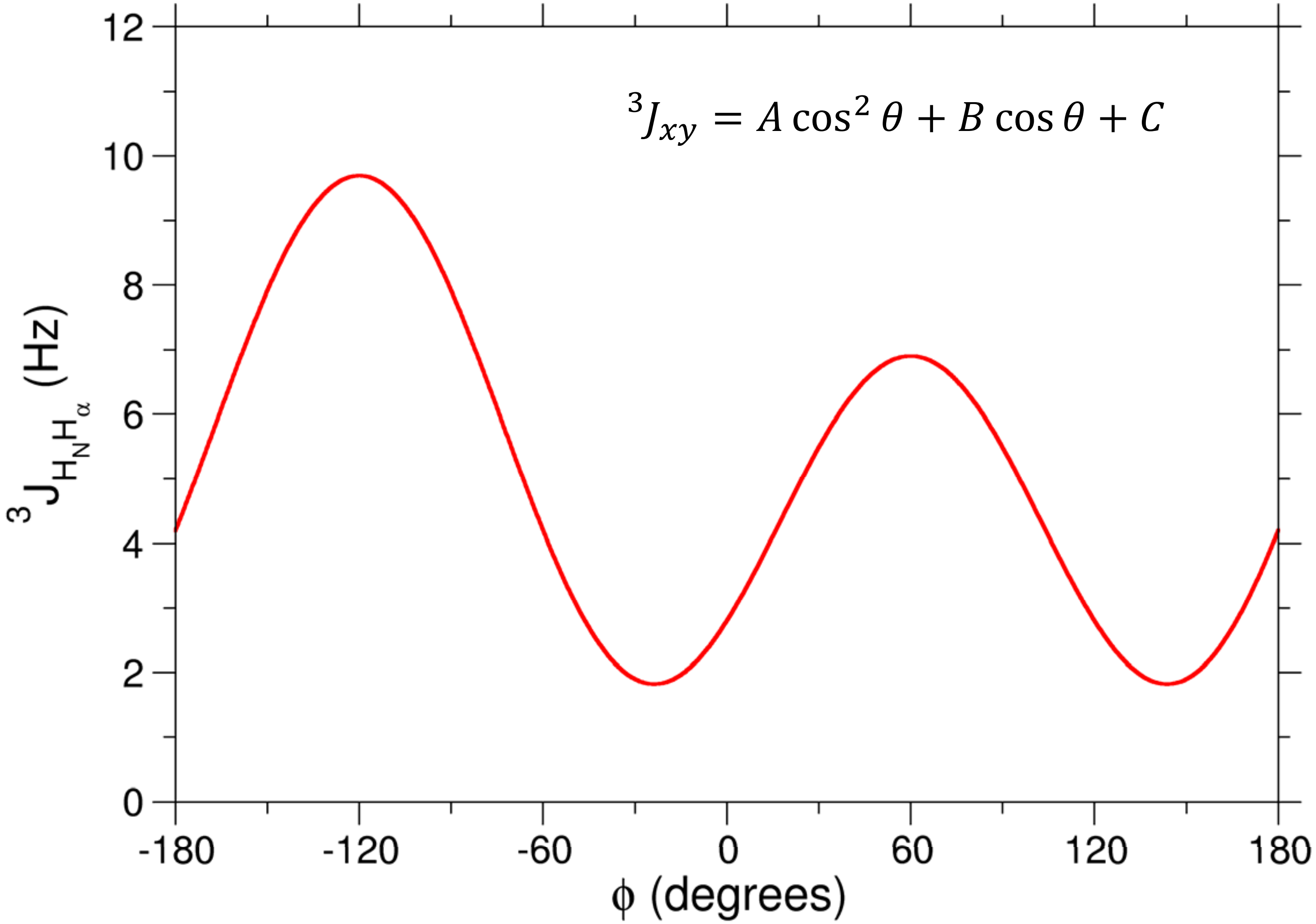
Karplus Equation



$$^3J_{xy} = A\cos^2\theta + B\cos\theta + C$$

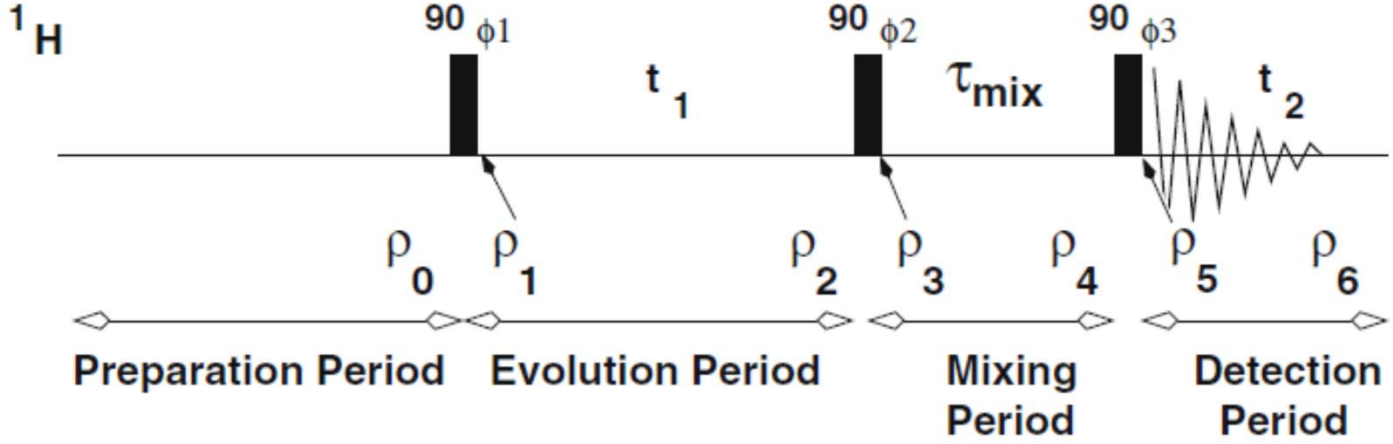
Nuclear Overhauser Effect (NOE)

- Dipole relaxation from nearby spins results in transfer of magnetization

- Heteronuclear NOE: limited distance (^{15}N-^{1}H bond), used mostly for dynamics experiments

- Homonuclear NOE (^{1}H-^{1}H): High γ value of proton → longer distances possible (~ 6 Å)

Homonuclear NOE Experiment (2D)

