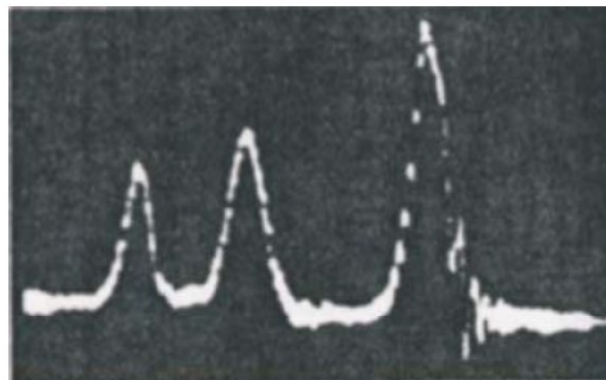


# History of NMR: how and when Scalar Coupling was discovered?

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The three peaks of ethanol observed by the Packard group in 1951.

Here is a photo of the first spectrum captured off an oscilloscope display:



The operating field of the magnet was 7,600 gauss. That gave a proton resonance at 32.4 MHz. It would be a few generations of instruments before plotters were integrated...

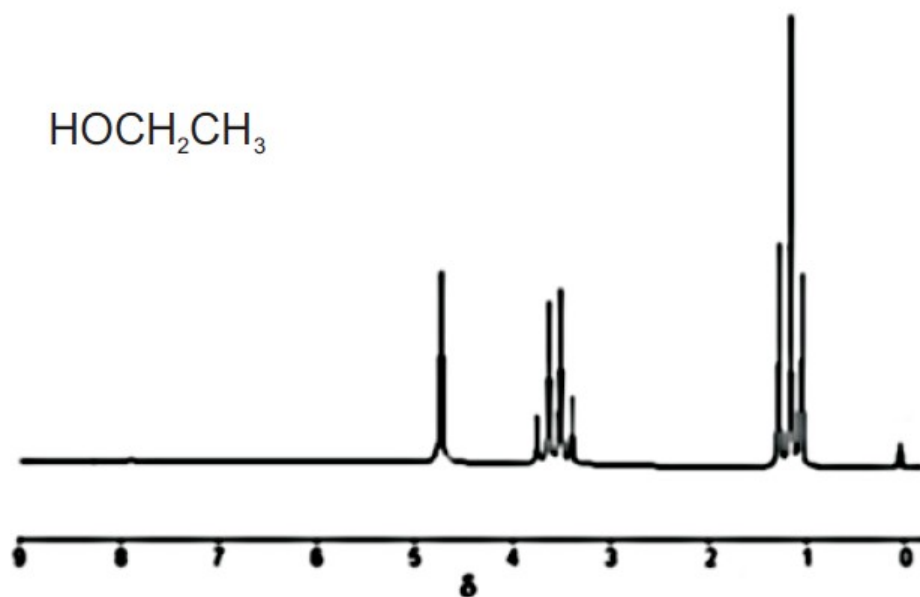
An early custom-built 30 MHz instrument:



# History of NMR: how and when Scalar Coupling was discovered?

1951 Herbert Gutowski et. al. find that NMR resonances split due to interactions between nuclei called **Spin-spin coupling**.

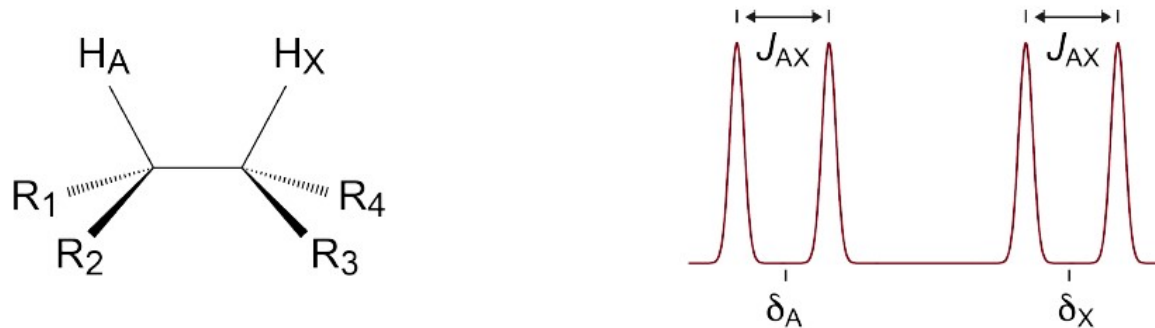
Spin-spin coupling is transferred through bonds.



For proton and other spin =  $\frac{1}{2}$  nuclei, the number of lines in a multiplet is equal to the number of *equivalent* neighboring spins plus one ( $n + 1$ ).

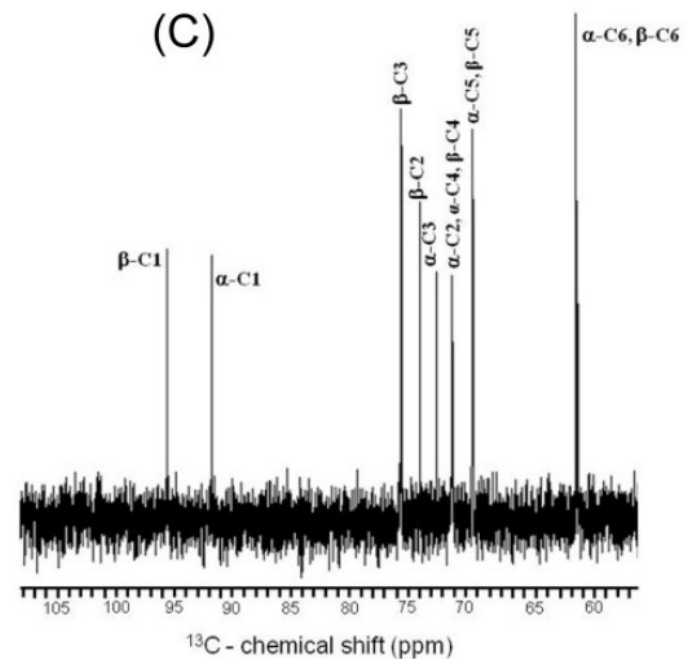
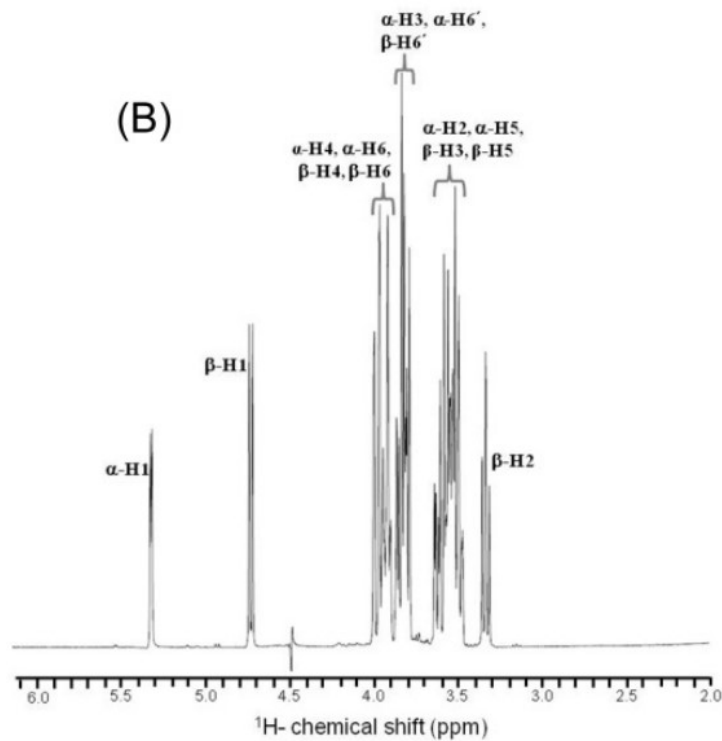
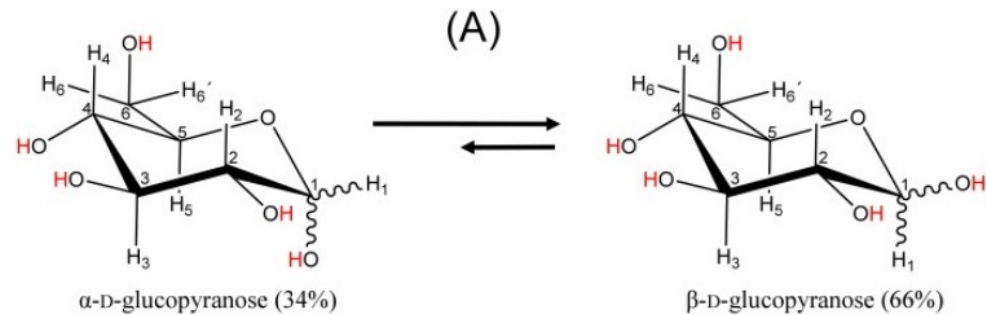
# Scalar Coupling ( $J$ ) - spin-spin coupling

