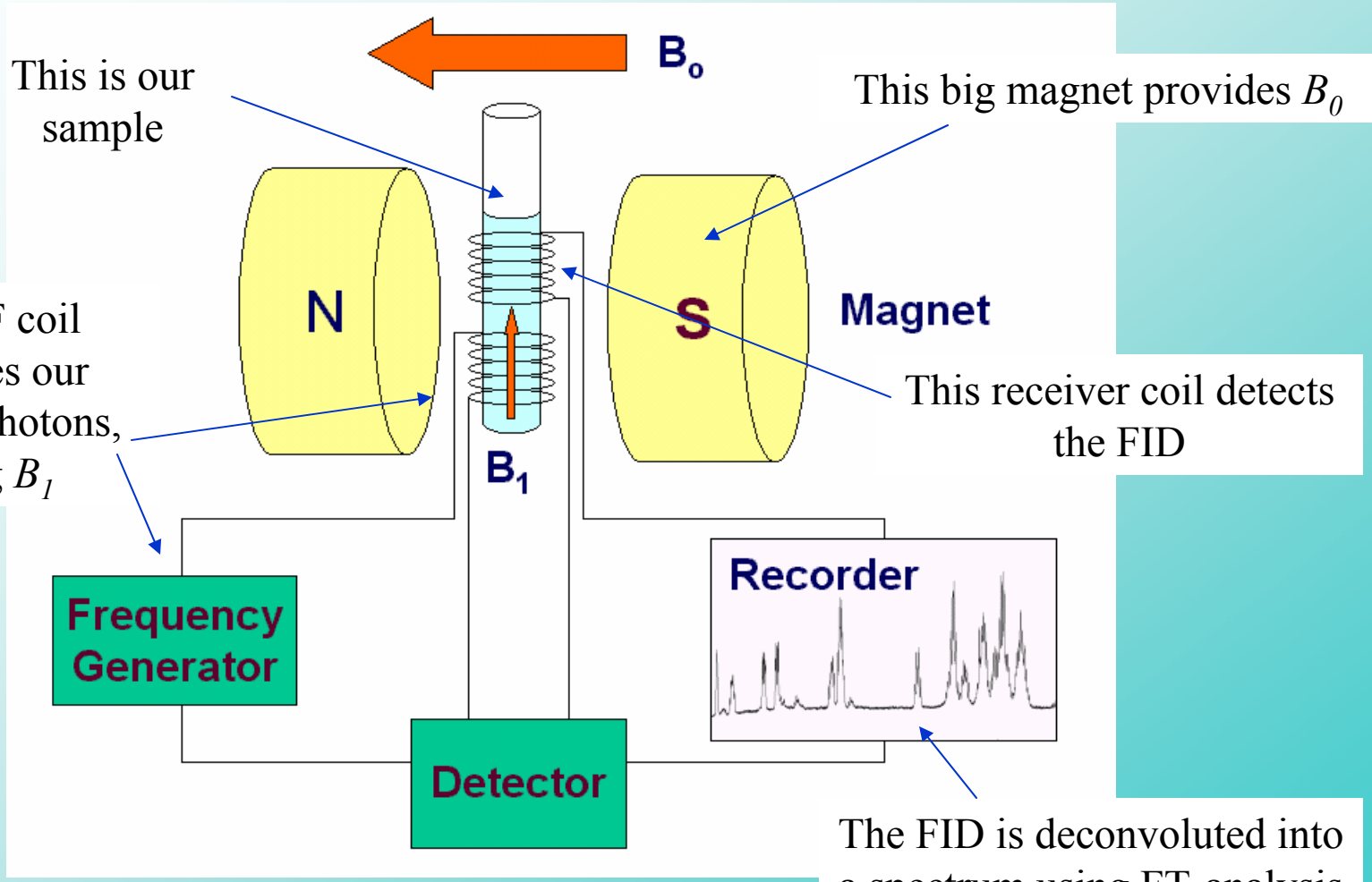
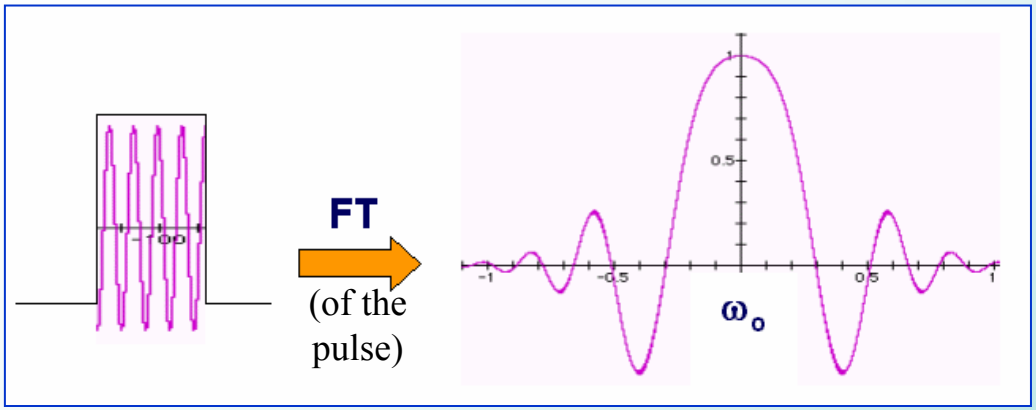
Lessons from previous lecture

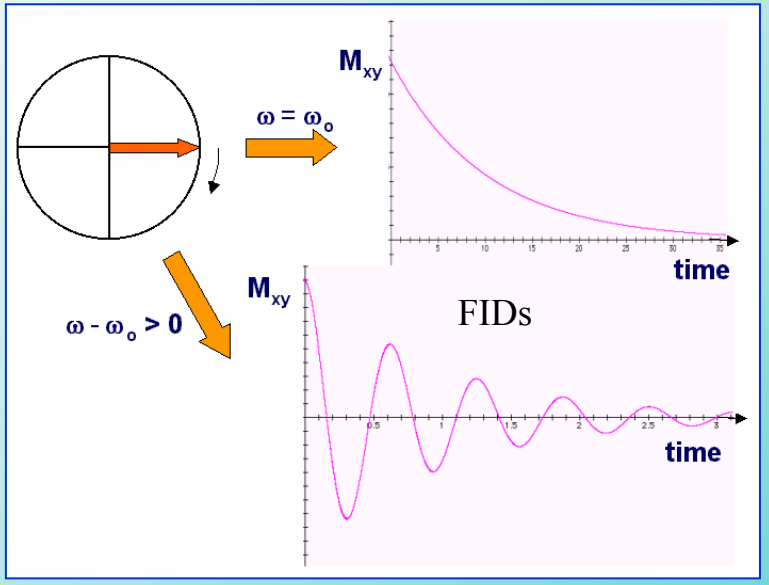
Frames of reference – the laboratory frame.

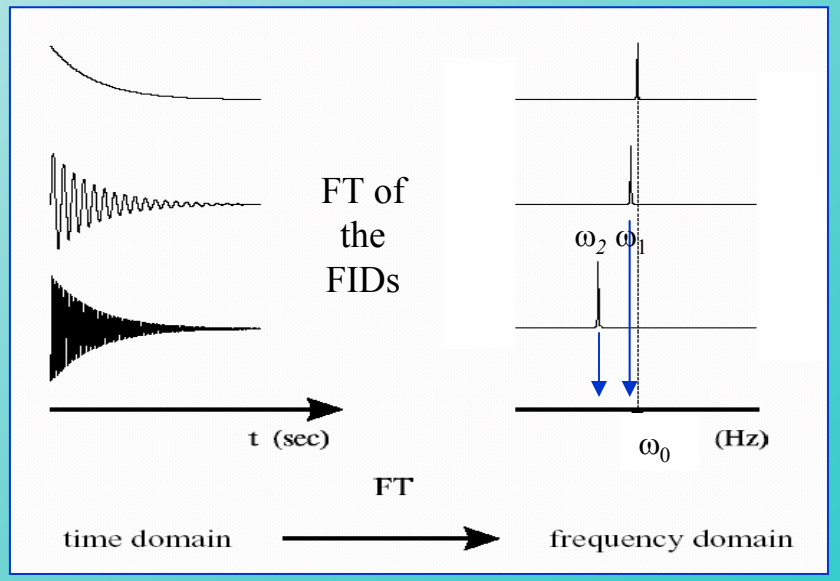


How do we sample transitions with a range of energies?

The square wave pulse centered at frequency ω_0 contains harmonics that effectively cover a range on either side of ω_0. The detector coil is tuned to ω_0, so the FT of the FID for a transition at the central frequency is a smooth curve. The transitions away from ω_0 show harmonics, and the frequency shows up as a shift away from ω_0 after Fourier transformation.







Pulsed NMR.

