called zero quantum transition and ω_2 is called double quantum transition, so intensity of this case like *A1* intensity will be dictated by what is the difference between *P1* and *P2*, similarly the intensity if *A2* will depend on population difference between 3 and 4, intensity of *X1* depends upon population difference between *P1* and *P3* and for *X2* it is *P2* and *P4*.

So at equilibrium all this will have equal population difference and resulting identical intensity of all four transition for a homonuclear system that we have seen previously so this is *A1*, *A2* and this is *X1*, *X2* all equal intensity because the difference between these populations are equal.

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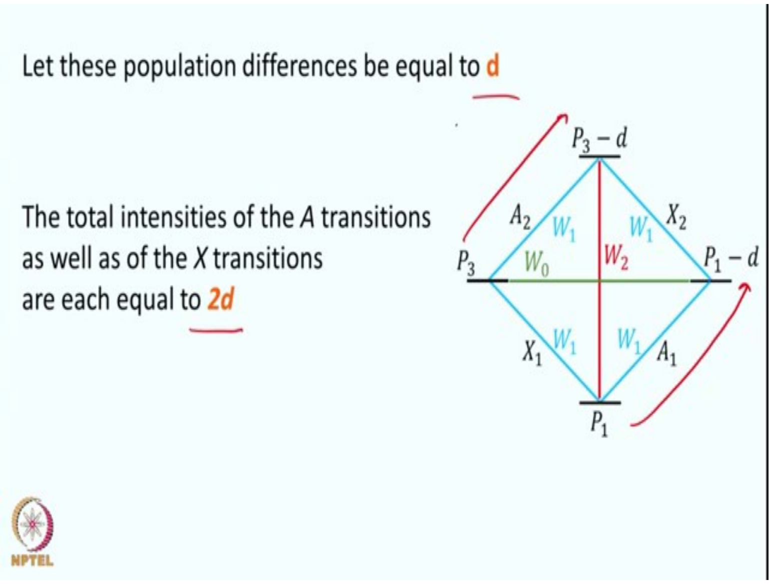

Let these population differences be equal to **d**

The total intensities of the *A* transitions
as well as of the *X* transitions
are each equal to **2*d***

Now let the population difference be equal to *d*, let us define that so total intensity of *A* transition here *A* transition here *A1* and *A2* will be equal to *2d,* so here that is what we measure the population difference lets define as d, so for this single quantum we have a *2d* intensity for *A* line.

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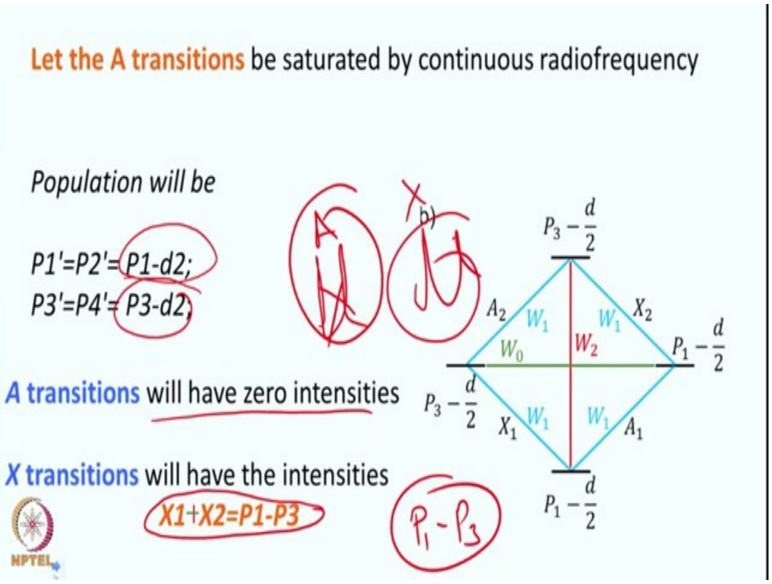

Let the A transitions be saturated by continuous radiofrequency

Population will be

P1'=P2'=P1-d2;
P3'=P4'=P3-d2;

A transitions will have zero intensities

X transitions will have the intensities

X1+X2=P1-P3

$P_1 - P_3$

Now let the A transition be saturated we are eradiating A transition by continuous RF, so now population between the perturbed state is going to be *P1* and *P2* will be *P1 - d2* here and *P3 P4* will be *P3 - d2* and *A* transition because we are eradiated *A* so like *A* transition will have 0 intensity and X transition will have like *X1 + X2* like here *X1 + X2 = P1 - P3*, so as we were saying we had a *A* and we had a *X* we eradiated this so this there is no signal which some signals which of equal intensity and - *P1 P2*.

But where is the enhancement of this signal so where is the signal enhancement, we got some intensity but can we quantify that signal enhancement, now that let us look at in the next lecture how we are going to get enhancement in the *X* signal, you think over it and we will continue from here, how using this concept we can enhance the signal of *X* spin if you are perturbing the *A* spin. So we will continue from here looking forward to have you in the next class and let us have some questions of you have any doubt do not hesitate to contact me again, thank you very much.