- Splitting of NMR signals due to through-bond interactions between nuclei is called *scalar coupling* (or *J coupling* or *through-bond coupling*)
- Scalar couplings are important:
  - the magnitudes of the splittings and splitting patterns contain useful structural information
  - they are central to magnetization transfer (COSY, HSQC)
  - readily measured (peak separation, 2D  $J$  resolved, intensity)
- Below, scalar coupling between  $H_A$  and  $H_X$  results in splitting of the signal from each into two peaks (doublets)

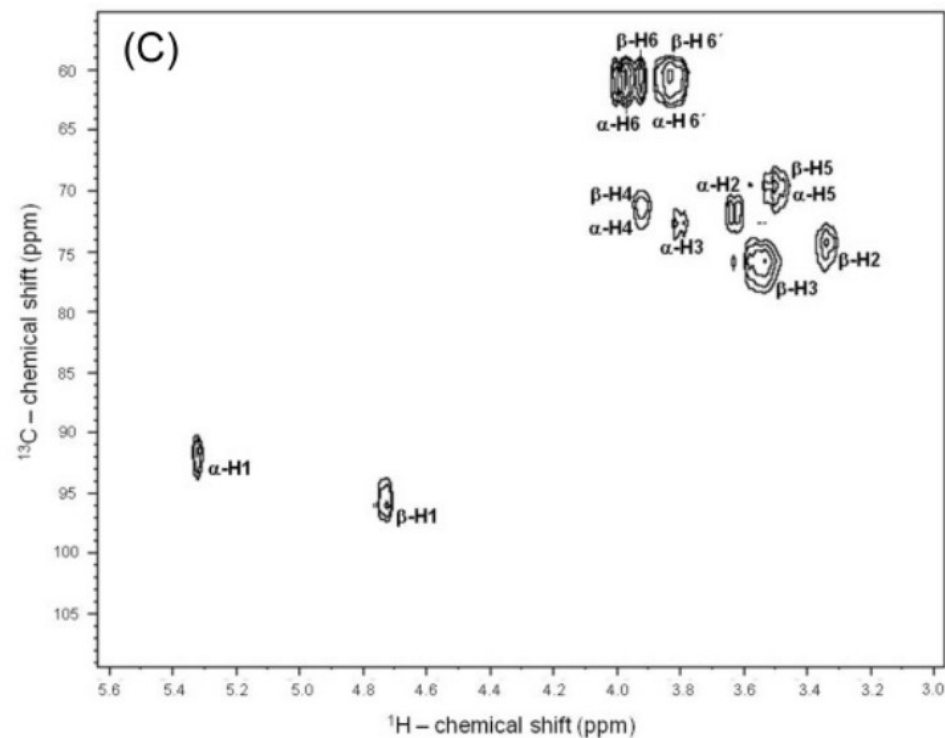
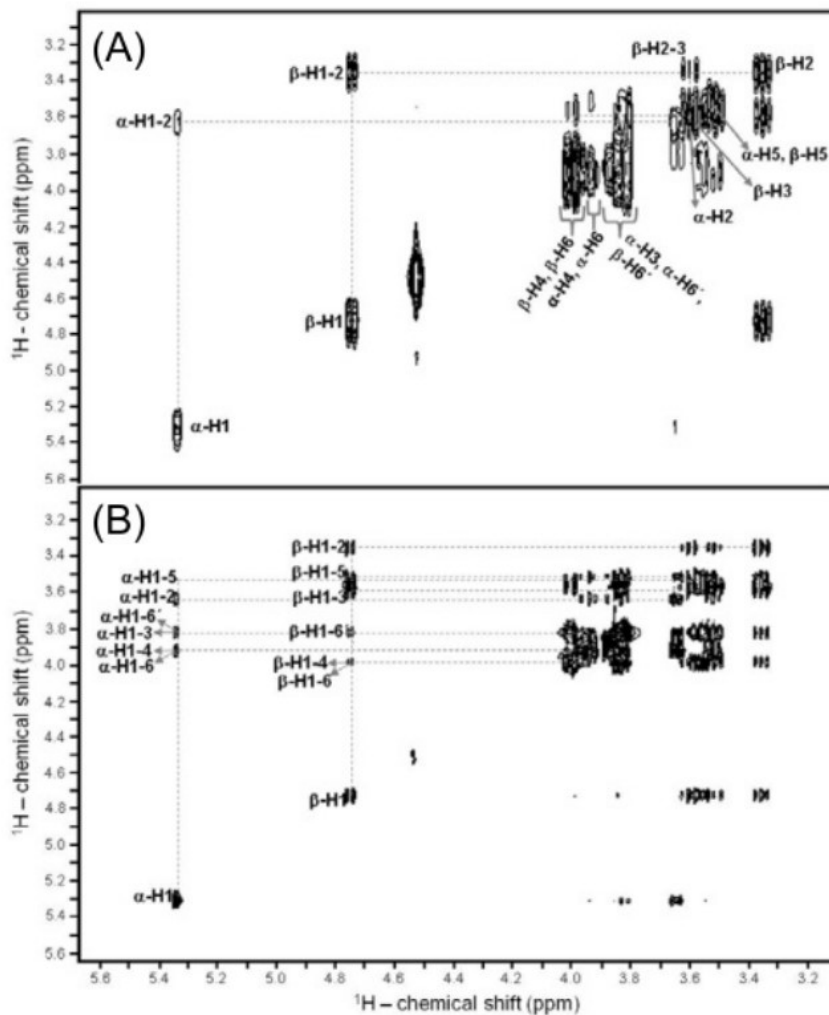


# 1D $^1\text{H}$ NMR spectrum of glucose:

$$^3J_{\alpha\text{H}1,\text{H}2} = 3\text{-}4 \text{ Hz and } ^3J_{\beta\text{H}1,\text{H}2} = 7\text{-}8 \text{ Hz}$$

