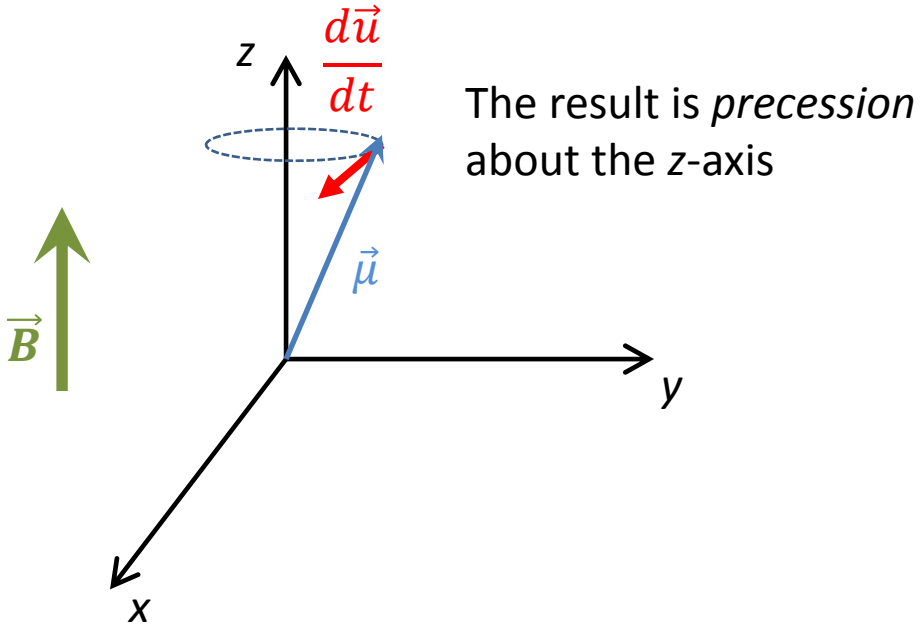
Why Fourier Transforms?

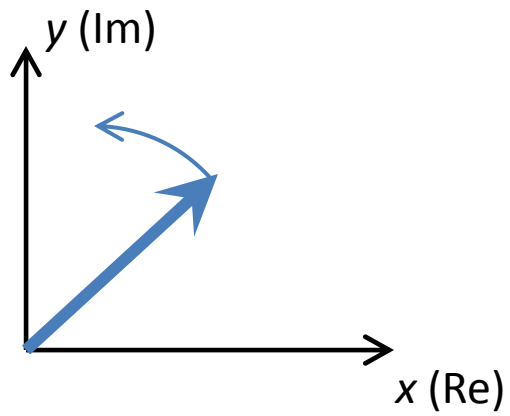
- In a magnetic field, individual nuclear spins feel a torque, and $\frac{d\vec{\mu}}{dt} = \gamma\vec{\mu} \times \vec{B}$



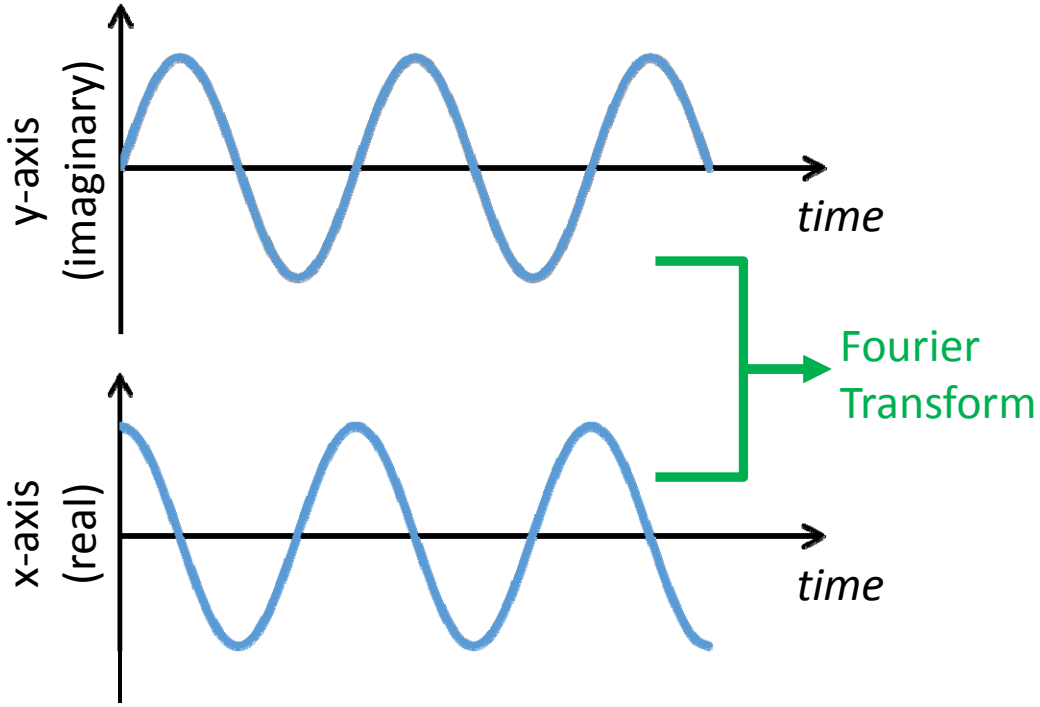
The result is *precession* about the *z*-axis

