

# **Introduction to Biophysical Chemistry**

**TIGP Academia Sinica Fall 2008**

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**Lecture 20 December 24, 2008**

**Magnetic Resonance Spectroscopy.**

# Nuclear Magnetic Resonance. II.

# Methods of Nuclear Magnetic Resonance

References: "Methods of Experimental Physics," 20, Academic Press, New York, pp 1-52.

CS II, pp. 481-525.

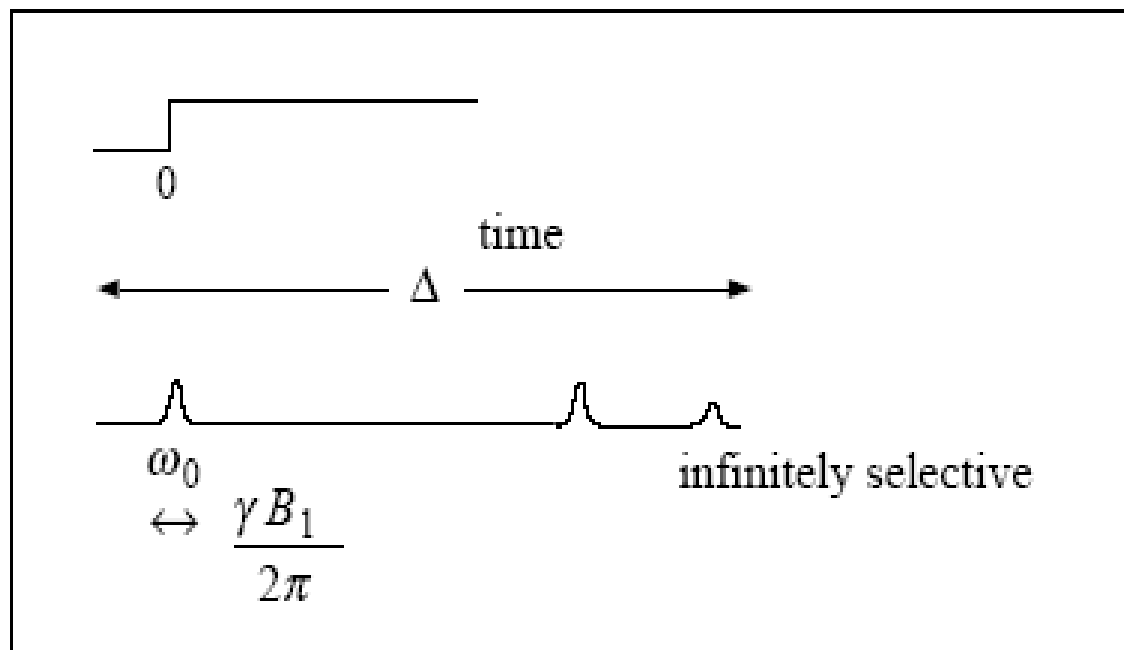
Principles of Magnetic Resonance, C. P. Slichter, Chapters 1 and 2

Nuclear Magnetic Resonance Spectroscopy, R. K. Harris, Pitman Books, London, 1983, Chapters 1 and 3

# 1946: Harvard ( $F(\omega)$ ) vs. Stanford ( $S(t)$ ) experiment

## Continuous Wave Experiment

A “CW” NMR spectrum is obtained by sweeping the rf frequency or the magnetic field through resonance slowly. In this experiment rf is on continuously. However,  $B_1$  is small, so that only one resonance is excited at a time.

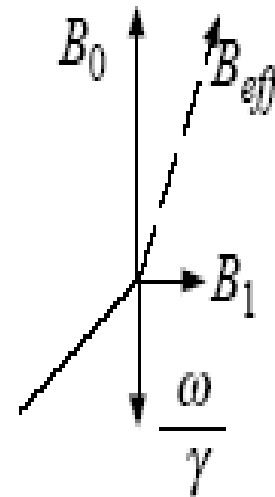


In a frame rotating with angular velocity  $\omega$ , the “effective field” is given by

$$\check{B}_{eff}^{\vee} = \left( B_0 + \frac{\omega}{\gamma} \right) \check{k}^{\vee} + B_1 \check{i}^{\vee}$$

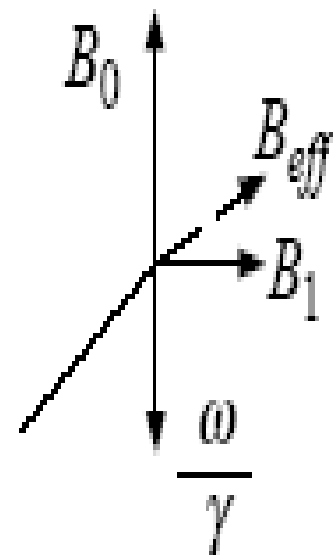
Since  $\|\check{B}_0^{\vee}\| \gg \|\check{B}_1^{\vee}\|$ , when  $\omega$  is far from  $\omega_0$ ,  $\check{B}_{eff}^{\vee} \approx \check{B}_0^{\vee}$  and  $M_z$  is unaffected, while when  $\omega \rightarrow \omega_0$ ,  $\check{B}_{eff}^{\vee} \approx \check{B}_1^{\vee}$  and absorption occurs.

Far from resonance  $\check{B}_{eff}^{\vee} = \left( B_0 + \frac{\omega}{\gamma} \right) \check{k}^{\vee} \approx B_0 \check{k}^{\vee}$



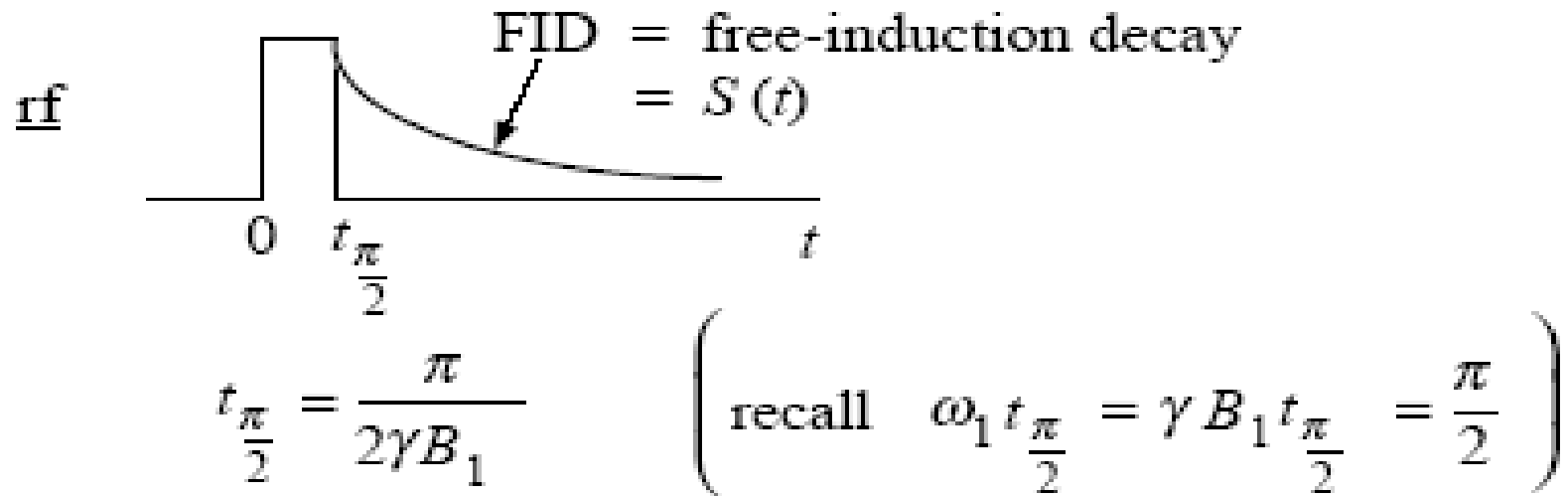
At or near resonance

$$\frac{\nu}{B_{\text{eff}}} = \left( B_0 + \frac{\omega}{\gamma} \right) + B_1 \approx B_1$$