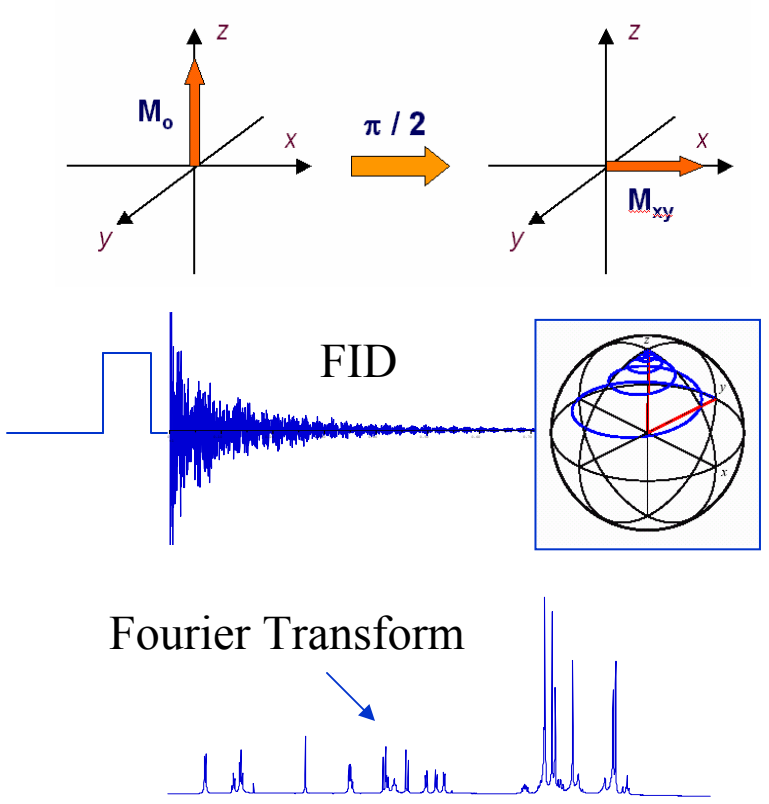
We defined two frames of reference:

1. The laboratory frame, - given by the geometry of the instrument (the parent's perspective in the kids' roundabout analogy).
2. The rotating frame, in which everything rotates at the precessing frequency of the system, ω_0 (the kids' perspective). This allows us to treat a **population** of spins, and represent it by a single vector given by their vector sum.
3. In the laboratory frame, our instrument has generator and receiver coils. These are oriented along the x or y axis, at right angles (orthogonal) to the field generated along the z-axis by the magnet.
4. For the rotating frame, we can choose only one value for the frequency of a precessing system, ω_0. However, we noted that the pulses contain a range of frequencies centered on ω_0, and that we excite transitions with frequencies in this range, close to ω_0. In all but the simplest systems, most spins will $\neq \omega_0$.
5. Since we are rotating at ω_0, these off beat transitions get out of phase at a rate determined by the difference from ω_0.
6. We can set up pulses so as to apply a torque to the spin system. Because of the orthogonal geometry, we initially flip our spins from the z-axis.

7. We can time our radio frequency (RF) pulses so as to generate a spin flip through 90° (with a $\pi/2$ pulse) or through 180° (with a π pulse), around the axis.

8. A $\pi/2$ pulse along y flips our spin vector into the x,y-plane along x. We can detect the transitions of spins in this plane.

9. After a pulse, the system relaxes back to its equilibrium orientation (with the spin vector in the z-axis). This occurs through reversal of spin flips, with emission of quanta at RF frequencies, - our FID.

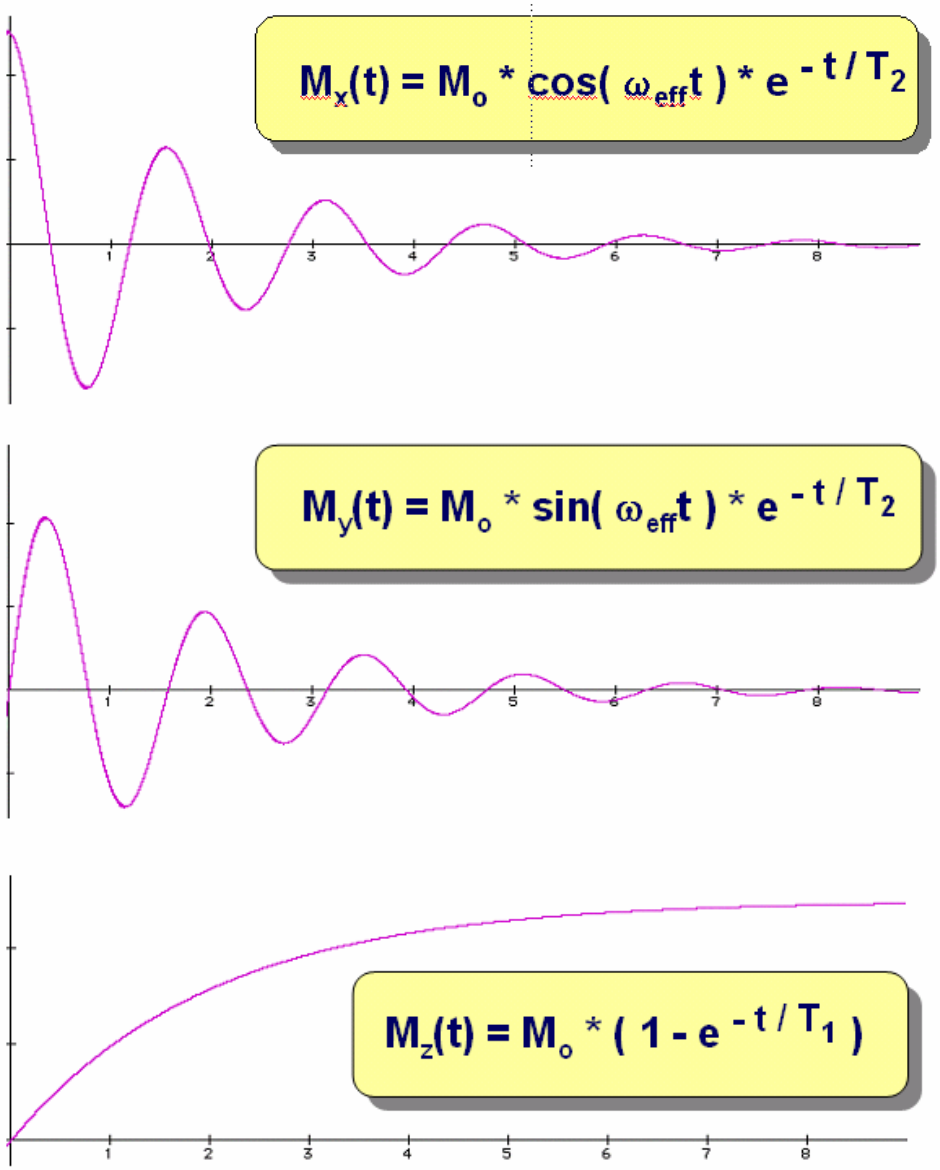


10. Relaxation occurs through two processes. One of these is out of the x,y-plane towards the z-axis, with time constant T_1. The second is relaxation in the x,y-plane with time constant T_2. The combination leads to a spiral precession for most spins.

11. Our detector, because of the geometry of the machine (laboratory frame), detects photons emitted in the x,y-plane. We translate the time data into energy by FT.

Solutions to the Bloch equations after $\pi/2$ pulse

- Graphically, we have the following:



Because of the orientation of the detector coils, we see magnetization in the x,y-plane

Magnetization disappears in the *x*-axis with an overall decay given by T_2, with harmonics. This is our FID

Magnetization disappears from the *y*-axis with the same T_2 depenedence, but out of phase with its disappearance from the *x*-axis.

The rate of appearance of net magnetization in the *z*-axis is given by T_1. We follow the time course in a double-pulse experiment, in which we assay the spin remaining in the x,y plane after varying the delay.

This is the main contribution if we flip our spin vector through 180° with a π pulse.

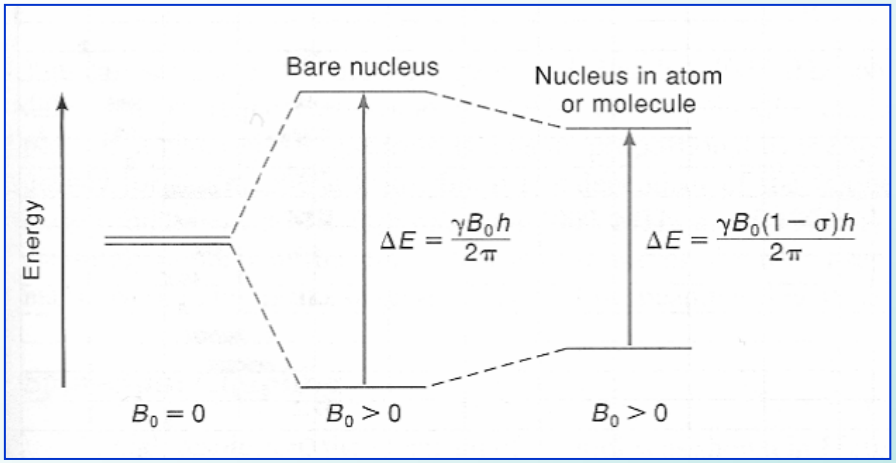
What information about structure can be obtained using NMR?
Structural information from chemical shifts, and spin relaxation.

Chemical shifts of nuclear spins

If our nucleus is in a molecule, the electrons in molecular orbitals will be distributed around it, and moving in molecular arbitals. Application of a magnetic field will induce a circulation change in electrons, which will itself induce a small change in magnetic field δB. The total field felt by a nucleus (the local field, B_{loc}) will then be:

$$B_{loc} = B_0 + \mathrm{d}B = (1 - \sigma)B_0$$

The factor σ is called the **shielding constant**, and it gives rise to a shift in the apparent value of the ν, or ω, or the energy for spin flipping, because all of these depend on the field felt by the nucleus.



$$\Delta E = h\nu = \frac{\gamma B_0 h}{2\pi}, \quad \text{or} \quad \nu = \frac{\gamma B_0}{2\pi}$$

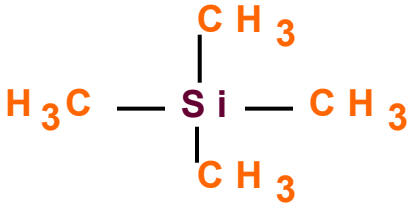
$$\nu_{precession} = \frac{\omega}{2\pi} = \frac{\gamma B_0}{2\pi}$$

$$B_{loc} = B_0 + \mathrm{d}B = (1 - \sigma)B_0$$

$$\Delta E = \frac{\gamma B_0 (1-\sigma) h}{2\pi}$$

Values for dB, and hence for σ, are small compared to B_0, and depend on the instrument used. A more convenient scale is the **chemical shift, δ**, which is the difference between the resonance frequency (ν or ω) and a reference standard, usually tetramethylsilane (TMS).

