NMR spectroscopy for Structural Biology NS

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Lecture: 13

Polarization Transfer Technique - 1

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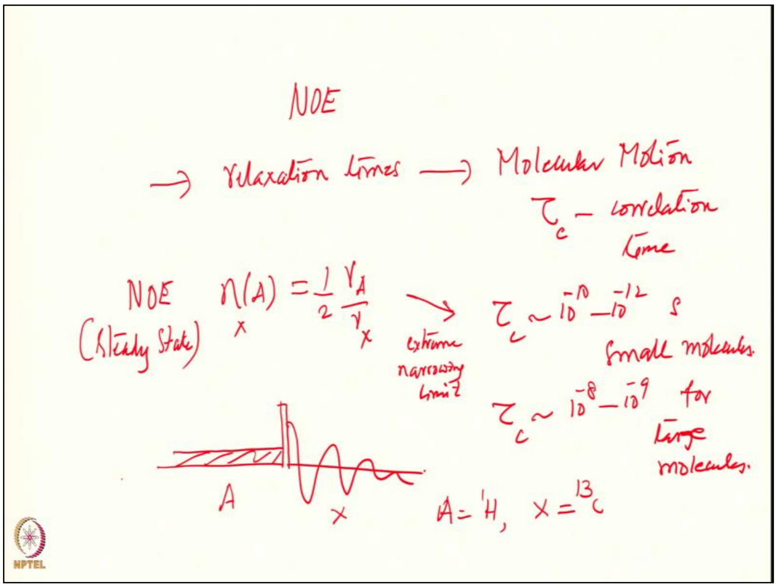

So, there have been detailed theoretical calculations for NOE we will not go into those detailed theories but I just want to show you the results. NOE as I said this will depend upon the relaxation times and this will depend upon the molecular motion how fast the molecule is tumbling inside the solution and that is characterized by a particular time constant known as the correlation time.

For small molecules correlation times is of the order of 10^{-10} to 10^{-12} this is for nanoseconds seconds or no therefore 10 to the minus this is extremely small time second. So, that means is order of nanoseconds right. So, and for this is for small molecules. And correlation time can be obtained of 10^{-8} to 10^{-9} for large molecules. So, this basically reflects how fast the molecule is tumbling inside your solution and the relaxation times will depend upon this.

Now according to that so, the theoretical calculations on NOE have been done and it turns out that the NOE is equal to if we want to represent this as n_XA and A is the one which is perturbed in the earlier case I wrote it as $n^i{}_X$A is the one which is perturbed and n_X is the X is the one which you are going to monitor and this is typically $n_x = \frac{1}{2}\frac{\gamma_A}{\gamma_X}$ where γ is the gyromagnetic ratio.

And it all depends upon the individual spins also and this will be different for different situations this is for this condition this is for this condition. So, therefore what will be the kind of you know this is the steady state NOE steady state and NOE means I do an experiment in the as I indicated to you before the experiment is done in this manner that I have FID collected here and I do a pre saturation perturbation.

I do a perturbation here on one particular line on a particular line let us say I do it is for the spin A and I monitor the spin X here. So, when I perturb here I monitor the intensity and without the perturbation amount of intensity and then I take the ratio get the NOE and this will. Now by calculations one can show that this is proportional to $\frac{1}{2}\frac{\gamma_A}{\gamma_X}$ what is the advantage here.

So, the advantage is suppose A is proton suppose A is proton and X is let us say carbon 13 what is the ratio of $\frac{\gamma_A}{\gamma_X} = 4$ right. So, therefore and therefore this NOE will be a factor of 2. If $\frac{\gamma_A}{\gamma_X}$ then of course it is a factor half. So, this is 0.5 this is an enhancement how much is the enhancement in the intensity of the lines then when you perturb one spin and observe the other spin.

And if you are perturbing proton and observing carbon 13 then means you are getting a factor of 2 enhancement in the in the intensity of the line. So, and that is the significant advantage that is the significant advantage because the factor of 2 enhancement meaning it is like a factor of four in terms of the time with respect to the signal averaging. So, therefore the steady state NOE this is the steady state NOE which you call steady state NOE.