Larmor Precession

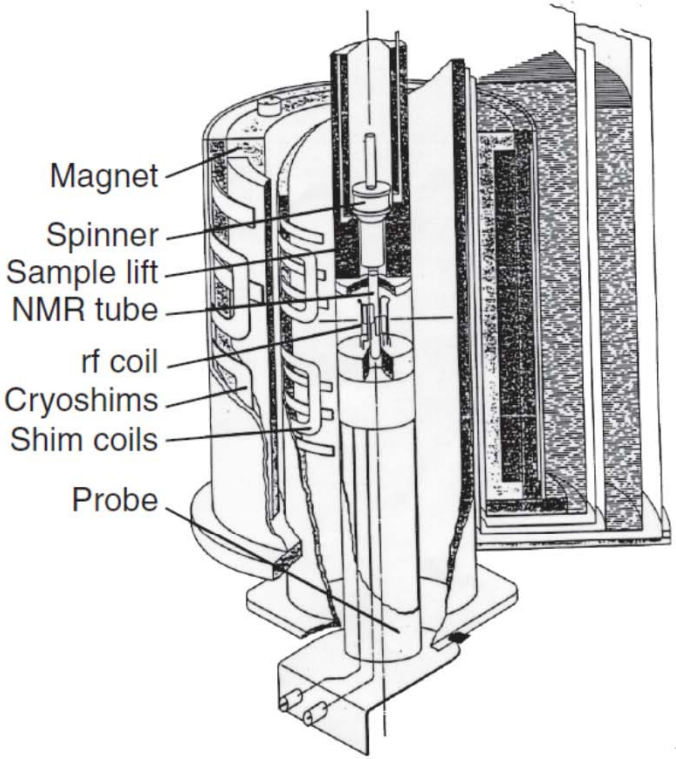
- When excited, spins will precess at the *Larmor frequency*, $\omega = \gamma B_0$. This is *identical* to the absorption frequency.

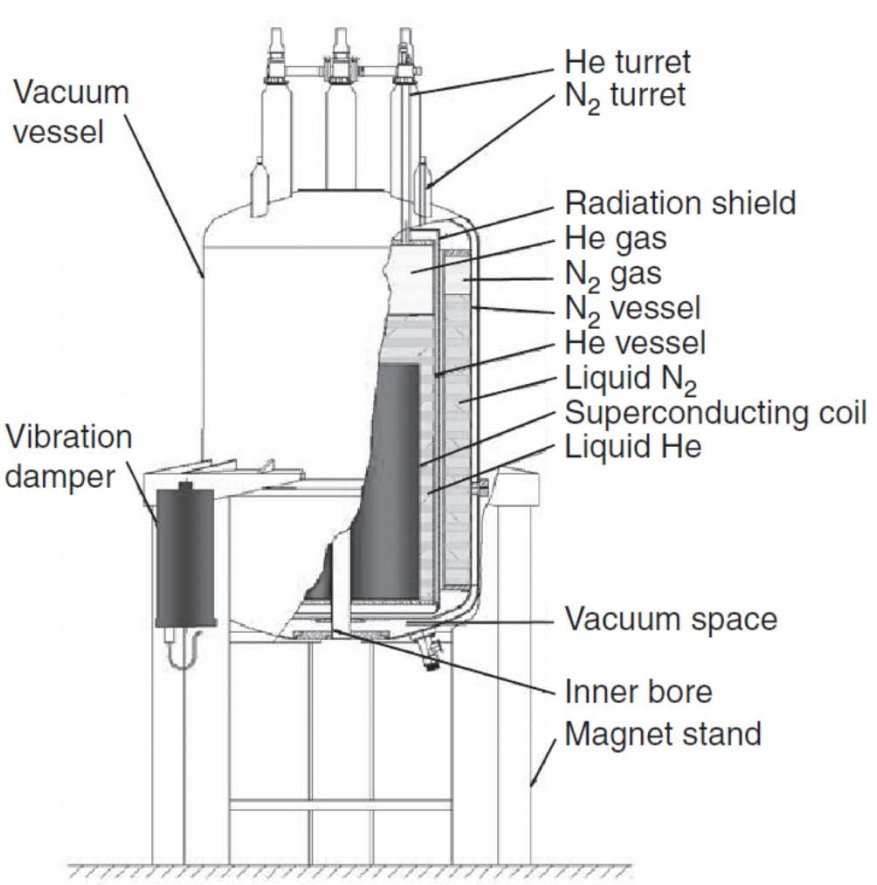


We detect magnetization as it precesses in the sample.