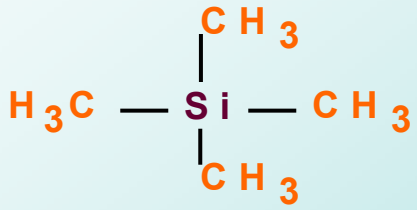
$$\delta = \frac{\omega - \omega^0}{\omega^0} \times 10^6$$

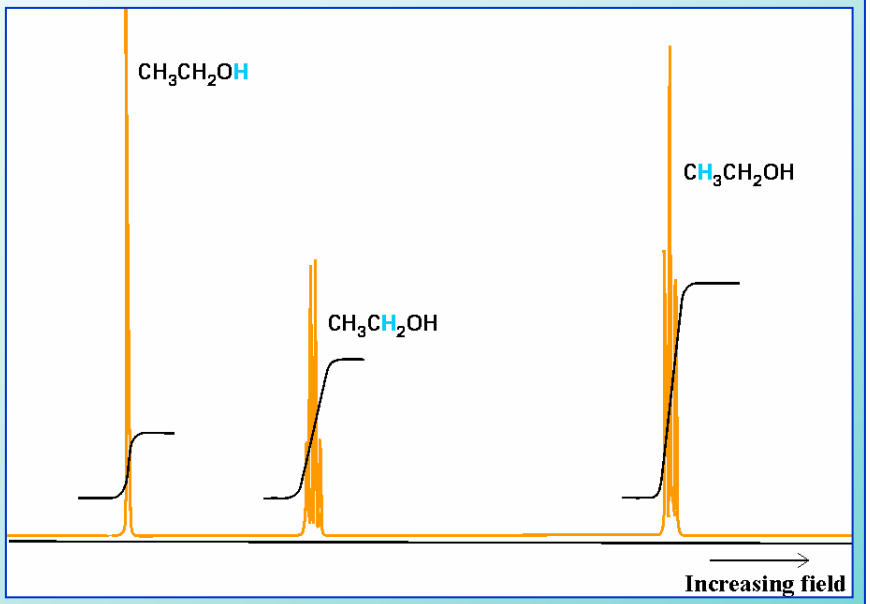


Chemical shifts are usually reported using the δ scale, in units of parts per million (ppm), using the relationship above.

Because different nuclei in a molecule experience different local fields, they all show different chemical shifts. These can be used to characterize chemical species.

$$\delta = \frac{\omega - \omega^0}{\omega^0} \times 10^6$$





$(CH_3)_4Si$
$CH_3—CH_2—$, $(CH_3)_3CH—$, $(CH_3)_4C$
$CH_3CH_2—$
R—SH
$—CH_2—$ in a ring
$(CH_3)_2CH$
$CH_3—X$, $—CH_2—X$, $>CH—X$ } CHO, COR, CHPh, COOH, $COOR$, $CONH_2$
$—CH_2—$ in ring ketones
$(CH_4CO)_2O$
$CH_2—X$, $—CH_2—X$, $>CH—X$ } N< (acyclic), N< (cyclic sec.), N< (cyclic tert.), $NHCOCH_3$, $NHSO_2Ph$, Quart. salt.
CH_3CN
$CH_2=C(CH_3)_2$
$CH_2=C(CH_3)—X$, $(CH_3)_2C=CH—X$ } CHO, $COCH_3$, $COOCH_3$, $OCOCH_3$, C≡CH, Ph, CN, Br, $CH=CH_2$
$—CH_2—NH_2$
CH_3Ph
CH_3CH_2Ph, $PhCH_2CH_2Ph$, $(CH_3)_2CHPh$
$HC≡C—$
$CH_2—X$, $—CH_2—X$, $>CH—X$ } OH, OR, OPh, OCOR, OCOPh, $OCOCF_3$
$>CH_2—X$, $—CH_2—X$, $CH—X$ } F, Cl, Br, I
PhSH
CH_2NO_2, $—CH_2NO_2$, $>CHNO_2$
$PhNH_2$
$—CH=CH—$ conjugated, $—CH=CH—$ nonconjugated } Olefins
$CH_2=C$ terminal
Cyclic
$CH_2=C=CH_2$
$CH_2=C(CH_3)_2$
$(CH_3)_2C=CHCH_3$
$CH_2=C(CH_3)—X$, $(CH_3)_2C=CH—X$ } CHO, $COCH_3$, $COOCH_3$, $OCOCH_3$, C≡C, Ph, CN
NO_2, COR, X, OH, NH_2, OR
RC—H, PhC(=O)H
RC—OH, PhC(=O)OH
RSO_3H, $PhSO_3H$

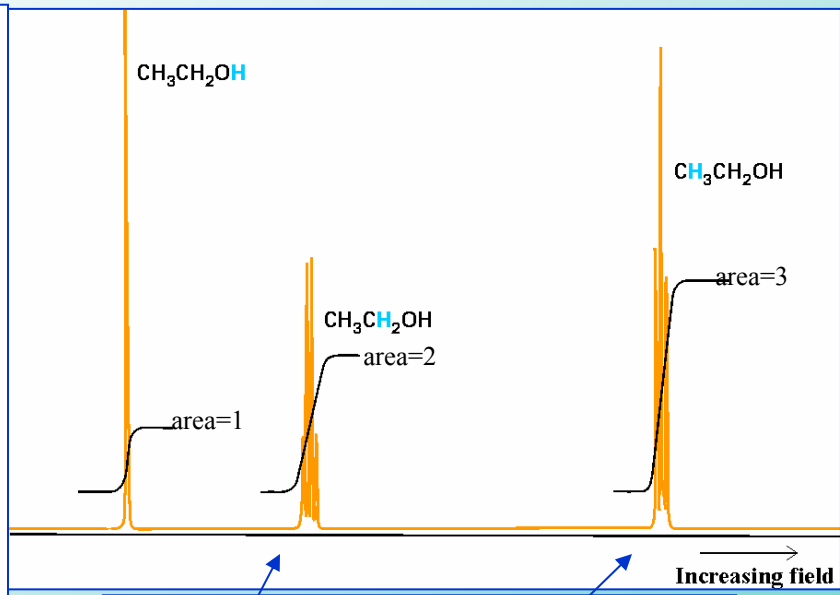
δ/ppm 11 10 9 8 7 6 5 4 3 2 1 0

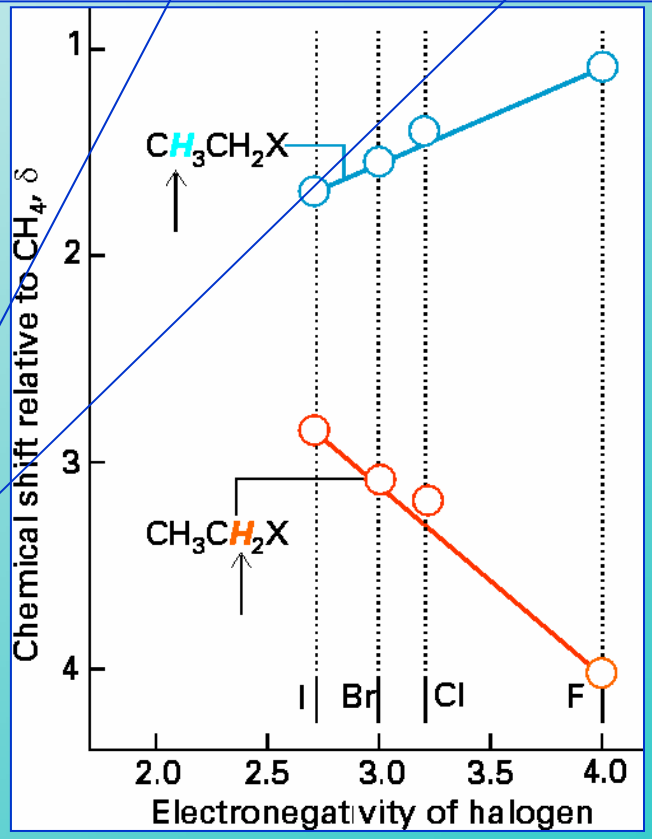
Figure 17.30
Chemical shifts (ppm) for various organic compounds. TMS has a chemical shift of zero because it is a reference compound. [From E. Mohacsi, *J. Chem. Educ.* **41**, 38 (1964).]

Chemical shifts of protons in ethanol.

Ethanol has three different types of protons, - those on -CH_3, -CH_2 and –OH groups. These all experience different chemical shifts, as shown on the right. The integrated area under the spectra (solid line) show the number of spins, - 3, 2 and 1 for the above groups. Different substituents cause different shifts (bottom right).The same substituent groups in general have a similar effect on the chemical shift in different molecules, so the position of a particular group on the δ (ppm) scale is diagnostic, as shown in the Table from Chang in the previous slide. Values for δ can therefore be used to characterize chemical compounds, or to identify particular groups in large molecules.

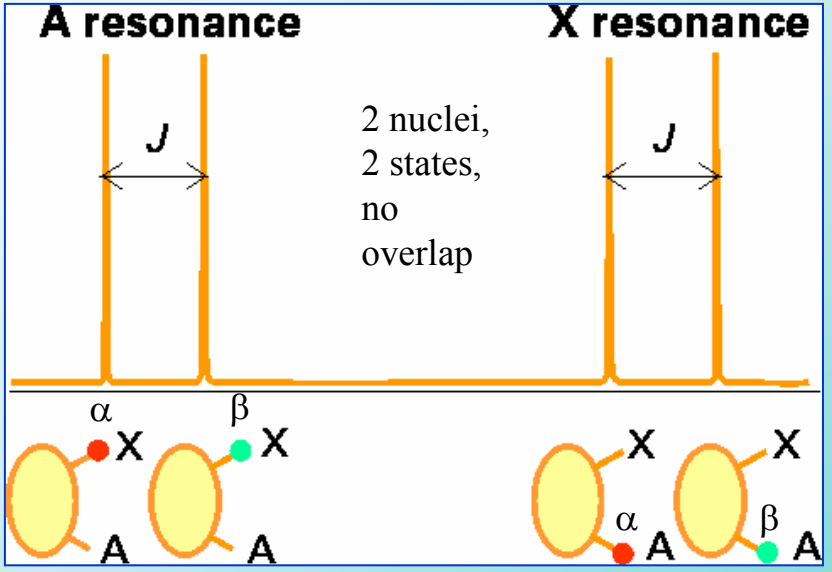
Note that the spectral lines associated with the different protons are subdivided into multiple lines. This is called fine structure, and is due to spin-spin interaction.



