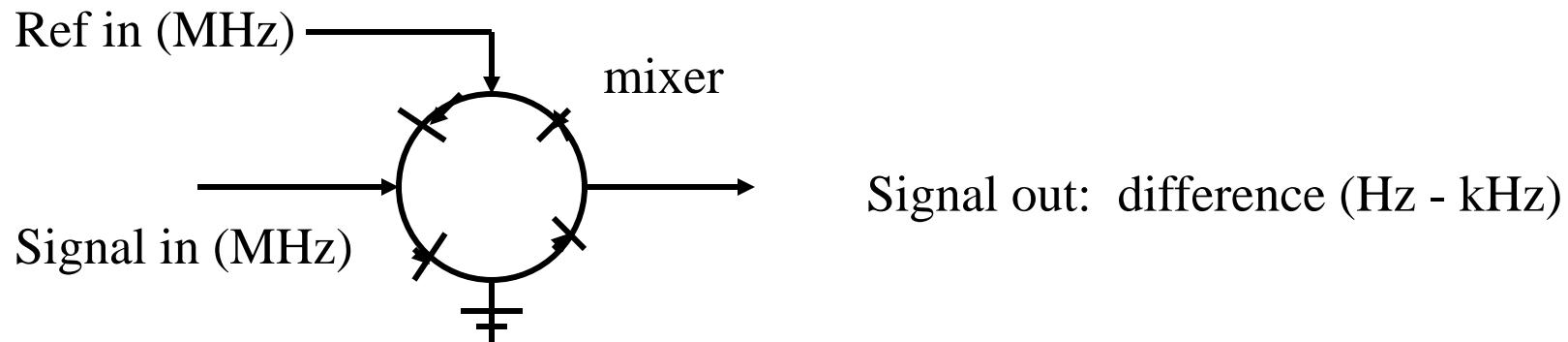


# NMR Spectroscopy:

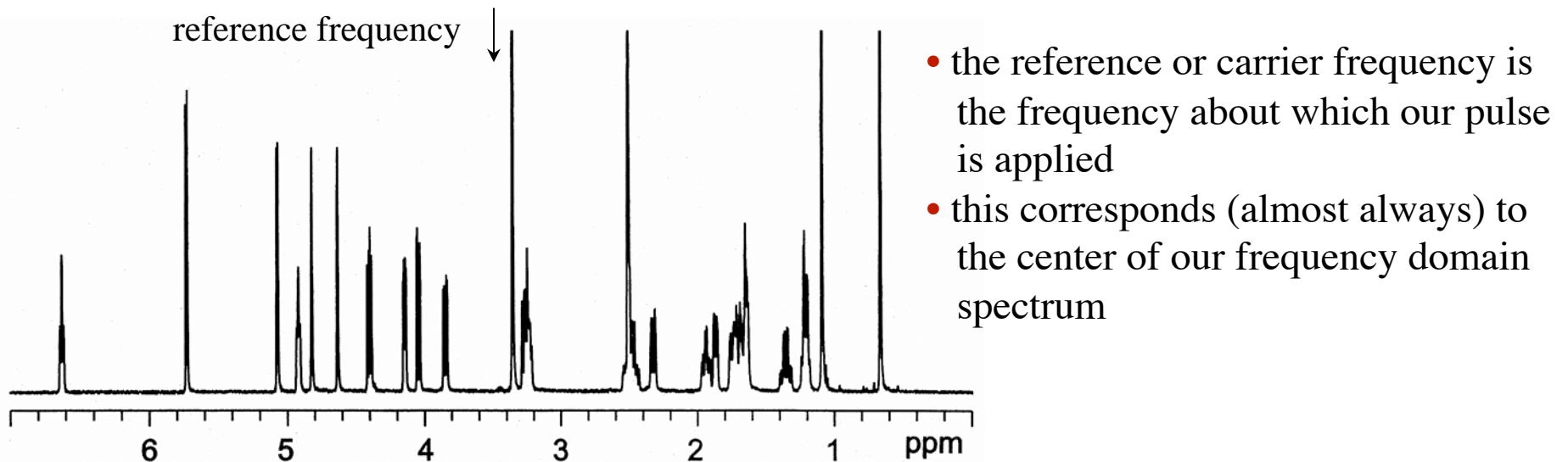
## 3

# Signal Manipulation

- time domain NMR signal in MHz range is converted to kHz (audio) range by mixing with the reference (“carrier”) frequency

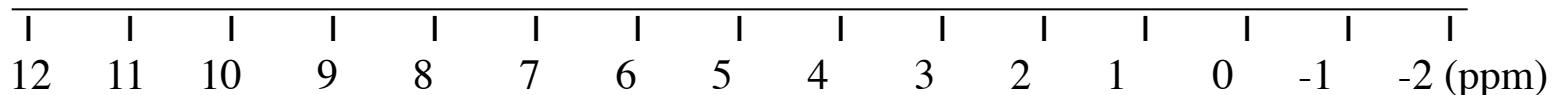


- thus, the frequencies that we observe are relative to a reference (and to one another)

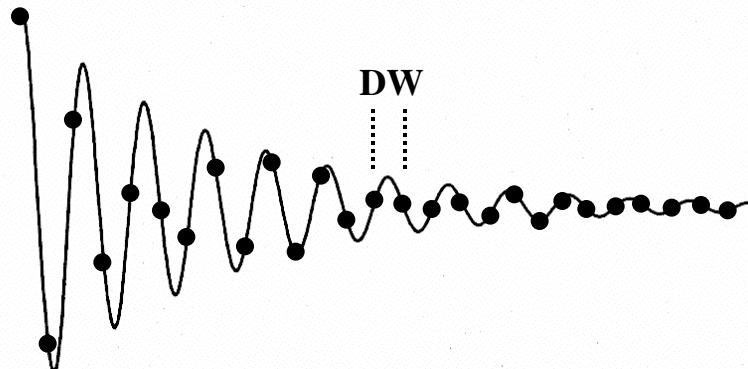


# Acquisition Parameters (Time Domain)

- the **spectral width** (in the frequency domain) or **spectral window** (SW) is determined by the rate of digitization of the time domain signal
  - for  $^1\text{H}$ , a typical SW might be 10 to 14 ppm (i.e., at 400 MHz, 4000 - 5600 Hz)



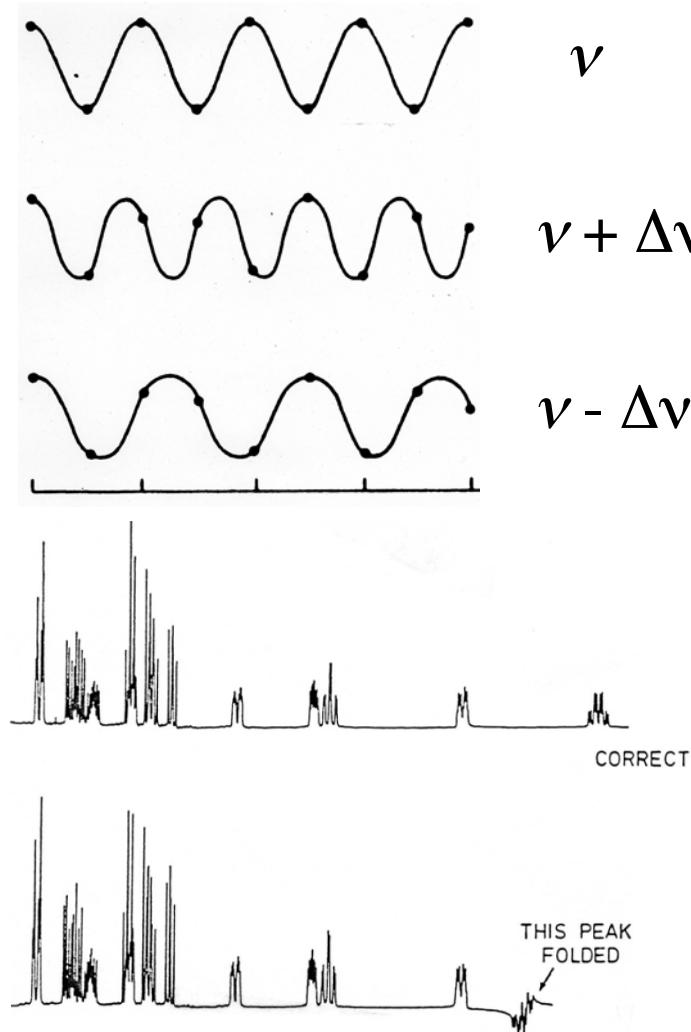
- the SW tells us the largest frequency *difference* that we can determine
- the **Nyquist Theorem** states that in order for a frequency difference of SW to be measured, the time domain data (FID) has to be sampled at a frequency not less than  $2 \times \text{SW}$ 
  - this frequency is called the **Nyquist frequency**
- the **dwell time** (DW) is the time between sampled points and determines SW



$$\text{DW} = \frac{1}{2 \times \text{SW}}$$

# Acquisition Parameters (Time Domain)

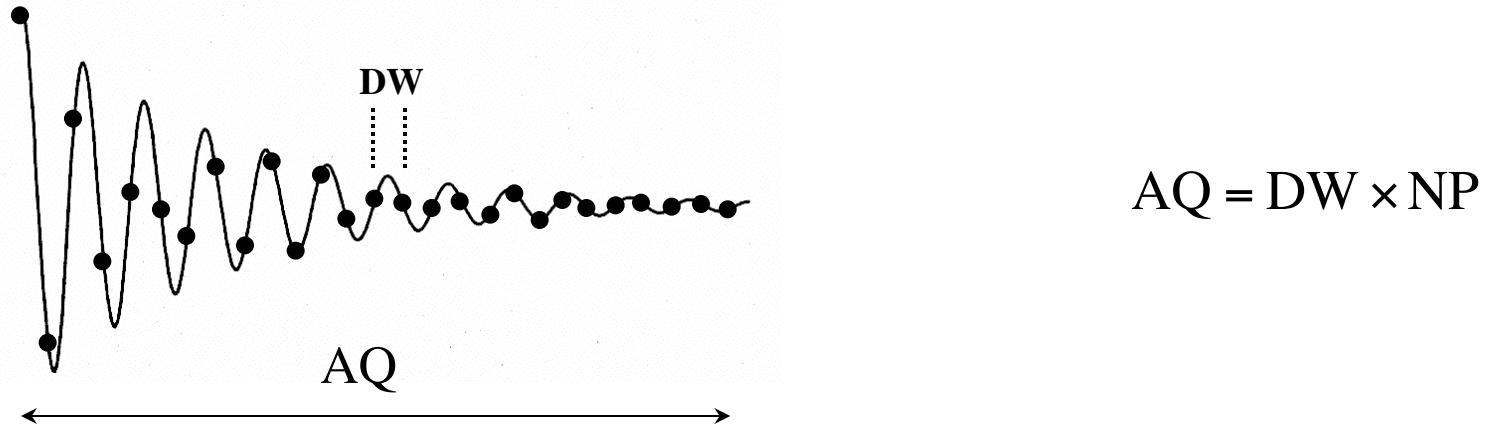
- if the sampling frequency is less than the Nyquist frequency (if  $DW > 1/(2 \times SW)$ ), then frequencies greater than or less than the reference frequency cannot be discriminated from one another



- at a frequency  $\nu$ , sampling occurs at  $1/(2 \times SW)$
- if the same sampling frequency is used to digitize two additional signals, one at  $\nu + \Delta\nu$  and one at  $\nu - \Delta\nu$ , we cannot discriminate between the faster and the slower signal
  - note that for the signals at  $\nu + \Delta\nu$  and at  $\nu - \Delta\nu$ , at each point sampled, the signal amplitudes are identical
- for signals outside of  $SW$  (for signals digitized at a frequency less than the Nyquist frequency), the peaks corresponding to the signals will be folded in the spectrum
  - folded peaks are often characterized by aberrant amplitude and phase characteristics

## Acquisition Parameters (Time Domain)

- the total **number of points digitized** (NP) multiplied by the dwell time (DW) represents the total time that the FID is sampled and is called the **acquisition time** (AQ)