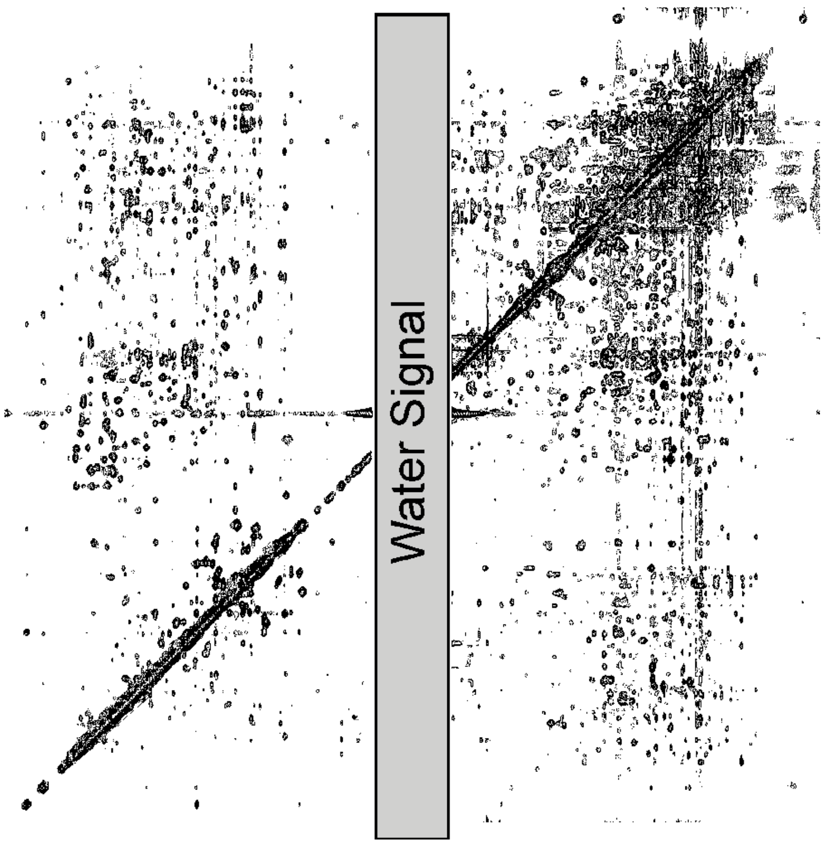


- Magnetization is transferred during τ_{mix} at a rate dependent on geometry ($r \propto r^{-6}$)
- Faster rate → more transfer → more intense crosspeaks

Rule & Hitchens, p. 360.

Homonuclear NOE

- Diagonal peaks: no transfer
- Cross peaks: geometrically close protons (unlike TOCSY, where cross peaks are bonded)



http://www.cryst.bbk.ac.uk/PPS2/projects/schirra/images/2dnosy_1.gif

NOE Transfer vs. Size

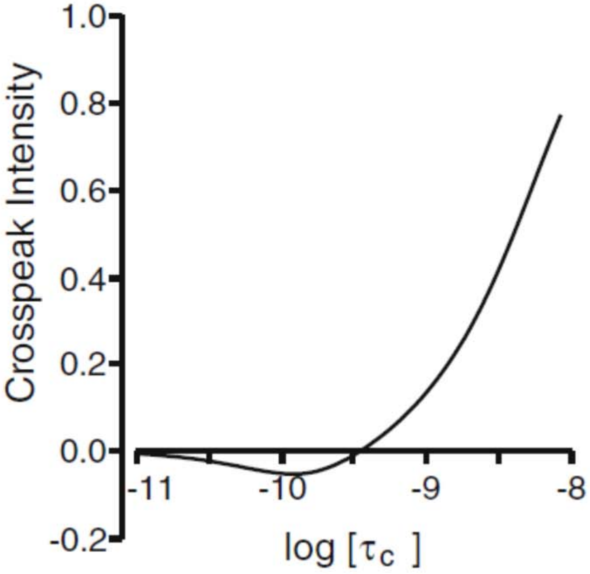


Figure 16.7 Effect of rotational correlation time on NOESY crosspeak intensity. The crosspeak intensity as a function of the rotational correlation time is shown. An inter-proton distance of 2 Å and a spectrometer frequency of 500 MHz was used in this calculation. For reference, a 20 residue peptide would have a rotational correlation time of ≈ 1 nsec ($\log \tau_c = -9$).