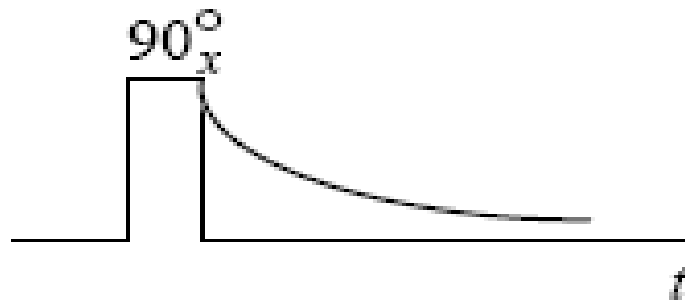


$F(\omega) \equiv$  NMR spectrum in frequency domain.

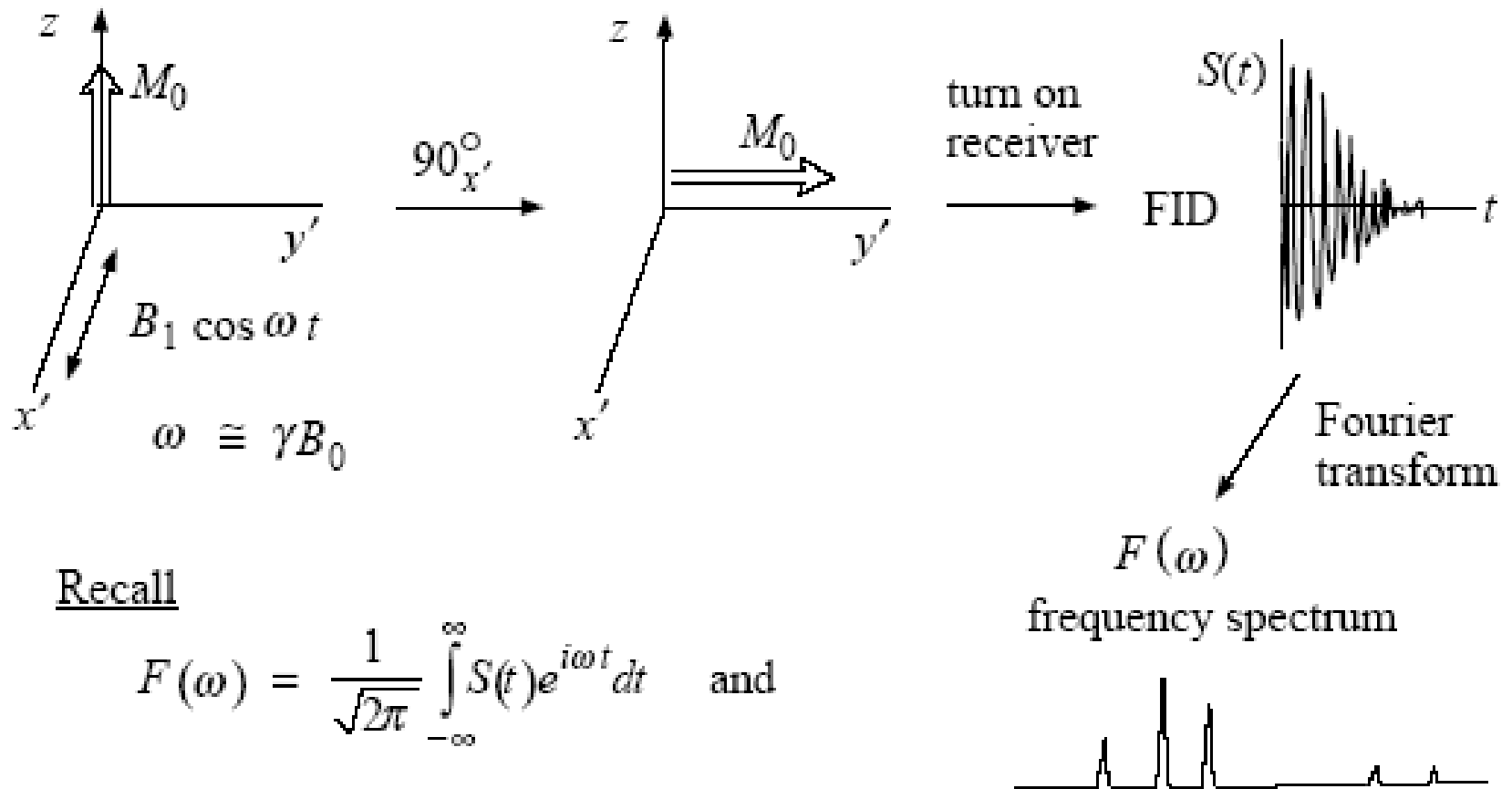
# Single-pulse experiment



This is usually written as



# Single-pulse experiment



## Recall

$$F(\omega) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} S(t) e^{i\omega t} dt \quad \text{and}$$

$$S(t) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} F(\omega) e^{-i\omega t} d\omega$$



# Fourier Transform NMR

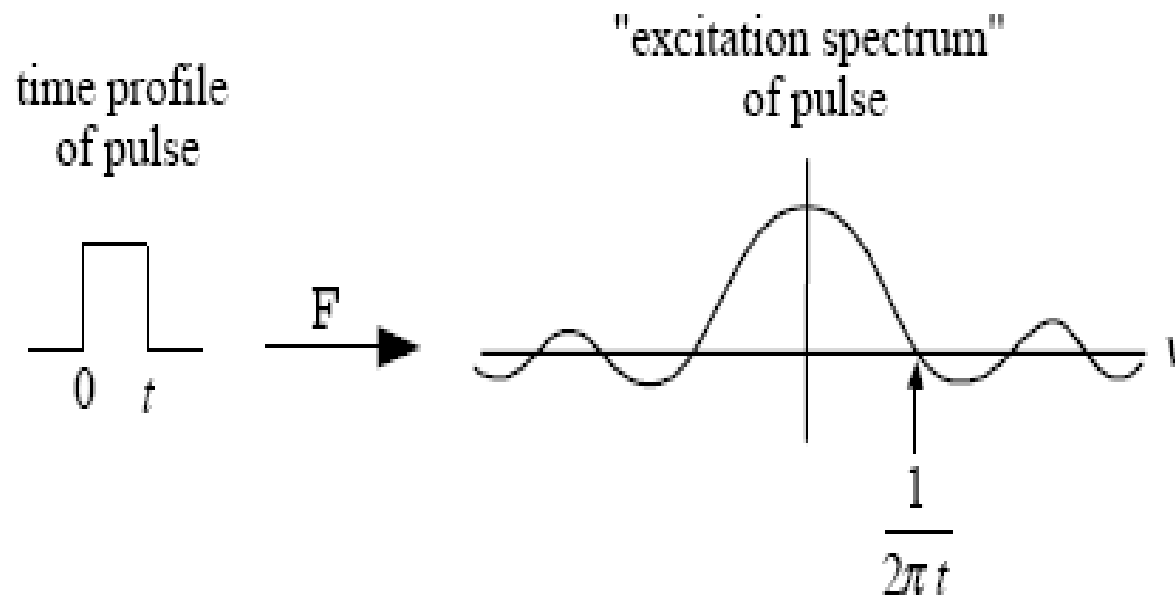
Same information in  $F(\omega)$  and  $S(t)$ .