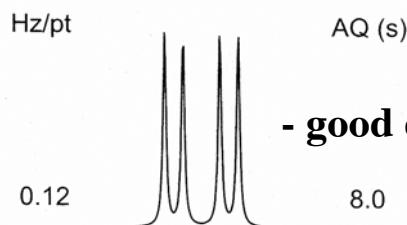
- thus, SW, DW, AQ and NP are all interrelated:

$$DW = \frac{1}{2 \times SW}$$

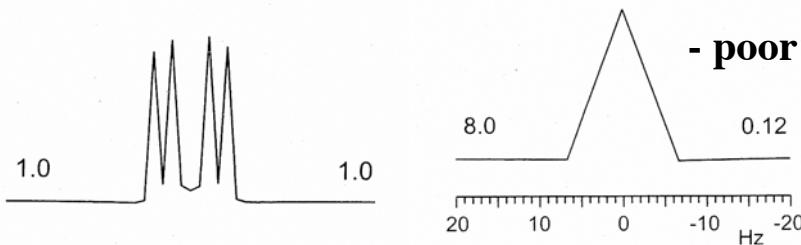
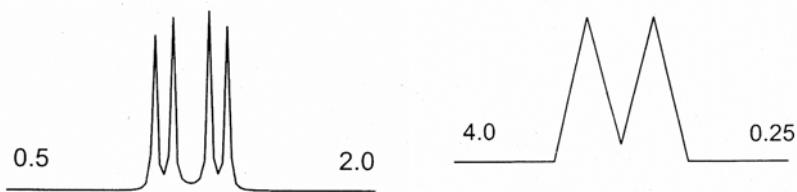
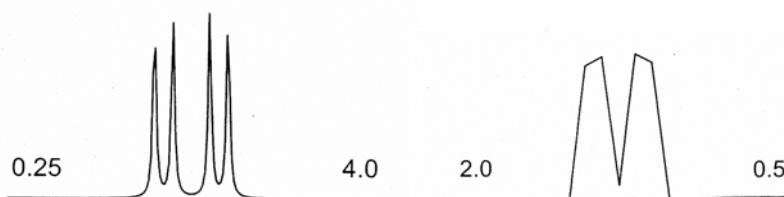
$$AQ = DW \times NP = \frac{NP}{2 \times SW}$$

# Acquisition Parameters (Time Domain)

- the **digital resolution** (DR) is defined as twice the spectral width (in Hz) divided by the number of digitized points (NP) (thus, units are Hz/point)



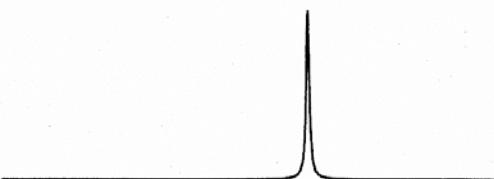
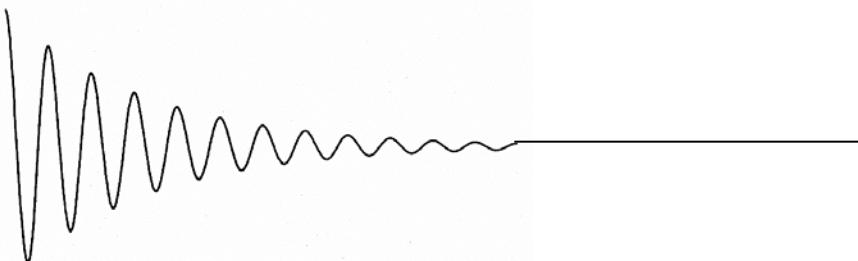
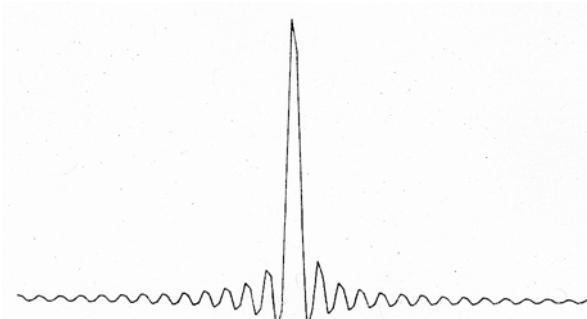
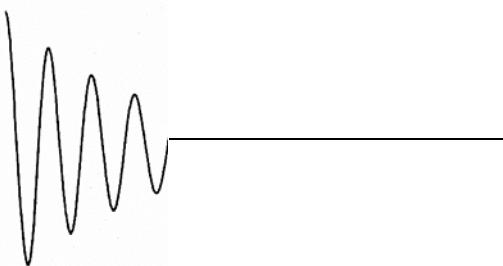
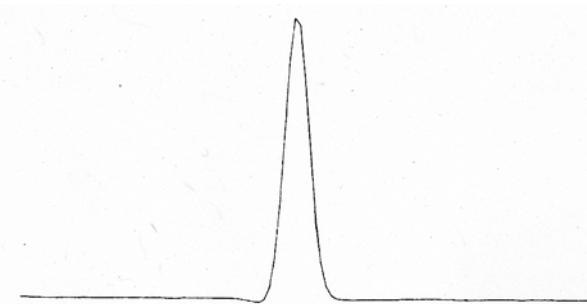
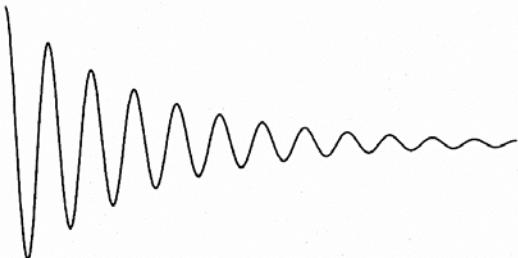
$$DR = \frac{2 \times SW}{NP} = \frac{1}{AQ}$$



- the digital resolution is also, thus, the reciprocal of the acquisition time
- better (*improved*) digital resolution means DR is smaller
- the digital resolution can therefore be *improved* by decreasing SW or increasing NP (i.e. increasing the acquisition time)

## Acquisition Parameters (Time Domain)

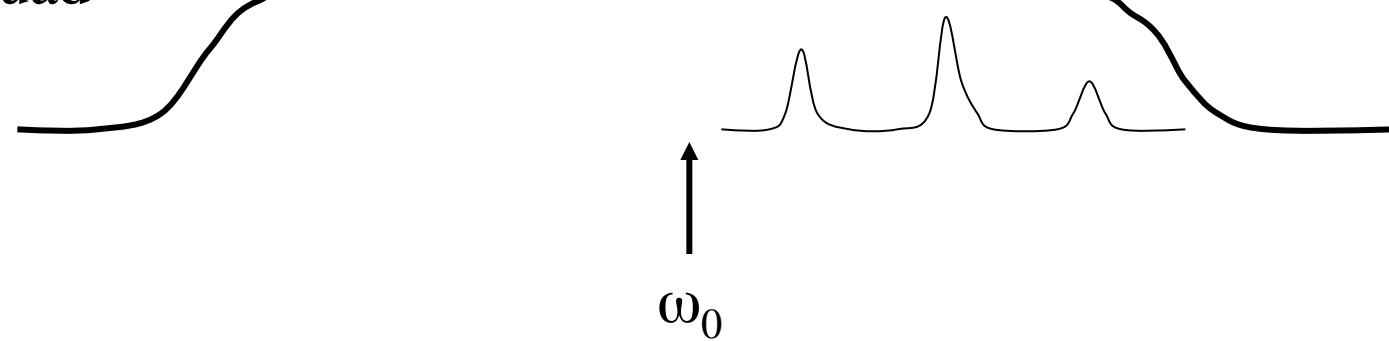
- increasing AQ leads to better digital resolution and sharper lines. Truncating the FID (very short AQ) leads to baseline artifacts
- a good rule of thumb is that  $AQ \approx T_2$  to  $2xT_2$  (acquisition times longer than  $2xT_2$  are not significantly beneficial)



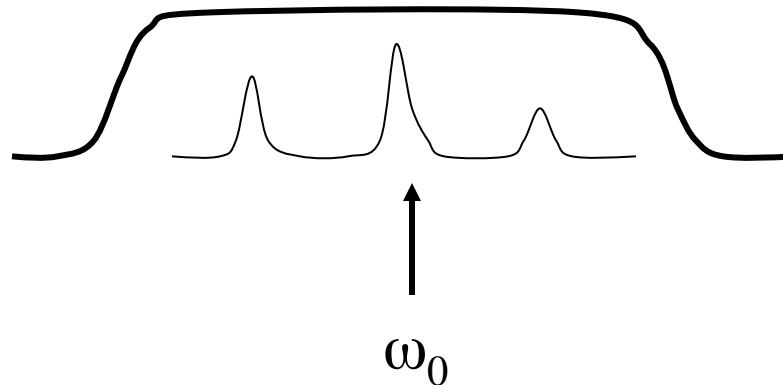
# Quadrature Detection

- if NMR signal is detected on a single axis ( $x$  or  $y$ ), the Fourier Transform cannot distinguish between signals that are larger than or smaller than the reference frequency by the same amount
- using such a single channel detection scheme, the carrier or reference frequency is placed at one edge of the spectrum of expected signals
- quadrature detection alleviates this problem and permits the reference to be placed in the center of the spectrum

No Quad

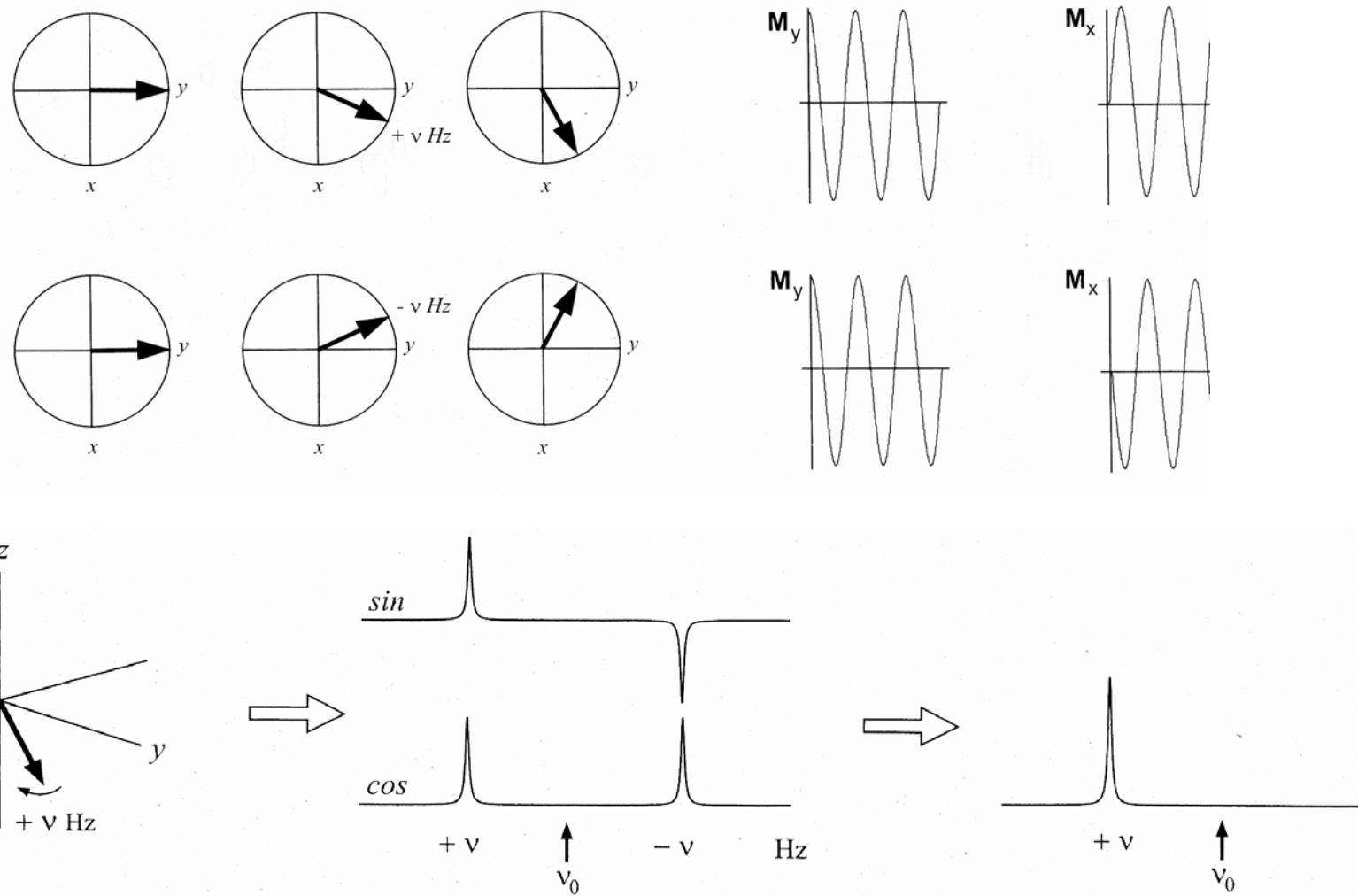


With Quad



# Quadrature Detection

- quadrature detection permits discrimination between positive and negative signals (below)
- quadrature detection permits a smaller SW, improved digital resolution and, as it turns out, an increase in S/N of  $\approx 2^{1/2}$



# Optimizing Pulse Width

What is the optimal pulse width/length/angle to use?