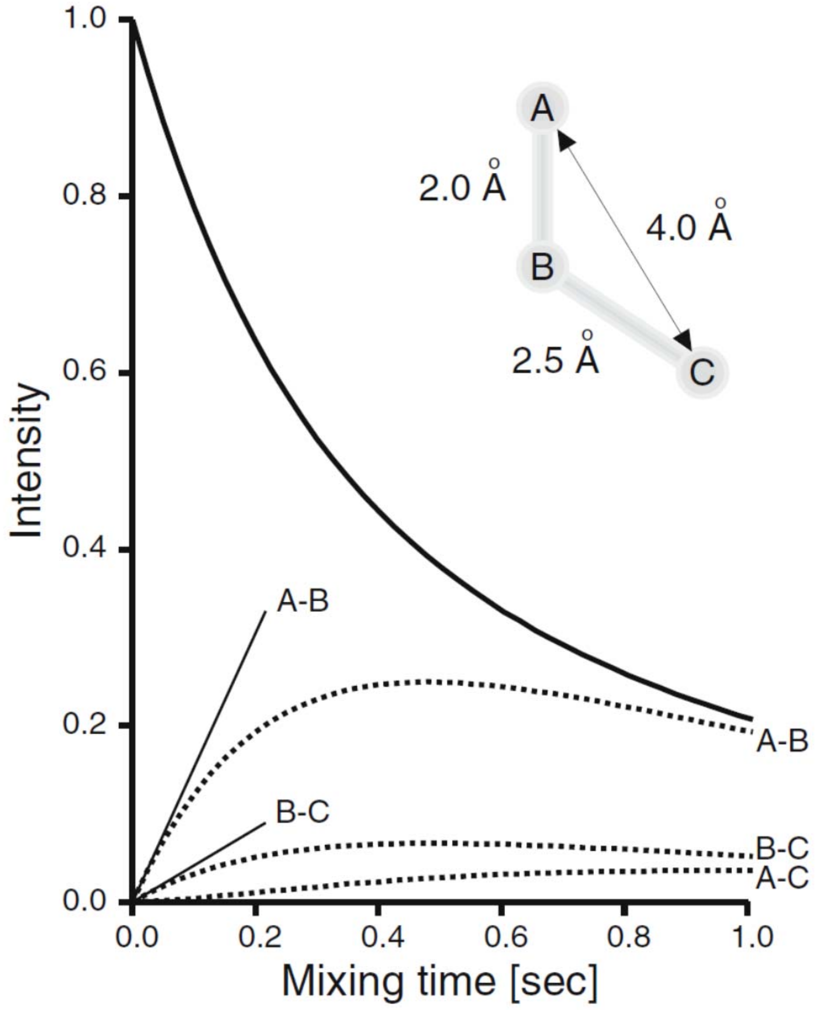
- Organic chemists often see negative cross peaks
- Some small peptides give no NOE cross peaks (need to use ROESY)

Rule & Hitchens, p. 364.

NOE → Distances

- Best approach: calibrate transfer rates using several mixing times (takes a lot of fitting)

- Commonly done: measure intensity, calibrate to known distance (i.e. helix H_N–H_N distance)

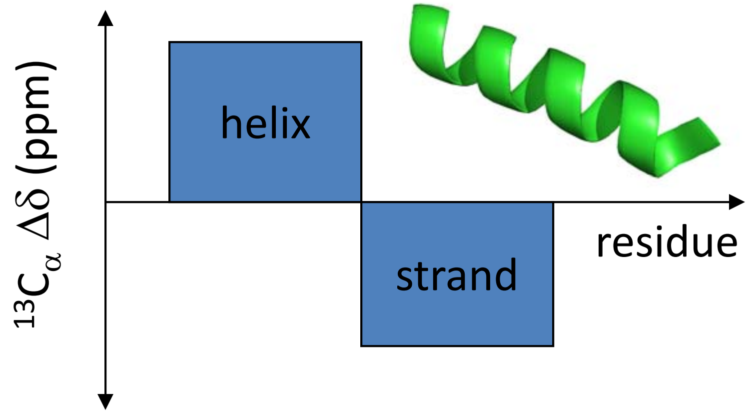


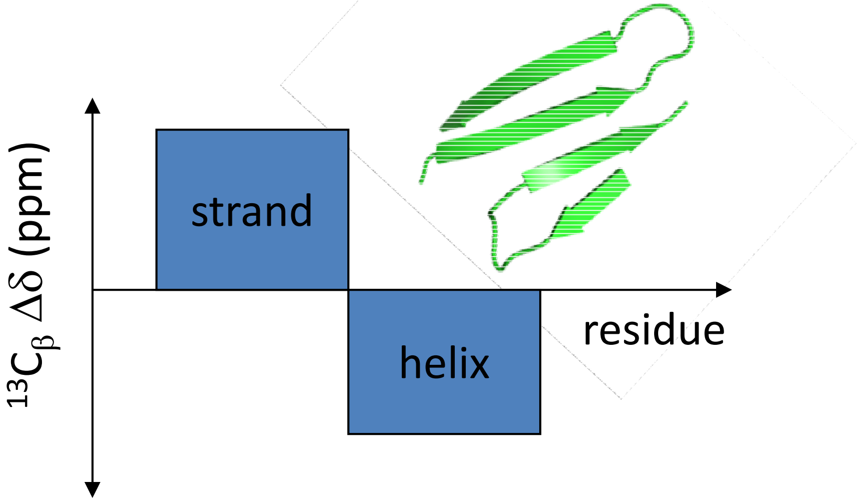
Rule & Hitchens, p. 368.

