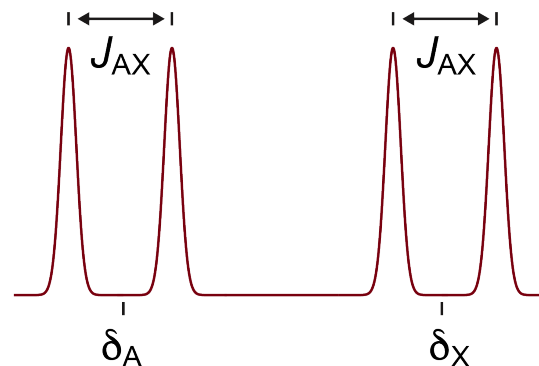
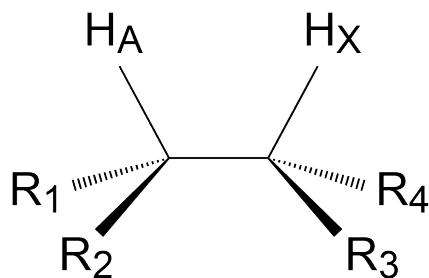


Origin of Scalar Couplings

BCMB/CHEM 8190

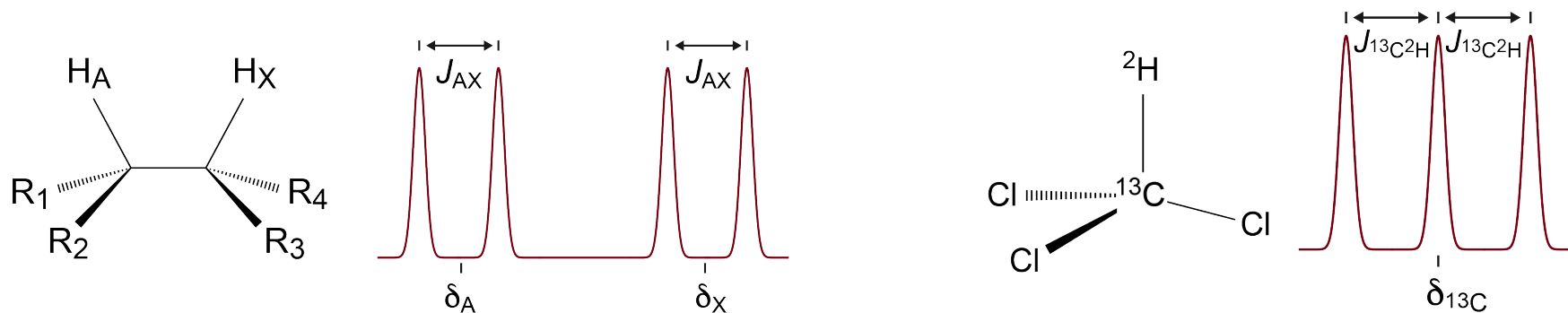
Traditional View of Scalar Coupling

- 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)



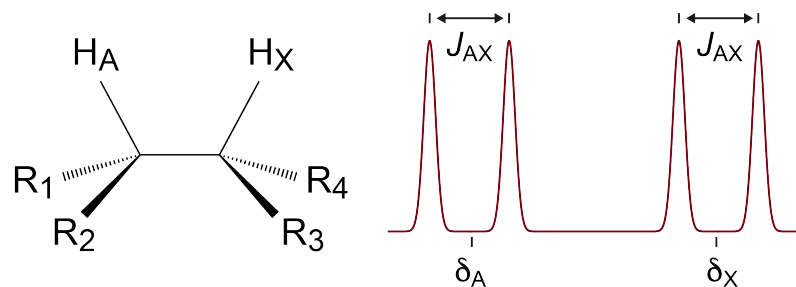
Traditional View of Scalar Coupling

- 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 ^1H nucleus ($I = 1/2$) is split into two peaks, depending on whether the coupled ^1H nucleus is in the $m=+1/2$ (α) or $m=-1/2$ (β) state
- Below (right), the ^{13}C nucleus is split into three peaks, depending on whether the coupled ^2H nucleus ($I = 1$) is in the $m=1$, $m=0$, or $m=-1$ state



Traditional View 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 $^3J_{H_AH_X}$)
- The magnitude of J depends on structural factors and the number of intervening chemical bonds:



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

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

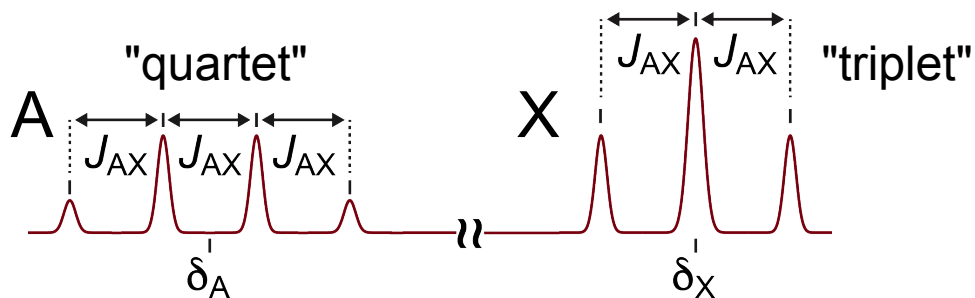
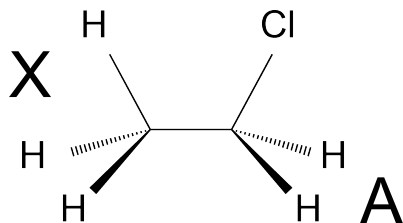
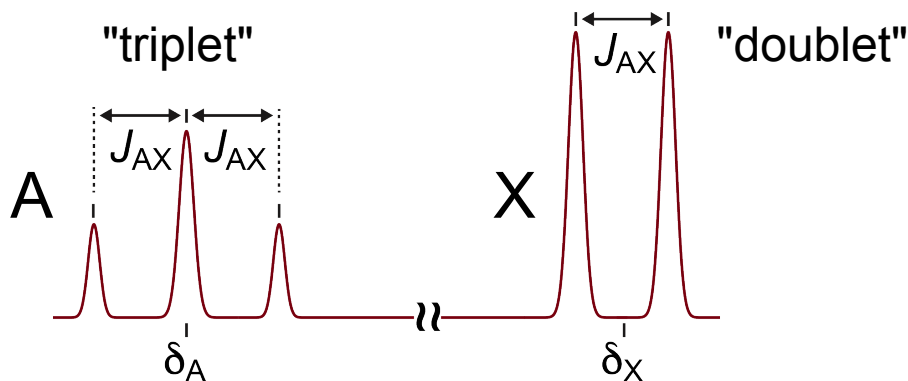
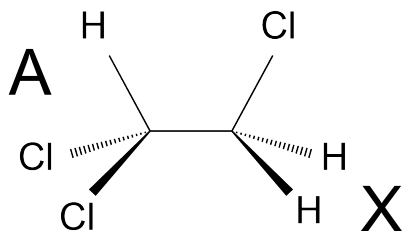
$$^3J_{H,H} \approx 5\text{-}20 \text{ Hz typically}$$