- a 90° pulse angle gives maximum S/N for one pulse, but the delay ( $d_1$ ) between successive pulses must be long for recovery of equilibrium magnetization
- following a 90° pulse, if  $(d_1 + AQ) = 5 \times T_1$ , then > 99% of equilibrium magnetization will be recovered before the next pulse
- thus, *for a given number of pulses* (without regards to time), 90° pulses will give maximum sensitivity (as long as  $(d_1 + AQ) \geq 5 \times T_1$ )
- other schemes, which permit faster pulsing (shorter  $d_1$ ) combined with smaller pulse angles are possible
- for optimizing the S/N and total experimental time, the best compromise for the pulse width/angle is the ***Ernst Angle***
- Ernst Angle is in degrees

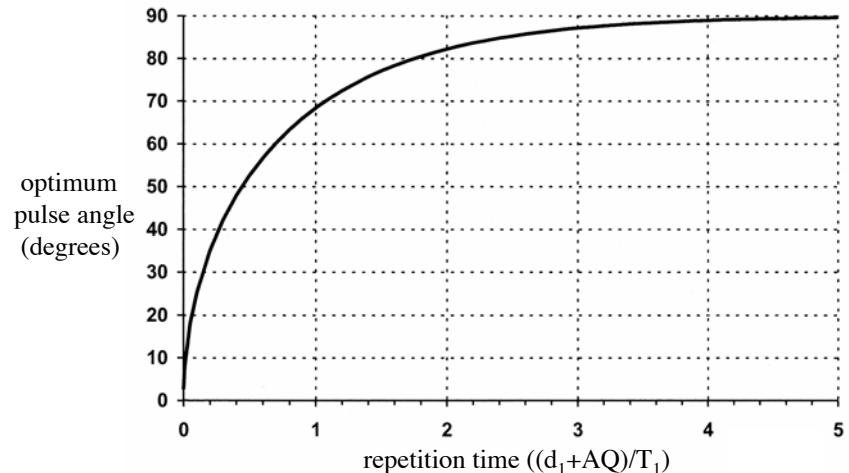
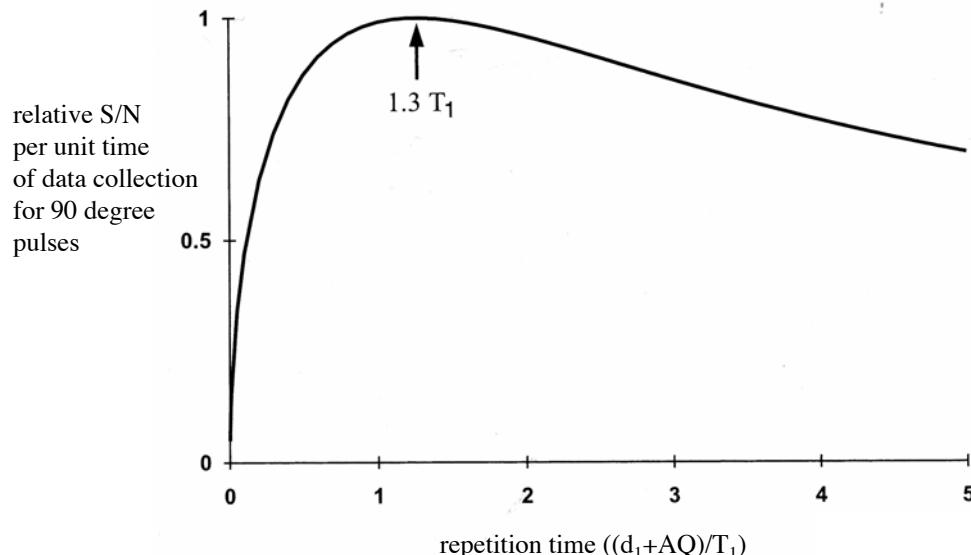
$$\cos \alpha_{\text{Ernst}} = e^{-(d_1 + AQ)/T_1}$$

# Optimizing Pulse Width

$$\cos \alpha_{\text{Ernst}} = e^{-((d_1 + AQ)/T_1)}$$

**What is the optimal repetition time (best S/N) for a given repetition time?**

- the optimal pulse angle is dependent on the repetition time
- as the repetition time shortens, so does the optimal pulse angle



**What is the optimal repetition time (best S/N) for a given experiment time using 90 degree pulses?**

- the optimal pulse angle is  $1.3 \times T_1$  for 90 degree pulses for a pre-determined total experiment time

# Resonance Frequencies and Shielding

- Thus far, we have specified the resonance condition as follows:

$$\nu_L = \nu_1 = \left| \frac{\gamma}{2\pi} \right| B_0, \Delta E = h\nu_1$$

- Because all nuclei of a particular type (i.e. all  $^1\text{H}$  nuclei) have the same  $\gamma$ , this suggests that the resonance frequencies of all  $^1\text{H}$  nuclei are the same (this would not be very interesting or useful)
- In molecules, nuclei are surrounded by electrons and other nuclei/atoms
- Thus, in diamagnetic molecules, the effective field ( $B_{\text{eff}}$ ) at a given nucleus is (almost) always less than the actual ( $B_0$ ) field

$$B_{\text{eff}} = B_0(1 - \sigma)$$

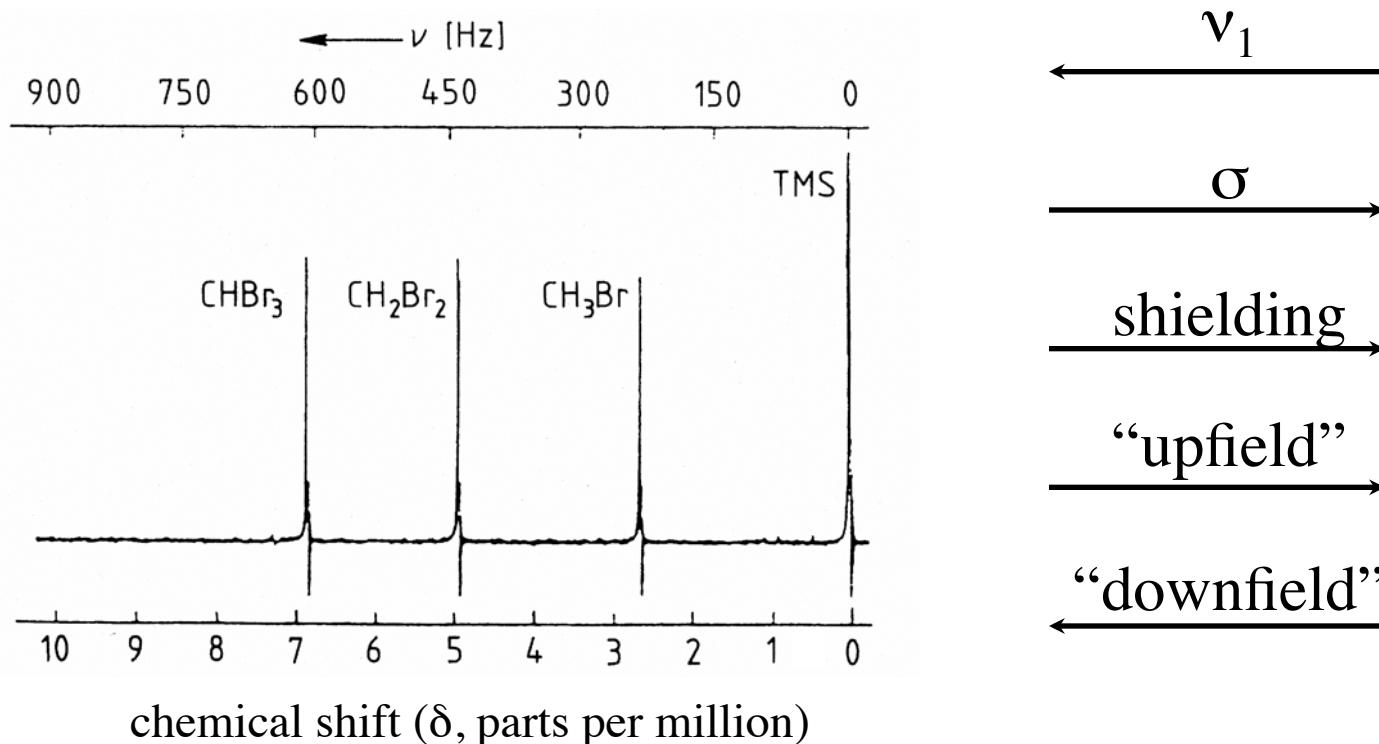
- Here  $\sigma$  (sigma) is the *shielding constant*
  - $\sigma$  is dimensionless and independent of magnetic field strength
  - $\sigma$  depends mostly on electronic structure: *highly shielded* means *high electron density*
- The new resonance condition thus is:

$$\nu_L = \nu_1 = \frac{\gamma}{2\pi}(1 - \sigma)B_0$$

# Resonance Frequencies and Shielding

$$B_{eff} = B_0(1-\sigma) \quad \nu_L = \nu_1 = \frac{\gamma}{2\pi}(1-\sigma)B_0$$

- Now,  $\nu_1$  is proportional to  $B_0$  and  $(1-\sigma)$
- When the effective field ( $B_{eff}$ ) that the nucleus experiences is significantly less than the actual field ( $B_0$ ), the nucleus is said to be **shielded** (i.e. shielded from  $B_0$ )
  - so, increased  $\sigma$  = increased shielding = decreased frequency ( $\nu_L$  or  $\nu_1$ )
- Example:  $^1\text{H}$  deshielding by Br (90 MHz spectrometer)



# Chemical Shifts and Shielding

- The frequency difference between two signals is dependent on  $B_0$ 
  - this is inconvenient for comparing spectra acquired on different instruments at different  $B_0$

$$\nu_{LA} = \frac{\gamma}{2\pi}(1-\sigma_A)B_0, \quad \nu_{LB} = \frac{\gamma}{2\pi}(1-\sigma_B)B_0, \quad \Delta\nu = \nu_{LA} - \nu_{LB} = \frac{\gamma B_0}{2\pi}(\sigma_B - \sigma_A)$$

- We can define a  $B_0$ -*independent* parameter for expressing relative frequencies called the ***chemical shift*** ( $\delta$ )

$$\delta_A = \frac{\nu_A - \nu_{\text{reference}}}{\nu_{\text{reference}}} \times 10^6$$

- The units of  $\delta$  are parts per million (ppm)
- The denominator can be replaced by the instrument frequency (without introducing any error), and then any chemical shift difference can readily be determined

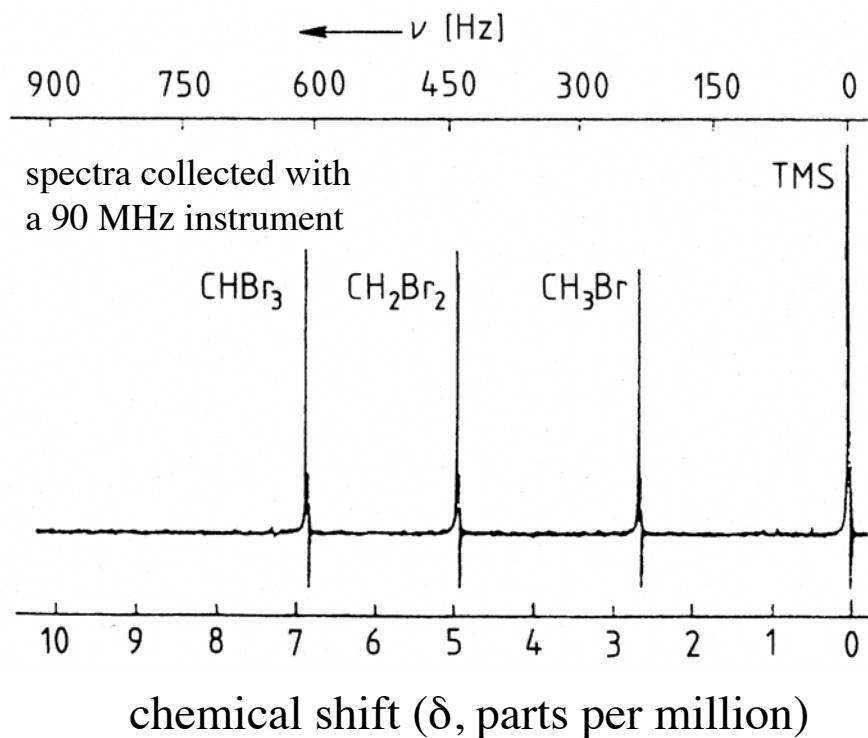
$$\Delta\delta = \frac{\Delta\nu}{\text{spectrometer frequency}} \times 10^6$$

- So, with a 400 MHz magnet, 1 ppm = 400 Hz, etc.
  - so  $\Delta\delta$  is independent of magnetic field strength