In fact

$$F(\omega) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} S(t) e^{-i\omega t} dt$$
$$S(t) = \left( \frac{1}{2\pi} \right)^{1/2} \int_{-\infty}^{\infty} F(\omega) e^{i\omega t} d\omega$$

# Constraints on rf pulse

- 1) Must be  $\ll T_2$
- 2) Must be short enough to evenly excite all resonances in spectrum. Pulse of length  $t$  contains these Fourier components:



# Constraints on rf pulse

Recall for a  $\frac{\pi}{2}$  pulse,  $t = \frac{\pi}{2\gamma B_1}$ . We require for uniform excitation that

$$\gamma B_1 \gg 2\pi\Delta \quad \text{where } \Delta \text{ is the spectral width}$$

$$\text{So } t_{\frac{\pi}{2}} \ll \frac{\pi}{2(2\pi\Delta)} = \frac{1}{4\Delta}$$

Representative numbers:

$$t_{\frac{\pi}{2}} \approx 5\mu\text{s} \quad \Delta \approx 5-10 \text{ kHz for } ^1\text{H}$$

$$\Delta \approx 20 \text{ kHz for } ^{13}\text{C}$$

# Free-induction Decay

After  $\frac{\pi}{2}$  pulse,  $M_{xy} \neq 0$ , nonequilibrium, so  $M_{xy}$  must decay with time. The rate of decay ( $1/T_2$ ) depends on how the individual spins that make up the magnetization can get out of phase, and this decay thus depends on

- (a) different frequencies of precession of various spins that are excited by rf:

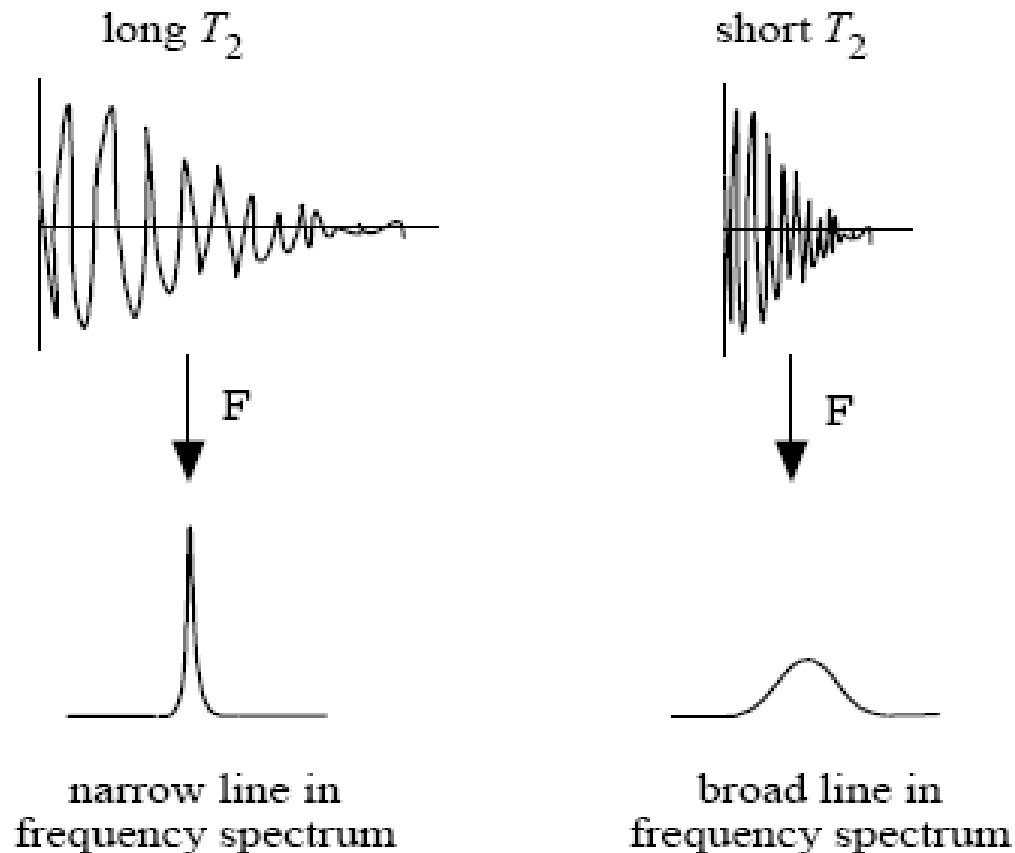
$$\Delta\omega_0 : \omega_1^0, \omega_2^0 \text{ K K}$$

- (b) magnetic field inhomogeneity over the sample

- (c) variations in the precession frequencies due to fluctuations in the local magnetic field at the nucleus:  $\Delta(\omega - \omega_0)^2$ <sup>1/2</sup>

# Free-induction Decay

In any case, free-induction decay (FID)  $\equiv$  NMR spectrum in the time domain. Long  $T_2$  transform to sharp lines in the frequency domain; short  $T_2$ 's yield broad lines.



# Fourier Transform NMR

Advantage of  $S(t)$ : it takes  $T_2$  to acquire  $T_2 \approx 20 - 100$  msec for small protons  
 $T_1$  per experiment  $T_1 \approx 1$  sec

so in the time it takes to do a CW experiment, say 250 sec, you can do 250 Fourier transform experiments.

$$\frac{S}{N} \text{ improvement } N^{1/2} = (250)^{1/2} = 16$$

# Fourier Transform NMR

Another important difference is that in FT NMR experiments, all spins are affected simultaneously by rf, and all contribute to detected signal simultaneously.

FT NMR experiments are typically shown as timing diagrams or “pulse sequences”, *e.g.* the simplest one-pulse experiment:

# Spin-lattice Relaxation

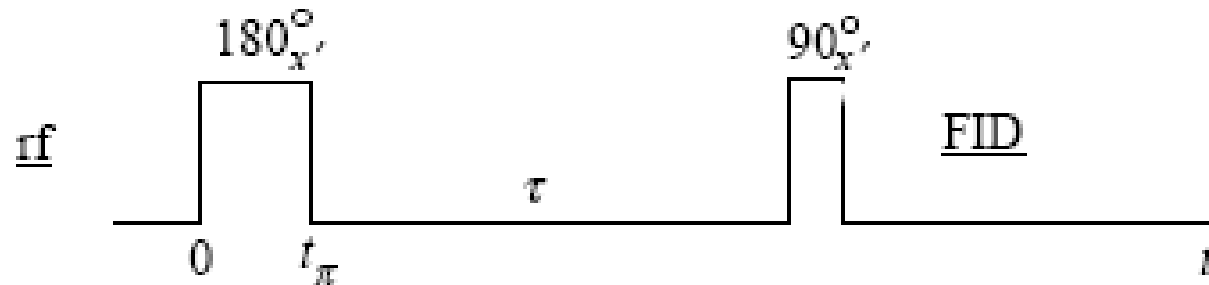
After a single pulse  $\neq 2n\pi$ ,  $M_z$  not at thermal equilibrium. Bloch postulated exponential recovery of  $M_z$  (justified for random isotropic rotational motion) with time constant  $T_1$  ... also called spin-lattice relaxation time ( $T_2$  processes are adiabatic ... spin-spin magnetization transfer without loss of energy to surroundings vs.  $T_1$  processes ... spin system loses energy to surrounding "lattice" as relaxation proceeds). So from Bloch, after a single pulse