Chemical Shifts

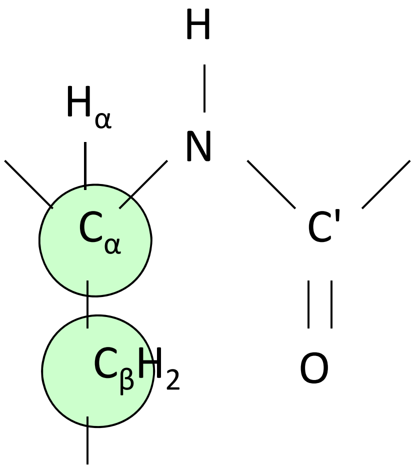
- Chemical shifts contain information on electronic environment
- Primary structure is most important, difficult to interpret by a computer (see CS-ROSETTA)
- Still not possible to look at HSQC and tell structure, although homologous proteins tend to give similar patterns

Secondary Chemical Shifts

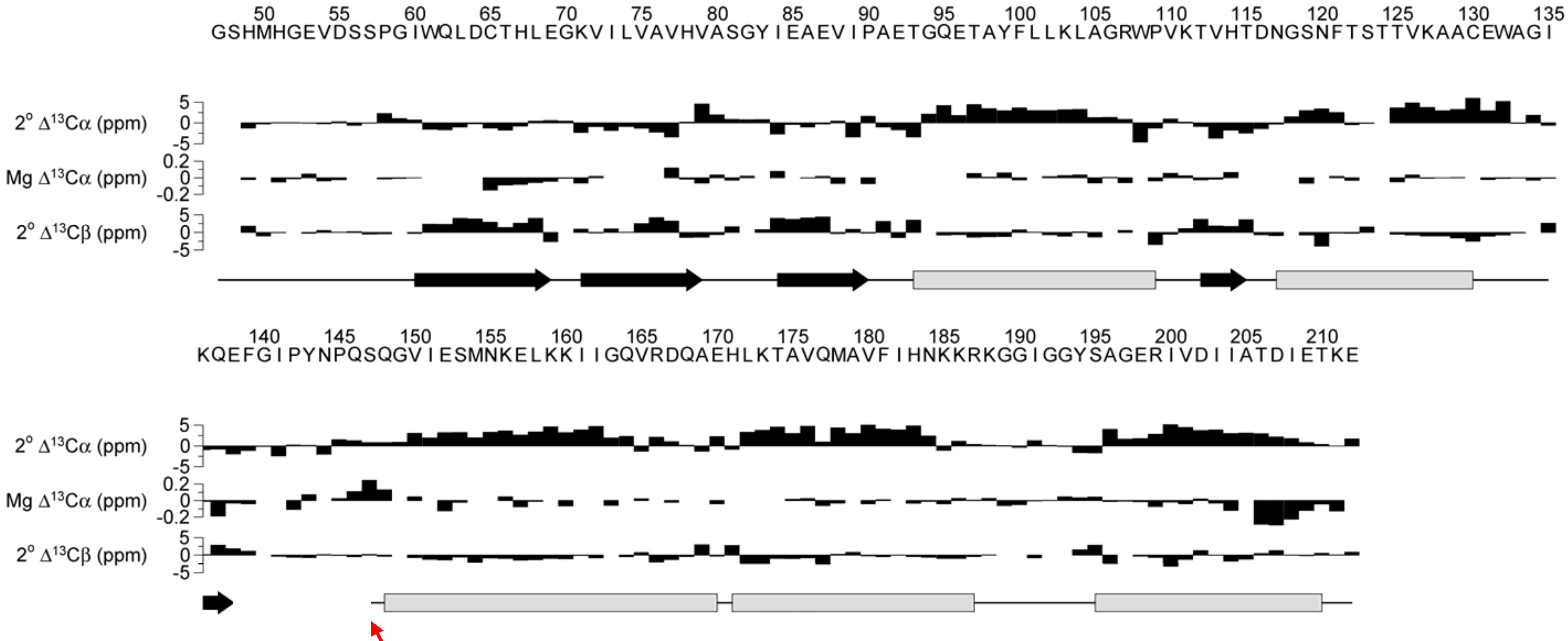




- Definition:
  $\Delta\delta = \text{ppm}_{\text{observed}} - \text{ppm}_{\text{random coil}}$