# Chemical Shift Referencing and Ranges

- Normally, chemical shifts are reported relative ( $\Delta\delta$ ) to a chemical shift standard  
-for  $^1\text{H}$ , this is usually TMS (tetramethylsilane)

$$\delta_{\text{TMS}} = 0$$



- Example:  $\text{CH}_2\text{Br}_2$

$$\Delta\delta = \frac{450 \text{ Hz}}{90 \text{ MHz}} \times 10^6 = 5 \text{ ppm}$$

# Origins of Shielding

- The  $B_0$  field induces current in the electron cloud surrounding nuclei, which in turn induces a local magnetic field
- The local induced magnetic field opposes the  $B_0$  field (thus,  $B_{\text{eff}} < B_0$ )
- Shielding is thus dependent on the electron density distribution around the nucleus
- Lamb's equation describes the electron density  $\rho$  at a distance  $r$  from the nucleus

$$\sigma = \frac{4\pi e^2}{3mc^2} \int_0^\infty r\rho(r)dr$$

- Only valid for spherical charge density / electrons near to nucleus ( $s$  electrons)
- Examples:

He (2 1s electrons)	$59.93 \times 10^{-6}$
H (~2 1s electrons)	$60 \times 10^{-6}$
Ne (10 s electrons)	$547 \times 10^{-6}$
- To account for non-spherical charge density (atoms in molecules,  $p$  orbitals, etc.), other terms are necessary.
- Thus, the term discussed above, which accounts for spherical charge density ( $s$  orbitals) and *opposes*  $B_0$ , is called the diamagnetic component ( $\sigma^{\text{dia}}$ ) of the shielding factor

# Origins of Shielding

- A term to account for non-spherical charge density is necessary
- This term is called the “paramagnetic” shielding term,  $\sigma^{\text{para}}$

$$\sigma = \sigma^{\text{dia}} + \sigma^{\text{para}}$$

- This shielding term is finite when the charge density is asymmetric
- The sign is opposite to  $\sigma^{\text{dia}}$  (so  $\sigma^{\text{dia}}$  and  $\sigma^{\text{para}}$  effects can attenuate or cancel one another)
- $\sigma^{\text{para}}$  is inversely proportional to the square of the mass and the electronic excitation energy for low-lying excited levels

$$\sigma^{\text{para}} \propto \frac{1}{m^2 \Delta E}$$

- In heavy atoms, including  $^{13}\text{C}$ , there are low-lying excited states, so that  $\Delta E$  is small (not so for  $^1\text{H}$ , for example), so this term can become important
- This is why the chemical shift ranges for heavier atoms are often much larger than for lighter atoms

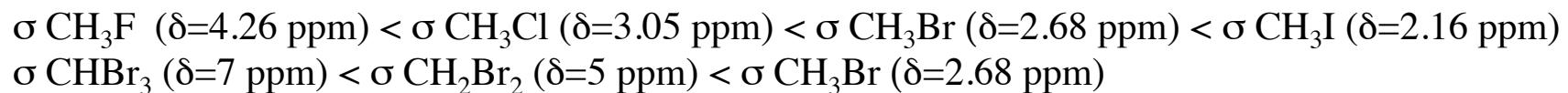
$^1\text{H}, ^2\text{H}$ : ~10 ppm  
 $^{13}\text{C}$  ~300 ppm  
 $^{15}\text{N}, ^{31}\text{P}$  ~500 ppm

$^{19}\text{F}$  ~1300 ppm  
 $^{59}\text{Co}, ^{195}\text{Pt}$  ~10,000 ppm

# Neighboring Group Effects on Shielding

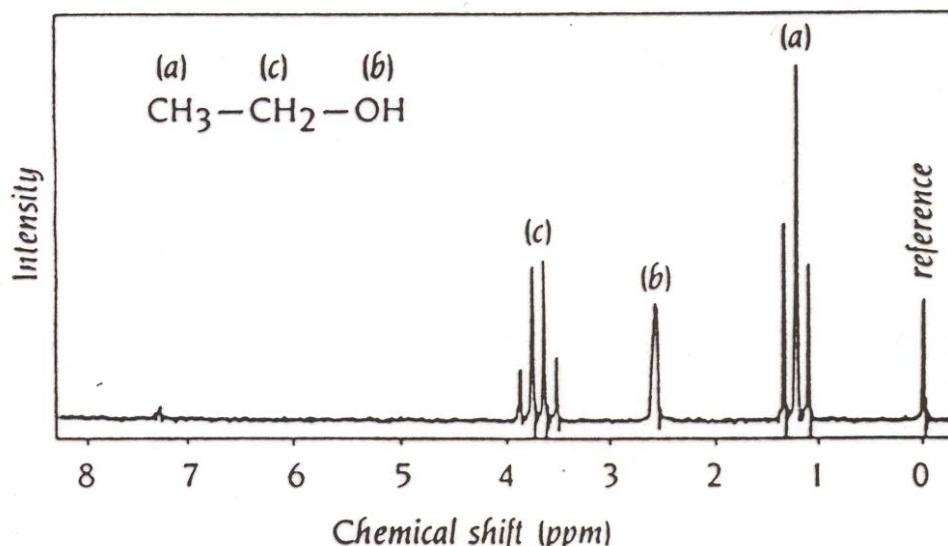
- Inductive Effects:

- chemical shift correlates with electronegativity of neighboring groups
- electronegative groups deshield neighboring nuclei



- Electronegativity of oxygen shifts  
-CH<sub>2</sub>- of ethanol downfield

- Electronegativity of carbon chain increases as chain length increases

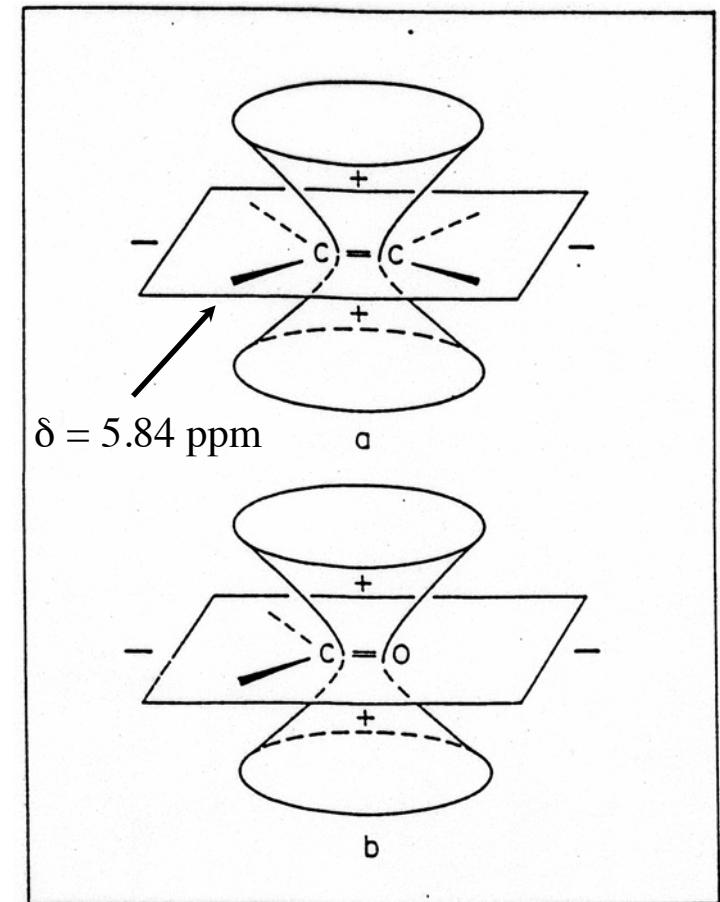
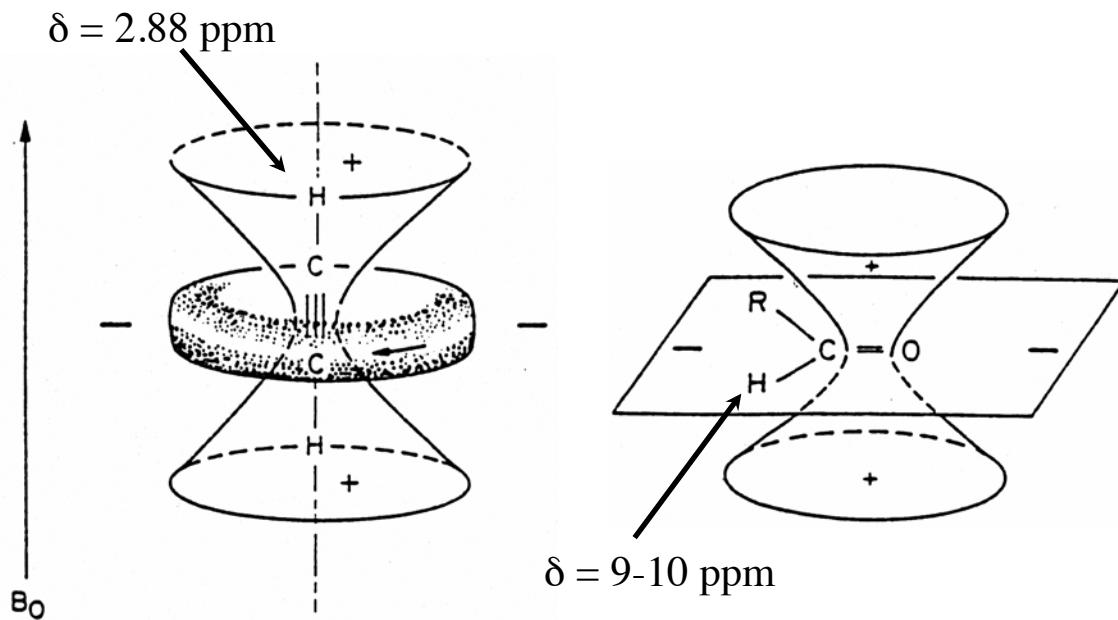


$\delta = 0.23 \text{ ppm}$	$\text{H}-\text{CH}_3$
$\delta = 0.80 \text{ ppm}$	$\text{H}-\text{CH}_2-\text{CH}_3$
$\delta = 0.91 \text{ ppm}$	$\text{H}-\text{CH}_2-\text{CH}_2-\text{CH}_3$

# Neighboring Group Effects on Shielding

- Magnetic anisotropy effects:
  - chemical bonds are often magnetically anisotropic
  - thus, magnetic moments in these bonds induced by the  $B_0$  field are not necessarily equal for different orientations of the bond
  - so, shielding of neighboring groups is dependent on their location with respect to the bond

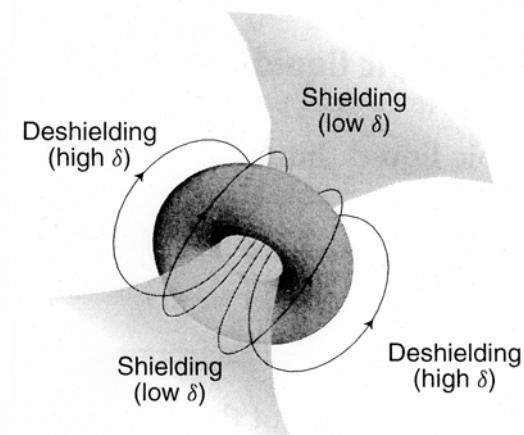
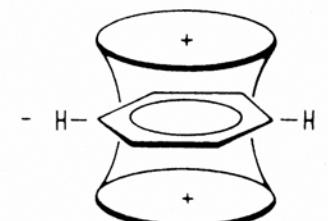
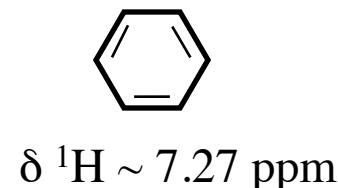
Ethane:	1.96 ppm
Ethylene	5.84 ppm
Acetylene	2.88 ppm



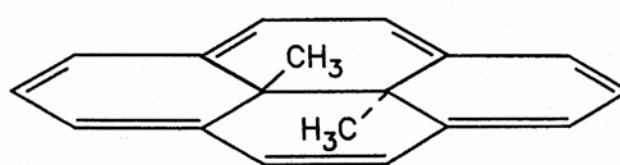
# Neighboring Group Effects on Shielding

- Magnetic anisotropy effects:

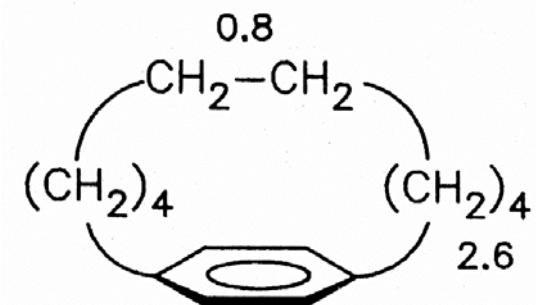
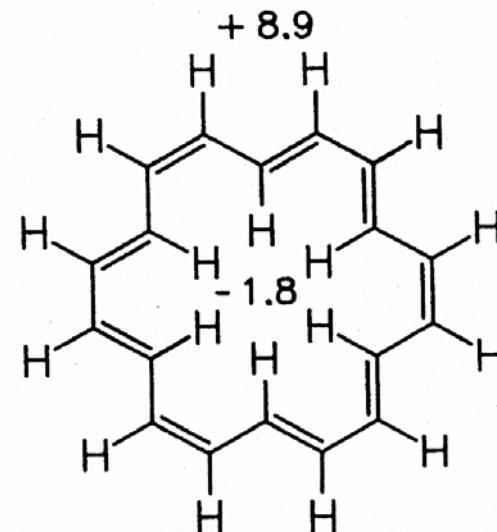
- $\pi$  electrons in ring systems (i.e. benzene) support induced electron currents that differentially shield/deshield neighboring nuclei depending on location (called *ring-current shift*)



$\delta(\text{H}) = 5.7$



$\delta(\text{CH}_3) = -4.25$



# Other Effects

- Isotope Effects:

- $^{12}\text{CHCl}_3$ ,  $\delta(^1\text{H}) = 7.240$  ppm

- $\text{C}_6\text{H}_6$ ,  $\delta(^{13}\text{C}) = 128.53$  ppm

- $^{13}\text{CHCl}_3$ ,  $\delta(^1\text{H}) = 7.237$  ppm

- $\text{C}_6\text{D}_6$ ,  $\delta(^{13}\text{C}) = 128.0$  ppm

- Hydrogen bonding / solvent effects

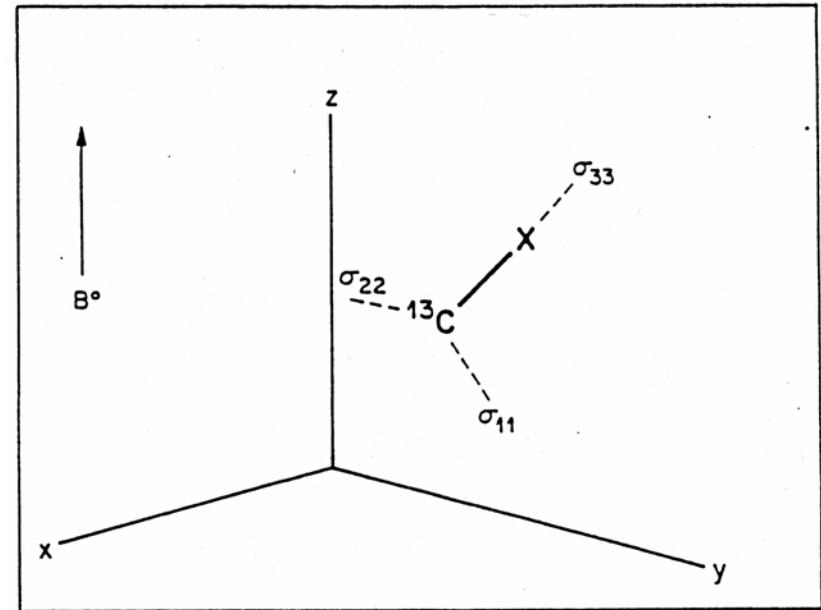
- $^1\text{H}$  chemical shifts of hydroxyl groups are highly variable due to interactions with solvent (are highly temperature and concentration dependent)
- protic solvent, polar vs non-polar solvents: cause chemical shift changes

- Electric field effects

- carbonyl groups, nitro groups

# Chemical Shift Anisotropy

- The electron density distribution around a nucleus (in a molecule) is generally not spherically symmetric
- Therefore, the value of the shielding constant  $\sigma$  is dependent on the orientation of the molecule with respect to  $B_0$
- This can be described by a tensor that relates the molecular orientation to the orientation of  $B_0$



- The average, or isotropic, shielding is defined as the average of the trace elements of the tensor:

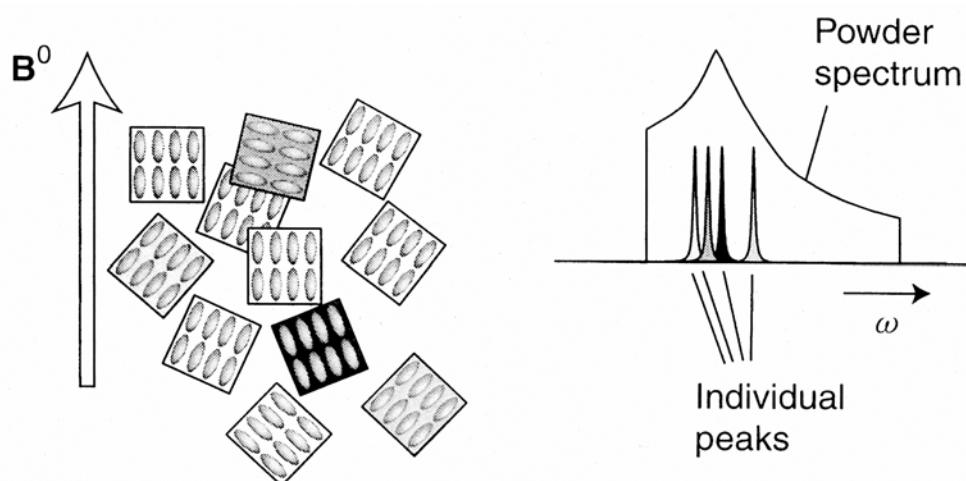
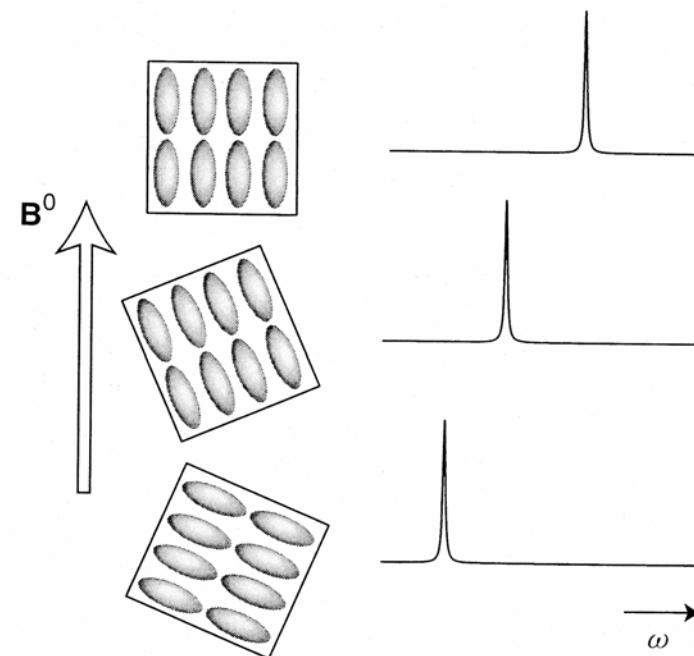
$$\sigma_{iso} = \frac{\sigma_{11} + \sigma_{22} + \sigma_{33}}{3}$$

- For molecules in solution, each molecule samples all possible orientations rapidly, so  $\sigma = \sigma_{iso}$

- Above: orientation defines effective field. If  $\sigma_{33}$ , for instance, is aligned with  $B_0$ , then  $B_0 = (1-\sigma_{33})B_0$

# Chemical Shift Anisotropy

- In a crystal (right), if all molecules are oriented in the same direction, the observed chemical shift will depend simply on the orientation of the crystal in  $B_0$

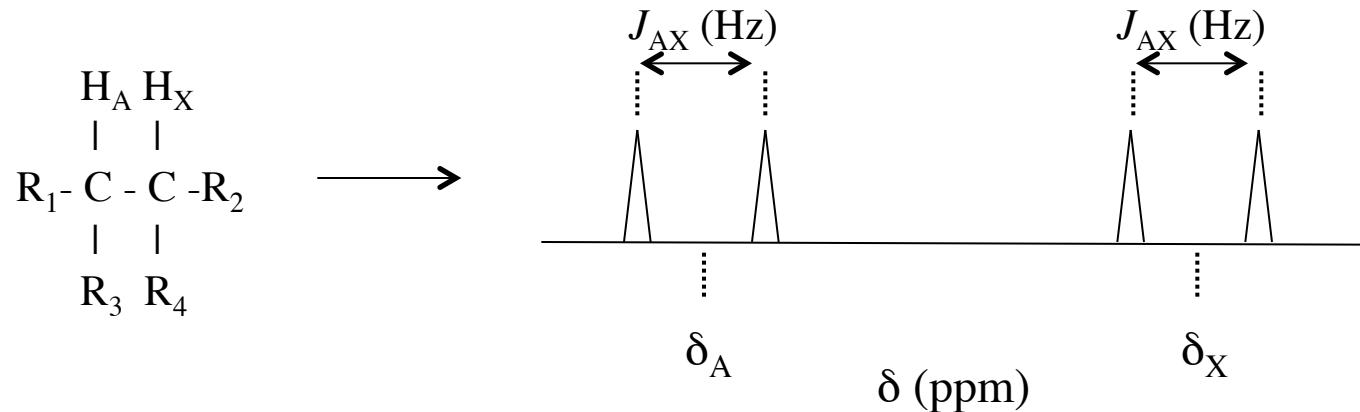


- In a powder (left), each crystallite contributes a peak at its own chemical shift, and the observed peak is a superposition of all peaks (and thus is very broad)



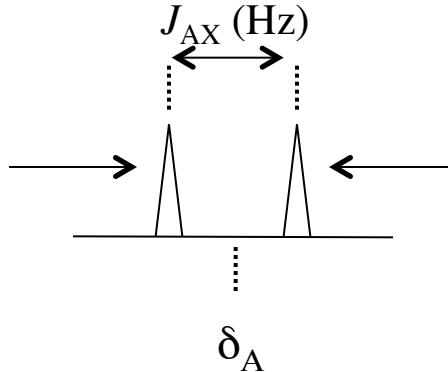
# Through-Bond Coupling

- Frequency-domain NMR signals are often split into two or more component peaks due to the effect of directly bonded nuclei or neighboring nuclei 2 or 3 bonds away



- This is due to ***through-bond coupling***, also known as ***scalar coupling***, ***indirect coupling***, ***spin-spin coupling***, or ***J coupling***.....
- In the example above, if we consider spin A (for instance) in an ensemble of molecules, in some of the molecules spin X will be in the  $\alpha$  ( $m = +1/2$ ) state, and in others spin X will be in the  $\beta$  ( $m = -1/2$ ) state

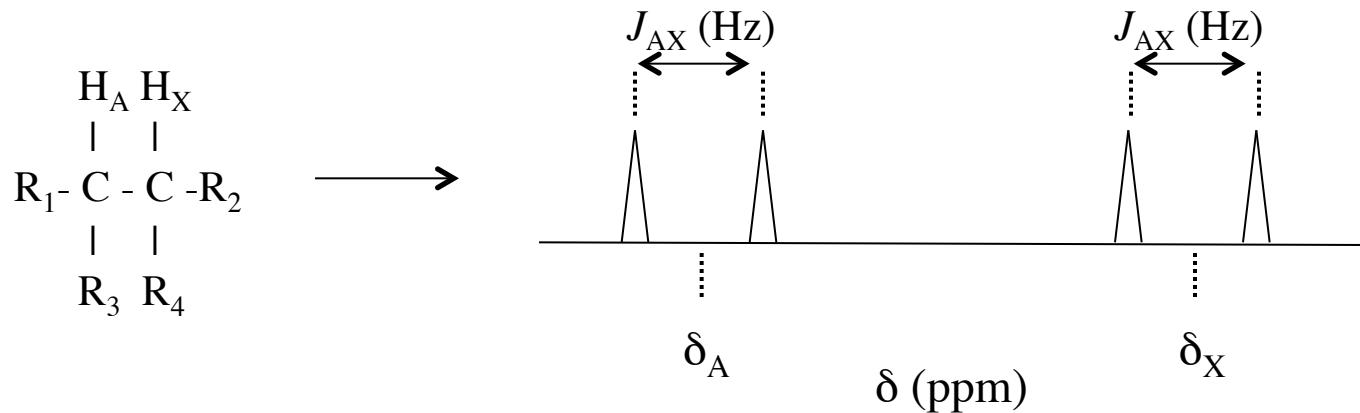
for spin X,  $m = +1/2$ ,  
which augments  $B_0$ ,  
and *deshields* spin A