$$\frac{d}{dt}M_z = -\frac{(M_z - M_0)}{T_1}$$

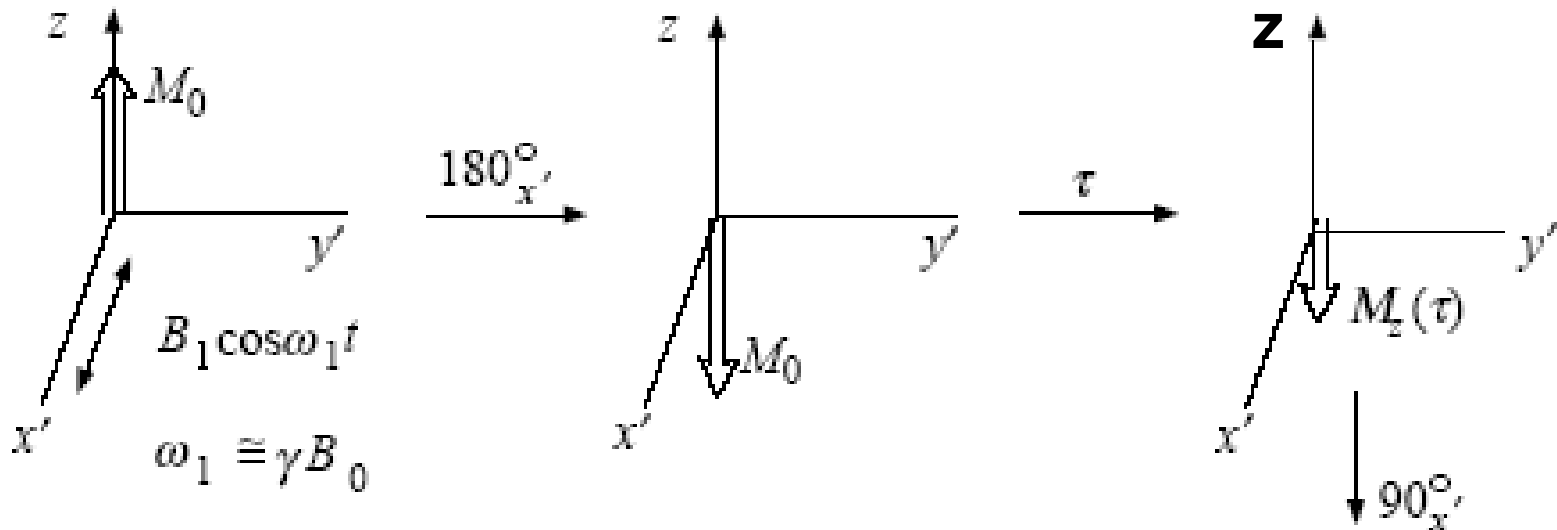
General solution:  $M_z(t) - M_0 = A e^{-t/T_1}$ . For a  $\pi$  pulse:  $M_z(t) = (1 - 2e^{-t/T_1})M_0$