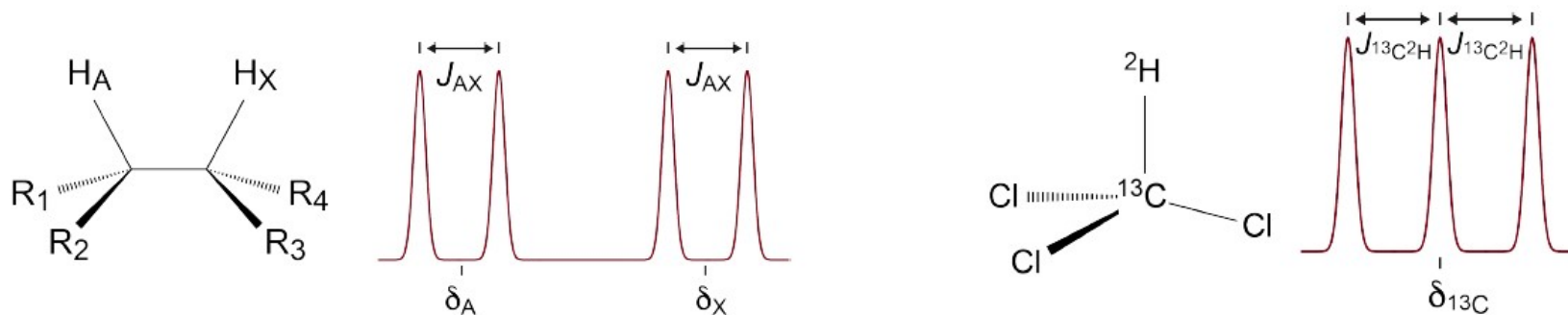


# 2D NMR cross-peaks rely on $J$



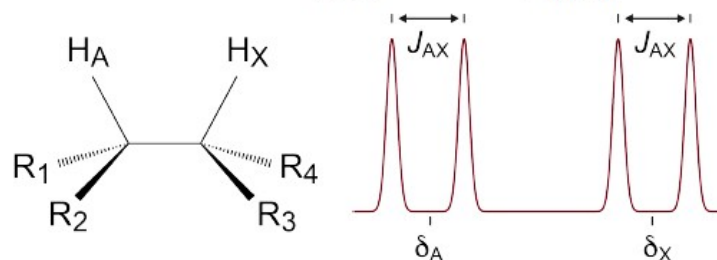
# Properties of Scalar Coupling

- Scalar coupling is mediated by bonding electrons
- The energy of a nucleus coupled to another will change slightly depending on the spin state of the coupled nucleus
- The energy difference results in small frequency changes
- Below (left), each  $^1\text{H}$  nucleus ( $I = 1/2$ ) is split into two peaks, depending on whether the coupled  $^1\text{H}$  nucleus is in the  $m=+1/2$  ( $\alpha$ ) or  $m=-1/2$  ( $\beta$ ) state
- Below (right), the  $^{13}\text{C}$  nucleus is split into three peaks, depending on whether the coupled  $^2\text{H}$  nucleus ( $I = 1$ ) is in the  $m=1$ ,  $m=0$ , or  $m=-1$  state



# Properties of Scalar Coupling

- The magnitude of the splitting is called the *coupling constant*, and is typically symbolized by  $J$
- $J$  is measured in Hz, and is magnetic field strength independent
- $J$  measured from the splitting of the signal from one of the coupled nuclei is 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
- $J$  is often given a superscript and subscript, the former indicating the number of bonds separating the coupled nuclei, the latter designating the atoms involved (i.e.  ${}^3J_{AX}$ , or  ${}^3J_{HAHX}$ )
- The magnitude of  $J$  depends on structural factors and the number of intervening chemical bonds:



$${}^1J_{H,H} \approx 275 \text{ Hz}$$

$${}^2J_{H,H} \approx 5\text{-}10 \text{ Hz typically}$$

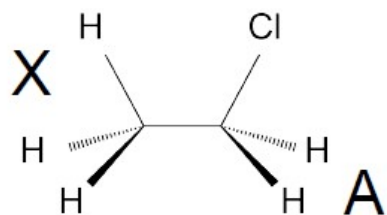
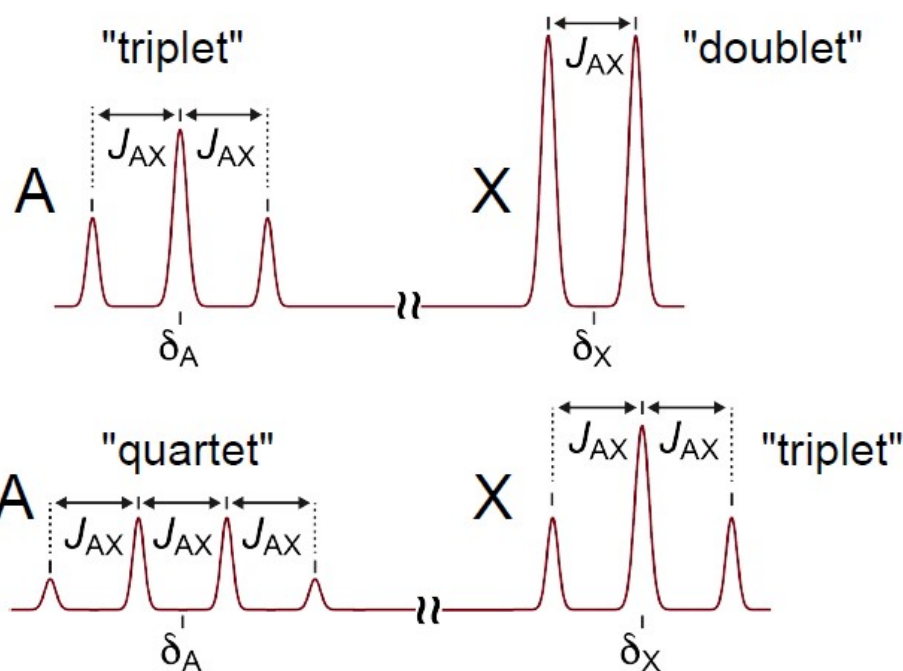
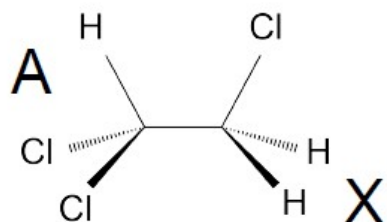
$${}^3J_{H,H} \approx 5\text{-}20 \text{ Hz typically}$$

$${}^4J_{H,H} \text{ often unobservable, but as large as } 7 \text{ Hz}$$

$${}^5J_{H,H} \approx \text{usually unobservable}$$

# Properties of Scalar Coupling

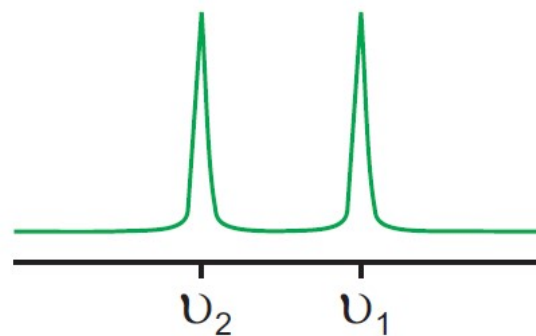
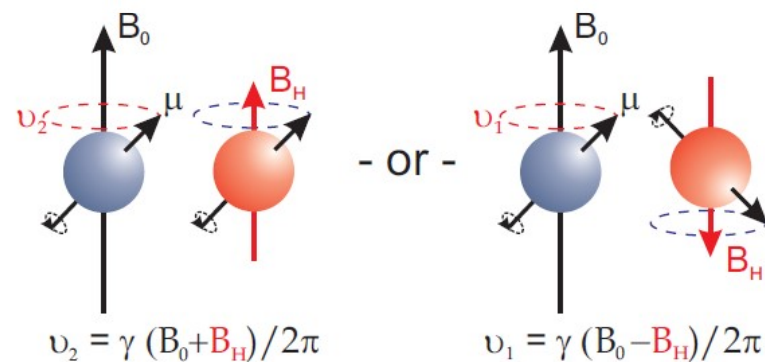
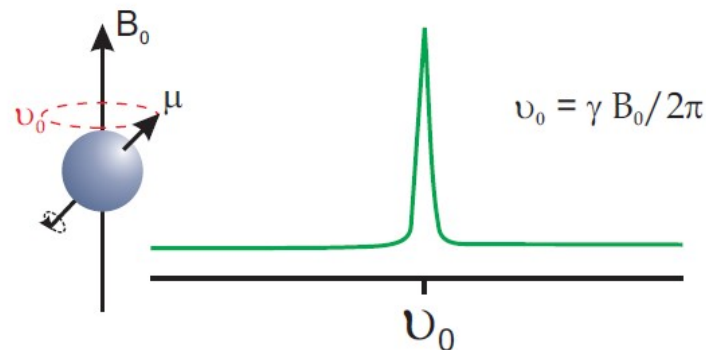
- The multiplet splitting pattern of a signal split by another nucleus (or group of equivalent nuclei) depends on  $n$ , the number of nuclei doing the splitting, and  $l$
- The multiplicity (number of peaks in the multiplet signal) is equal to  $2nl+1$ . For spin  $\frac{1}{2}$ ,  $l = \frac{1}{2}$ , and this reduces to  $n+1$ .