for spin X,  $m = -1/2$ ,  
which opposes  $B_0$ ,  
and *shields* spin A

# Through-Bond Coupling

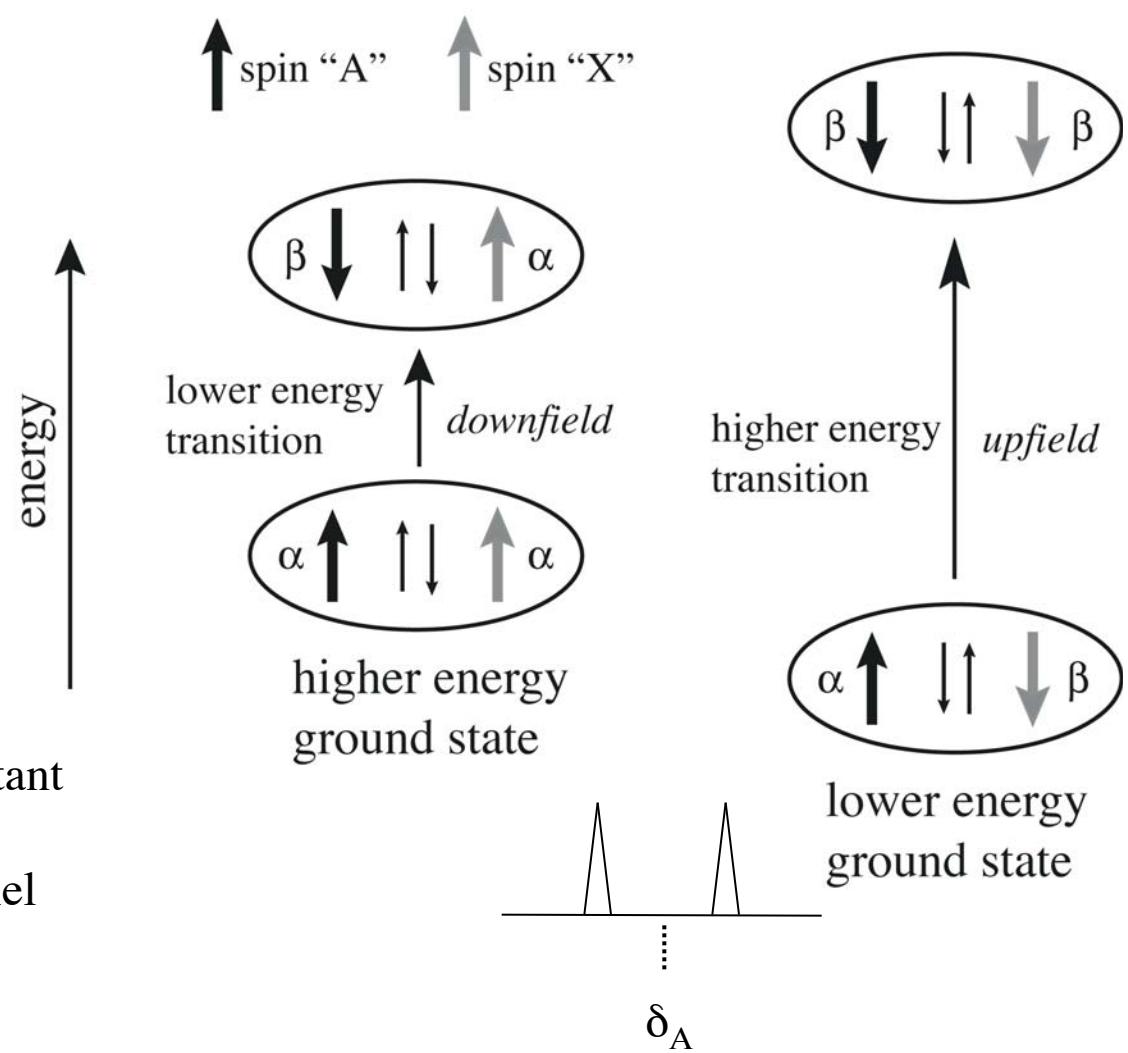
- The splitting between the components of the signal is called the *coupling constant*, or  $J$



- $J$  is usually given a superscript and subscript, the former describing the number of bonds separating the coupled nuclei, the latter designating the two atoms involved
  - for the example above,  $^3J_{\text{AX}}$  or  $^3J_{\text{H}_\text{A}\text{H}_\text{X}}$
- $J$  is *always* measured in Hz
- $J$  (in Hz) is *independent* of the magnitude of  $B_0$
- The value of  $J$  measured from the splitting of the signal from one of the coupled nuclei is always the same as the value measured at the signal from the other coupled nucleus
- The chemical shift of the nucleus is the center of the multiplet

# Mechanism of Through-Bond Coupling: Dirac Model

- Coupling occurs through bonds, i.e. via interactions with bonding electron spins
- Consider the one-bond coupling ( ${}^1J_{AX}$ ), for instance  ${}^1\text{H}-{}^{13}\text{C}$ 
  - Energetically preferred states are normally those where nuclear and electron spins are *antiparallel*
  - Electron spins of bonding pair are antiparallel (*Pauli exclusion principle*)
  - Thus, for low energy states, if nuclear spins are *antiparallel*, then couplings are said to be **positive**
  - In other words, the coupling constant is positive if it stabilizes the state where nuclear spins are antiparallel
  - Normally, for  $\gamma > 0$ ,  ${}^1J$  is positive



# Mechanism of Through-Bond Coupling: Dirac Model

- Coupling occurs through bonds, i.e. via interactions with bonding electron spins
- Consider the two-bond coupling ( $^2J_{AX}$ ),  
for instance  $^1\text{H}-^{12}\text{C}-^1\text{H}$  (geminal  $^1\text{H}-^1\text{H}$  coupling)

-Energetically preferred states are normally those where nuclear and electron spins are *antiparallel*

-Electron spins of bonding pair are antiparallel (*Pauli exclusion principle*)

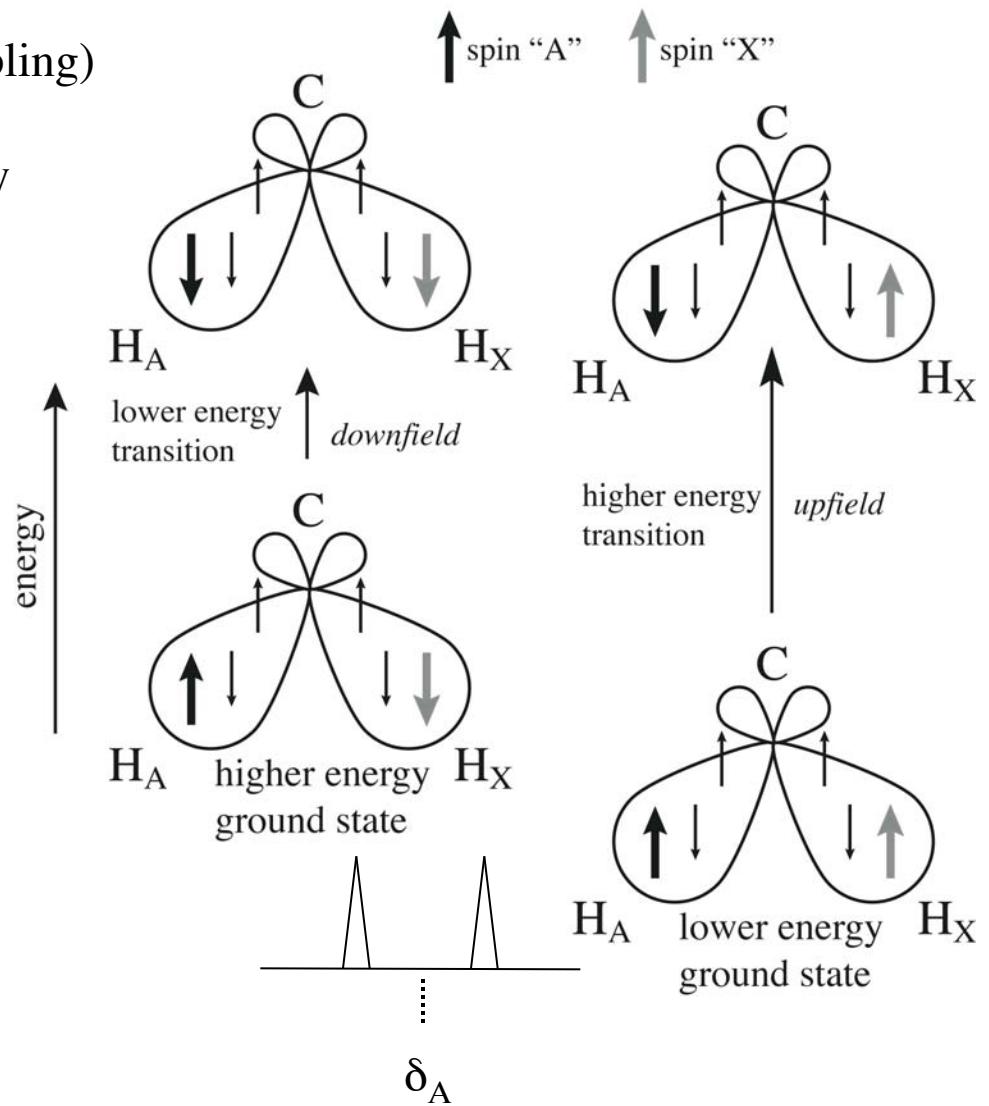
-The energetically preferred state for the bonding electrons on the carbon atom is when these are parallel (*Hund's rule*)

-Thus, for low energy states, if nuclear spins are *parallel*, then couplings are said to be ***negative***

-In other words, the coupling constant is negative if coupling stabilizes the state where nuclear spins are parallel

-Normally, for  $\gamma > 0$ ,  $^2J$  is negative

$\alpha \rightarrow \beta$  transitions for spin "A"



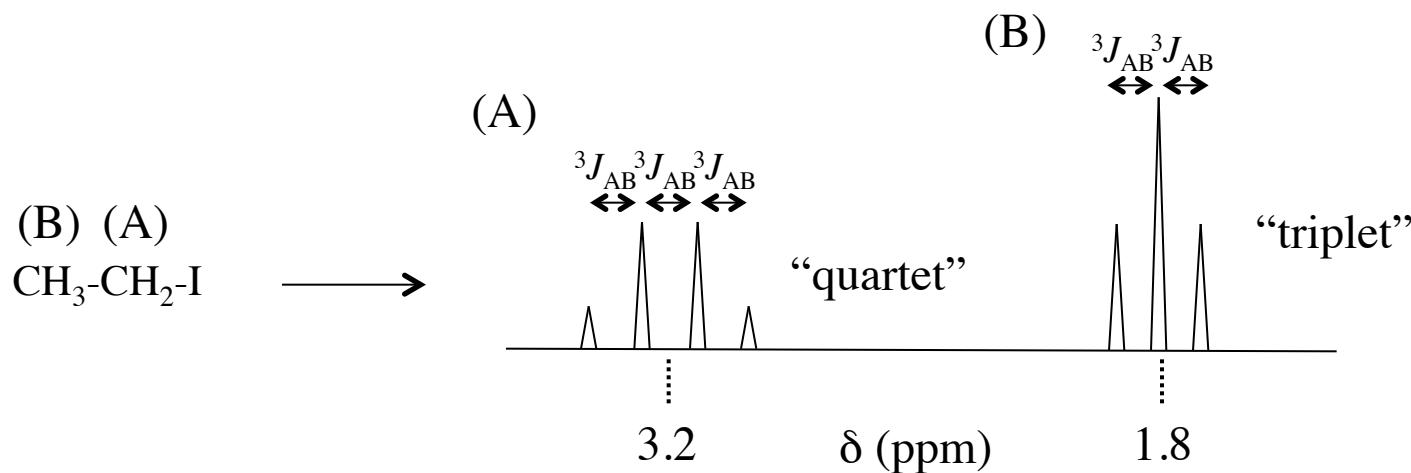
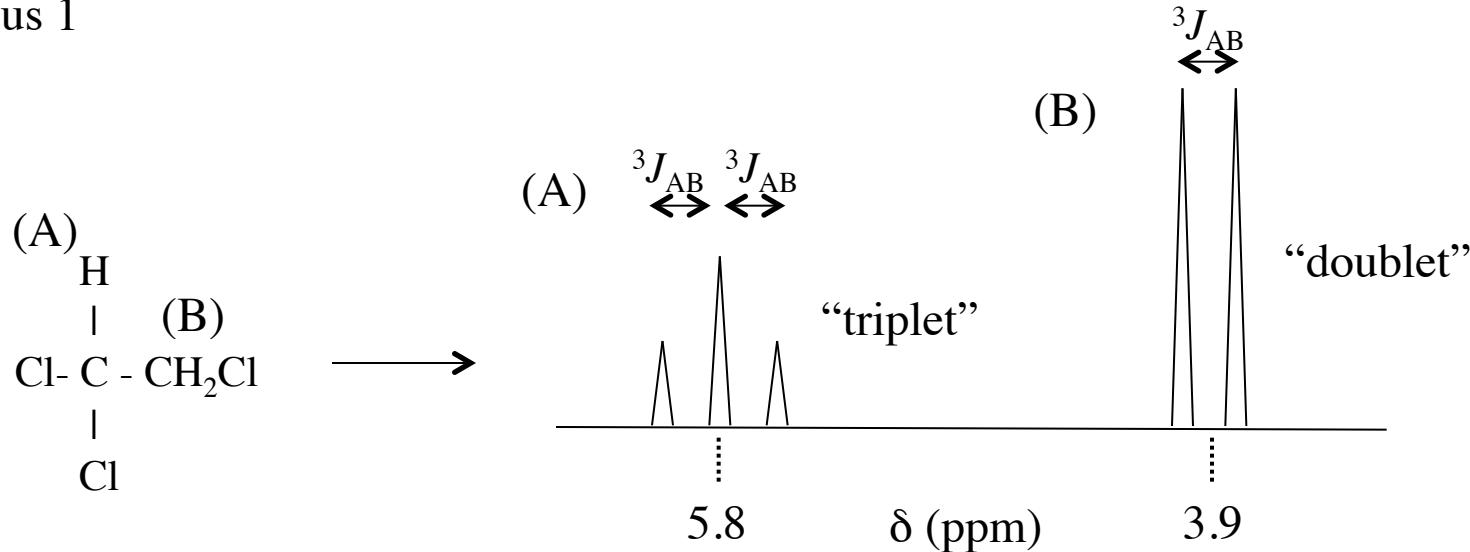
# Mechanism of Through-Bond Coupling