IN CCD Secondary Shifts



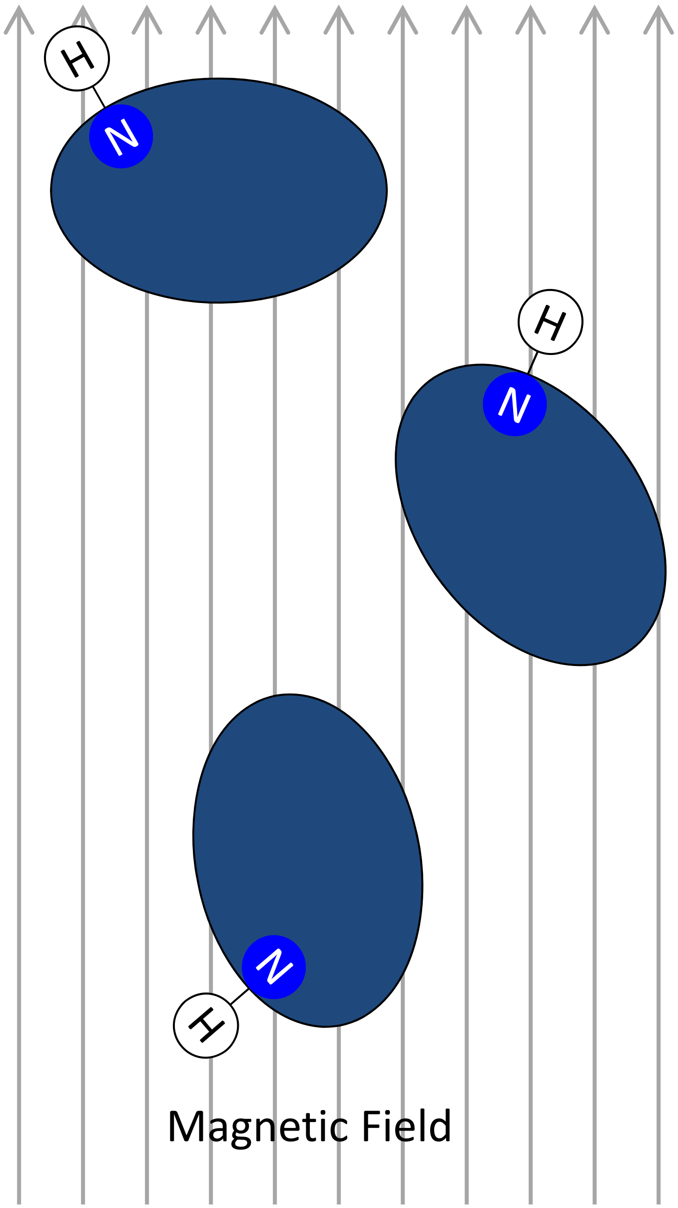
Active site loop

Residual Dipolar Couplings