# Origin of Scalar Coupling

The simplistic explanation for scalar coupling is that the neighboring spin's magnetic moment acts to either add to or subtract from the main field. The resulting resonance frequency changes due to the change in the effective field at the nucleus. For spin =  $1/2$  nuclei there are two possible orientations for the magnetic moment and the result is two possible frequencies.

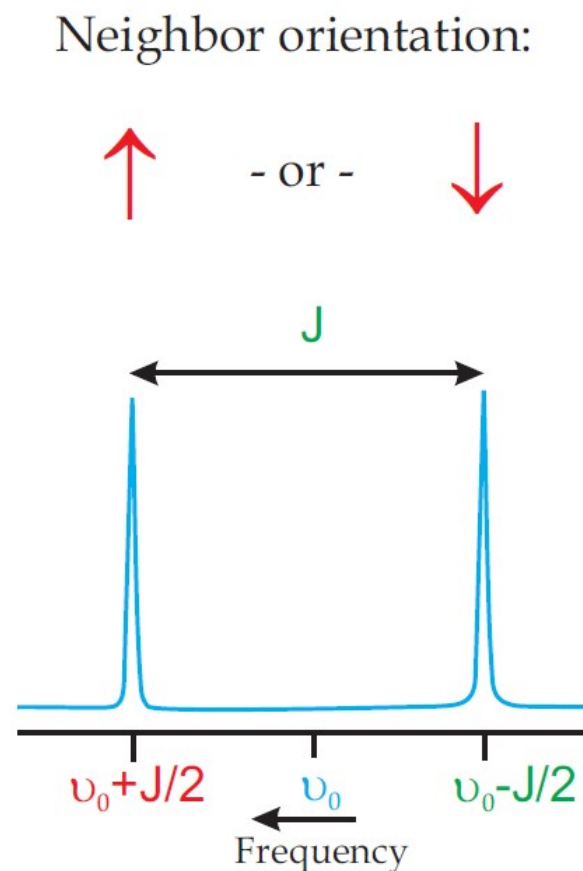


# Origin of Scalar Coupling

The number of lines for a resonance depends on the number of *equivalent* neighbors. The intensity of each line is the result of the statistical probability of the possible orientation of the magnetic moments of the neighboring spins.

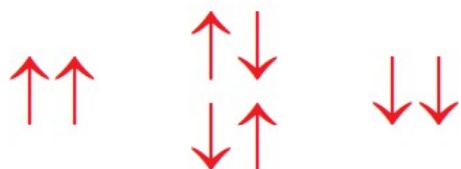
For the case of a single (spin =  $\frac{1}{2}$ ) neighbor there are two orientations that the neighbor could be in. The population of each state is essentially equal. (We will see why this is true in a later chapter).

The spacing between the lines is called the coupling constant ( $J$ ) and is measured in Hz.