- Fermi contact: interaction of nuclear spins via bonding electrons
  - depends on electron density at the pair of nuclei
  - depends on the “s” electrons, or s character of the bond(s) between the nuclei
  - electron spins must be correlated, i.e. there must be a bond
- Dependence on “s” character
  - consider one bond  $^{13}\text{C}$ - $^1\text{H}$  couplings:

$\text{CH}_3\text{-CH}_3$	$s, sp^3$	$1 \times 1/4 = 1/4$	125 Hz
$\text{CH}_2=\text{CH}_2$	$s, sp^2$	$1 \times 1/3 = 1/3$	156 Hz
$\text{C}_6\text{H}_6$	$s, sp^2$	$1 \times 1/3 = 1/3$	158 Hz
$\text{HC}\equiv\text{CH}$	$s, sp$	$1 \times 1/2 = 1/2$	249 Hz

# Spin-Spin Splitting: $n+1$ Rule

- Multiplet splitting patterns are determined by the number of nuclei ( $n$ ) doing the splitting plus 1



# Spin-Spin Splitting: Intensities and Pascal's Triangle

- The relative intensities of the multiplet components in a signal split by spin-spin coupling can be obtained from Pascal's triangle

singlet	1
doublet	1 1
triplet	1 2 1
quartet	1 3 3 1
quintet	1 4 6 4 1
sextet	1 5 10 10 5 1
septet	1 6 15 20 15 6 1

relative intensities (in the spectrum)

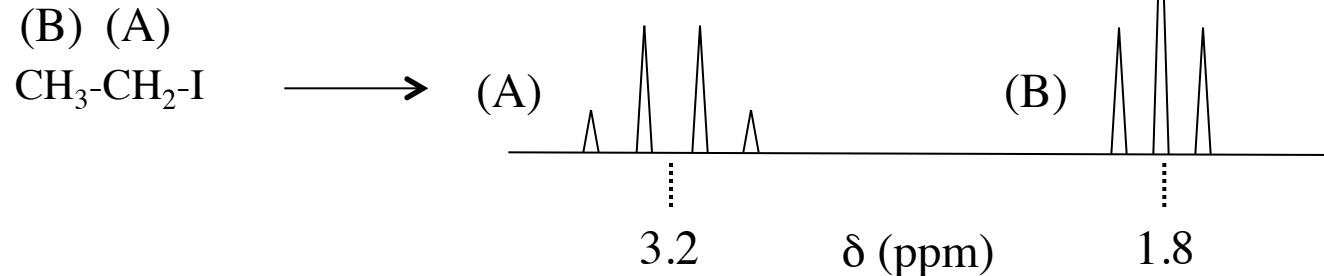
1 3 3 1

3 6 3

relative intensities (in the multiplet)

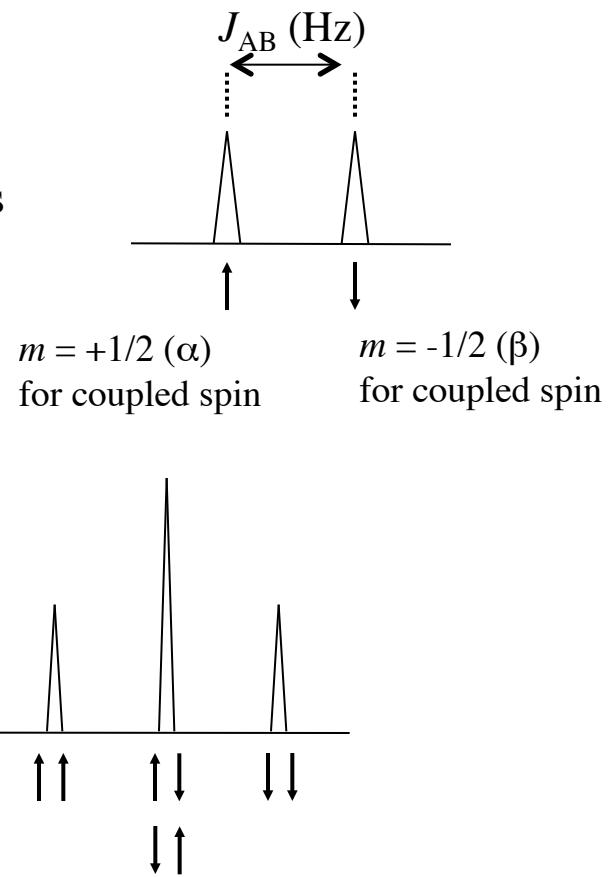
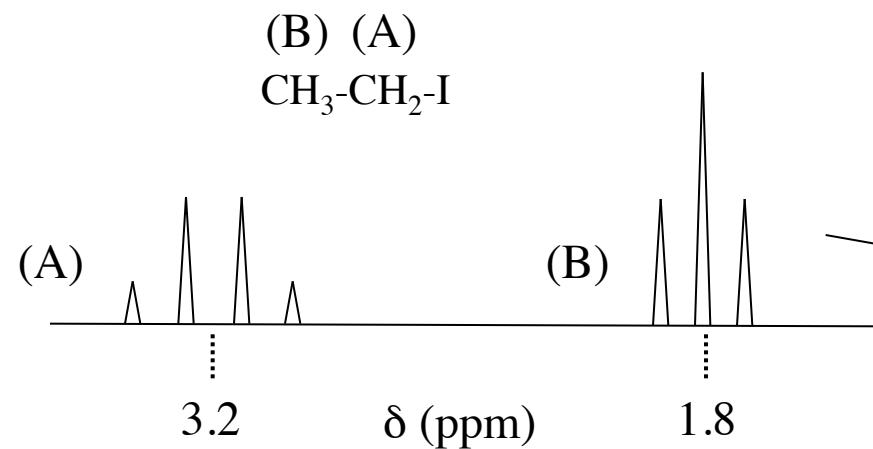
1 3 3 1

1 2 1



# Spin-Spin Splitting: Intensities and Pascal's Triangle

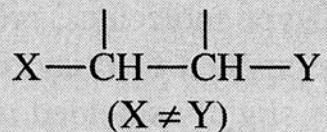
- For a doublet, we saw that there are two peaks, because the coupled spin can be in either the  $\alpha$  or  $\beta$  state
- The *peak heights are equal* because, for the coupled spin, there are essentially equal numbers of spins in  $\alpha$  and  $\beta$  states



- For a triplet, there are three peaks, because the two coupled spins can both be in the  $\alpha$  state, they can both be in the  $\beta$  state, or one can be  $\alpha$  and the other  $\beta$
- The *relative peak heights are 1:2:1* because there are 2 ways that one spin can be  $\alpha$  and the other  $\beta$ , and only one way both can be  $\alpha$  and only one way both can be  $\beta$