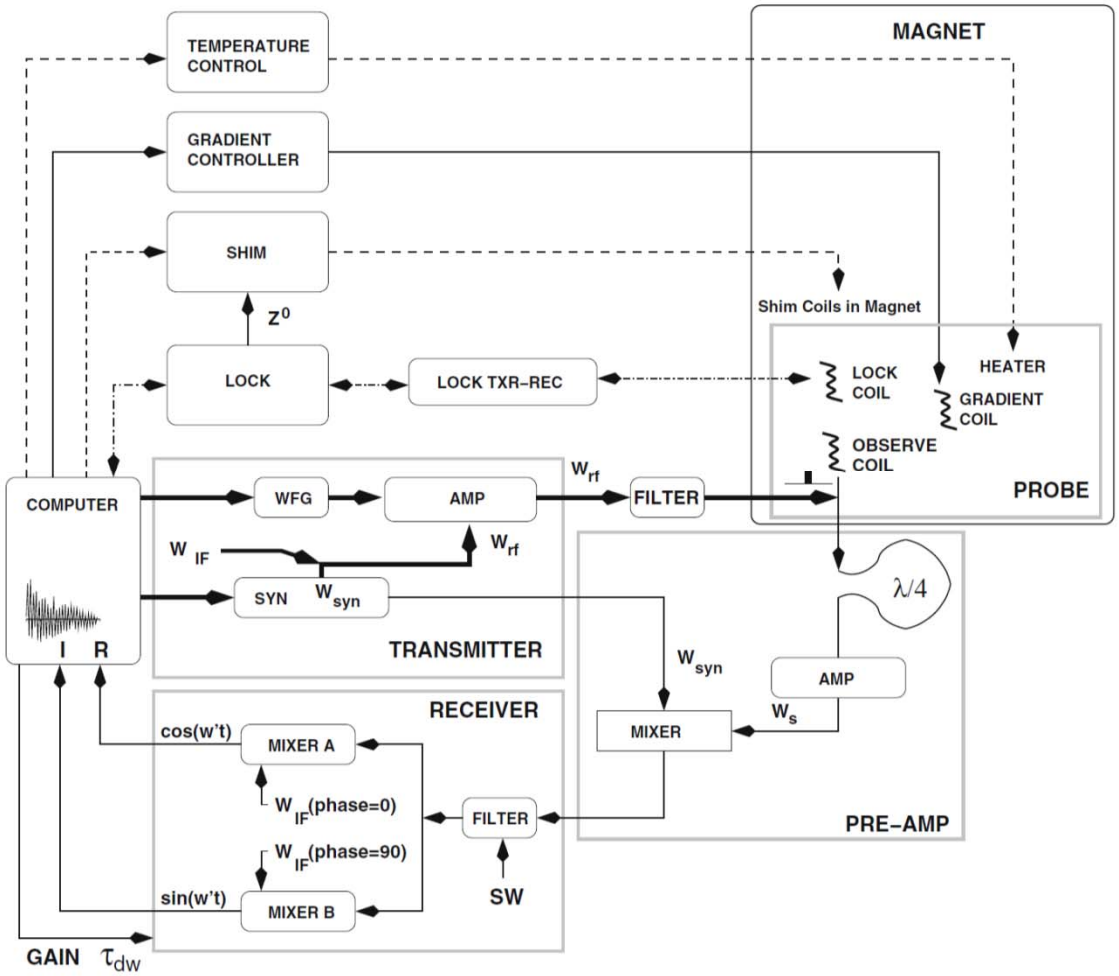
NMR Instrumentation





From *Protein NMR Spectroscopy*
Cavanagh *et al.*, Chapt. 3, p. 116

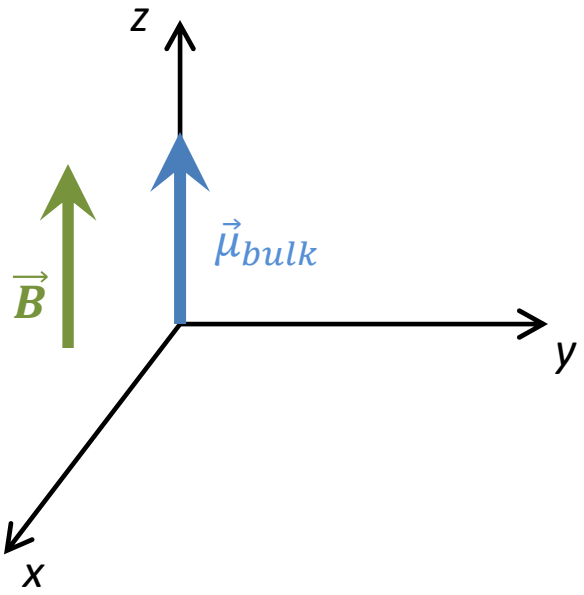
Block Diagram



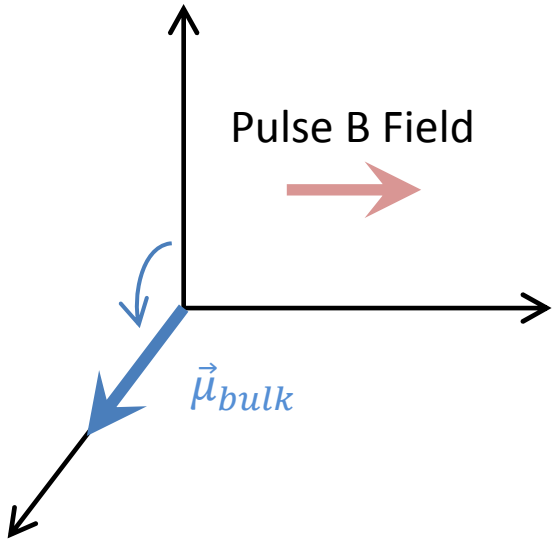
From *Fundamentals of Protein NMR Spectroscopy*
Rule & Hitchens, Chapt. 2, p. 30

1-Dimensional Pulse Program

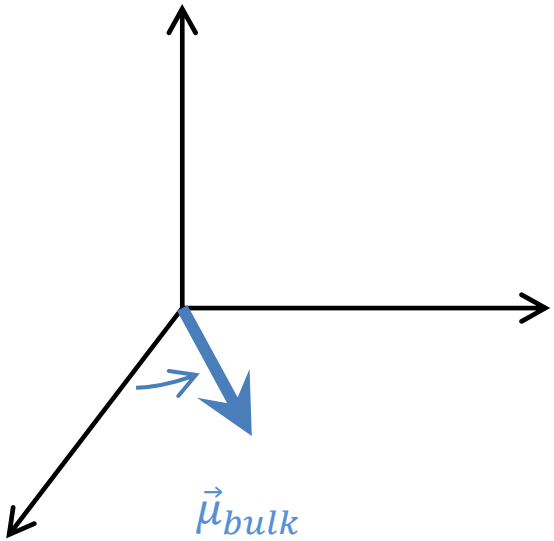
(assumes off-resonance spin in a rotating x, y, z frame)



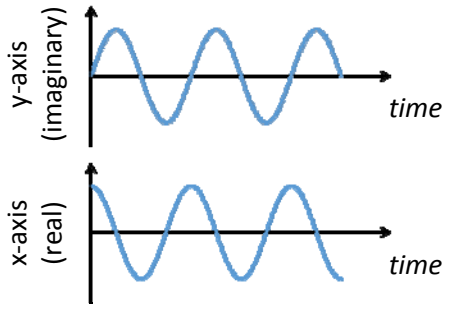
x, y components in a bulk system average out, so bulk magnetization points along z.