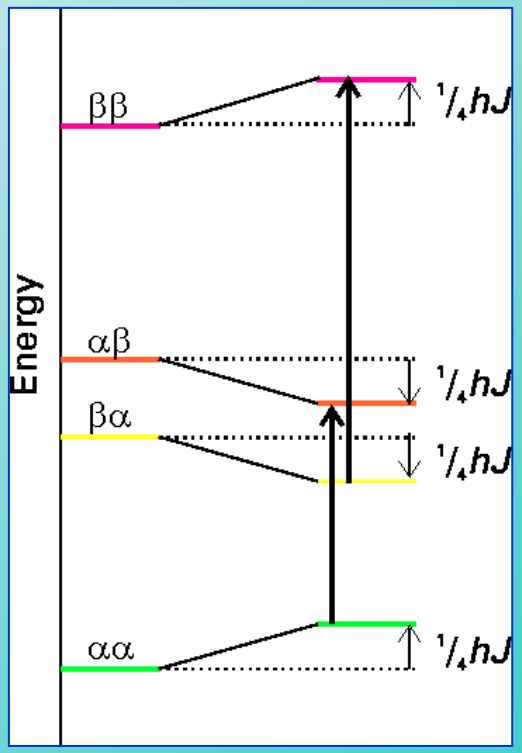


Spin-spin interaction

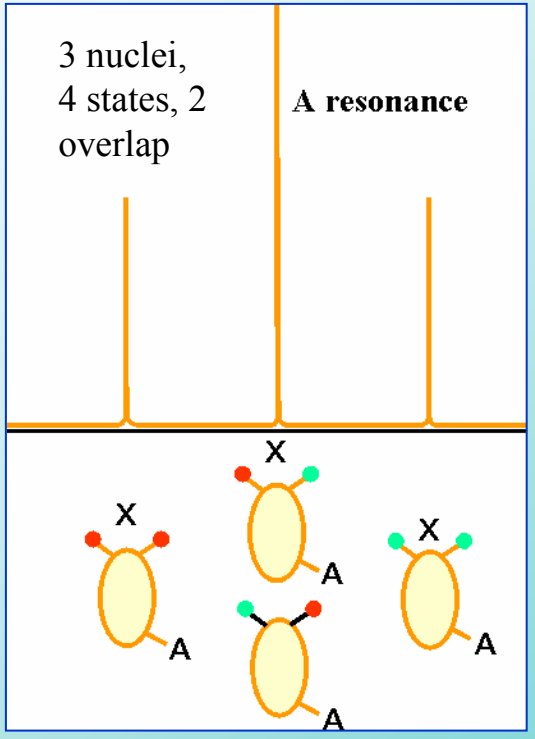
In addition to the chemical shift due to the molecular magnetization from electron circulation, the nuclei also experience the local field from neighboring nuclei.



If we consider a molecule with two spins, A and X, A will see the α and β populations of X, and vice versa. These will shift the energy levels (right) in an exactly equal fashion for the two spins, by an amount *J*.



In general, *n* equivalent spin-½ nuclei split the energy of a neighbor into *n*+1 lines. The intensities of the lines depend on overlap of energies for symmetrical states. The intensities of different lines follow the distribution in Pascal's triangle,



n = no. of spin-½ nuclei

1
2
3
4
5
6
7
8

			1				
		1		1			
	1		2		1		
1		3		3		1	
	4		6		4		1
5		10		10		5	
	6		15		20		15
7		21		35		35	

4 states, middle two overlap to give double intensity

Spin-spin coupling rules:

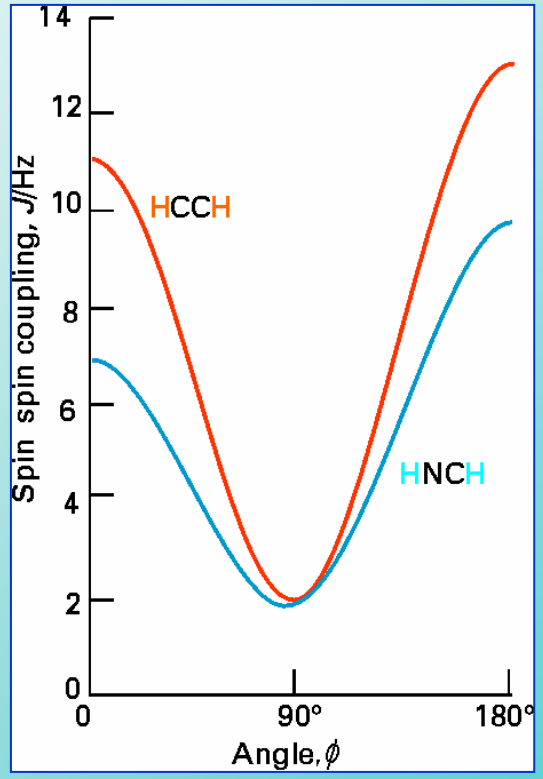
1. Nuclei must be non-equivalent to spin couple. For example, the three methyl protons are magnetically equivalent, so don't interact with each other. However, they are different from the protons on the -CH_2 group, so they split the single resonance into a quartet with 1:3:3:1 intensities.
2. Coupling is through bond, - 3 bonds is maximal separation to see effect (maybe 4).
3. Nomenclature – ${}^{n}J_{AX}$, n is no. of bonds, A and X are nuclei involved. So ${}^{3}J_{HH}$ is coupling between two protons 3 bonds apart; ${}^{1}J_{CH}$ is coupling between a ^{13}C carbon and a ^{1}H proton, joined by a single bond; etc.
4. Typical values: ${}^{1}J_{CH}$ ~ 100-1000 Hz; ${}^{2}J_{CH}$ ~ 10-100 Hz

5. Couplings over 3 bonds are dependent on torsion angle around the middle bond, according to the Karplus equation:

$$^{3}J_{HH} = A + B\cos\phi + X\cos 2\phi$$

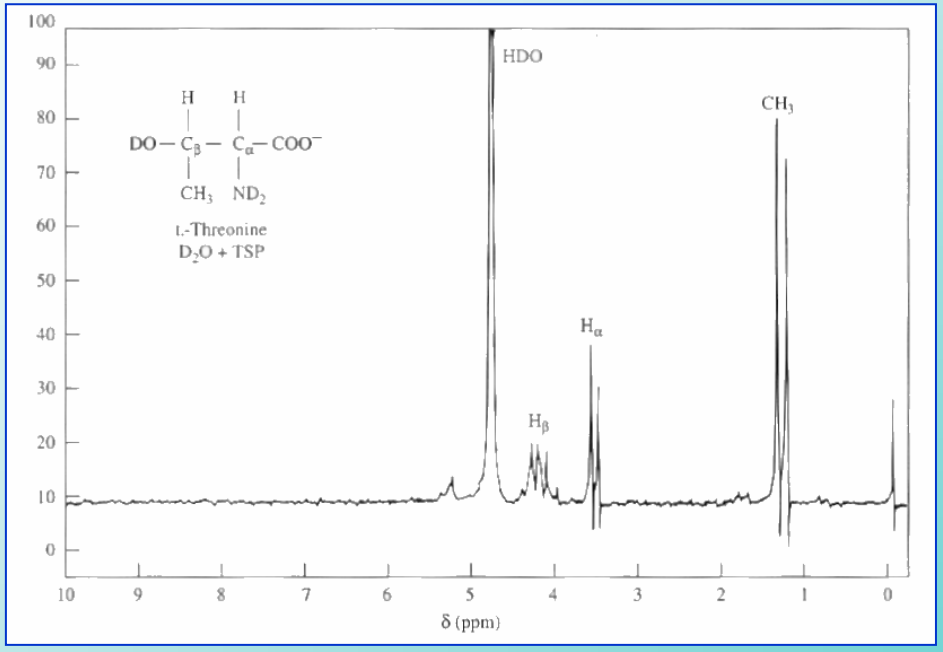
Typical values for A, B, and C are 7, -1, 5 Hz.

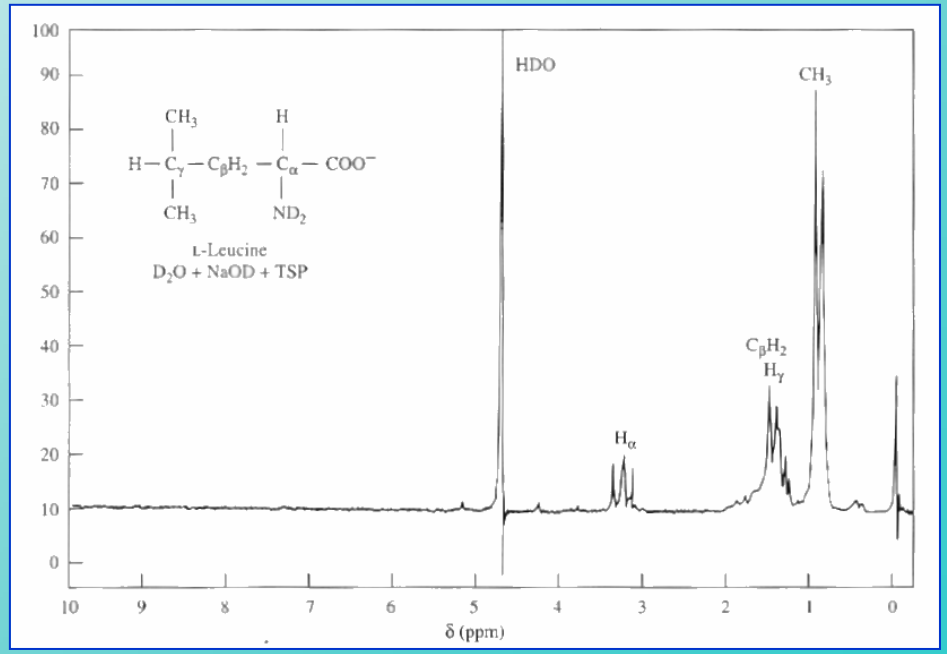
A point of practical interest is the difference in values for HCCH and HNCH bonding (the latter is part of the peptide bond (see right).



1D-NMR. In combination, the data about nuclear (chemical) species (from chemical shifts), connectedness and orientation (from *J* splittings, and relaxation through spin-spin coupling), and distances and angles (from dipole-dipole interactions), provide the information on distances and connections necessary for determination of chemical structure and bond orientation.