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

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

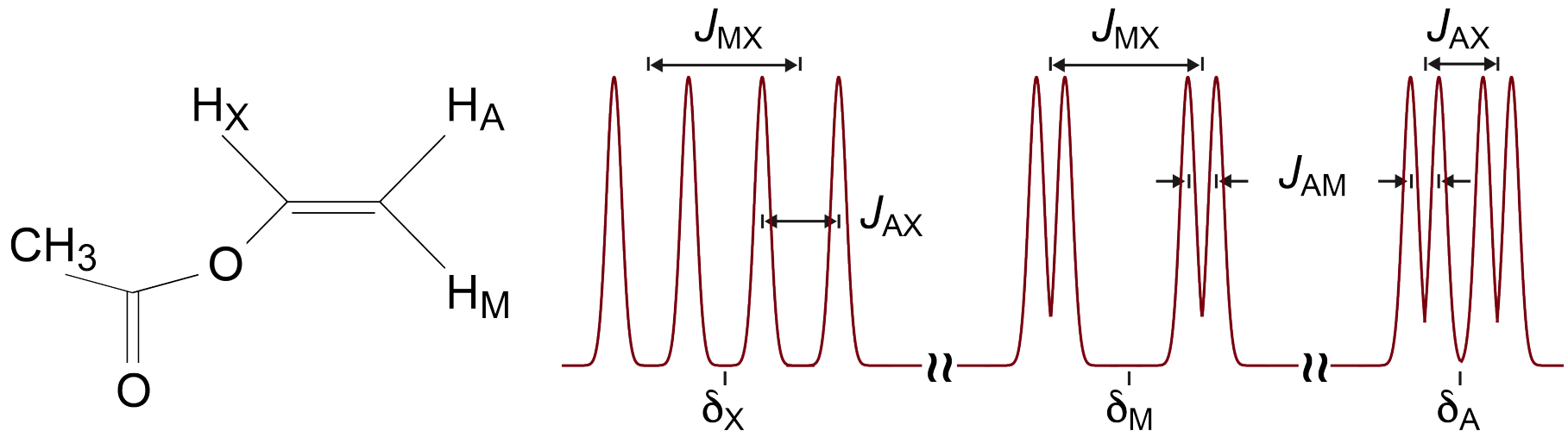
Traditional View of Scalar Coupling

- 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 $n+1$.



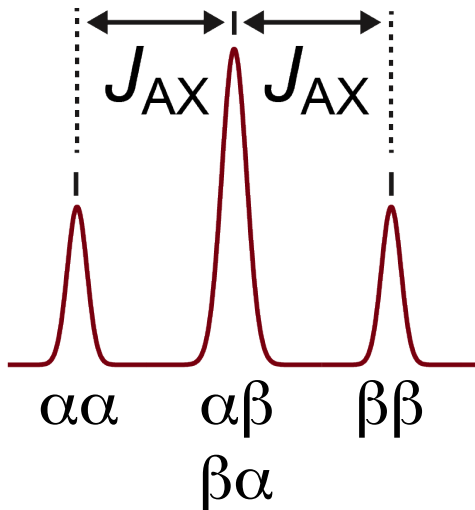
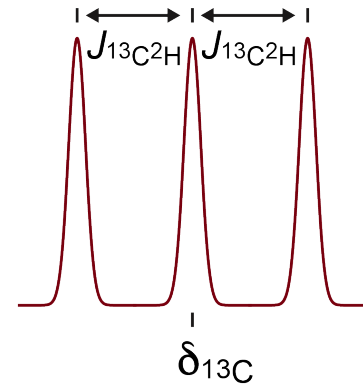
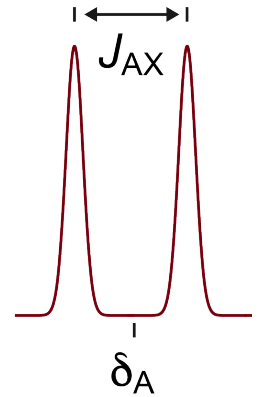
Traditional View of Scalar Coupling

- Split signals can be split again by coupling to additional nuclei
- In vinyl acetate (below), the signal from "X" is split into a doublet by "M", and this doublet is split into a doublet of doublets by "A"
- Likewise, "M" is split by "A" and "X", and "A" is split by "M" and "X"



Traditional View of Scalar Coupling

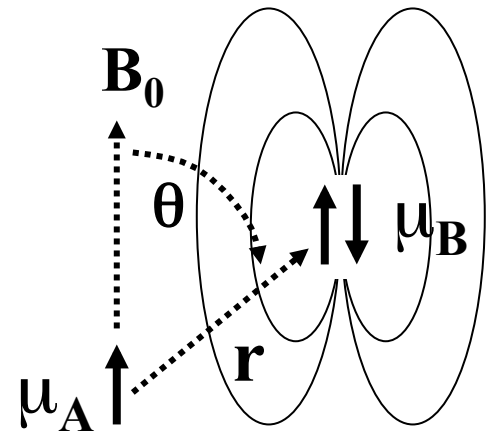
- For a doublet, the two peaks signify that the coupled nucleus ($I = 1/2$) is in either the $m=+1/2$ (α) or $m=-1/2$ (β) state
- The intensities of the peaks are the same, because the numbers of coupled nuclei in the α and β states are virtually
- For coupling to a spin 1 ($I = 1$) nucleus, the intensities of the three peaks are the same, because the $m=1$, $m=0$, and $m=-1$ states are virtually equally populated



- For coupling to equivalent nuclei ($I = 1/2$) intensities are not equal: peaks from states with nearly identical energies ($\alpha\beta$, $\beta\alpha$) overlap (^1H next to methylene, triplet with 1:2:1 intensities)