And in the this limit is called as the extreme narrowing limit this is the small, small molecules very rapid motions and this is also called as extreme narrowing limit. Here the tumbling is very rapid the motions are very fast. So, the averaging happens much much better and the relaxation times are also very are different. So, therefore without going into the theoretical detail this is to just to show you what kind of a thing is it will look like. Now what will be the situation when we are in this large molecule range?

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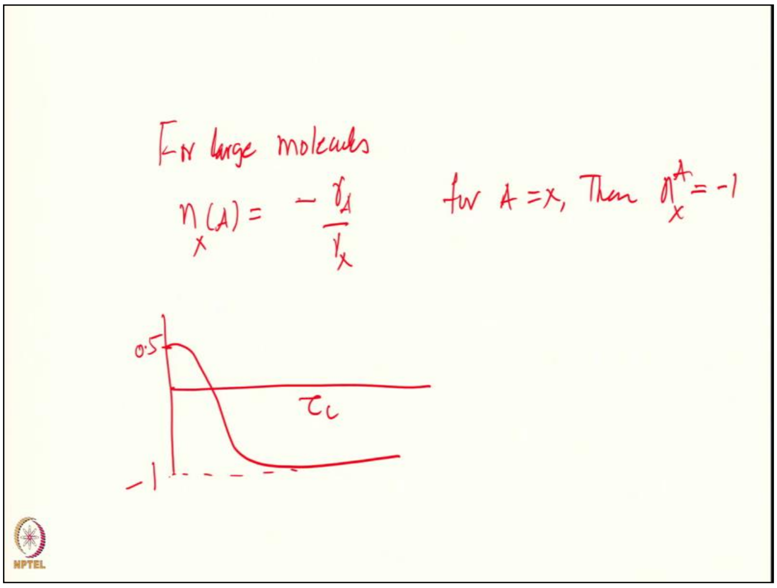

For large molecules

$n_x(A) = -\frac{\gamma_A}{\gamma_X}$ for A = X, Then $n_x^A = -1$

The large molecule range it turns out that this is equal to $-\frac{\gamma_A}{\gamma_X}$ for large molecules. $n_x A = -\frac{\gamma_A}{\gamma_X}$ So, therefore you can see for proton if A = X then this $n_x A = -1$. And if I want to plot here the NOE as a function of the correlation time here it will be it will look like this and this is my 0.5 and this is my -1. So, this is the case for homonuclear systems. So, you have -1. So, the negative NOE you will get this is called as negative NOE.

But nonetheless it is it is a kind of a significant change in the intensity and you when you take the difference you will get a substantial enhancement and for large molecules you will get a -1 as a this one but if it is not homonuclear then of course you are $\frac{\gamma_A}{\gamma_X}$ that is proton and nitrogen or something then you get a factor of half also and then you will get -5. So, you get heteronuclear ones, of course you will have a different number for it will be -4 something like that -5 for proton or nitrogen.

Because this is well nitrogen has another complications in the sense that the gyromagnetic ratio for nitrogen is negative. So, therefore there even for the small molecules there is the $\frac{\gamma_A}{\gamma_X}$ in the case of nitrogen it will be negative. So, this is what I showed here in the previous slide when I said $\frac{1}{2}\frac{\gamma_A}{\gamma_X}$ you see here if you are talking about nitrogen proton nitrogen NOE.

And then let me also draw that here if let us say A is equal to proton then X is equal to nitrogen 15 then this nitrogen 15 has γ of nitrogen is negative. So, therefore you will get a negative enhancement there and that will be a factor of 5 because $\frac{\gamma_A}{\gamma_X}$ there is 10 and therefore divided by multiplied by half then you get a -5. So, even in the extreme narrowing limit when you are talking about heteronuclear NOE proton nitrogen it is -5 and proton carbon it is +2.

So, these are the factors one has to remember one studying these experiments in the steady state NOE. Now there is another experiment. So, far I talked about the; what is called as the saturation pressure this is called as one saturation was done on the spin A and we are monitoring the spin X. There is another way one can do and that is called as transient NOE.