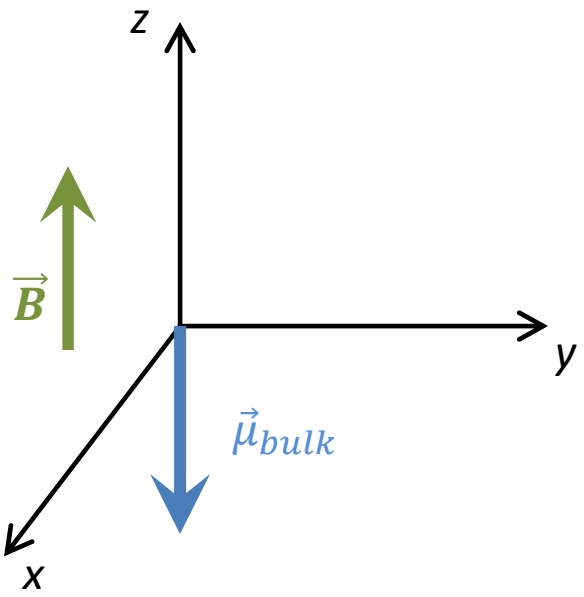
90° pulse along y rotates bulk magnetization to x axis



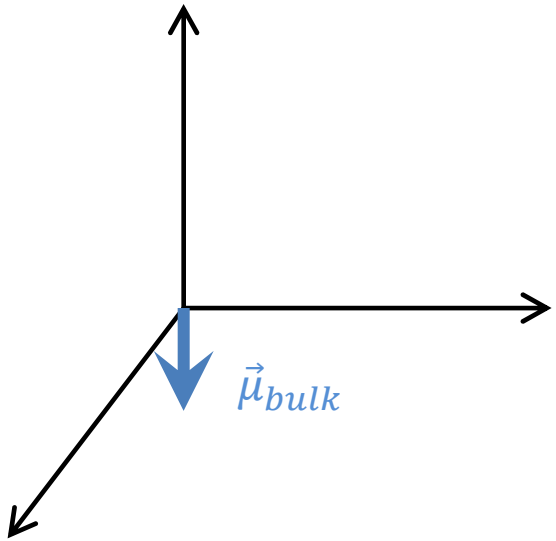
Bulk magnetization precesses in xy plane



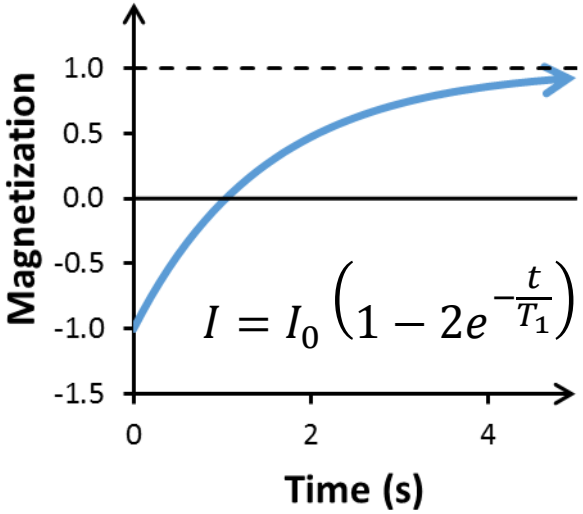
Relaxation: T1 Relaxation



After a 180° pulse, bulk magnetization points along –z, but it is not at equilibrium



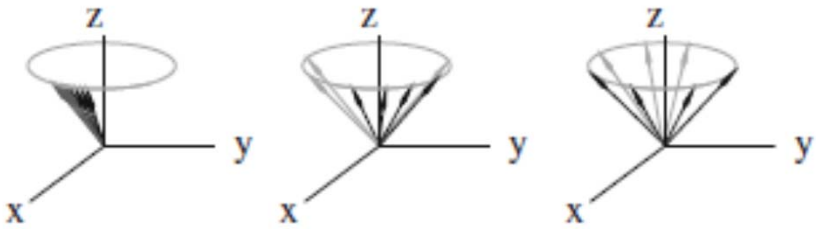
Over time, bulk magnetization returns to +z axis (equilibrium)



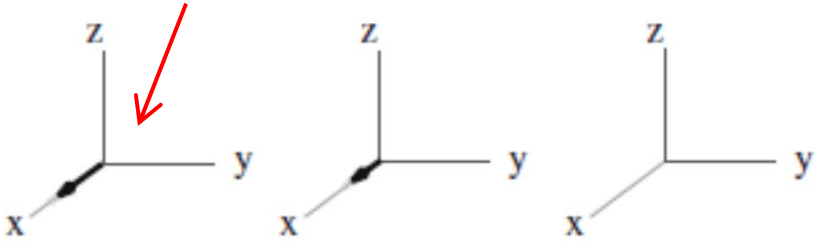
Relaxation with exponential time constant T_1

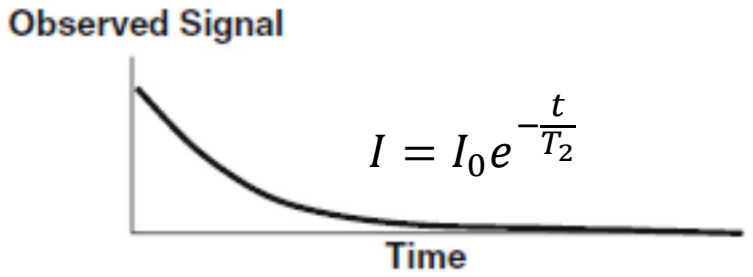
Relaxation: T2 Relaxation

- Bulk magnetization is sum of many individual magnetic dipoles
- Over time dipoles "diphase" – they become randomized
- Once de-phased, there is no net signal to measure