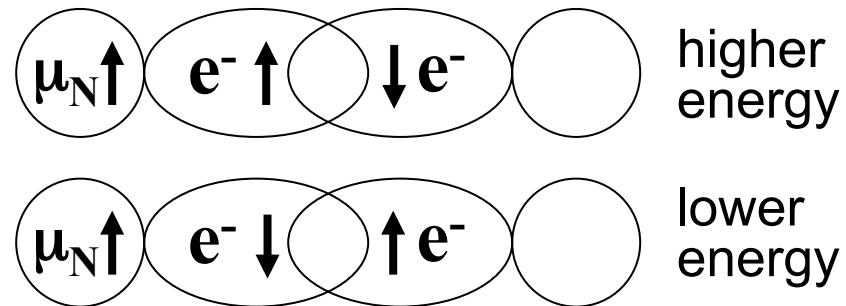
The Origin of Scalar Couplings

- Dipolar coupling model ($E \propto (3\cos^2\theta - 1) / r^3$) *cannot* account for scalar coupling (r is fixed, molecule tumbles in space, result is average over all values of θ , result = 0)

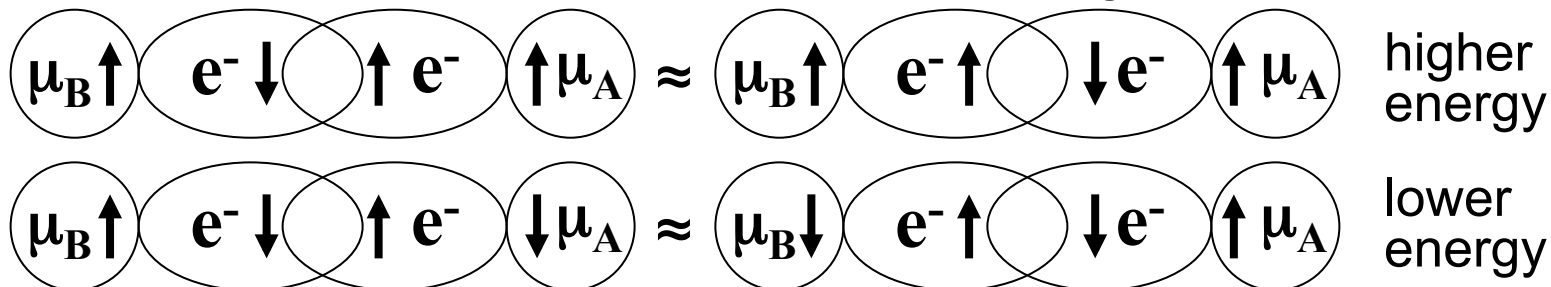


- Scalar coupling is due to a *Fermi Contact* mechanism (interactions between nuclear and electron magnetic moments)

- Electron moments in bond are antiparallel (Pauli). Lower energy state is when nuclear moment is antiparallel to adjacent electron moment



- When coupling lowers the energy of the state where nuclear moments are antiparallel, the coupling is said to be *positive*



Fermi-Contact Expression for Scalar Coupling

- In the limit of weak coupling ($v_i - v_j \gg J_{ij}$) the scalar coupling term in the Hamiltonian reduces to products of I_z (simple α and β spin functions, ignore x- and y-components of the dot product)

$$E_J = \sum_{i \neq j} J_{ij} I_i \cdot I_j \approx \sum_{i \neq j} J_{ij} I_{zi} I_{zj}$$

- Expression for Fermi contact energy comes from second order perturbation treatment of spin contributions to electronic energy

$$E = (4g\beta\gamma_N h / 3)^2 \sum_{j,k} \langle \psi_0 | \delta(r_{kA}) \delta(r_{kX}) S_{jz} S_{kz} | \psi_0 \rangle I_{zA} I_{zX} \quad (\text{homonuclear case})$$

- $\gamma_A \gamma_X$ (i.e. γ_N^2) and $I_{ZA} I_{ZX}$ dependence as expected
- Dirac delta functions ($\delta(r=0)=1$, $\delta(r \neq 0)=0$) at the positions of the nuclei: probability of finding electron k at nucleus A ($\delta(r_{kA})$) and nucleus X ($\delta(r_{kX})$) (*only s orbitals are non-zero at the nucleus*)
- If the electrons are not correlated (i.e. there is no bond between the nuclei), the expression for the average of the $S_{jz} S_{kz} = 0$
- So, there has to be a bond and the magnitude of the coupling constant depends on the 's' character of the bond

Some predictions for one-bond couplings

Couplings are short range and depend on electron densities at the coupled nuclei (ie, the product of “s characters”) as well as the values of γ for the nuclei.