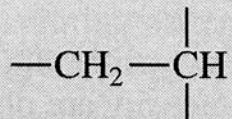
# Common Splitting Patterns

II



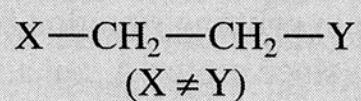
II

II



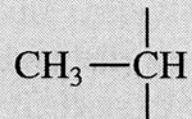
III

III



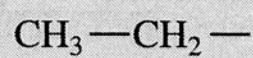
III

II



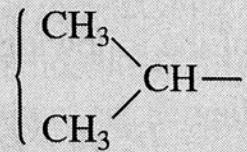
III

III



III

II

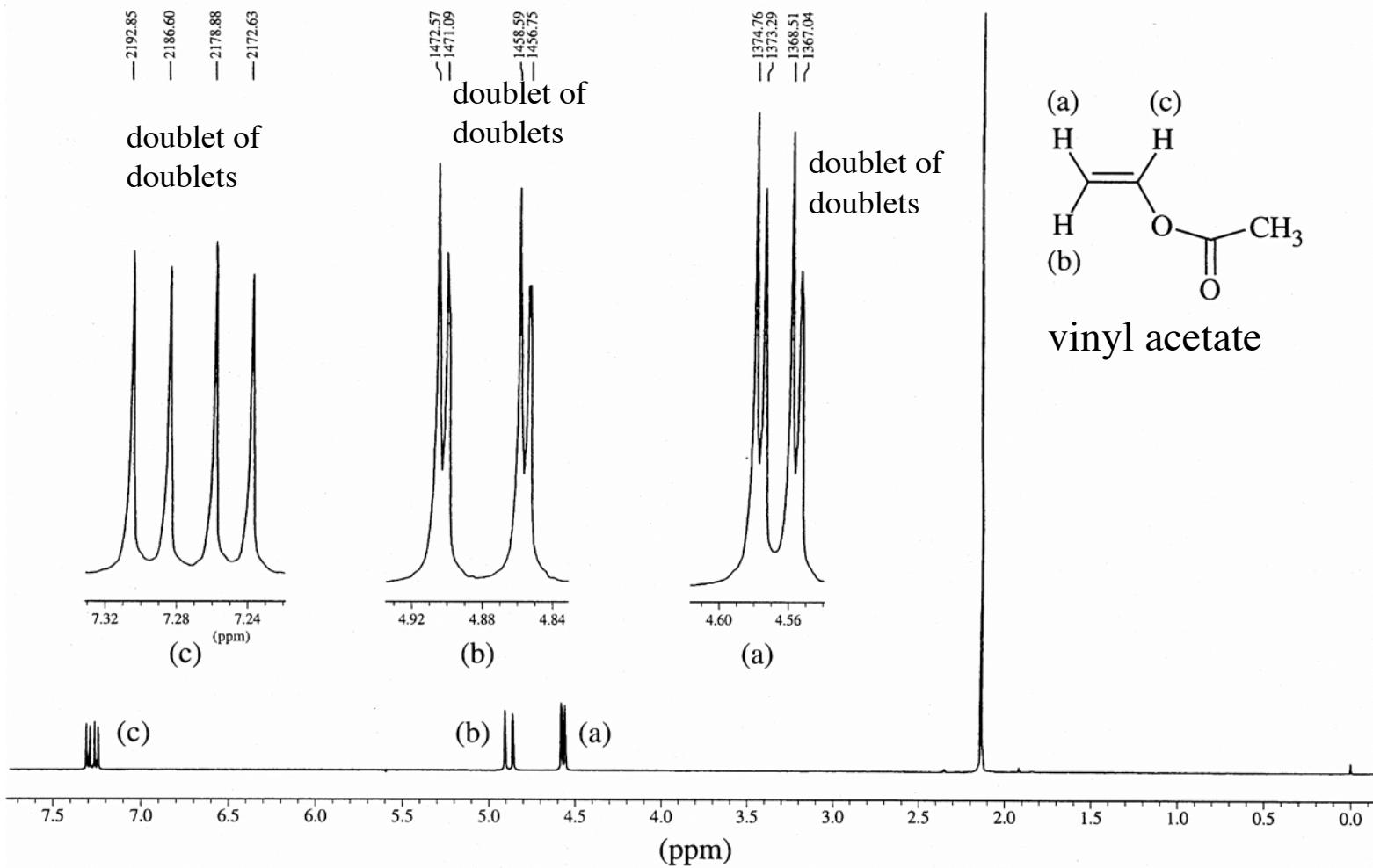


IV

- Some commonly observed splitting patterns

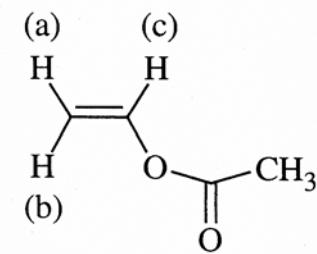
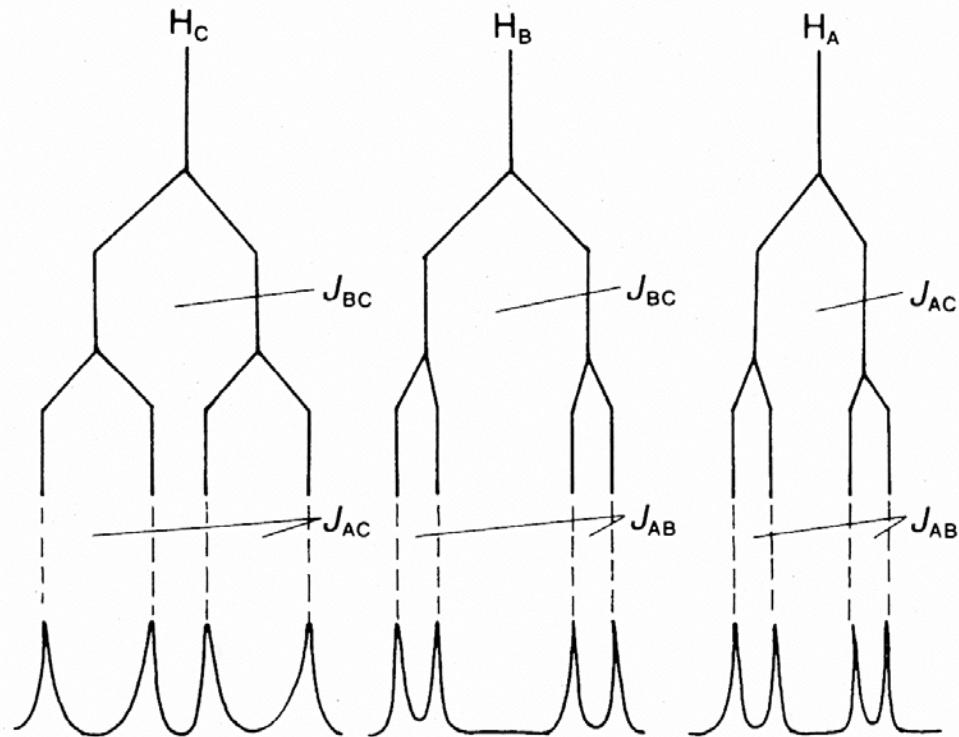
# Multiple/Complex Splittings

- Split signals can be split again by other spins
  - in vinyl acetate, for instance, the signal from “c” is split into a doublet by “b”, and this doublet is split into a doublet of doublets by “a”
  - likewise, “b” is split by “a” and “c”, and “a” is split by “b” and “c”



# Multiple/Complex Splittings

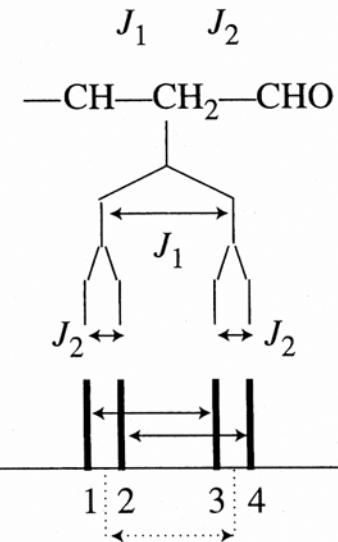
- The coupling constant between any two spins will be observed in the signal from each of the spins
- Observation of a coupling in more than one signal identifies coupled spins



vinyl acetate

# Extracting the Coupling Constants

- Analysis and determination of coupling constants

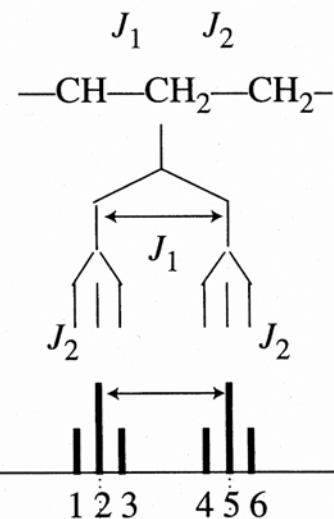


## Doublet of Doublets (dd)

To obtain  $J_1$  measure the difference between lines 1 and 3, or 2 and 4, in Hz.\*

\*Do not try to find the centers of the doublets!

$J_2$  is the spacing between lines 1 and 2, or 3 and 4



## Doublet of Triplets (dt)

To obtain  $J_1$  measure the difference between the most intense lines (2 and 5) in Hz

$J_2$  is the spacing between lines 1 and 2, or 2 and 3, or those in the other triplet.

# Spin Decoupling

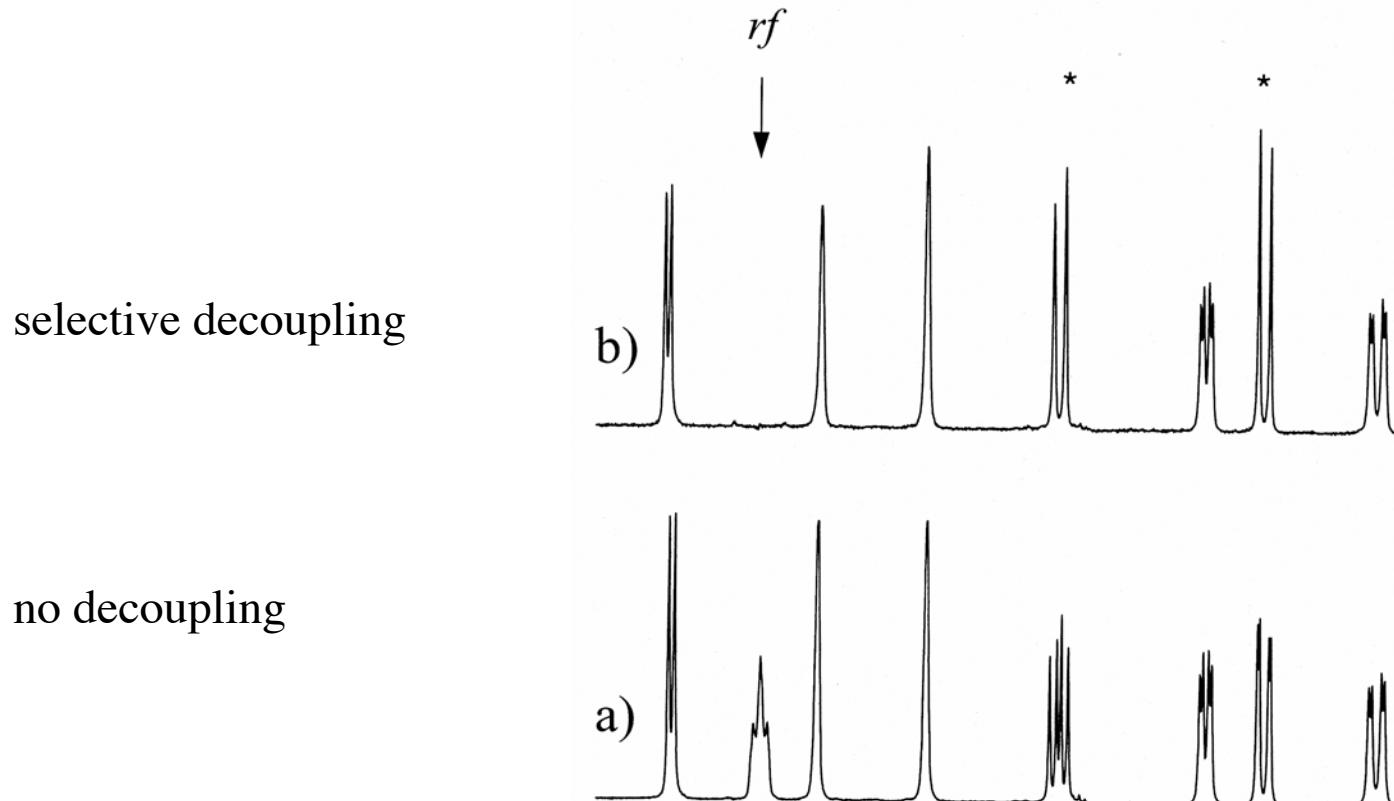
- It is often advantageous to reverse the splitting caused by spin-spin coupling by *spin decoupling*
- Spin decoupling (or just “decoupling) can be used for several reasons
  - to simplify spectra
  - to assist in identification of coupling between nuclei
  - to improve signal-to-noise
- How do we decouple spins from one another?
- Remember, coupling occurs because the transitions of one spin ( $\alpha$  to  $\beta$  or  $\beta$  to  $\alpha$ ) occurs when the other (coupled) spin is in either the  $\alpha$  or  $\beta$  state
  - thus, for one spin to couple to another, the lifetime of the coupled spin in a given state must be long enough for the transitions of the other to occur
  - this lifetime is proportional to  $1/J$

$$\tau_1 = 1/J$$

- if this lifetime is significantly shortened, the coupling will not occur, and the splitting of the signal will not occur

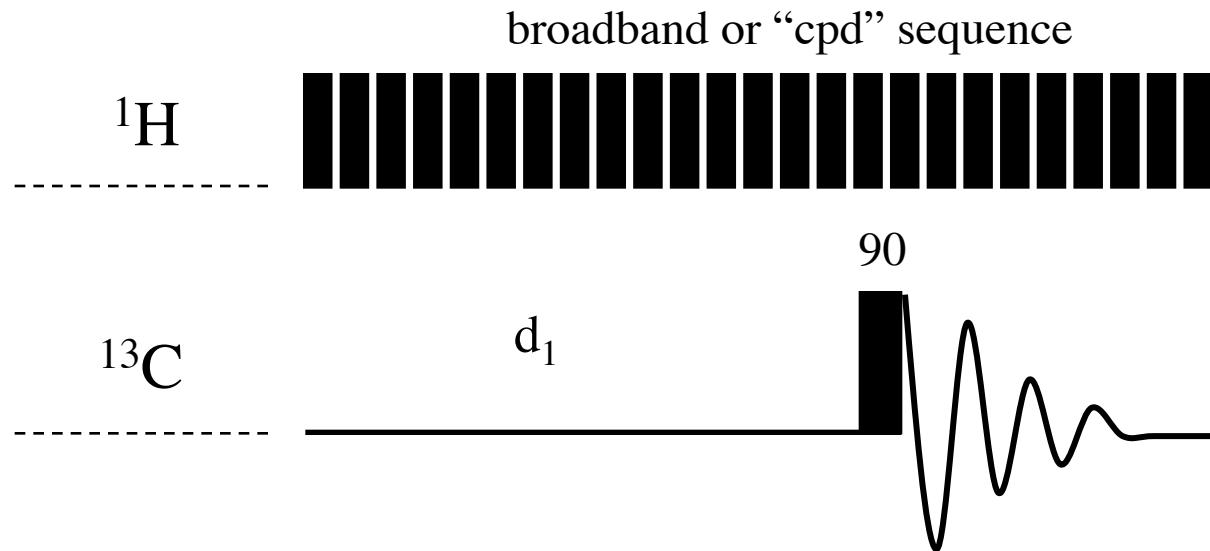
# Selective Spin Decoupling

- Selective excitation of one signal (spin) in an NMR spectrum leads to simplification of the spin-spin splitting patterns to which the spin is coupled
  - selective excitation is accomplished by a very long pulse centered at the Larmor frequency of the signal of interest
  - the long pulse causes rapid  $\alpha \rightarrow \beta$  and  $\beta \rightarrow \alpha$  transitions such that the lifetime of any particular spin in any given state is short (i.e.  $\tau_1 < J$ )



# Broad-Band Decoupling

- Often it is useful to remove *all* coupling of a given type
- For instance, it is often useful in  $^{13}\text{C}$  NMR/spectra to remove all coupling from  $^1\text{H}$
- This is accomplished by *broad-band decoupling* methods (also called *composite pulse decoupling* or “cpd”)
- In this case, a large series of pulses is rapidly applied to the nuclei to be decoupled so that all spins undergo rapid  $\alpha \rightarrow \beta$  and  $\beta \rightarrow \alpha$  transitions



# Broad-Band Decoupling

- Substantial signal-to-noise gains can accompany broad-band decoupling
  - collapse of the multiplet into a singlet
  - nuclear Overhauser effect