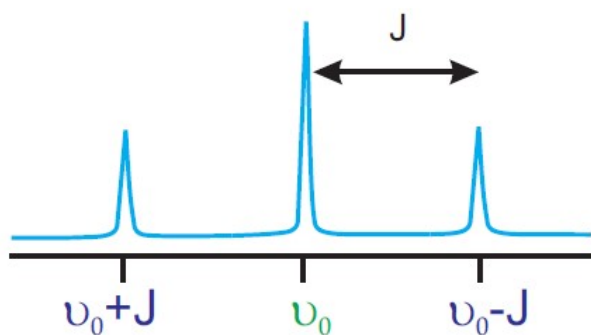


# Splitting pattern!

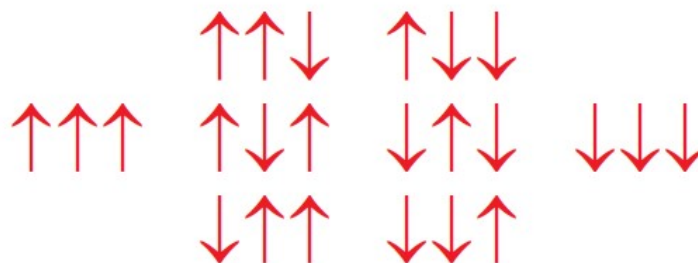
For two equivalent  
Neighboring spins



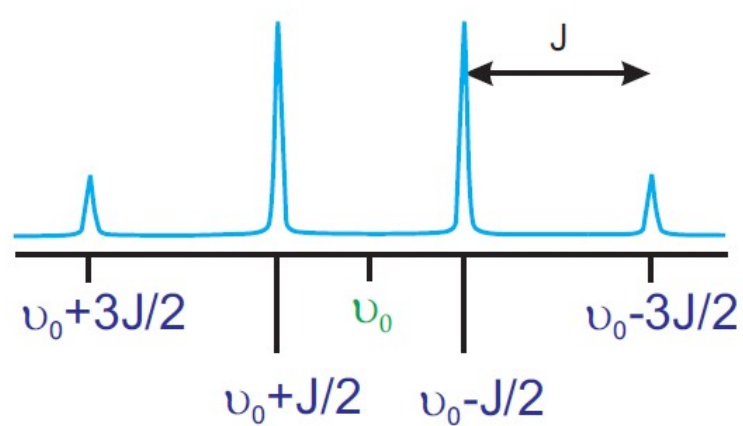
1 : 2 : 1



For three equivalent  
Neighboring spins

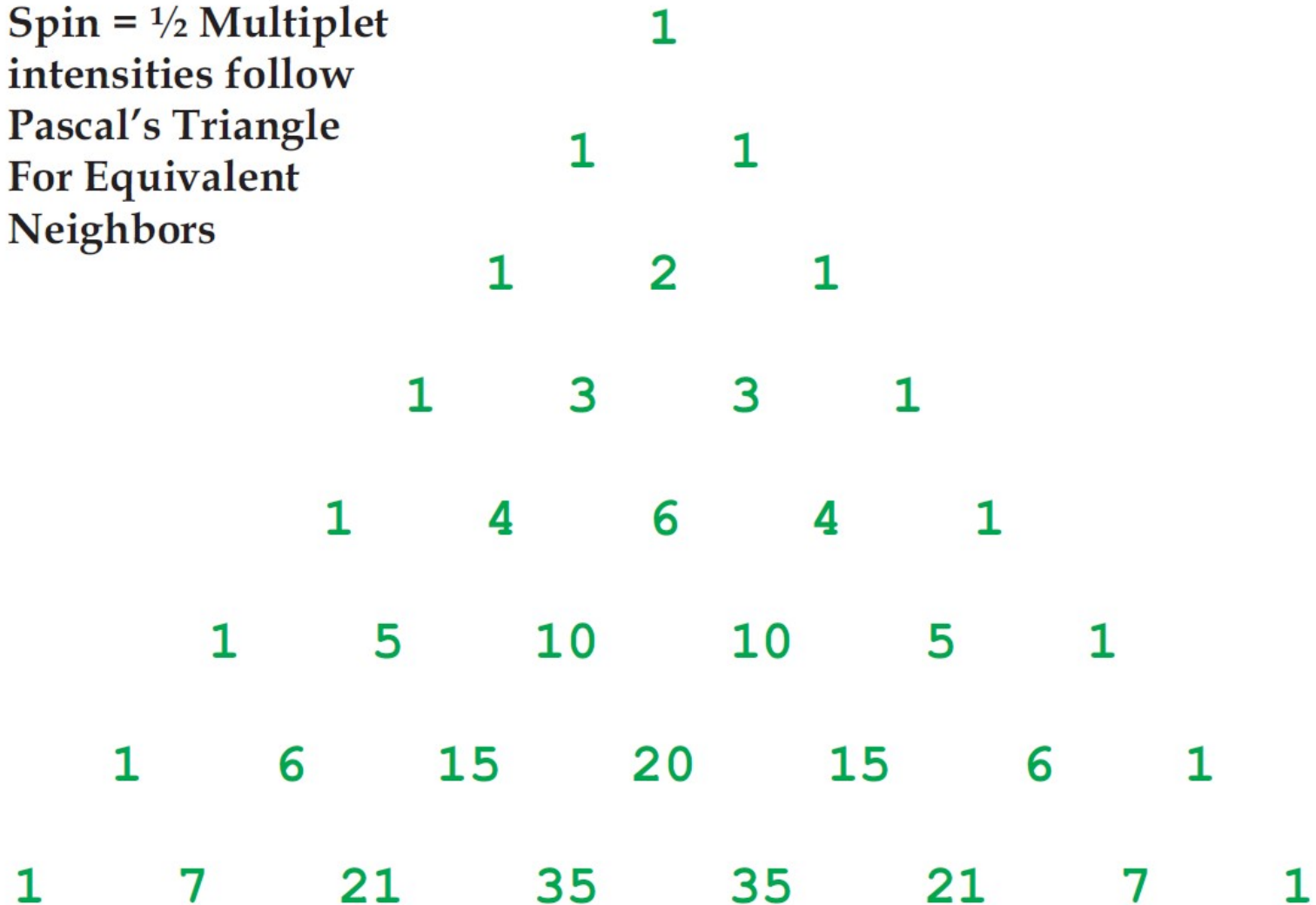


1 : 3 : 3 : 1



# Pascal's Triangle in Multiplet pattern of $I = 1/2$

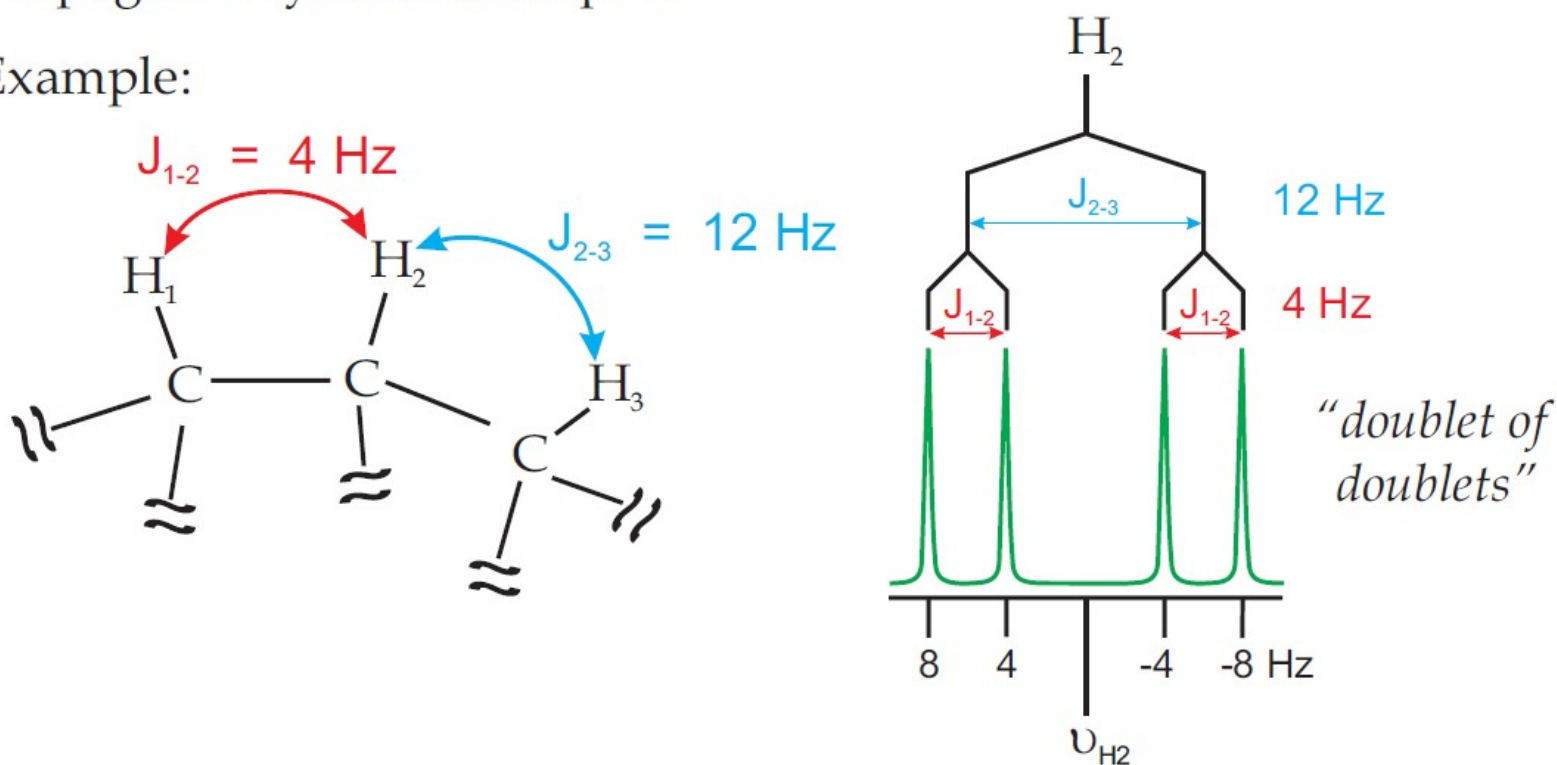
Spin =  $1/2$  Multiplet  
intensities follow  
Pascal's Triangle  
For Equivalent  
Neighbors