$^{13}\text{C}-^{13}\text{C}$

CH_3-CH_3	35 Hz	$\text{sp}^3 - \text{sp}^3$	$1/4 \times 1/4 = 1/16$
$\text{CH}_2=\text{CH}_2$	67 Hz	$\text{sp}^2 - \text{sp}^2$	$1/3 \times 1/3 = 1/9$
C_6H_6	57 Hz	$\text{sp}^2 - \text{sp}^2$	$1/3 \times 1/3 = 1/9$
$\text{HC}\equiv\text{CH}$	171 Hz	sp-sp	$1/2 \times 1/2 = 1/4$

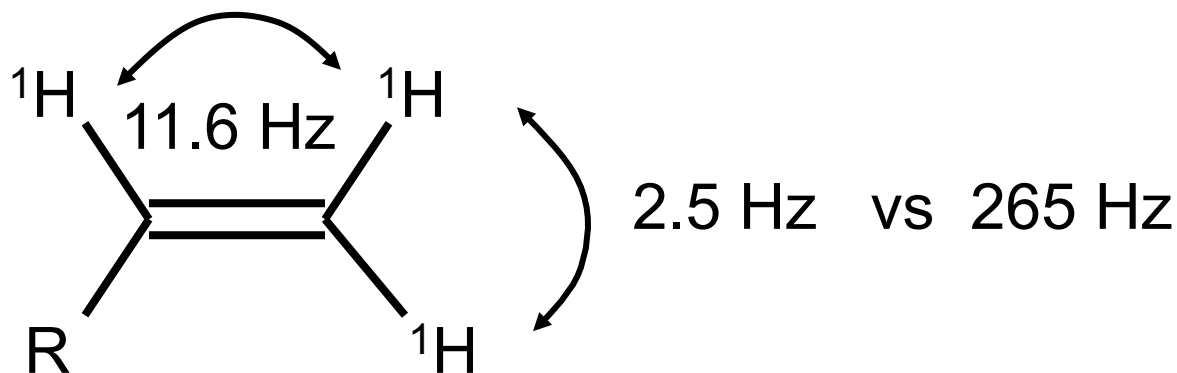
$^{13}\text{C}-^1\text{H}$

CH_3-CH_3	125 Hz	$\text{s} - \text{sp}^3$	$1 \times 1/4 = 1/4$
$\text{CH}_2=\text{CH}_2$	156 Hz	$\text{s} - \text{sp}^2$	$1 \times 1/3 = 1/3$
C_6H_6	158 Hz	$\text{s} - \text{sp}^2$	$1 \times 1/3 = 1/3$
$\text{HC}\equiv\text{CH}$	249 Hz	s-sp	$1 \times 1/2 = 1/2$

- Knowledge of C-C and C-H couplings important for magnetization transfers in heteronuclear experiments

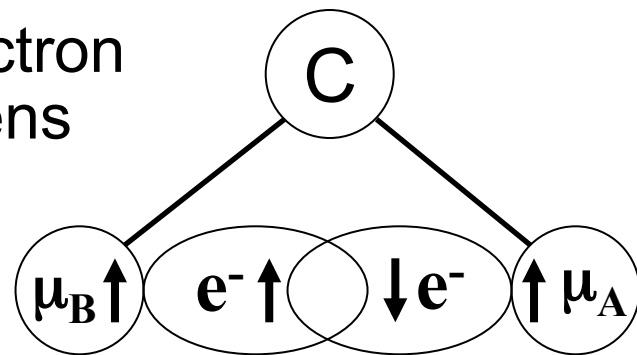
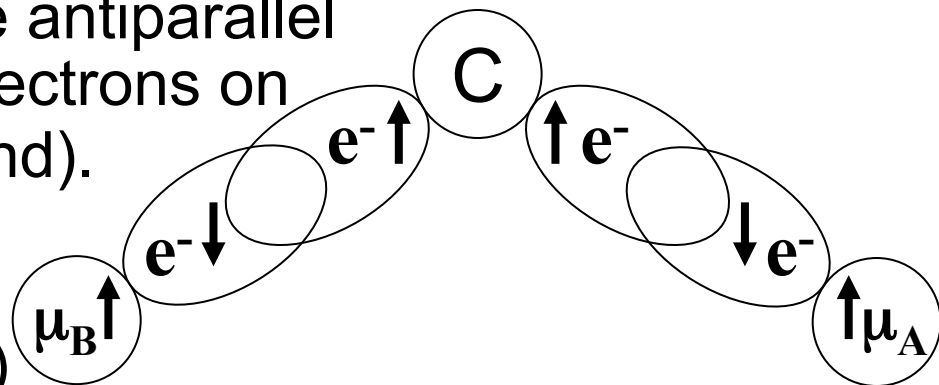
Predictions about Magnitudes of Scalar Couplings