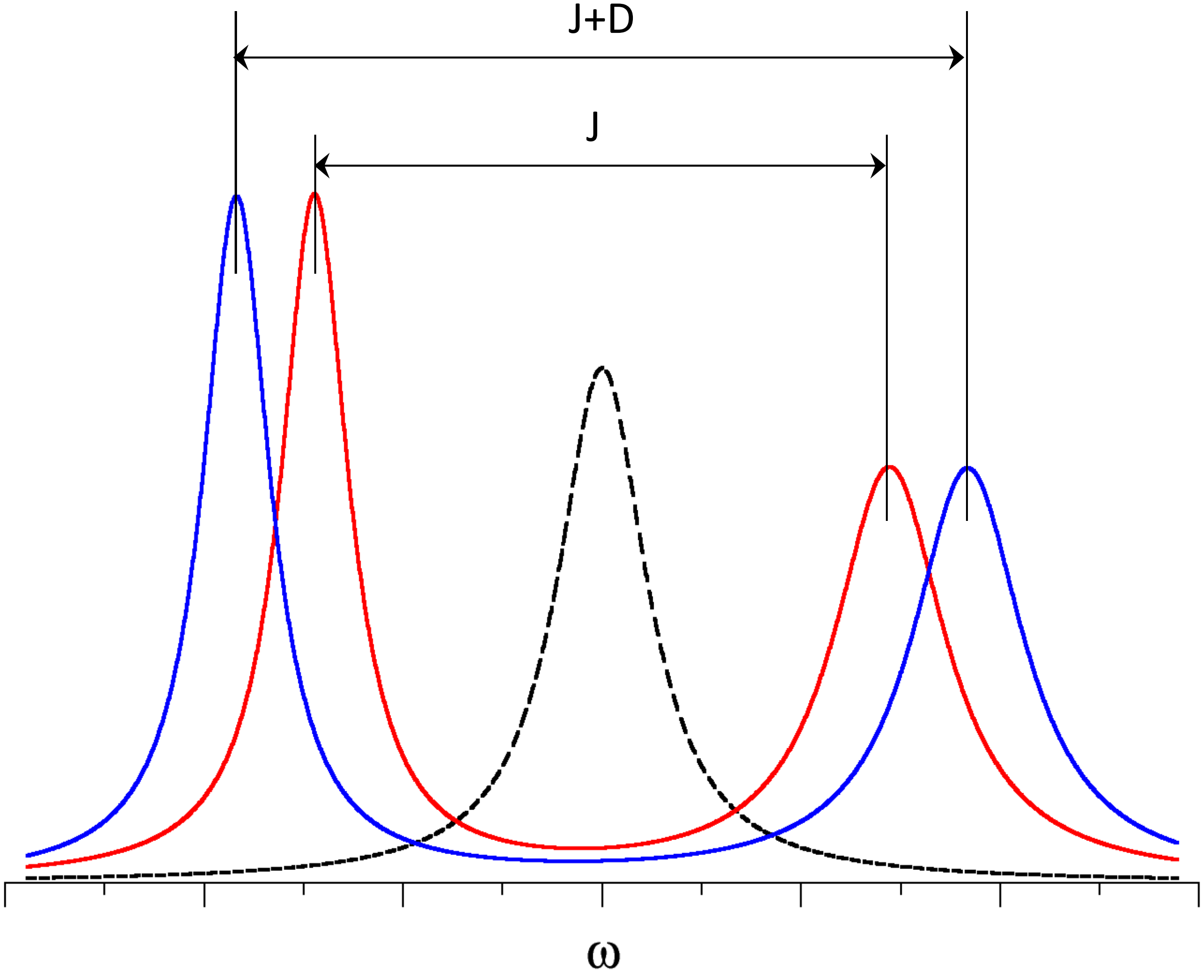
- Energetic coupling between bond vector and B field