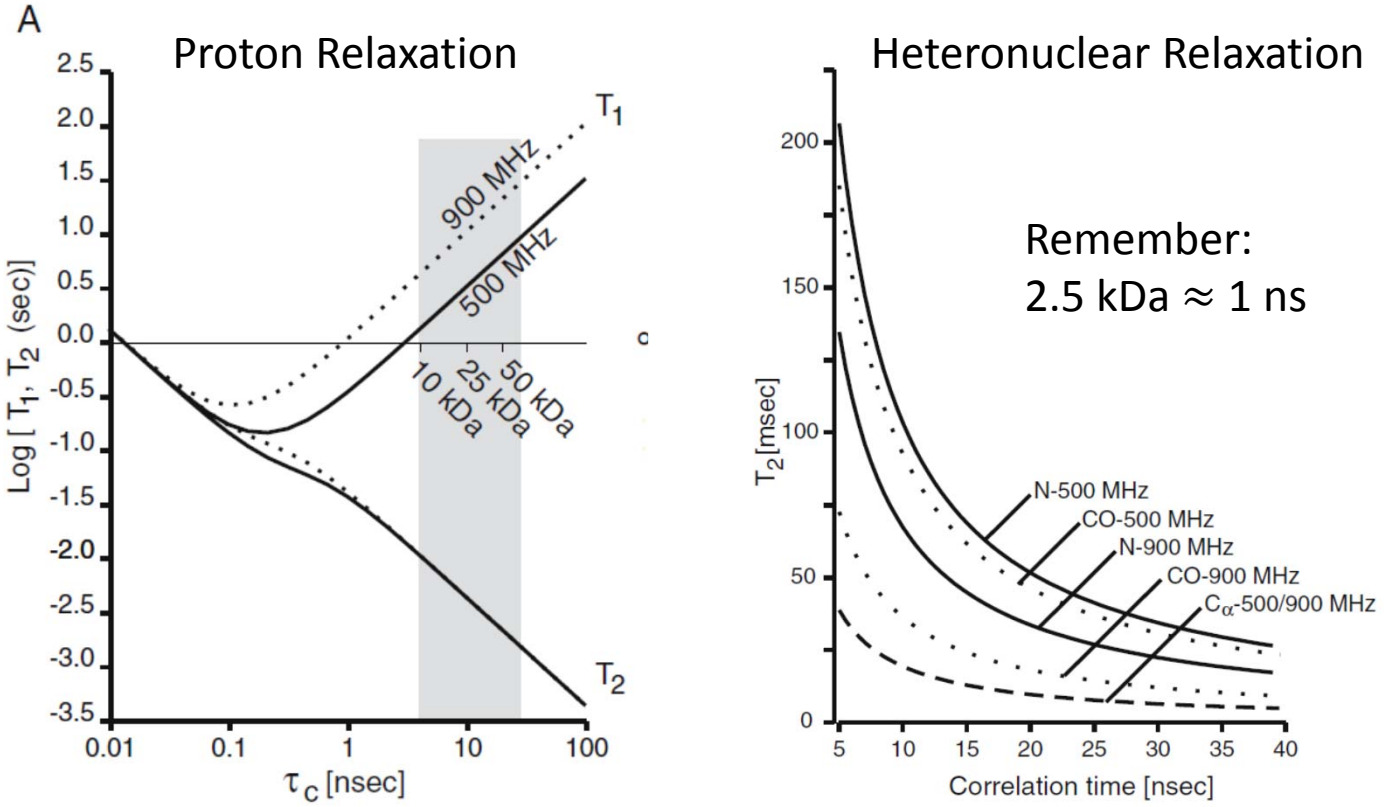
Note: On-resonance magnetization rotates at the same rate as the rotating xyz frame; therefore, it appears static.