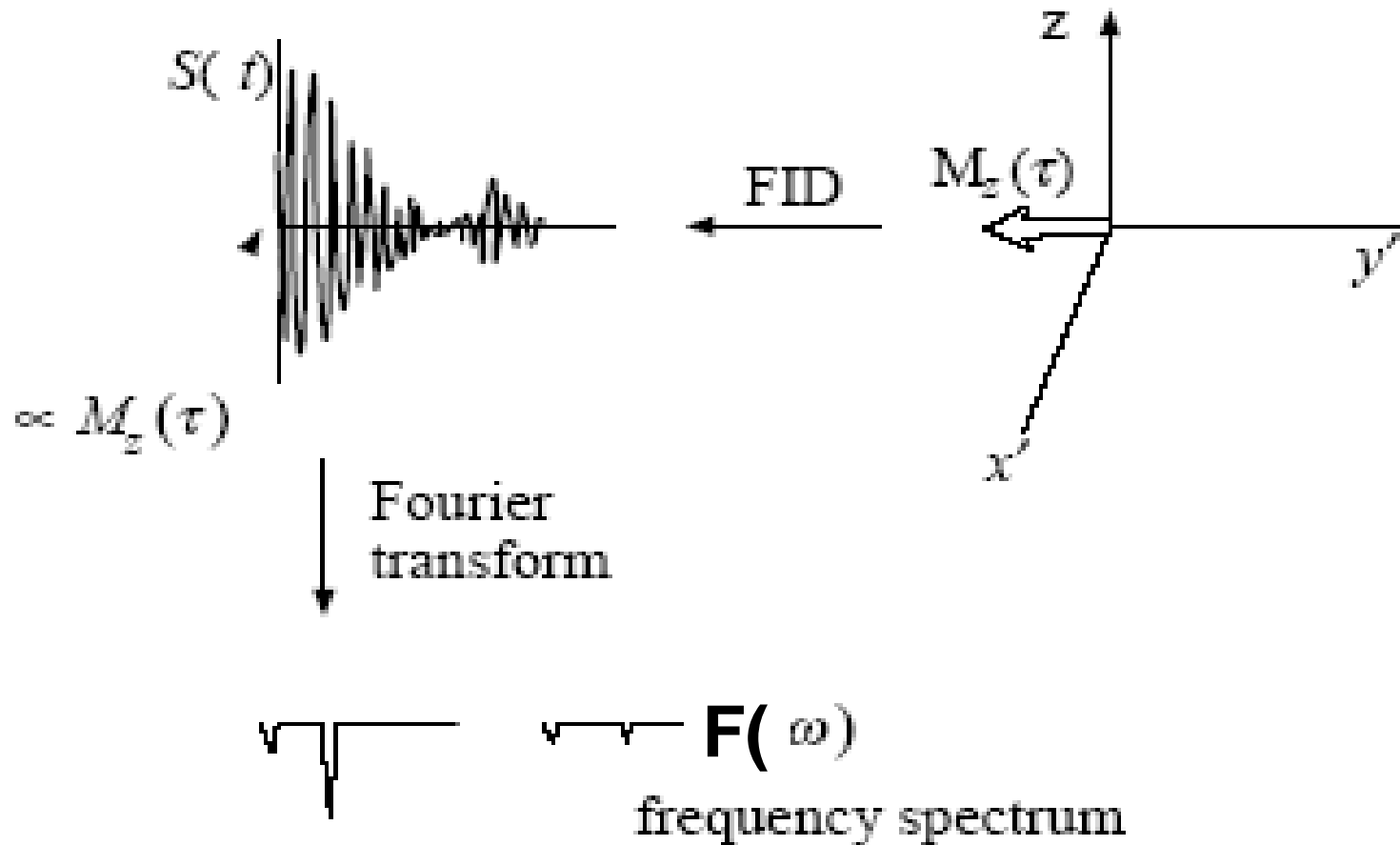
# Spin-lattice Relaxation



$$t_\pi = \frac{\pi}{\gamma B_1}$$

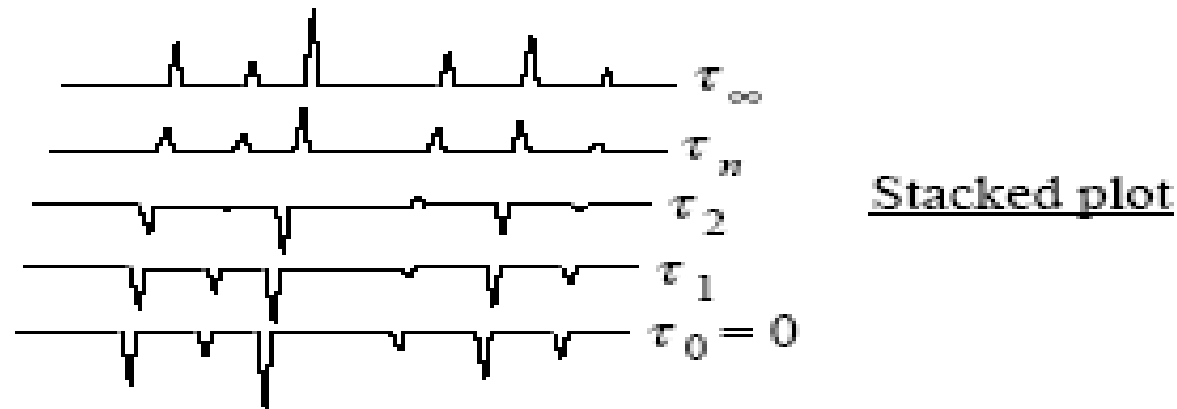


# Spin-lattice Relaxation



# Spin-lattice Relaxation

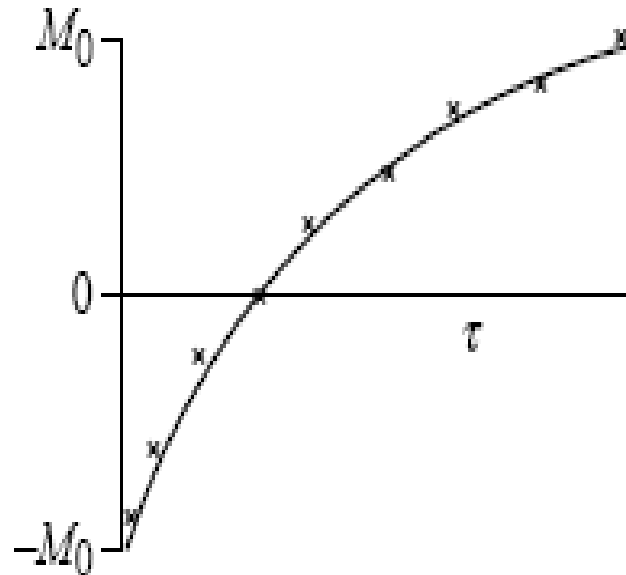
Vary  $\tau$  and stack  $F(\omega)$



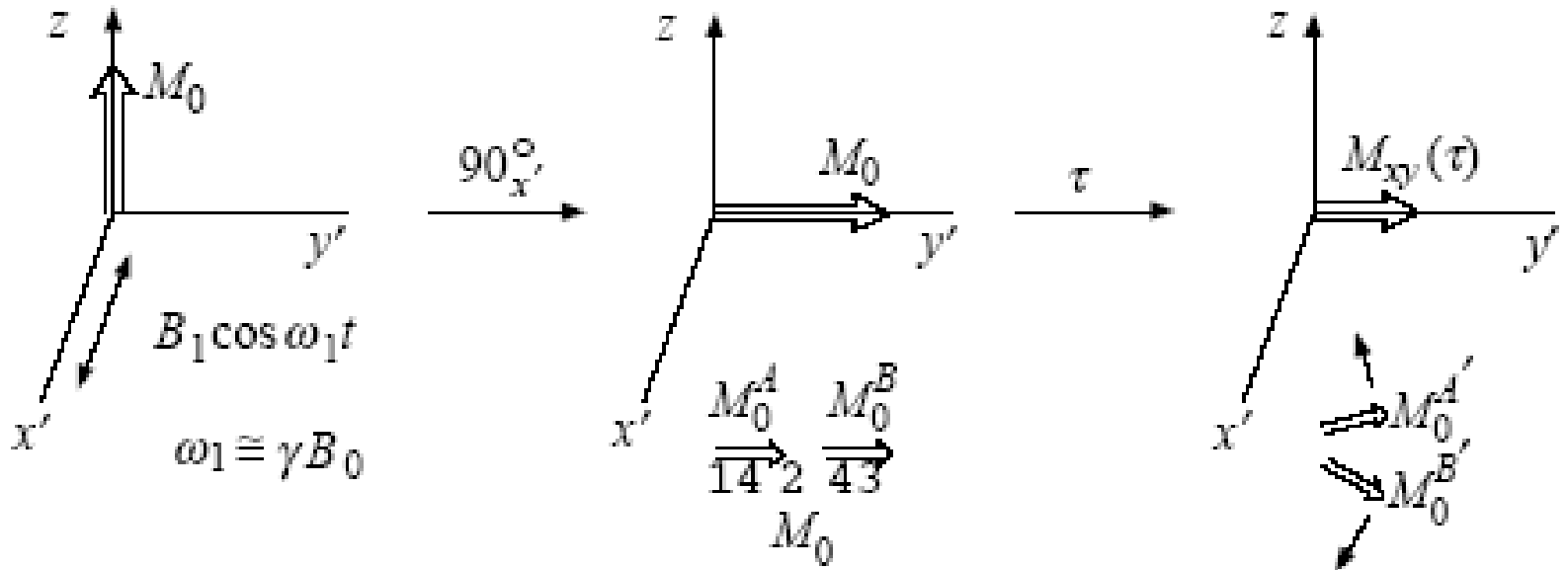
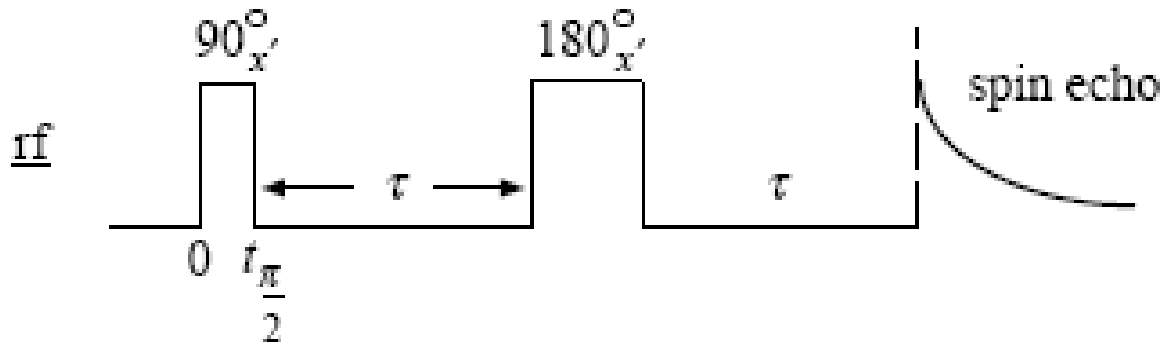
This is called  $\left( \pi_{x'} - \tau - \frac{\pi}{2_{x'}} - \text{FID} - \text{RD} \right)_n$   
 $\uparrow$   
 relaxation delay