# Multiplet pattern of in-equivalent neighbors

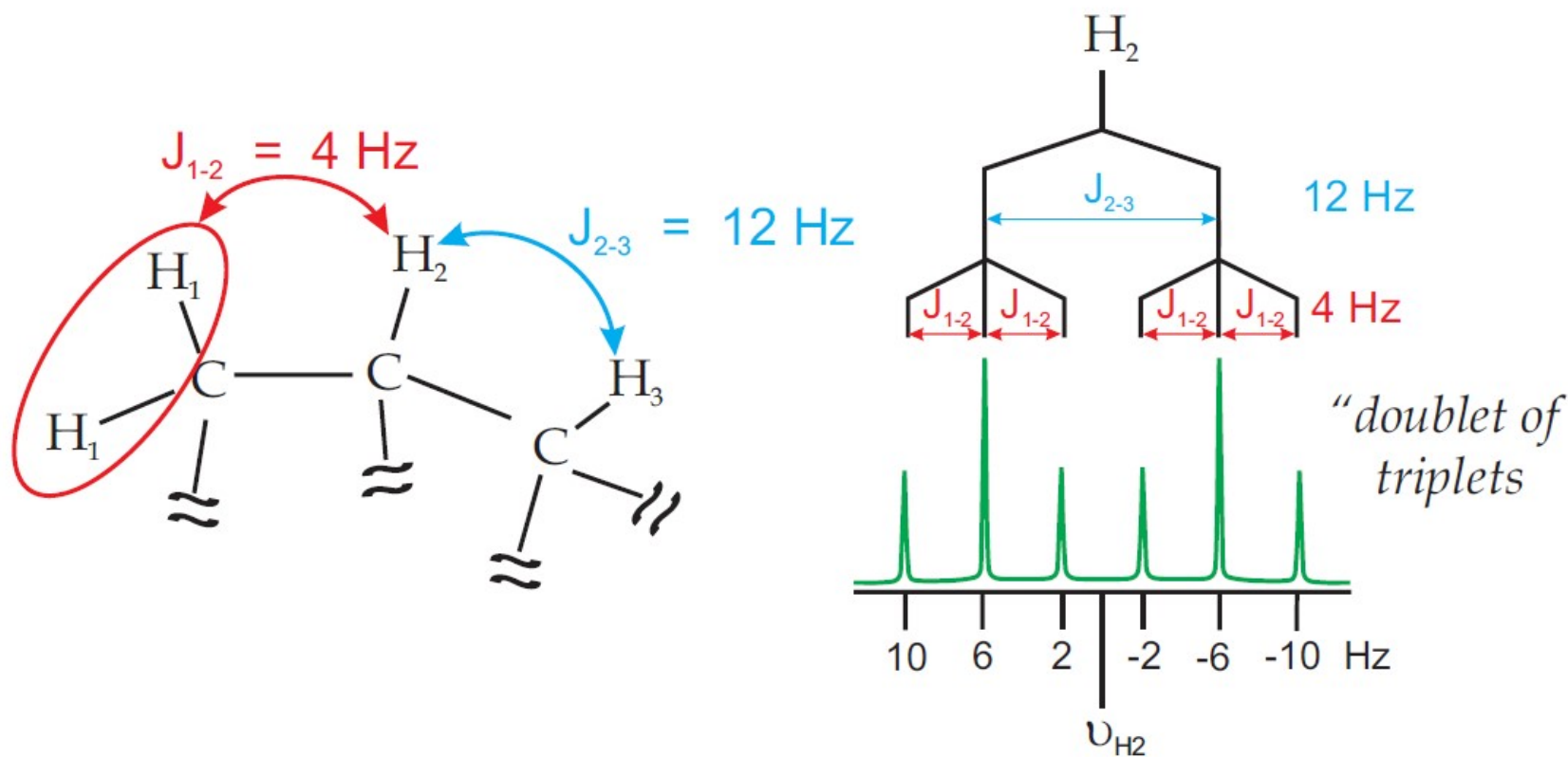
All neighboring spins may not couple to the same extent. In this case the coupling patterns are more complex than the number and intensities predicted by Pascal's Triangle. For a spin with more than one in-equivalent neighbor each individual coupling will propagate to yield a multiplet.

Example:



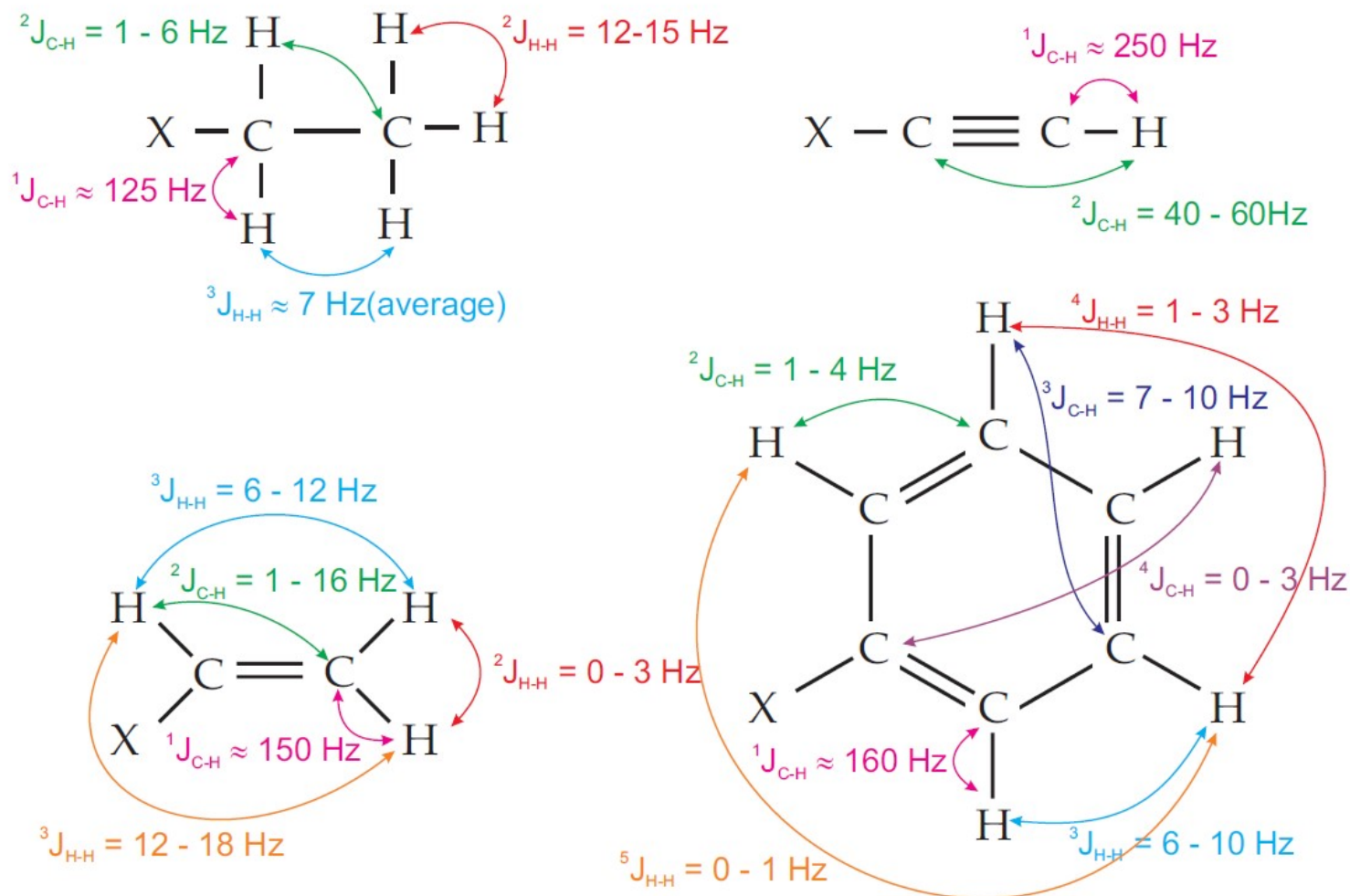
# Complexity in multiplet pattern increases with equivalents and non-equivalents

The coupling pattern can be built up just like the simple doublet of doublets. The triple pattern due to the two equivalent protons will have the 1:2:1 ratio predicted by Pascal's Triangle.