From *Fundamentals of Protein NMR Spectroscopy*
Rule & Hitchens, Chapt. 1, p. 16

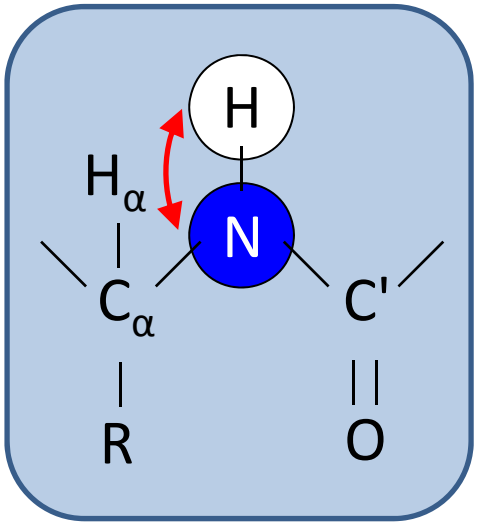
Relaxation for Proteins



- Big proteins have faster T_2 values

From *Fundamentals of Protein NMR Spectroscopy*
Rule & Hitchens, Chapt. 1, p. 16

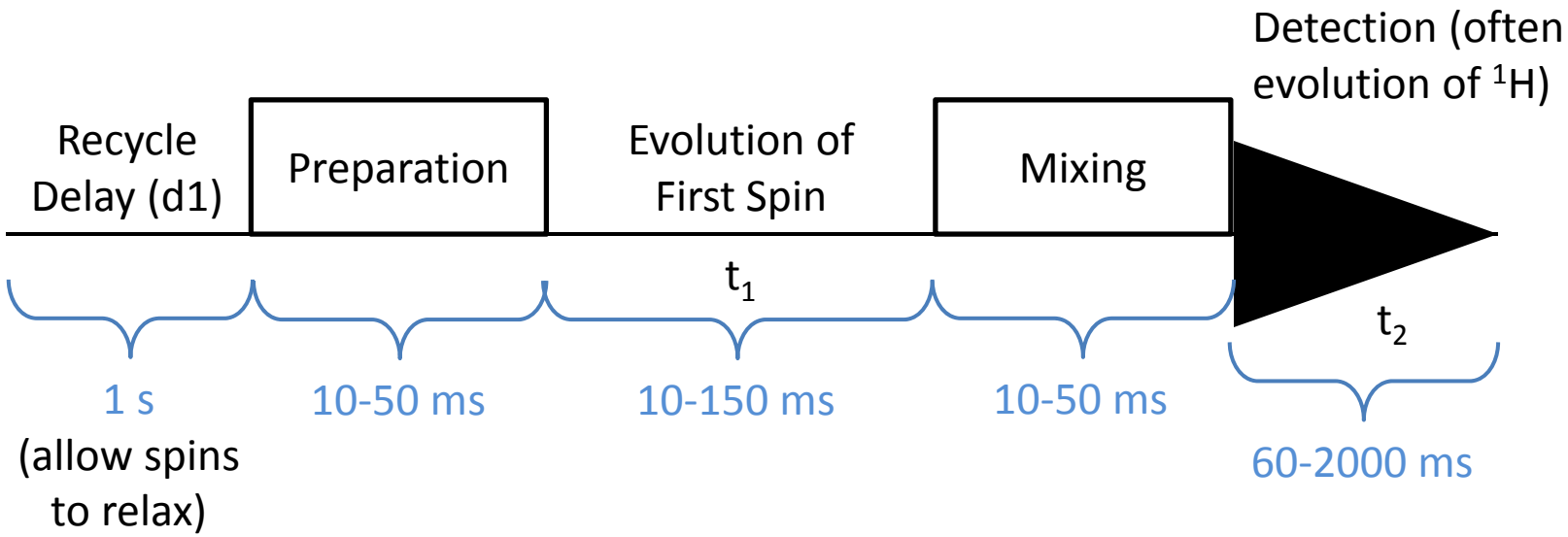
Multidimensional NMR



Idea: Correlate spins through *magnetization transfer*. This relies on quantum mechanics (and is beyond the scope of this class)

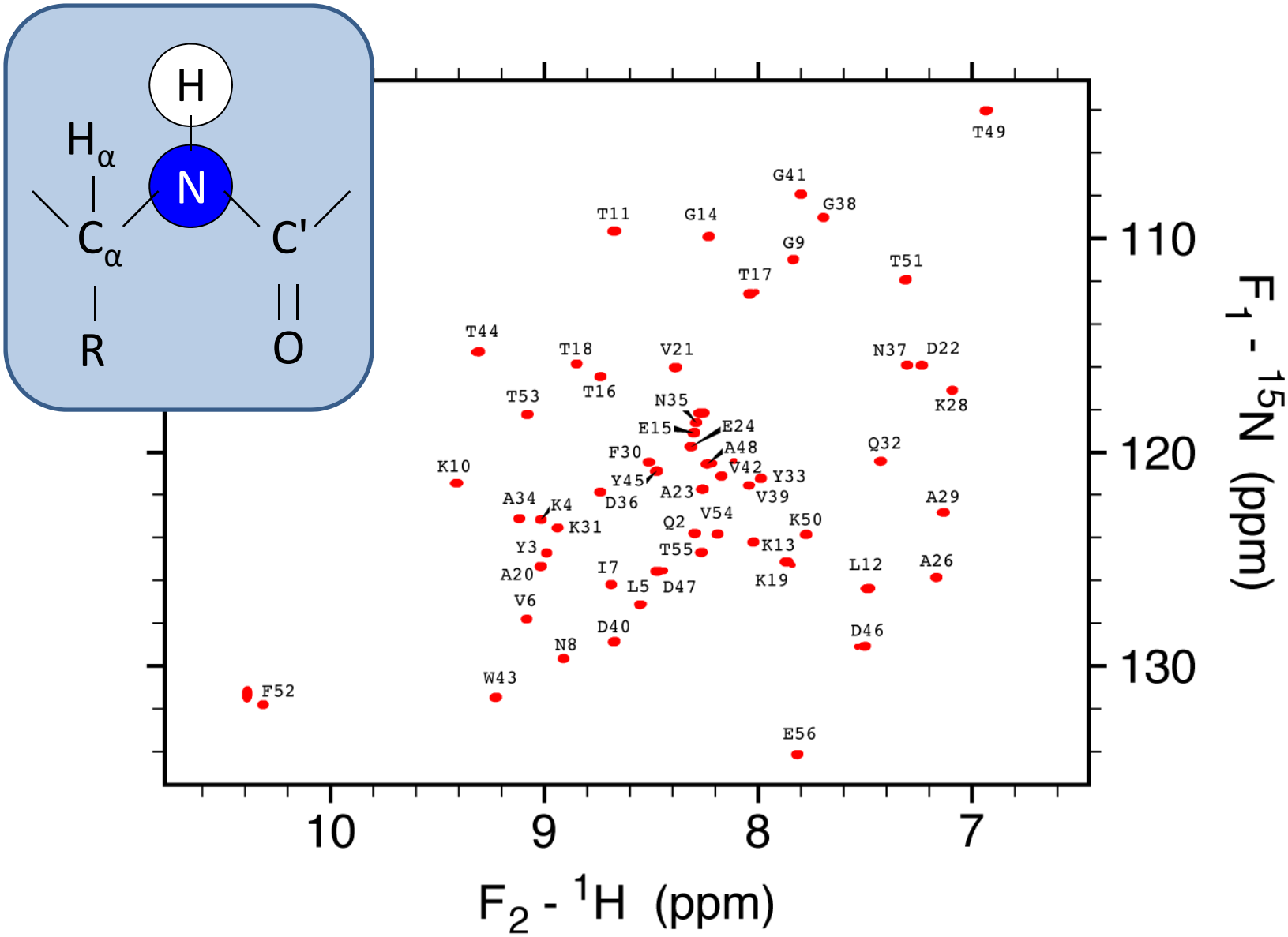
- Start magnetization on nucleus A and evolve chemical shift (a)
- Transfer magnetization to nucleus B and evolve chemical shift (b)
- Plot a peak at coordinates (a, b) corresponding to *spin system*