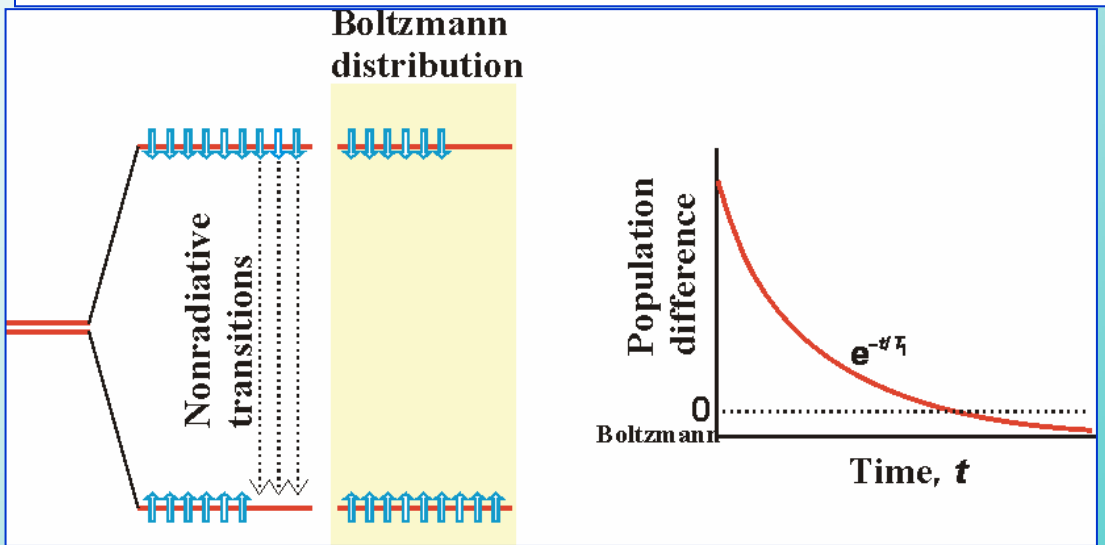
The spectra below demonstrate application of 1D-NMR to chemical structural studies. The samples are threonine (left) and leucine (right) dissolved in D_2O (^2H_2O). Because ^2H has a different γ, its spectra are off scale, and we see only ^1H here. Protons that can easily exchange are diluted out by ^2H, so we see only lines associated with the non-exchangeable protons (and a small amount of HDO). The fine structure and areas under the curves identify the two amino acids.





Relaxation through T_1 and T_2.

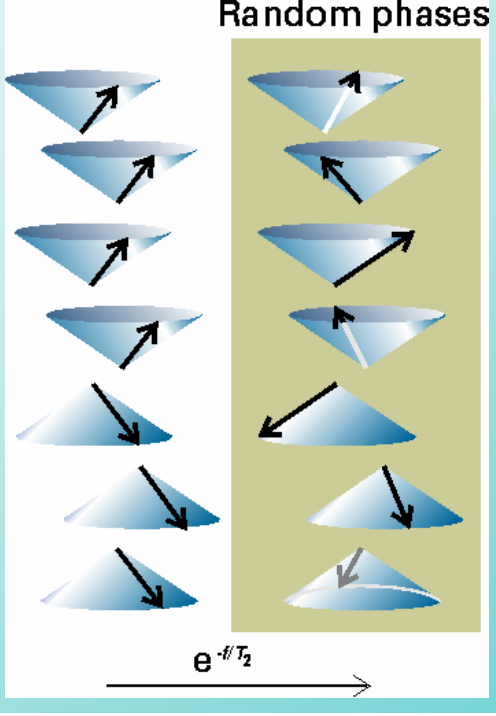
The power needed to saturate a transition is not constant. This implies that some variable pathway for relaxation from the energized state exists. Since we don't see RF photons from this decay, the relaxation must be by non-radiative or lower energy pathways. As we have seen, the relaxation rate constant back to the equilibrium state (z-axis) following a spin flipping pulse is called T_1. This is called a spin-lattice relaxation because it involves predominantly magnetic fields associated with the tumbling of the surrounding molecules and their nuclear spins. The magnetic fields of the lattice encourage relaxation only if they are in the right frequency range, - that is when the time scales are more or less matched. As a consequence, T_1 is very dependent on local motion, determined by several factors, for example local viscosity, temperature, molecular size. The curve for dependence of T_1 on local motion shows a minimum (fastest rate) in mid range. We'll see this in the next slide.

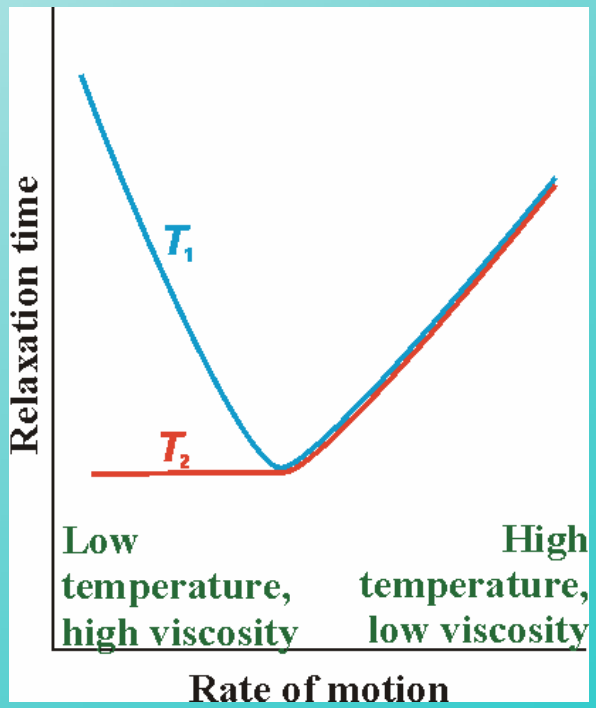


Since spin-lattice relaxation is by definition the return to the equilibrium state with orientation along z in an applied field, it is measured in pulsed NMR by following the relaxation in the axis of the applied field, B_0, - the z-axis.

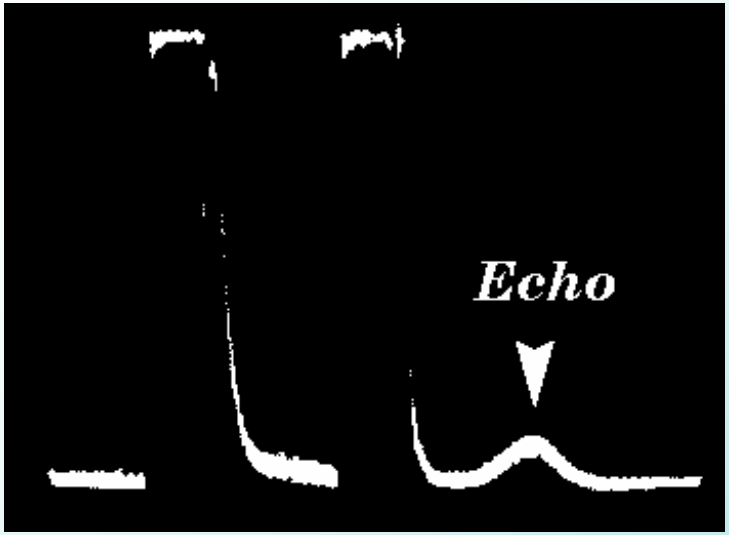
In contrast, spin-spin relaxation, with T_2, is due to relaxation from an ordered to a random configuration by **local** effects, and is unperturbed by the external field. It is measured in the plane orthogonal to the applied external field from the big magnet. If the latter is along the z-axis, we detect the spin-spin relaxations in the x,y-plane. T_2 is best detected in the range of local motions in which it is independent of T_1. This is when T_1 is slow, and the random relaxations are not scrambled by molecular tumbling. As local motions increase, T_2 follows T_1 because of randomization due to tumbling (see below, right).

In order to follow T_2, we need to be able to first put our system into a state in which the spin vector is in the x,y-plane, and then measure the time for evolution to the random state. In pulsed NMR, this can be achieved by using a two pulse protocol, $\pi/2$ followed by π, and varying the time between them.

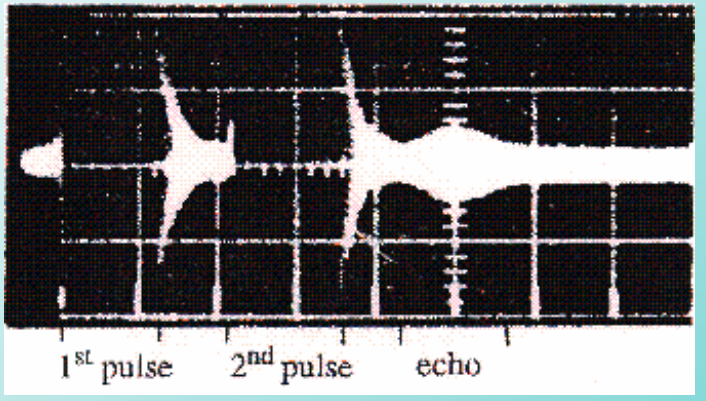




Spin-echo pulsed magnetic resonance



E. Hahn, "Spin echoes", Phys. Rev. 80, 580 (1950)



R.J. Blume, "Electron spin relaxation times in sodium-ammonia solutions", Phys. Rev. 109, 1867 (1958)

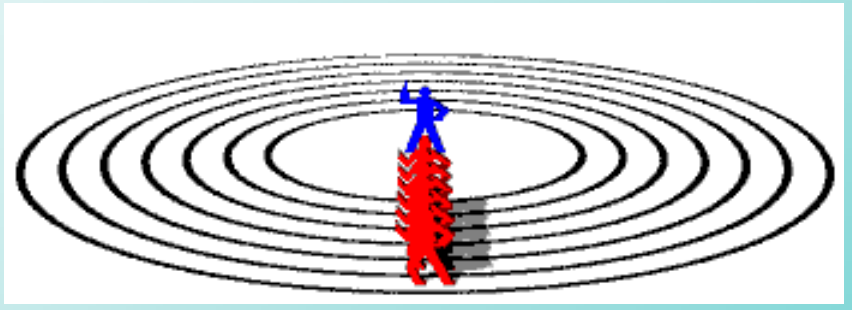
NMR spin echoes were discovered by Hahn in the early 1950s, and EPR echoes almost two decades later by Blume. The time lag reflects the much higher frequencies of EPR spectroscopy, and the need for development of improved electronics to follow the time course of EPR spin echoes.