- Wave functions (electronic) for ^1H - ^1H can be calculated at a high level of theory – even at VB *prediction* of 200 Hz is good.
- *Experimental* ^1H - ^2H is 44 Hz; correcting for γs , $J_{\text{H-H}} = \gamma_{^1\text{H}}/\gamma_{^2\text{H}} \times 44 = 6.5 \times 44 = 265\text{Hz}$
- Directly bonded pairs should have much larger couplings:

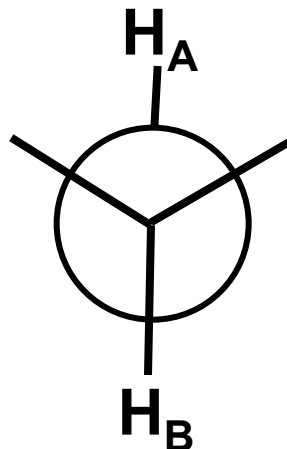
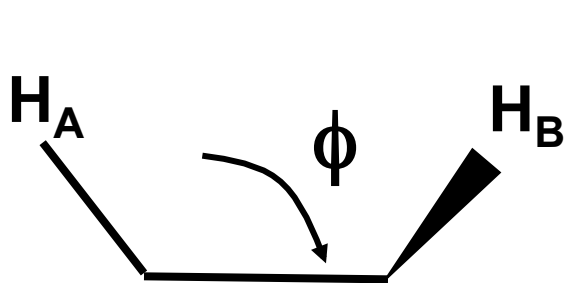


A two-bond coupling is small and sometimes negative – why?

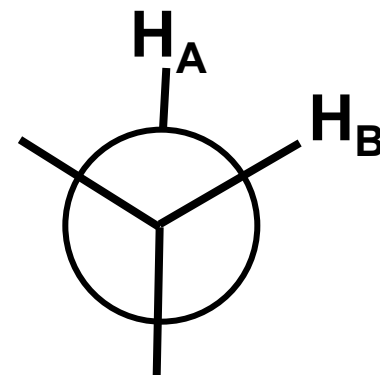
- Electron moments in bonds are antiparallel (Pauli). Moments of bonding electrons on central carbon are parallel (Hund).
- Low energy state is now when nuclear moments are *parallel* (definition of *negative coupling*)
- Thus, the coupling would be negative and could have a large magnitude
- Consider the possibility of correlated electron density directly between the two hydrogens (i.e. not via C)
- This coupling would be positive, and high energy
- Addition of the negative and positive contributions to the coupling constant would then tend to make the absolute value of the magnitude small



Vicinal Couplings ($^3J_{HH}$)