Multidimensional NMR Experiment

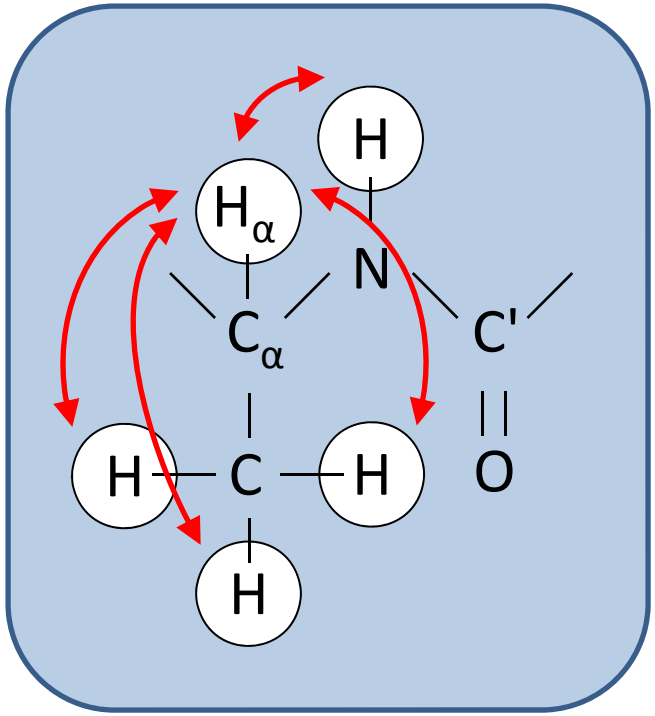


- Multiple evolution/mixing times may be included (3D, 4D experiments)
- **Consideration:** Relaxation times