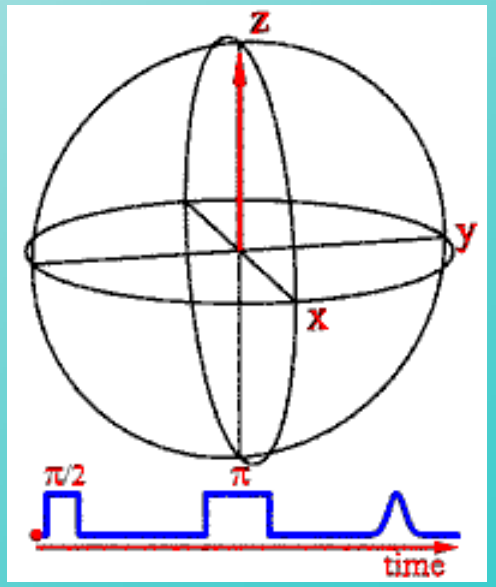
The general idea of the spin echo approach is to use π and $\pi/2$ pulses to bunch all the spins together, so that they are synchronized. Suitable choice of pulse, and the time between pulses, makes it possible to explore in detail the evolution of the spin states, and their detailed kinetics.

The synchronization can be visualized in terms of the cartoons below.

On the left, we have a representation of the "racetrack" synchronization model. The first BANG is the pulse that starts the transitions moving. The second BANG is a pulse that sends them back along the same path. Since the rates of transition (≡ frequencies) are characteristic for each "player", each goes back at the rate they started. As a result, they're in synch when they get back to the start.

The animation on the right shows a "pancake" model. The $\pi/2$ pulse flips the spin population to the x, y plane where it starts to precess. The π pulse flips all the spins over (like tossing a pancake). They continue to evolve, but the orientation is changed, so they end up back in sync, and generate the echo.





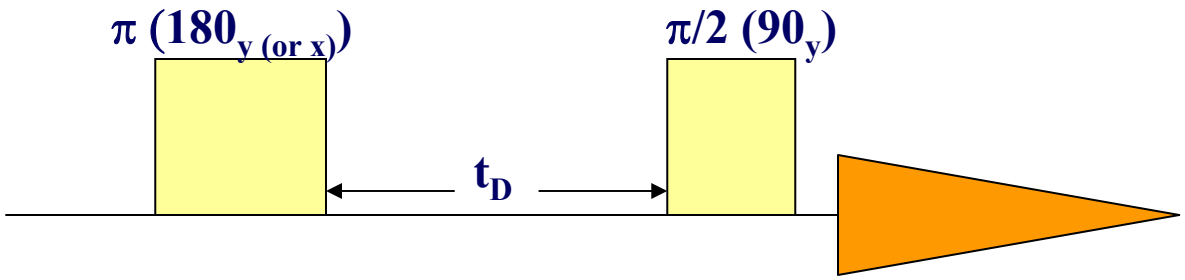
What does spin-echo gives us that the 1-pulse FID doesn't?

1. By synchronization of the spins, we increase the sensitivity.
2. By judicious use of pulse length, we can select orientations in our rotationally frozen frame of reference.
3. By varying the time between pulses, and looking at the effect on the echo (the synchronized population), we can explore the time-course of the different components of the relaxation process.
4. This allows us to look at each different spin (which is seen at a different frequency in the spectrum after FT), and the effect of local environment on their T_1 and T_2 relaxations.
5. The T_1 and T_2 components of relaxation contain information about the structural neighborhood of each species in our spectrum.
6. Structural information includes (a) nature of neighboring atoms; (b) bond lengths and their angular components; (c) distances; (d) energies.
7. From this information we can build 3-D structures at atomic resolution.

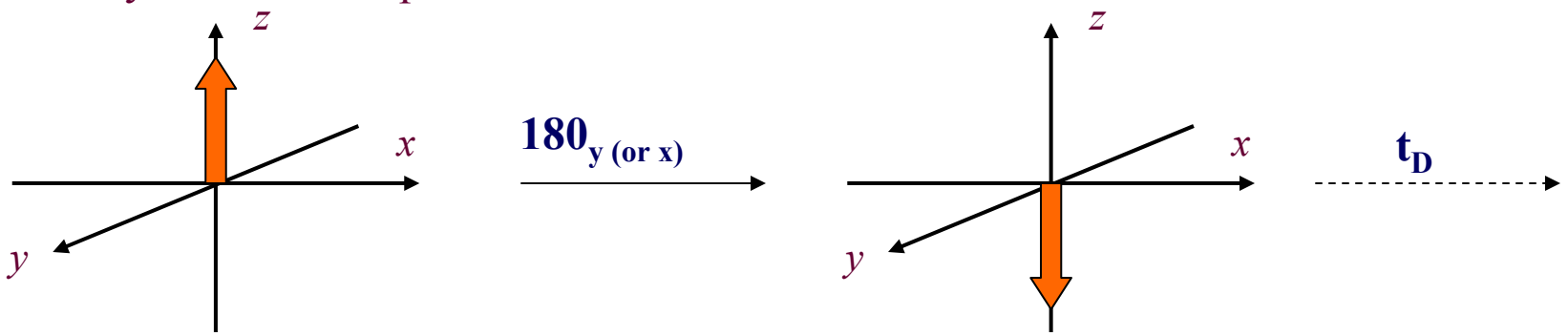
Inversion recovery – this is how we measure T_1

• Measurement of T_1 is important, as the relaxation rate of different nuclei in a molecule can tell us about their local mobility. We cannot measure it directly on the signal or the FID because T_1 affects magnetization we don't detect.

• We use the following pulse sequence:

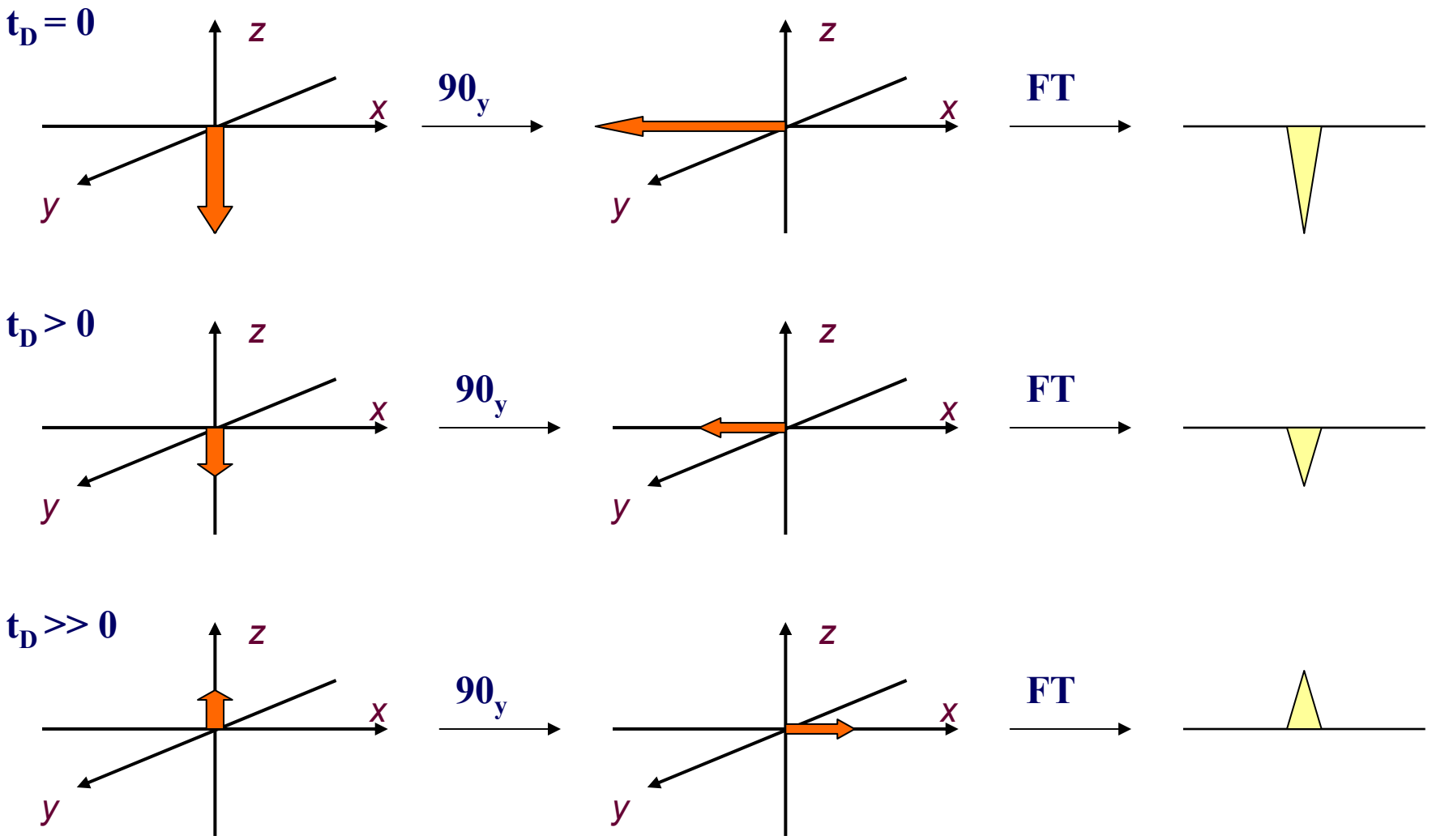


• If we analyze after the π pulse:



• Since we are letting the signal decay by different amounts exclusively under the effect of longitudinal relaxation (T_1), we'll see how different $\mathbf{t_D}$'s affect the intensity of the FID and the signal after FT.