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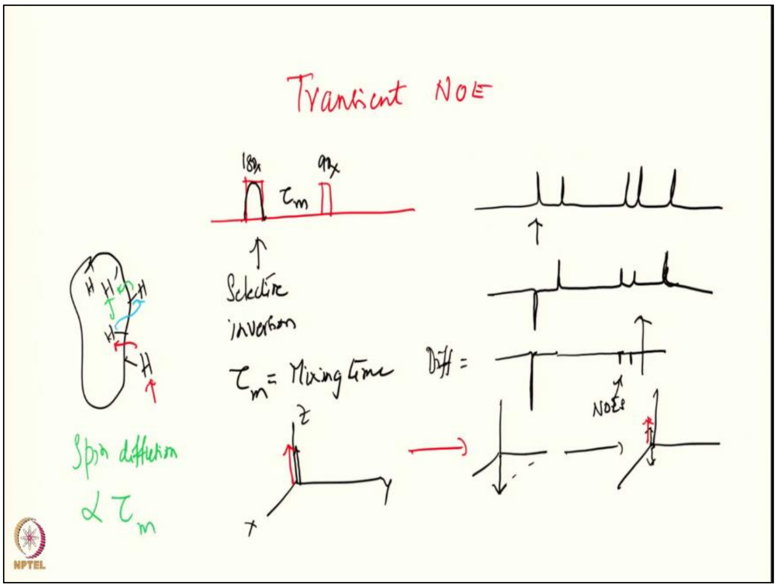

Transient NOE what you do here is your perturbation will be like this you do the experiment like this let me draw this by different colour this one I will put like this. So, this is a selective perturbation selective inversion and this is a hard 90° pulse. This is a 90 hard pulse what does that mean. So, then let us say I have a spectrum something like this. So, what I will do is I will selectively invert this line I will selectively invert this line after 180° pulse this is 180°.

So, I will selectively invert this. So, then I will get here this one like this and this remains like that and whatever happens here and I give a time here called τ_m and τ_m is the is the called the so, called mixing time. So, what I have achieved here let me explain what we have achieved here. So, this is this is the experiment what happens. So, let us assume that I have the initial magnetization of all the spins all of these have different magnetization components right let me draw the x, y, z.

So, draw the magnetization of this particular spin here and the magnetization of the other spins also will be here let us see. So, during the period when I apply selective 180° pulse what I am achieving is I am inverting this magnetization I am selectively applying a selective 180° degree pulse on the black line which means the magnetization which was here is now like this.

When you measure it of course I will see it as this as a negative signal. Now this is the perturbation this is the perturbation this perturbation has to recover back to equilibrium the system will have to recover back to equilibrium what it will do? It will relay this perturbation to some other spins which are close by in space. So, then this will relay. So, as it recovers as it recovers like this it will relay this magnetization to some other spins.

So, therefore what will happen is these components which are present here they will also get little bit of that component the red one which was there; red one we will also get certain contribution from the let us say like this a contribution and the black of course is recovering down there. So, the black is slowly recovering. So, it is it has come let us say then of course certain contribution of the magnetization has gone do not assume that the certain portion of the blanket it is transferred that to the red.

So, therefore this will show up when I measure it, it will show up in this some intensity of the red one if this were the red one that would have gone down or it would have gone up either way this can happen for both positive and negative NOE's. You saw already that for small molecules it is positive NOE which means there is an enhancement for large molecules it is the negative energy means that it will decrease intensity will decrease.

So, therefore there can be changes in these other portions of the spectrum as a result of the inversion here and I give a certain amount of time here tau m because I am allowing it the magnetization to transfer this is this is dependent on the relaxation time. Depending upon the relaxation time T_1 the efficiency of transfer will vary therefore this can go to different ones as a result of the dipolar coupling between the 2 spins.

So, therefore when I measure this I will get different intensities of these lines here not all of them but some. Some I will get some I do not get no something will be perturbed some will not be perturbed. So, now if I take the difference here if I take the difference between these 2 this fellow has not perturbed this has perturbed this has perturbed and maybe this is not perturbed let me take it up there this is not perturbed.

So, then I take the difference, difference is equal to what I will get I will get a thing here much larger let us say this minus this then I will have a 0 there and this I will see minus there because this is increase this is small interpret and this also I get a minus here and then I get 0 here again no perturbation right. These are the perturbed ones these are the NOEs and this will depend upon the mixing time.