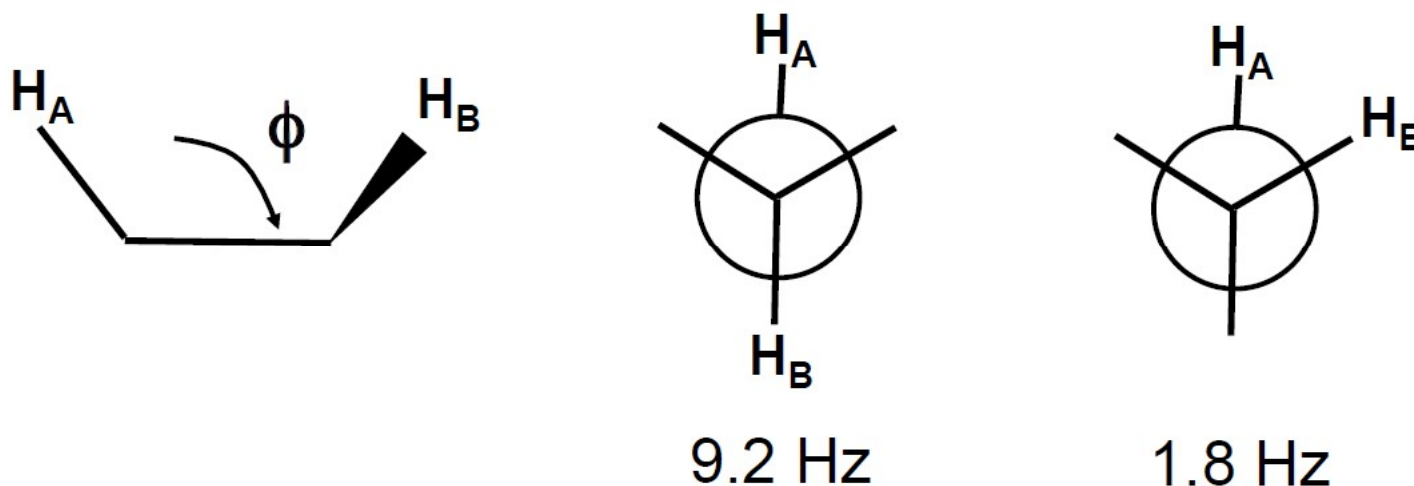
# Coupling constants depend on types of coupled nuclei, bonds and distance ranges.

Examples: Comparing  $^1\text{H} - ^1\text{H}$  and  $^{13}\text{C} - ^1\text{H}$  Coupling



# Vicinal couplings ( ${}^3J_{\text{HH}}$ ) are informative of conformation

## Vicinal Couplings ( ${}^3J_{\text{HH}}$ )



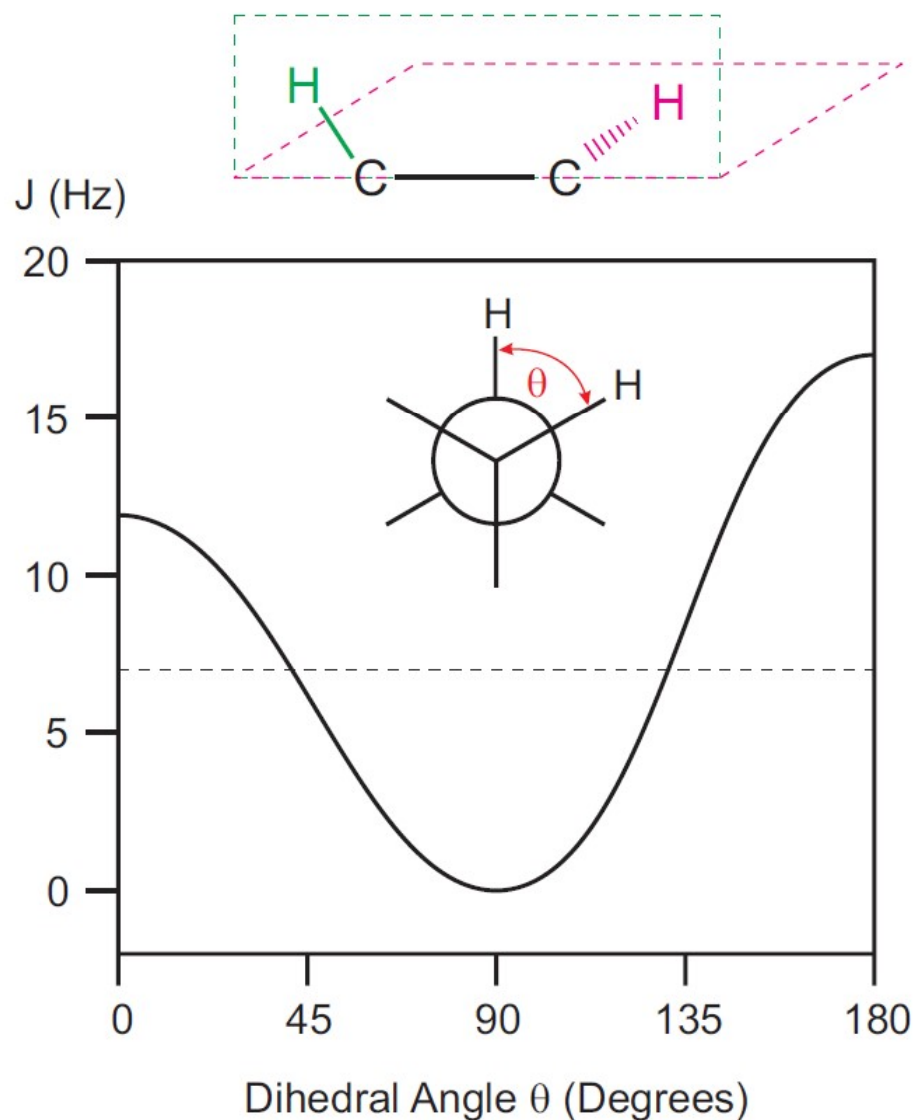
- Originally for H-C-C-H
- M. Karplus (1959) JCP **30** 11 - Karplus Equation
- $J_{\text{HNHA}} = 7.0 \cos^2(\phi) - 1.4 \cos(\phi) + 1.7$  for peptides
- (Bax and Wang (1996) JACS **118** 2492)