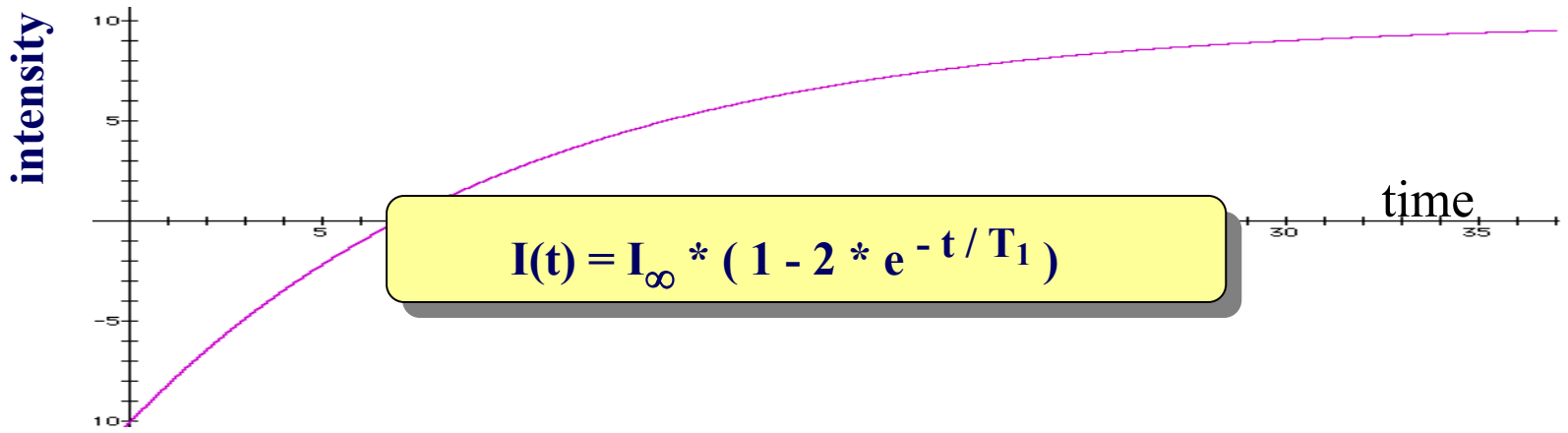
Inversion recovery (continued)



Depending on the t_D delay we use we get signals with varying intensity, which depends on the T_1 relaxation time of the nucleus (peak) we are looking at.

Inversion recovery (continued)

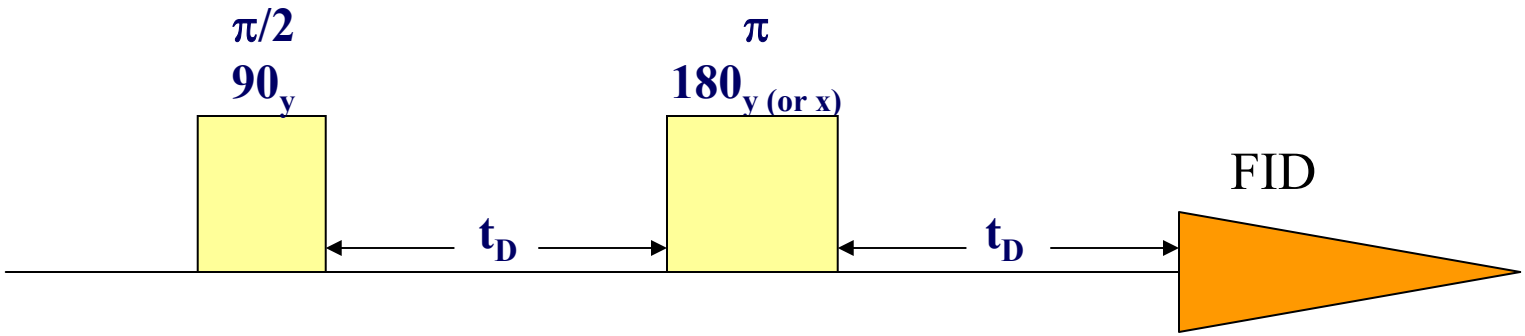
- If we plot the intensity versus time we get the following curve:



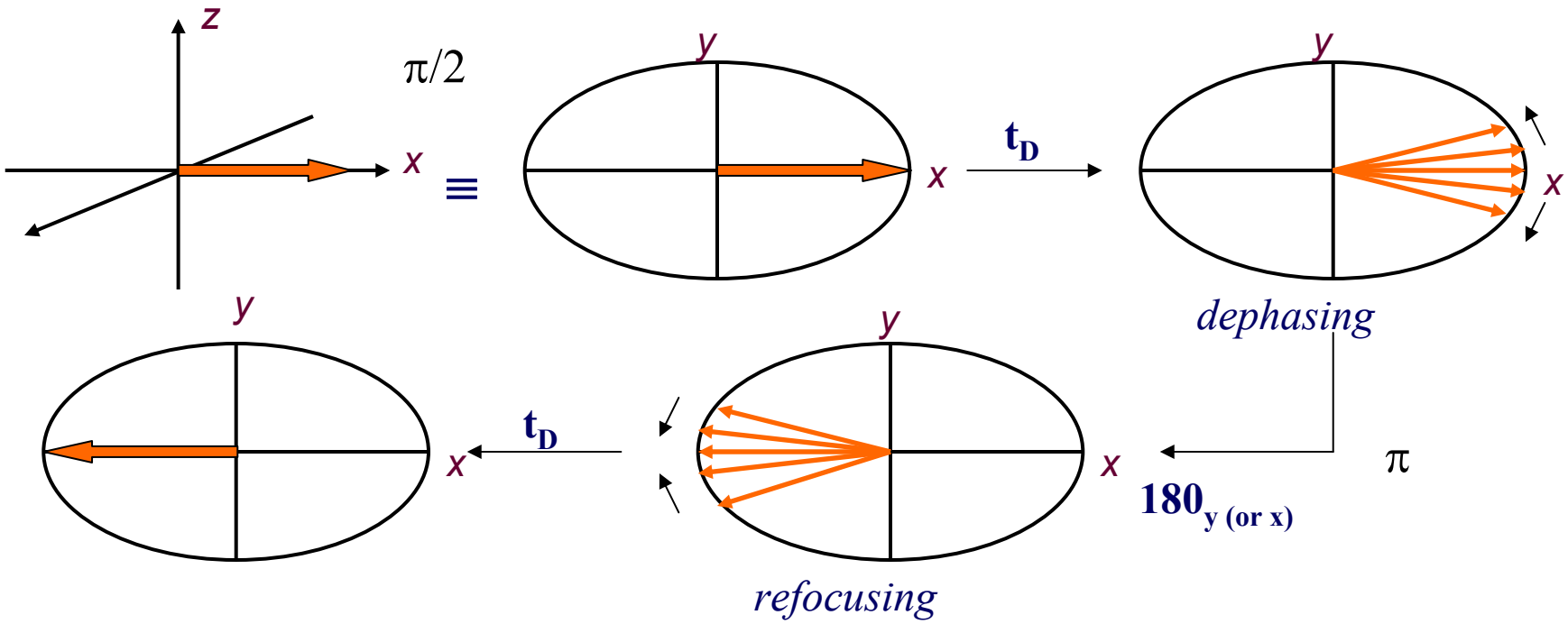
- It is an exponential with a time constant equal to the T_1 relaxation time.

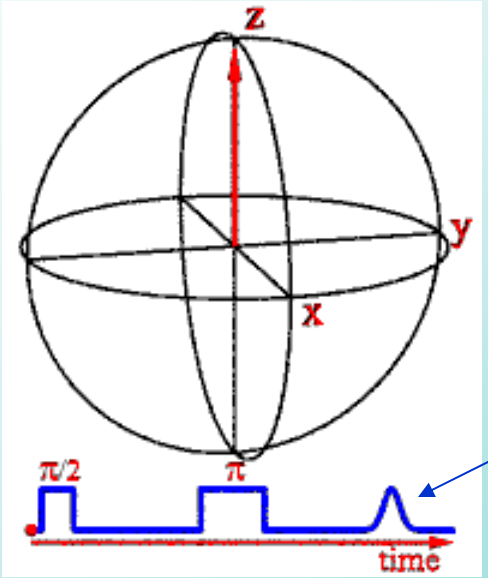
- To measure T_2 we have to first put our system into the x, y plane, and then sample it at different times.

Two-pulse spin-echo – this is how we measure T_2. How long does it take to dephase completely? We look to see how much magnetization we have left in the x.y plane as we vary t_D. This is determined by T_2.



A plot of intensity against $2t_D$ will gives us T_2.



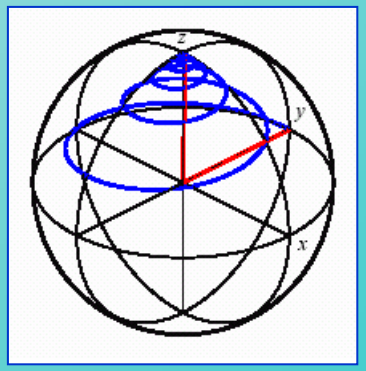


pulse timing

area under spin echo tells us how many spins remain in x, y plane

t_D t_D

Plot area under spin echo against $2t_D$ to measure T_2



The Nuclear Overhauser Effect, NOE:

A third type of relaxation process is important in macromolecular structural work

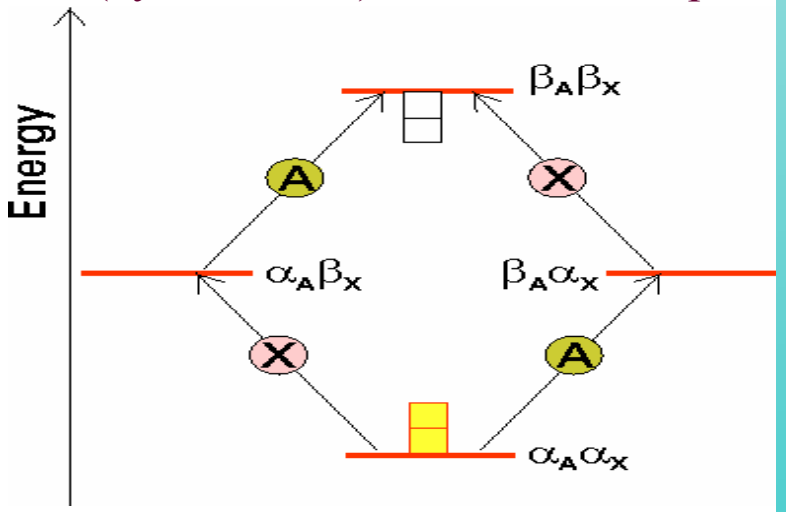
The NOE is a relaxation pathway due to dipole-dipole interactions. As such it is very distance dependent, falling off with $1/r^6$. It is this distance dependence that makes it particularly useful from the point of view of structural determination. The NOE relaxation is different from spin-spin relaxation associated with T_2. In NOE, ${}^{n}J_{AX}=0$, and the effect is not dependent on bonding. It therefore provides information about the distances of non-bonded interactions that determine secondary and tertiary structure.