How much time do I give why does it depend on the mixing time because it depends on the relaxation time of the perturbed spin and the relaxation times the other ones also. Now once I have done this will it always remain there? Will it remain at that point only question is let us say I have a molecule like this and I have a proton here a proton there a proton here a proton here a proton there so and so forth.

I perturbed one of these fellows I perturb this fellow then it will relay from here to here because it is close directly during the day of T_1 but this is also not perturbed as a result of this, this is also perturbed therefore it will also have to relax what it will do it will also transfer to something which it is close by. So, let us say I have I take that. So, from here it will go here take one more or it can go from here to here or it can go from here to here and so on so forth.

So, what is this phenomenon called this phenomenon is called as spin diffusion. Spin diffusion means the magnetization which is perturbed at one point it will keep diffusing through your network of coupled spins and that will obviously depend upon the time tau m. So, this will depend upon proportional to tau m larger the τ_m you give longer will be the spin diffusion and therefore if you restrict the towing to very small value then it will go only to the directly neighbouring spin.

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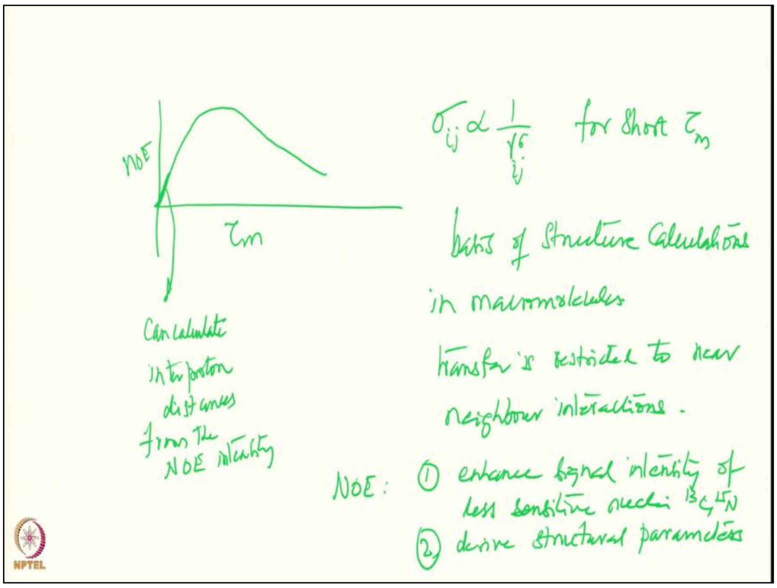

Can calculate interproton distances from the NOE intensity

$\sigma_{ij} \propto \frac{1}{r_{ij}^6}$ for short τ_m

basis of structure calculations in macromolecules

transfer is restricted to near neighbour interactions.

NOE: ① enhance signal intensity of less sensitive nuclei ^{13}C, ^{15}N

② derive structural parameters

So, if I were to plot. So, so if I want to plot the NOE as a function of tau m then it will go like this it will come down. So, this is the linear this is called the linear regime during which time it is directly proportional to the neighbouring spin only going to the neighbouring spin only and it is in this region one can calculate inter proton distances from the NOE intensity. Here the NOE intensity is proportional to what is called as the cross relaxation rate which is represented as σ.

$\sigma_{ij} \propto \frac{1}{r_{ij}^6}$ in this approximation in this region in this region it is proportional. If we go beyond that because the intensity NOE intensity is no longer just restricted to that it will start diffusing to other ones. So, the relaxation individual coupling between all the spins will come into the picture and you may not be able to do it.

Therefore transient NOE is an experiment which is done with a controlled manner you can control the extent of diffusion that can happen from one spin to another spin and that is the extremely useful parameter for calculating the structures of molecules and this is the basis of structure calculations in macromolecules. This is for short τ_m and there it is restricted to the near neighbour interactions only.

For short τ_m the transfer is restricted to near neighbour interactions therefore. So, polarization transfer has 2 objectives right. So, 2 objectives polarization transfer by NOE has 2 objectives. So, therefore NOE in summary other times NOE has 2 gains one is enhanced signal intensity of less sensitive nuclei this is like carbon 13 nitrogen 15 and the second is derived structural parameters of macromolecules.