# Spin-lattice Relaxation

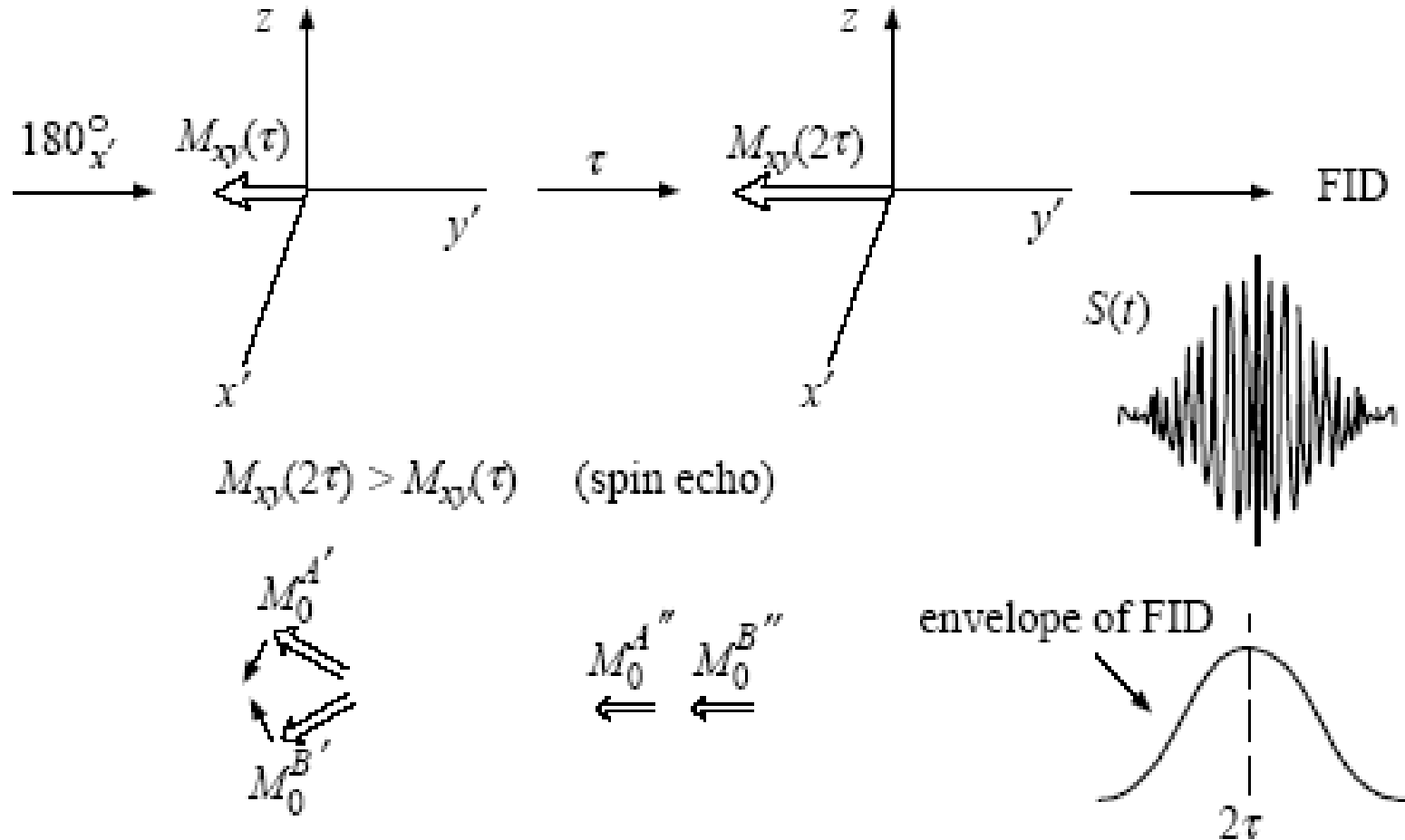
Note that different resonances have different  $T_1$ 's! For each of the resonances, obtain a curve. Use nonlinear least squares analysis to extract  $T_1$ .



# Another Two-pulse Experiment: "The Spin-Echo"



# The Spin-Echo Experiment



# The Spin-Echo Experiment

$M_0^A > M_0^{A'} > M_0^{A''}$  due to dephasing by fluctuating magnetic fields.

Vary  $\tau$  and obtain  $S(t, \tau)$  for a given  $\tau$ .

The above pulse sequence or experiment can also be written

$$\left( \frac{\pi}{2}_{x'} - \tau - \pi_{x'} - \tau - \text{spin echo} - \text{RD} \right)_n$$

This two-pulse experiment is a prelude to 2D NMR.

# The Spin-Echo Experiment

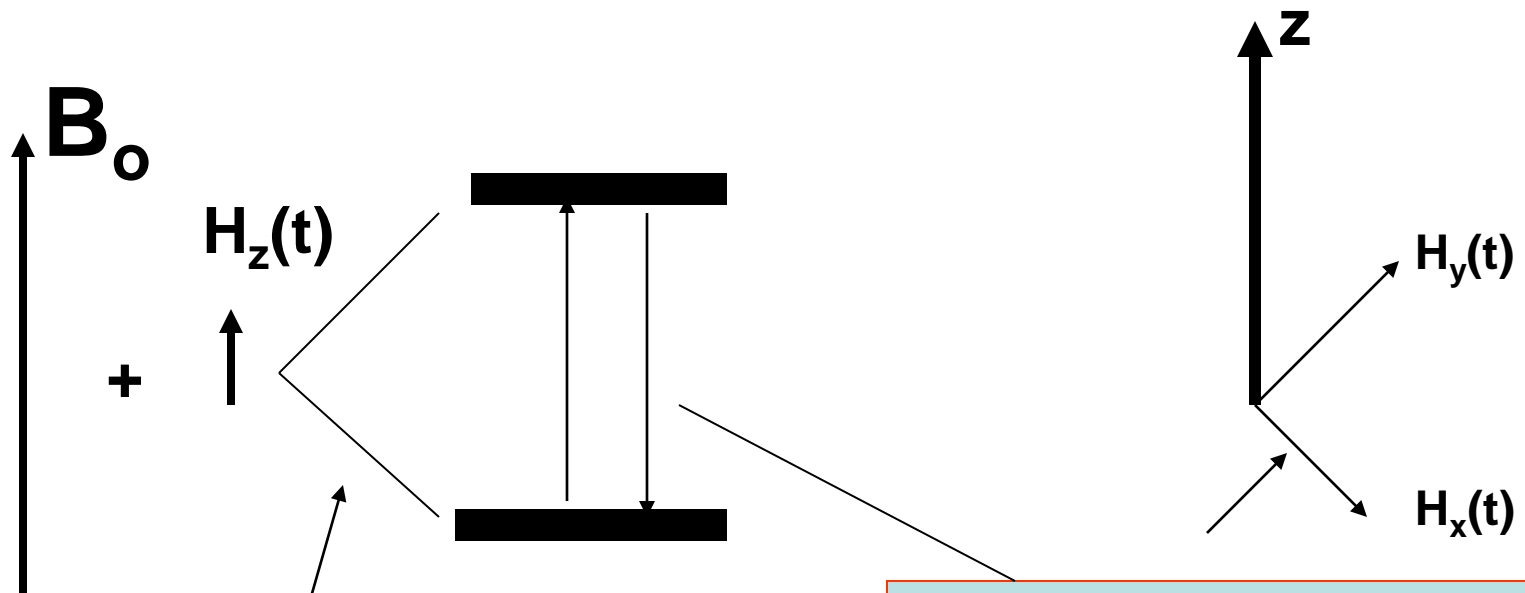
The spin echo is a component of many more complicated pulse sequences. By itself, it can be used to measure  $T_2$  ... by varying  $\tau$  and looking at envelope intensity (or signal intensity in  $F(\omega)$ ). Furthermore, Hahn showed an effect of diffusion on NMR spectra: in an inhomogeneous field, the spin echo amplitude diminishes according to:

$$M(2\tau) = M_0 \exp\left[\frac{-2\tau}{T_2}\right] \exp\left[-\gamma\left(\frac{\partial B}{\partial z}\right)^2 \frac{2}{3} D\tau^3\right] \text{ where } D = \text{diffusion constant}$$



# Random fields: Effects on dephasing of magnetization and spin-lattice relaxation

$$\mathbf{H}(t) = i H_x(t) + j H_y(t) + k H_z(t)$$



Introduces broadening of energy levels and increases spectral widths; contributes to dephasing of magnetization.