9.2 Hz

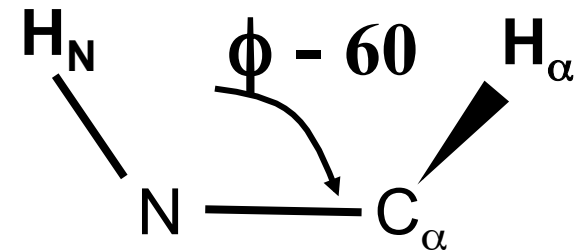
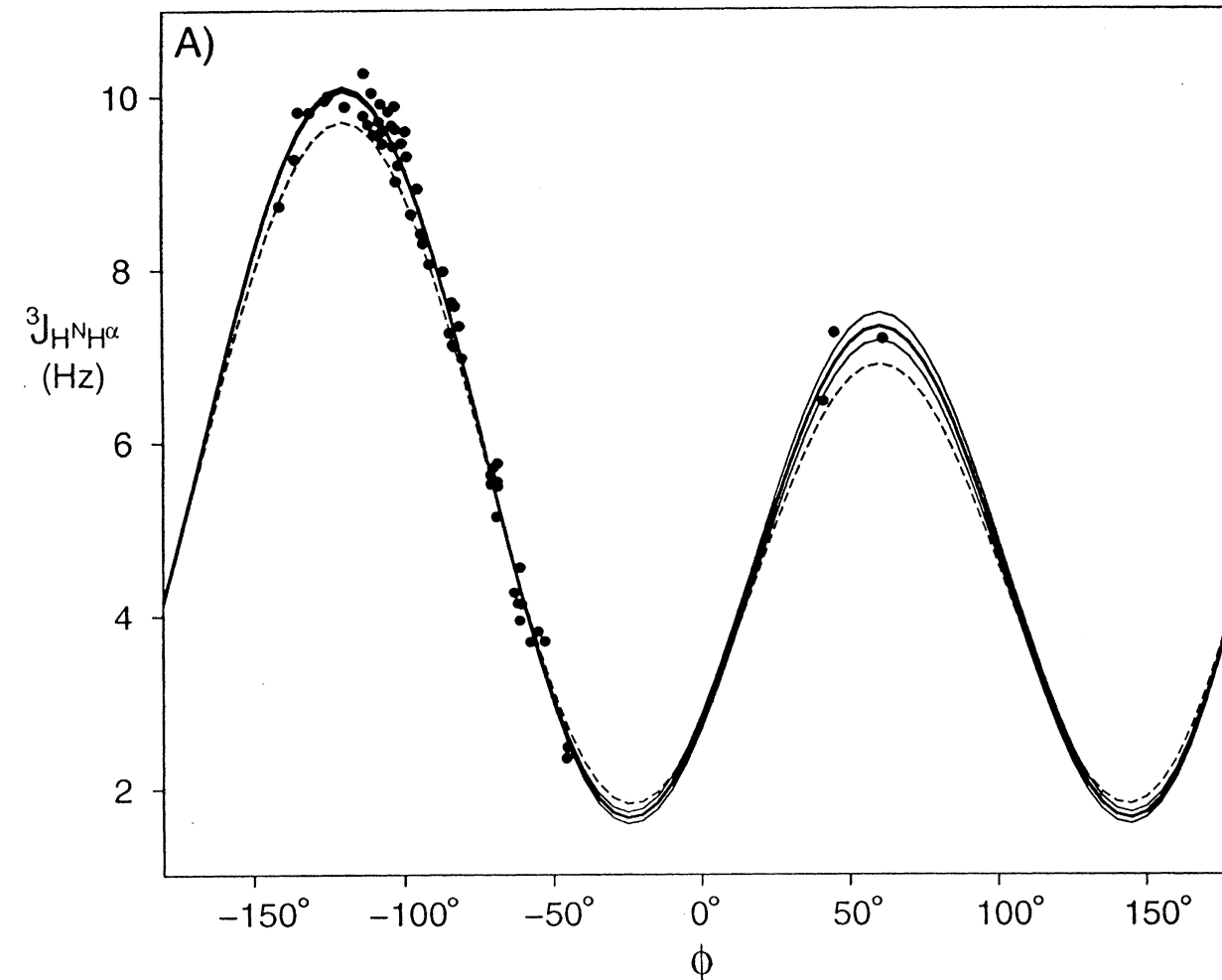