So, these are 2 important applications of NOE and we will not go into the theoretical details of this much more than this I think that is that is adequate for our purpose of structural biology which is the focus of this because otherwise it can get very complex. So now let me take one more topic.

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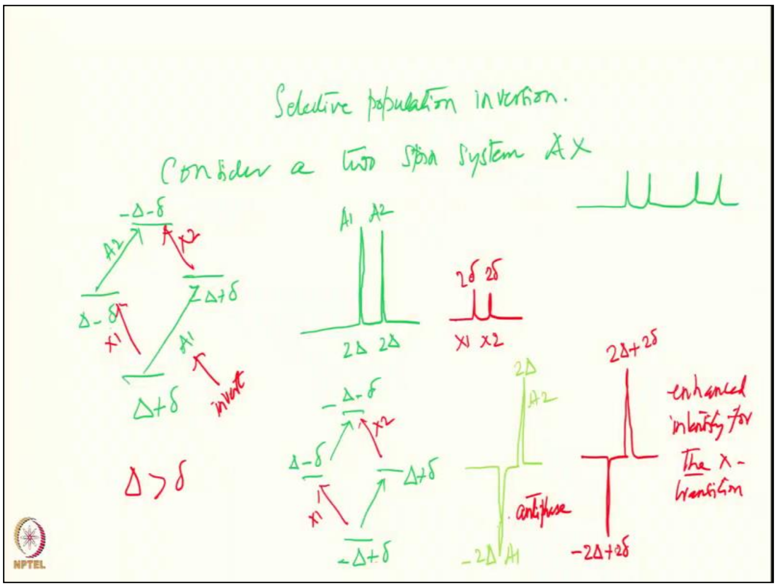

So, we will take another topic here which is called as selective population inversion. Consider a 2-spin system AX, A and X this can be heteronuclear movement nuclear also and what I will have I will have here a simple 2X system will have a will have 2 lines and X also will have 2 lines. Let me draw the energy level diagram for this I will have four states let us say the population of this is $\Delta + \delta$ population of this is $-\Delta + \delta$ and this one is $\Delta - \delta$ and this one is $\Delta + \delta$.

So, now I call this transition as A_1 this transition as A_2 call this as X_1 and this as X_2. Now let me draw the intensities of the A transitions intensity of the A transition is population this population minus this. So, therefore that will be 2δ and similarly the other one also A_2. So, if I call this as A_1 this is a 2 and both these have intensities of 2δ and what about X_1 and X_2 this is this minus this.

So, I will have smaller intensities and notice here $\Delta > \delta$ this is 2Δ and this is also 2δ and this is my X_1 and this is my X_2. So, now what I do is I invert this transition invert select your population inversion this is what I said select your population inversion. So, now what will be my intensities of the four levels. Now I will draw this levels again here this population becomes this.

So, this will be $-\Delta + \delta$ this will go there $\Delta + \delta$ I am inverting only this I am not changing anything else. So, this will be $\Delta - \delta$ and this will be $\Delta - \delta$ I am not doing anything here I am not perturbing these populations at all. Now I see what happens to the transitions what happens to the transitions the 4 transitions these were my A_1 A_2 transitions and the X_1 X_2 transitions are this X_1 X_2 what will be the intensities here.

Let me draw the ones first and this is now -2δ the A_1 will become -2δ delta change the colour here ok this became -2δ and this one is this minus this, this will be $+2\delta$. So, this is my A_1 and this is my A_2 what about the red ones? Red ones are these. So, $\Delta+\delta$, $-\Delta+\delta$ so, $\Delta-\delta$, $-\Delta - \delta$. So, that is $-2\Delta +2\delta$.

So, this is $-2\Delta +2\delta$ and the X_2 is $-2\Delta +2\delta$. So, that is this plus this minus this. So, $-2\Delta +2\delta$ see look at this whatever 2δ here 2Δ here has become this large quite a substantial enhancement in the index although the k gain is not the same in both cases there is but nonetheless each one of them has gained substantially in terms of the intensity of the lines.