Fourier components of these fluctuating magnetic fields at  $\omega_0$  induce transitions among these energy states and result in spin-lattice relaxation.

# Origin of random fields

## Any process that produces fluctuating magnetic fields

- **Magnetic field homogeneities** (modulated by translational diffusion)
- **Dipolar fields produced by other spins** (modulated by relative motions, including translational and rotational diffusion, and any dynamical physical/chemical process that affects the distances between spins)
- **Other interactions with a magnetic component**

$$H(t) = (1/2\pi) \int a(\omega) \exp i\omega t d\omega$$

**Contribution to spin-lattice relaxation rate ( $1/T_1$ )**

**proportional to  $|a(\omega_0)|^2$**

↙ at Larmor frequency

# Pulsed-Field Gradients

Recently (past 5 years) great use has been made of short duration (*i.e.* “pulsed”) field gradients. A linear gradient applied along the  $z$  axis modifies the Larmor frequency for different volume elements along the length of the sample tube:

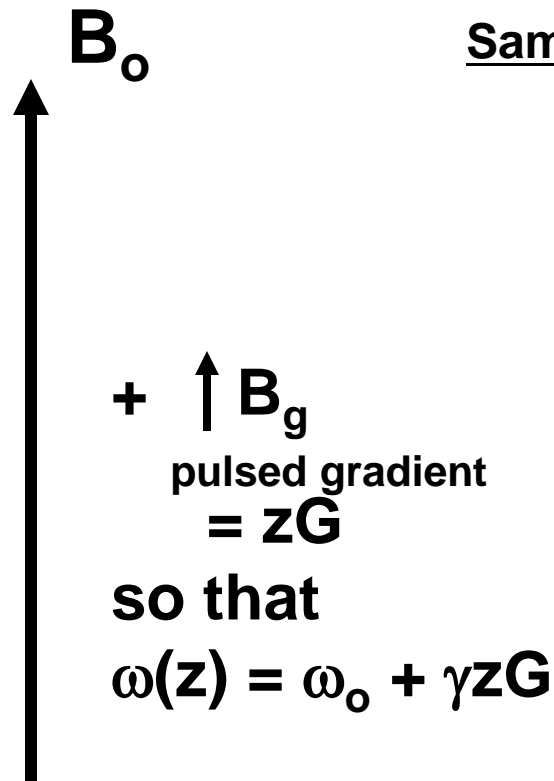
$$\begin{aligned}\omega(z) &= -\gamma [B_0 + B_g(z)] \\ &= \omega_0 - \gamma z G \quad \text{where } G \text{ is a constant } \propto \text{gradient strength}\end{aligned}$$

If  $M_{xy} \neq 0$  when the gradient is applied, the spins become “phase-encoded”... their phase

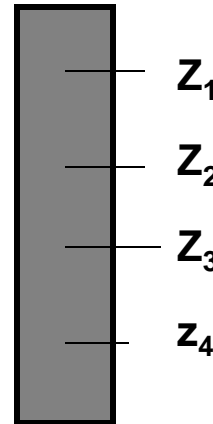
$\phi(\mathbf{z})$  in the  $xy$  plane depends on their location along the  $z$  axis:

$$\phi(z) = \gamma z G t \quad \text{where } t = \text{duration of gradient pulse}$$

# Pulsed-field gradients and phase encoding



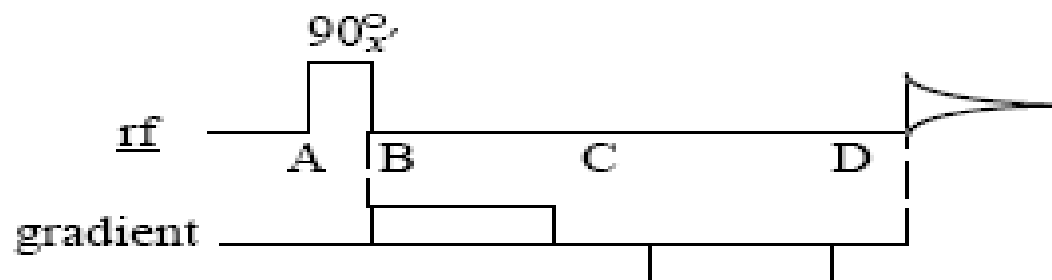
Sample: protein solution



Diffusion of small molecules (e.g.,  $H_2O$ ) up and down sample rapid and dephases magnetization of these spins

Macromolecules, e.g., proteins, diffuse much more slowly and their spins precess at  $\omega(z)$ .

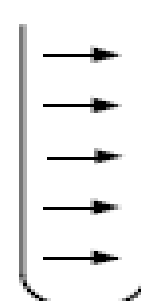
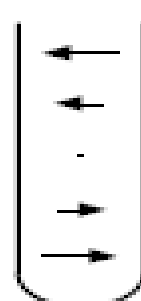
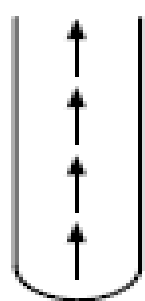
If after the spins of the small molecules have dephased, a reverse pulsed-field gradient is applied, the magnetization of the spins associated with the macromolecules will be refocused. A way to remove strong  $H_2O$  signal.



net moment  
spectrum



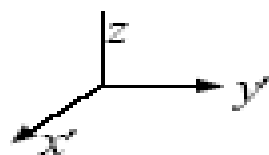
$B_0$



$z$  gradient  
coil

30 mm  $\frac{dM_x}{dz}$

"phase  
labeled"

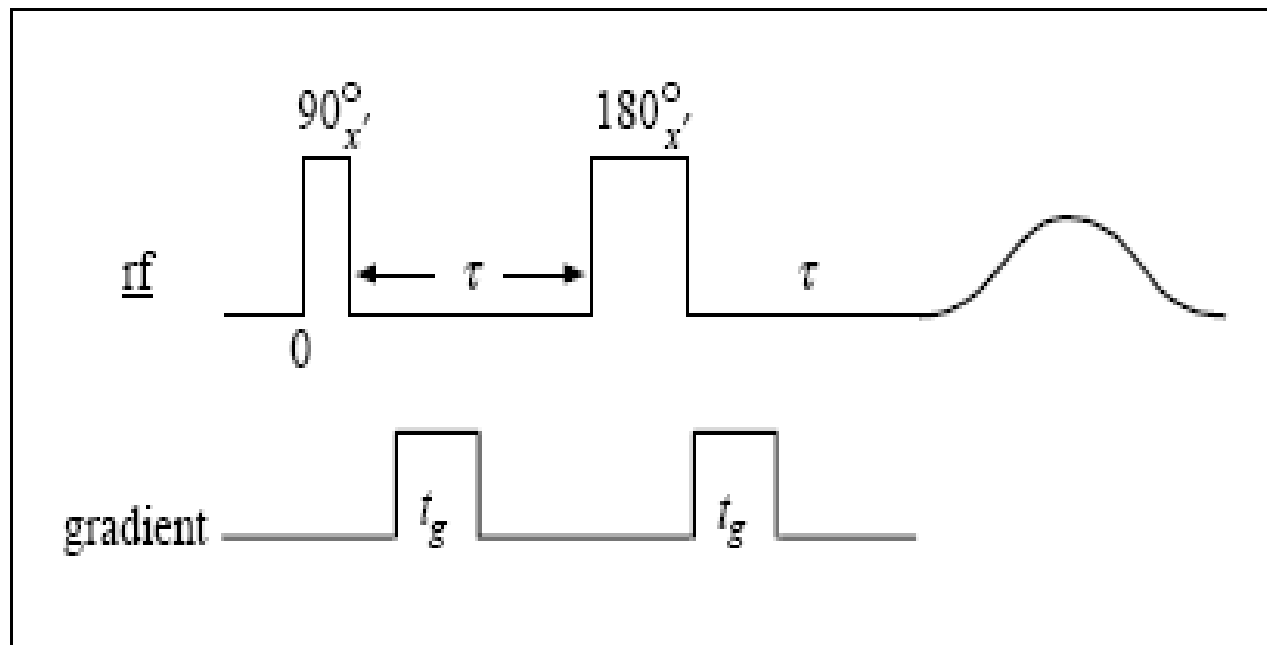


# Pulsed-Field Gradients

The initial 90° pulse generates transverse magnetization. The first gradient pulse phase-encodes the magnetization. The 180° rf pulse then inverts all spins ... so the second gradient pulse should reverse the phase-encoding *unless* the molecules diffuse out of their original volume element during the first echo delay  $\tau$ . This will diminish the signals of small molecules preferentially to those of big molecules (since the latter diffuse more slowly) ... particularly, this provides a way of eliminating the solvent signal in aqueous solutions of biological macromolecules.