# Karplus equation

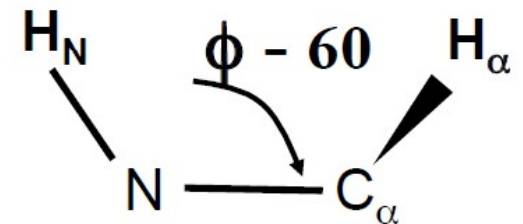
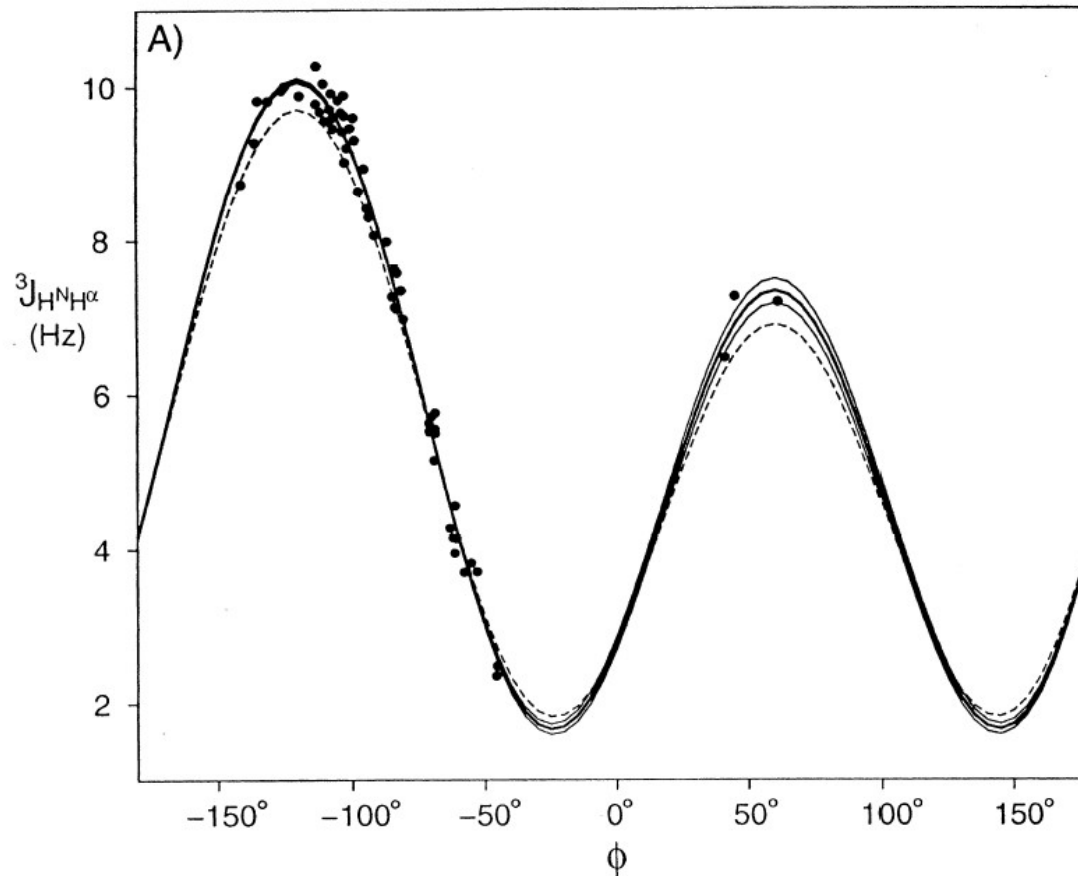
## Karplus Curve:

The magnitude of three bond coupling between two protons on adjacent aliphatic carbons follow a function first described by Karplus in 1959. The minimum (usually zero) occurs when the dihedral angle is near  $90^\circ$ . The  $180^\circ$  angle yields the largest coupling. The average coupling is about 7 Hz and is observed when rotation about the dihedral angle is free (and rapid).



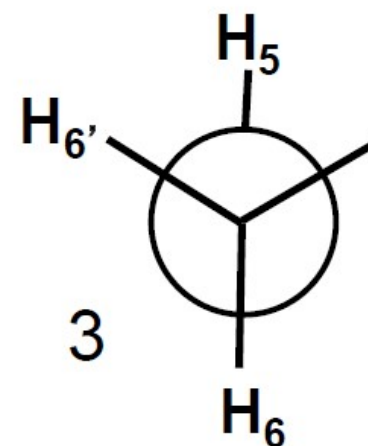
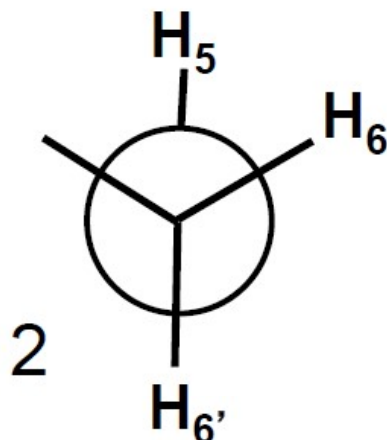
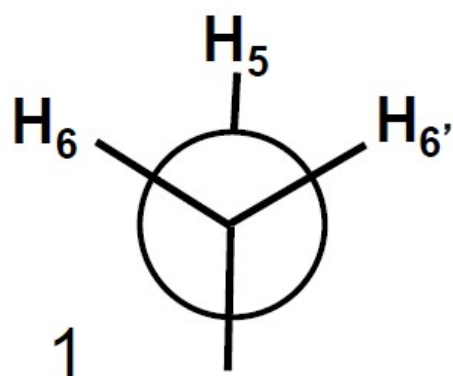
# Karplus relationship as diagnostic for dihedral angles

- Empirical parameterization of Karplus coefficients for proteins (Wang and Bax, 1996, J. Am. Chem. Soc. 118, 2483-2494)

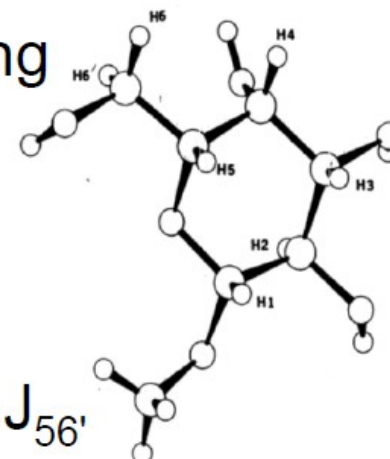


$\phi$  measured as  
C'-N-C<sub>α</sub>-C'

# Populations of Rotomeric States from Couplings: An Application of the Karplus Equation



- Occurs in pyranosides, furanosides, and nucleosides
- Assume only minimum energy states populated
- Assume rapid equilibration – averaging of coupling
- $J_{56} = 1.8 F_1 + 1.8 F_2 + 9.2 F_3$
- $J_{56'} = 1.8 F_1 + 9.2 F_2 + 1.8 F_3$
- $F_1 + F_2 + F_3 = 1$
- 3 equations, 3 unknowns, can solve with  $J_{56}$  and  $J_{56'}$



# Populations of Rotomeric States from Couplings: An Application of the Karplus Equation

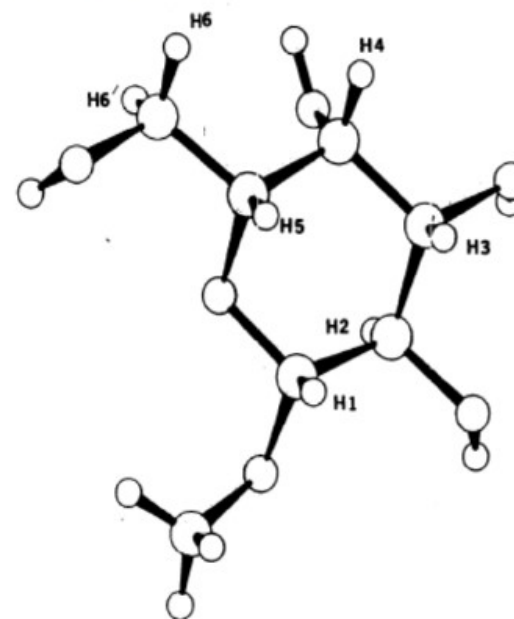
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Measure  $J_{65}$  and  $J_{6'5}$  for  $\beta$ -Me-galactose

7.0 Hz, 3.0 Hz

Using averaging formula find:

0.15, 0.7, 0.15 for states 1, 2, and 3



## Importance and influential factors of $J$

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Scalar coupling yields information of the *number, position* and *type* of neighboring spins. The splitting (line spacing) is measured in Hertz (Hz) and the magnitude is dependent on several factors including:

- 1) Type of bonds present between spins
- 2) Number of intervening bonds between spins (1 - 6 bonds)
- 3) Bond (dihedral) angles between spins
- 4) The magnetic moments of the nuclei (i.e., the nuclei's  $\gamma$ )

The spacing is *independent of field strength*. Coupling constants measured on a compound will be the **same** on any instrument.