- Normally averages to zero

- **Aligned media:** recover the coupling

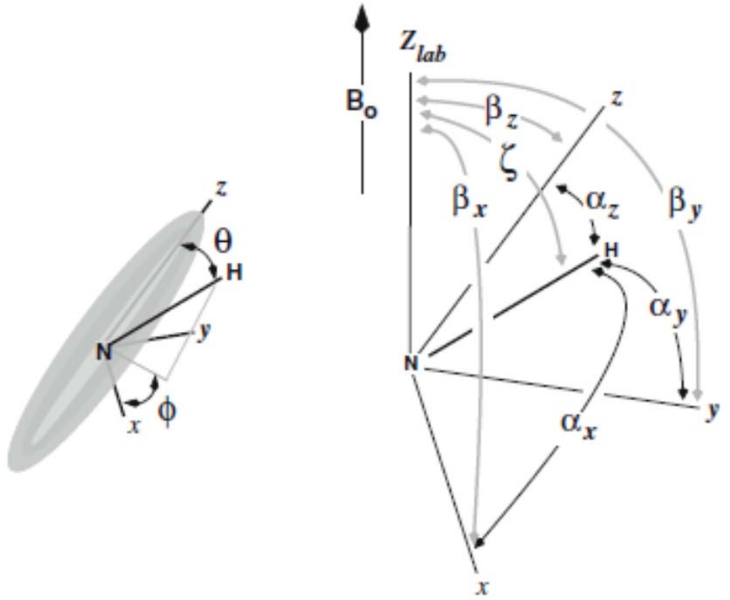


Residual Dipolar Couplings



RDCs and Structure

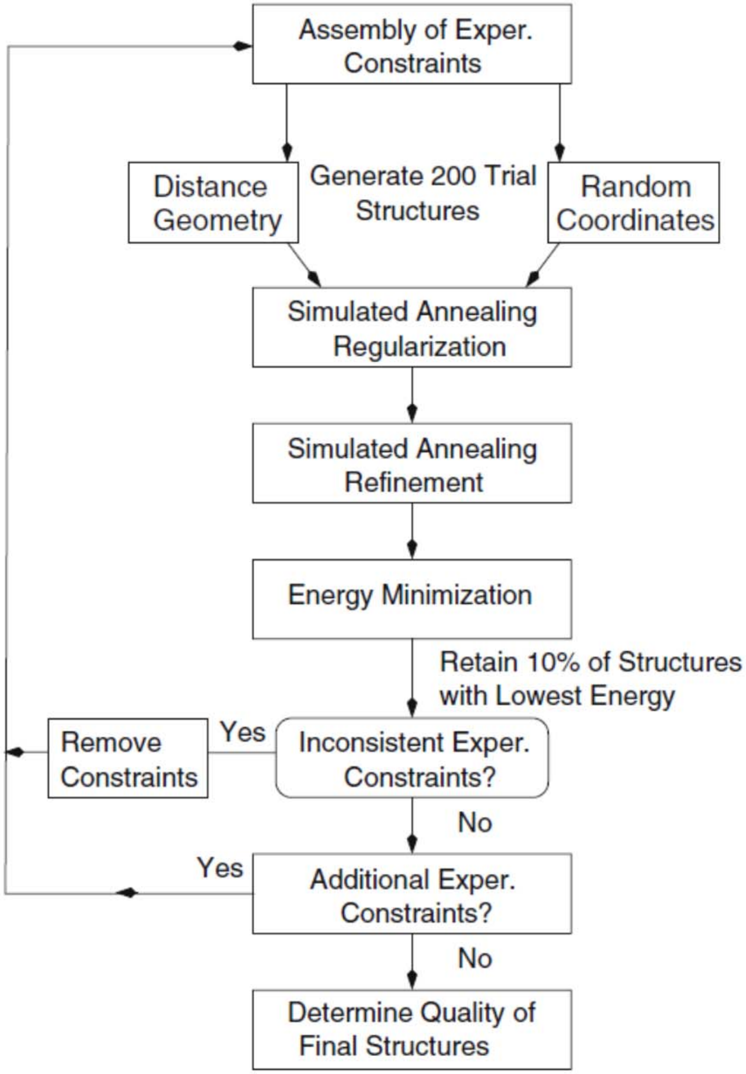
- **Determine alignment frame:** how does the molecule align in the media?
- Not easy if you don't know the structure!
- Then, can interpret all RDCs as an angle relative to that frame



Rule & Hitchens, p. 371.

Putting it all Together

- Computer-intensive process
- Use molecular force fields with experimental data



Rule & Hitchens, p. 383.