1.8 Hz

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

Wang-Bax Equation Compared to Ubiquitin Data

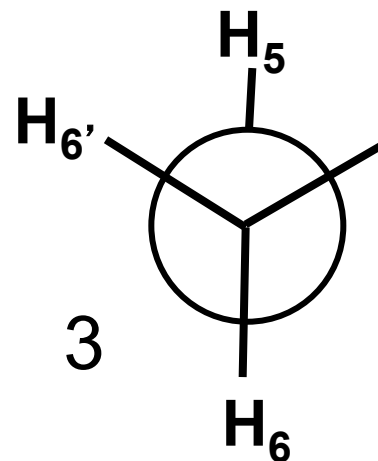
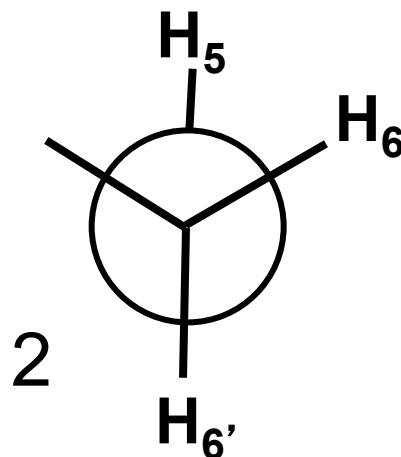
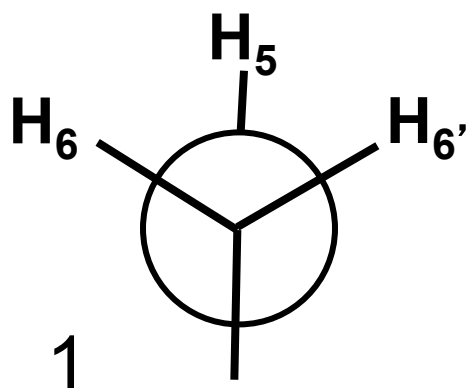
- Empirical parameterization of Karplus coefficients for proteins (Wang and Bax, 1996, J. Am. Chem. Soc. 118, 2483-2494)



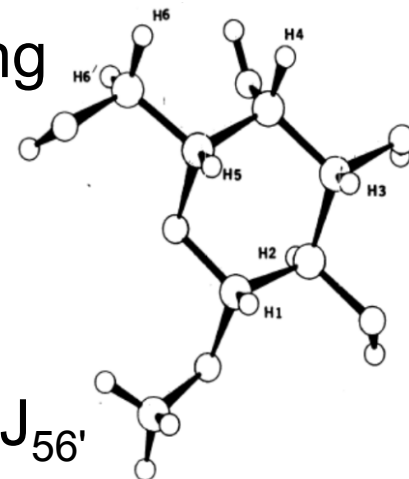
ϕ measured as
C'-N-C $_{\alpha}$ -C'

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 $J_{56'}$



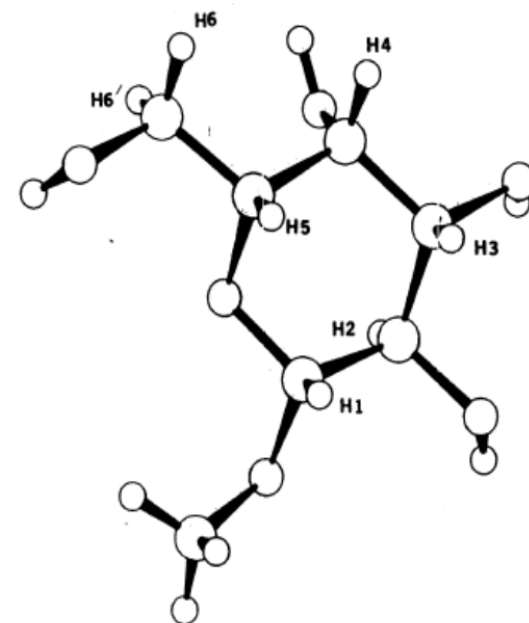
Application of Karplus Equations (cont)

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

7.0 Hz, 3.0 Hz

Using averaging formula find:

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



Reference on scalar coupling in Levitt: 217-223

Scalar Couplings Can be Predicted Theoretically

- “Interpretation of chemical shifts and coupling constants in macromolecules” D.A. Case, Curr. Opin. Struc. Biol. 10, 197-203 (2000).
- “Density functional calculations on disaccharide minics: studies on molecular geometries and spin-couplings”, F. Cloran, I Carmichael, A.S. Serianni, JACS, 121, 9843-9851 (1999).

Couplings And Hydrogen Bonds

- “Direct observation of hydrogen bonds in nucleic acid base pairs by internucleotide $^2J(\text{NN})$ couplings”, A.J. Dingley, S. Grzesiek, JACS, 120, 8293-8297 (1998).
- “NMR scalar couplings across Watson-Crick base pair hydrogen bonds in DNA ...”, K. Pervushin, ... K. Wuthrich, PNAS, 95, 14147-14151 (1998).



- “N-15-H-1 Scalar Coupling Perturbation: An Additional Probe for Measuring Structural Changes Due to Ligand Binding, Ma JH, Gruschus JM, Tjandra N, J. AM. CHEM. SOC., 131, 9884 (2009)

Chemical shifts and Coupling Constants: How Many Peaks can you Assign?