NOE, adapted from Moyna, and from Atkins (Figs.)

• The ***NOE*** is a different way in which particular spin states in the system can release energy and relax. It is another relaxation pathway, and a component of the overall relaxation processes. In particular, the NOE is related to exchange of energy between two spins that are not scalar (through-bond) coupled ($J_{AX} = 0$), but have ***dipolar coupling***.

• The NOE is evidenced by enhancement of certain signals in the spectrum when the equilibrium (or populations) of other nearby spins are altered (by saturation). We use a two spin system energy diagram with four states to explain it:

The boxes represent ***equilibrium occupancies***
A filled box shows excess occupancy of the favored spin state, relative to the empty boxes. As we expect, the $\alpha_A\alpha_X$ state is favored over the $\beta_A\beta_X$ state

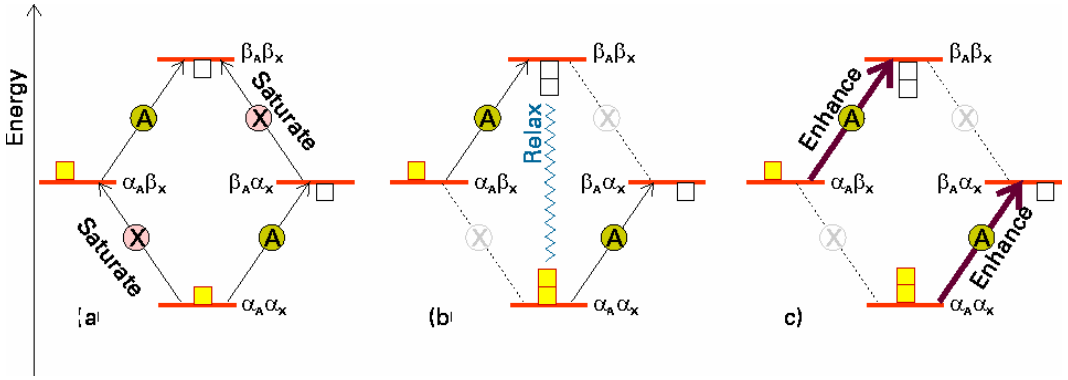


• **The diagonal arrows** represent ***transition probability***, or the rate at which certain allowed transition can take place. For the system in equilibrium we can have **A** and **X** transitions, which represents ***single quantum*** α to β transitions of spins A and X.

•The vertical and horizontal transitions are omitted because they are ***double quantum*** transitions, are forbidden, and normally have a much lower probability.

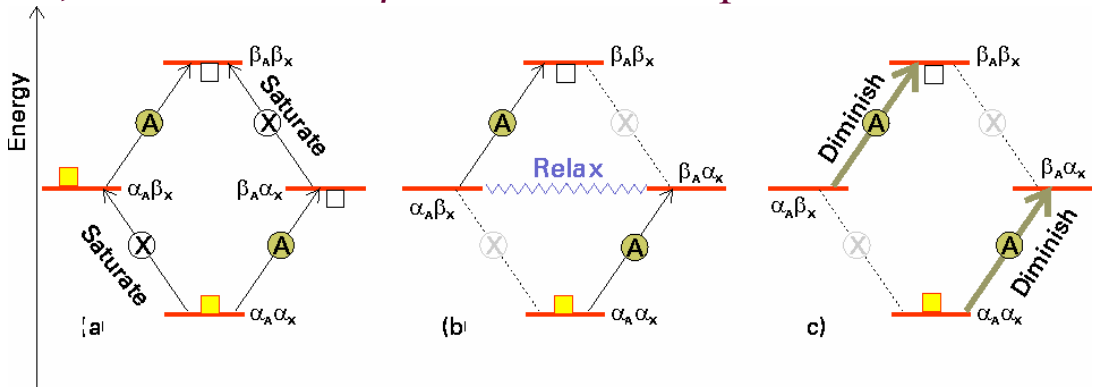
We now ***saturate*** the **X** transition with a pulse, which means that we make the populations on either side of each transition equal. The forward and back **X** transition rates are now the same, so they are effectively frozen:

- In this diagram, the changes in levels affecting the **A** transitions favor an increase in rate.



The **A** transitions no not occur during the pulse because we do not excite them, so the populations in the levels that affect their rates change only due to **X**. The **horizontal** and **vertical transitions,** though normally disallowed, become the only way **the system** can relax back to equilibrium. They can occur through dipole-dipole interaction. This will flip **both** spins, causing a **double quantum** transition, between α and β states for both spins.

- In this diagram, the changes in levels affecting the **A** transitions favor a decrease in rate.



These relaxation pathways deplete or enhance populations involved in A transitions, and thus the enhance their signals. **Vertical transitions** will give positive enhancement of **A**, and **horizontal transitions** will diminish **A** (negative enhancement).

We cannot detect these **double quantum transitions (dq)**, but they affect the way the spin system relaxes, so we can see their effects on the signals due to the allowed transitions. The two dq transitions have different rates, - one has a rate close to twice ω, while the other one is almost zero. So one will be related to very slow motions, and the other one to fast tumbling...

• If we now put all this in a big equation (the Solomon equation) we get something that will help us see several things. Using $\mathbf{V_{dq}}$ and $\mathbf{H_{dq}}$ for the rates of the vertical and horizontal dqs we have the enhancement given by η:

$$\eta = \gamma_A / \gamma_X * \frac{V_{dq} - H_{dq}}{2 * X + V_{dq} + H_{dq}}$$

• First, if the molecule tumbles rapidly (all small organic gunk) we find that under saturation of the **X** transitions **vertical dq** will dominate, so the maximum enhancement for **A** is γ_A / γ_X. If we are looking at the ^{13}C signal while decoupling (saturating) ^{1}H, we can get an enhancement of ~ 4.

• If the molecule tumbles slowly, as for a protein, **the horizontal dq** dominates, and we have a maximum NOE of - γ_A / γ_X. Since here we are interested in ^{1}H - ^{1}H NOE, the theoretical enhancement will be ~ -1.

Nuclear Overhauser Effect (summarized)

•It is useful to compare the frequency of the spin system to the molecular tumbling rate or ***correlation time***, τ_c.

• $\omega * \tau_c << 1$ -This means that the molecule tumbles fast, and we have positive enhancements. It is called the ***extreme narrowing condition*** (small molecules, non-viscous solvents).

• $\omega * \tau_c >> 1$ -This means that the molecule tumbles slowly, and we have negative enhancements. It is called the ***diffusion limit*** (proteins, viscous solvents).

• $\omega * \tau_c \approx 1$ - These are the "in the middle's", and we can have situations in which the NOE goes to zero. It will happen for certain medium sized molecules and it depends on the base frequency of the NMR

•There is one important point that we left out from our treatment, which is the dependence of the NOE on the distance between our A and X spins. Because of the **$1/r^6$** dependence, we will only see an NOE of our spins are **5 Å or less apart**. This therefore becomes a powerful tool for assaying distance, and therefore the "connectedness" of macromolecular structure in secondary and tertiary folding.

NMR acronyms

COSY – a 2D homonuclear **co**rrelated **s**pectroscop**y** and its variants. This explores the *J* couplings between nuclei in a complex mixture such as a protein. Since *J* couplings depend on bonded connections (out to 3 bonds), this allows us to measure the backbone connections of a macromolecule. If we know the sequence, we can identify splittings, and assign them to specific residues.

NOESY – a 2D **n**uclear **O**verhauser **e**ffect **s**pectroscop**y**. This explores the dipolar interactions in a complex mixture. These allow us to explore tertiary structure.

These are the two most important types of 2D-NMR. However, there are many variations on each, and many other pulse sequences that have their own acronyms. In addition, there are many protocols for refinement of the FID, - they get rid of useless noise, and enhance the resolution of the FT algorithm, also with their own acronyms.

The Nerds meet the Seven Dwarves in the shrinks office.

- ***COSY*** - 2D homonuclear **co**rrelated **s**pectroscop**y**
- ***TCOSY*** - 2D homonuclear **t**otal **co**rrelated **s**pectroscop**y** (Mad Hatter, too)
- ***NOESY***
- ***DQF-COSY*** - **d**ouble **q**uantum **f**iltered COSY
- ***MQF-COSY*** - **m**ultiple **q**uantum **f**iltered COSY
- ***SPI*** - **s**elective **p**opulation **i**nversion, - heteronuclear polarization transfer
- ***INEPT*** - **i**nsensitive **n**uclei **e**nhanced by **p**olarization **t**ransfer. It is used to increase the sensitivity (polarization) of nuclei such as ^{13}C and ^{15}N.
- ***APT -*** **a**ttached **p**roton **t**est. Simplification of ^{13}C by decoupling ^{1}H using saturation.
- ***DEPT*** - **d**istortionless **e**nhancement by **p**olarization **t**ransfer
- ***HETCOR*** - **het**eronuclear **cor**relation spectroscopy).
- ***HOMO2DJ*** – **homo**nuclear **2D J**-correlation spectroscopy
- ***INADEQUATE*** - **i**ncredible **n**atural **a**bundance **d**oubl**e** **qua**ntum **t**ransfer **e**xperiment
- ***CYCLOPS***
- ***EXORCYCLE***

No need to remember these, - just examples from the Moyna lectures.