You have at the cost of the magnetization of the A_1 transition you have got the enhanced intensity for the X transitions enhanced intensity for the X transitions notice here these A_1 and A_2 transitions are become completely opposite to each other anti-phase this is called as antiphase. They have become antiphase the A transitions will become antiphase because they inverted only one of them and therefore its intensity is changed.

So, 1 is -2Δ other one is $+2\delta$ in the earlier case both were 2δ 2Δ and I have inverted one of them therefore that one of them became -2δ delta other one became remain the same as 2δ delta and with the X_1 and the X_2 transitions which were initially both equal as 2Δ and 2δ. Now one of them has become $-2\Delta +2\delta$ and the other one will become $+2\Delta +2\delta$ and you see substantial gain in the intensity right.

So, therefore this obviously will depend upon how much is the individual difference between $\Delta$and $\delta$and that is dependent on the γ of the $\frac{\gamma_A}{\gamma_X}$. So, that is proportional to the ratio of the gyromagnetic ratio. So, the gain is proportional to this is this depends upon $\frac{\gamma_A}{\gamma_X}$. So, the gain will be proportional to the ratio of these gyromagnetic ratios of the 2.

So, substantial gain can be obtained if it is heteronuclear. For single homonuclear systems you may not be that much of an advantage but heteronuclear it will be great advantage this principle is used in other techniques which will take up next time and that is particularly useful for all multi-dimensional multiple experiments. The important thing of course to notice here is that you may call this as a disadvantage while there is an advantage which you already discussed the disadvantage could be that you have positive negative signals.

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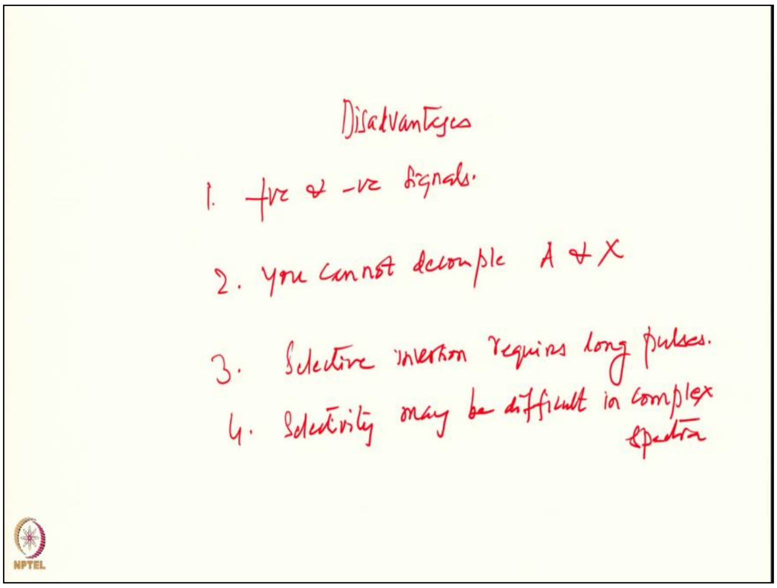

Disadvantages

1. +ve & -ve signals.
2. you cannot decouple A & X
3. Selective inversion requires long pulses.
4. Selectivity may be difficult in complex spectra

You can say the disadvantages would be you have one positive and negative signals second you cannot decouple A and X because if you decouple the 2 will collapse when the collapse you will get back the same intensity only when the coupling is present the 2 lines are separately observable then you gain the advantage. So, therefore and you want to get rid of both of these kind of a situations and that leads us brings us to other techniques which is which is called as insensitive neck.

And of course in the third disadvantage also could be selective inversion requires long pulses requires long pulses that means it will not be 10 microsecond pulse it may be millisecond pulse if it depends on the selectivity what you will want to observe. So, better the selectivity you want the longer the pulse length will be therefore and this is a difficult thing to achieve. And then the four selectivity's selectivity may be difficult in complex spectra.

So, while the gains are there with regard to the intensities we also have certain disadvantages here to get over this we go into some other techniques which are called as inept and that will be will be taken up next and in fact that will be the most crucial thing for all multi-dimensional NMR experiments. So, I think we will stop here.