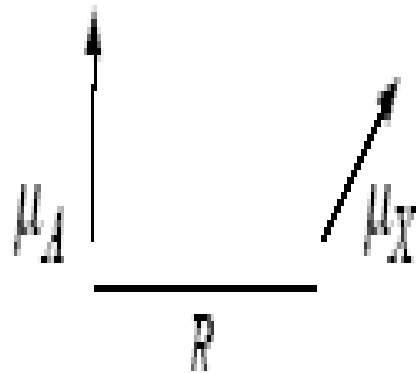
# Pulsed-Field Gradients

As an example, a pair of gradients can be combined with a Hahn spin echo to create a diffusion-selective (therefore molecular weight-selective) echo sequence.



# Nuclear Dipole-Dipole Interaction

Two magnetic dipoles will interact through space (contrast with the J-coupling interaction, which occurs only through the intervening electron density of bonds).  
Detection of this interaction is evidence for spatial proximity of the spins (measurable for distances  $\leq 5 \text{ \AA}$ )





# Nuclear Dipole-Dipole Interaction

Let's call  $A$  spin  $J$  and  $X$  spin  $S$ . . . . conventional nomenclature for what follows.

$$\begin{aligned} E_{dd} &= \frac{\vec{\mu}_A \cdot \vec{\mu}_X}{R^3} - \frac{3(\vec{\mu}_A \cdot \vec{R})(\vec{R} \cdot \vec{\mu}_X)}{R^5} \\ &= \gamma^2 \hbar^2 \left[ \frac{\vec{I}_A \cdot \vec{I}_X}{R^3} - \frac{3(\vec{I}_A \cdot \vec{R})(\vec{R} \cdot \vec{I}_X)}{R^5} \right] \quad (\text{for } \gamma_A = \gamma_X) \\ &= \gamma_I \gamma_S \hbar^2 \left[ \frac{\vec{I} \cdot \vec{S}}{R^3} - \frac{3(\vec{I} \cdot \vec{R})(\vec{R} \cdot \vec{S})}{R^5} \right] \quad (\text{generally}) \end{aligned}$$

# Nuclear Dipole-Dipole Interaction

This interaction provides the main means by which spins- $\frac{1}{2}$  exchange magnetization ( $T_2$  processes — called cross-relaxation) or dissipate magnetization to the lattice in longitudinal relaxation.

The motion of  $I$  relative to  $S$  sets up local field fluctuations at  $S$  (or, equivalently, modulates  $E$  levels of  $S$ ). There exists a component of this modulation at  $S$  transition frequency, so the fluctuations can induce transitions.

# Solomon's Equations

The Solomon equations are a phenomenological description of the relaxation behavior of the  $Z$ -components of  $I$ ,  $S$  magnetization in a two-spin dipolar-coupled system.

$$\frac{d}{dt}\langle I_Z \rangle = \frac{1}{T_{1II}} (\langle I_Z \rangle - I_0) - \frac{1}{T_{1IS}} (\langle S_Z \rangle - S_0)$$

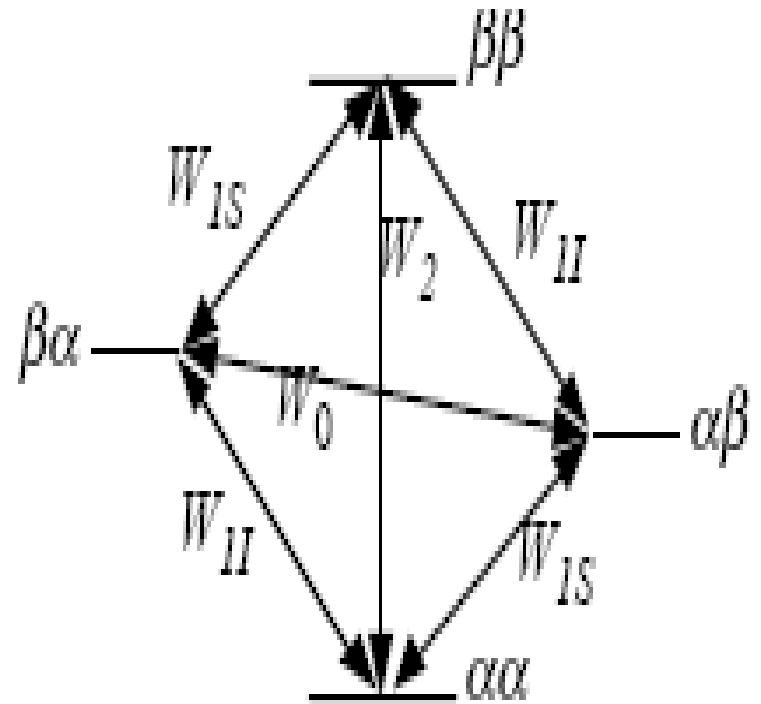
$$\frac{d}{dt}\langle S_Z \rangle = \frac{1}{T_{1SI}} (\langle I_Z \rangle - I_0) - \frac{1}{T_{1SS}} (\langle S_Z \rangle - S_0)$$

# Solomon's Equations

where  $\frac{1}{T_1^H} = W_0 + 2W_{1I} + W_2$

$$\frac{1}{T_1^{SS}} = W_0 + 2W_{1S} + W_2$$

$$\frac{1}{T_1^{IS}} = W_2 - W_0$$



# Solomon's Equations

Here  $W$ 's are transition probabilities, related to molecular geometry and motion by time-dependent perturbation theory.

$$W_0 = \frac{1}{10} \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{R^6} \frac{\tau_c}{1 - (\omega_I - \omega_S)^2 \tau_c^2}$$

$$W_{1I} = \frac{3}{20} \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{R^6} \frac{\tau_c}{1 + \omega_I^2 \tau_c^2}$$

$$W_{1S} = \frac{3}{20} \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{R^6} \frac{\tau_c}{1 + \omega_S^2 \tau_c^2}$$

$$W_2 = \frac{3}{5} \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{R^6} \frac{\tau_c}{1 + (\omega_I + \omega_S)^2 \tau_c^2}$$

where  $\tau_c$  = rotation correlation time for  $\vec{R}$

$$(\omega_I \tau_c \approx 1 \text{ for } \tau_c \approx 10^{-9} \text{ s for } ^1\text{H at 500 MHz})$$

# Magnetization Transfer

Main Result If in a two spin system, there exists nonequilibrium  $Z$ -magnetization, there will be magnetization exchange between  $A$  and  $X$ .

## Reading assignment

Eisenberg and Crothers (EC), Chapter 13, pp. 617-626.