2D NMR ^{15}N HSQC: GB3

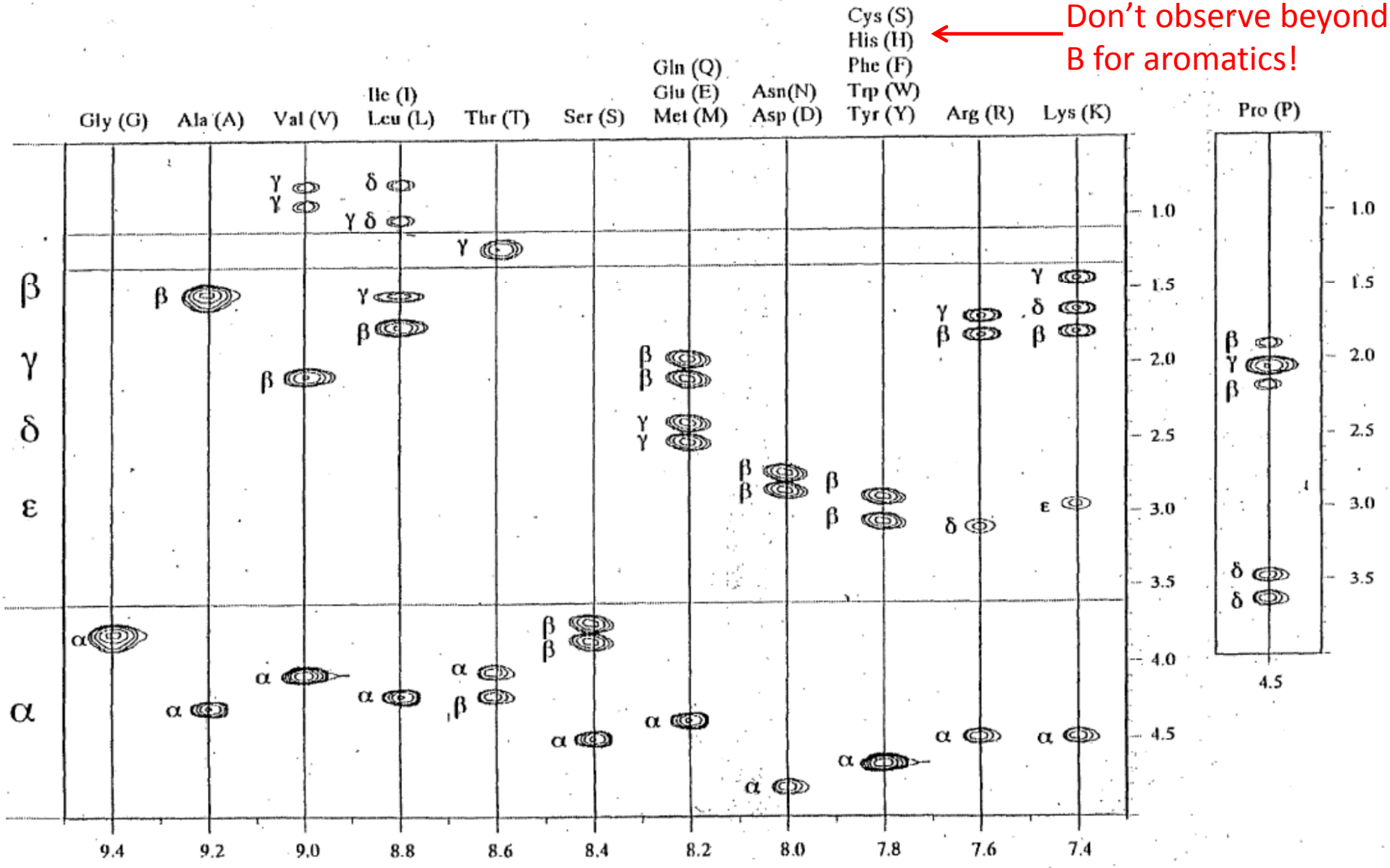


^{1}H TOCSY



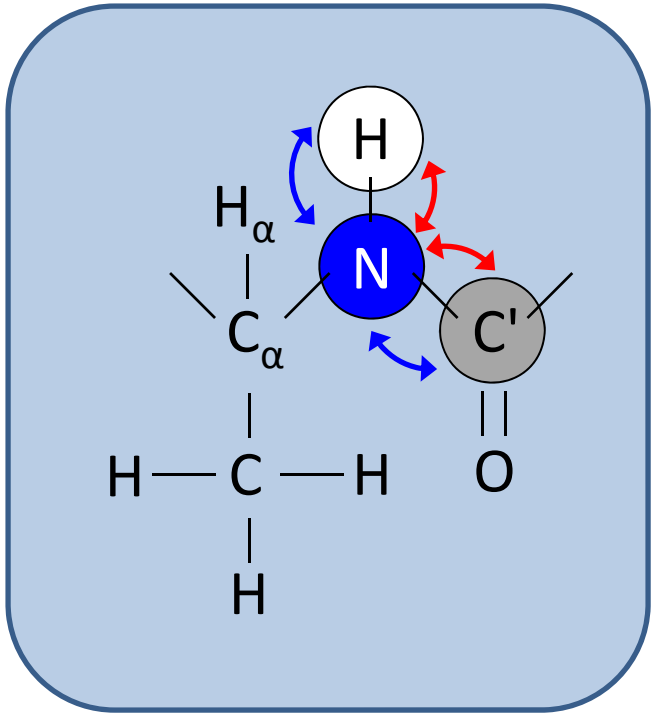
- Magnetization “hops” from nucleus to nucleus
- Example: Crosspeaks observed between H_N and: H_α, H_β, H_γ (gets weaker for more hops)

^{1}H TOCSY Fingerprints



Don't observe beyond B for aromatics!

3D HNCO

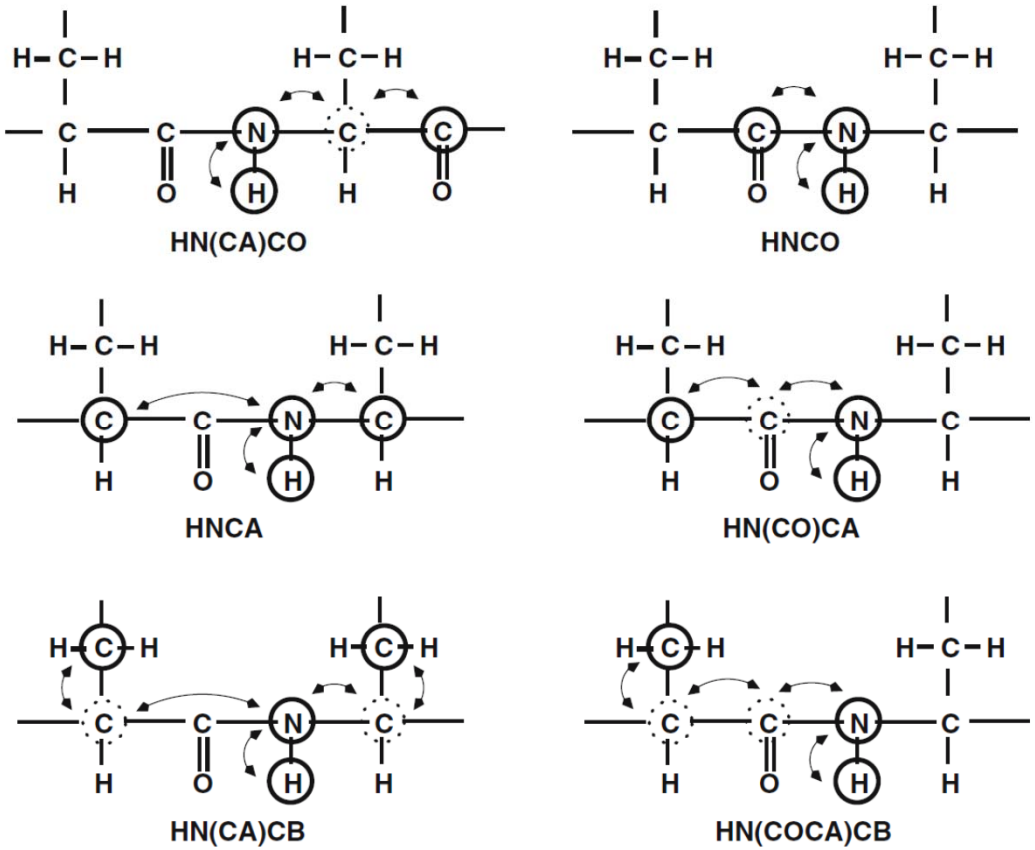


Out: Red transfers occur during preparation period

Back: Blue transfers occur between chemical shift labeling steps on the way back

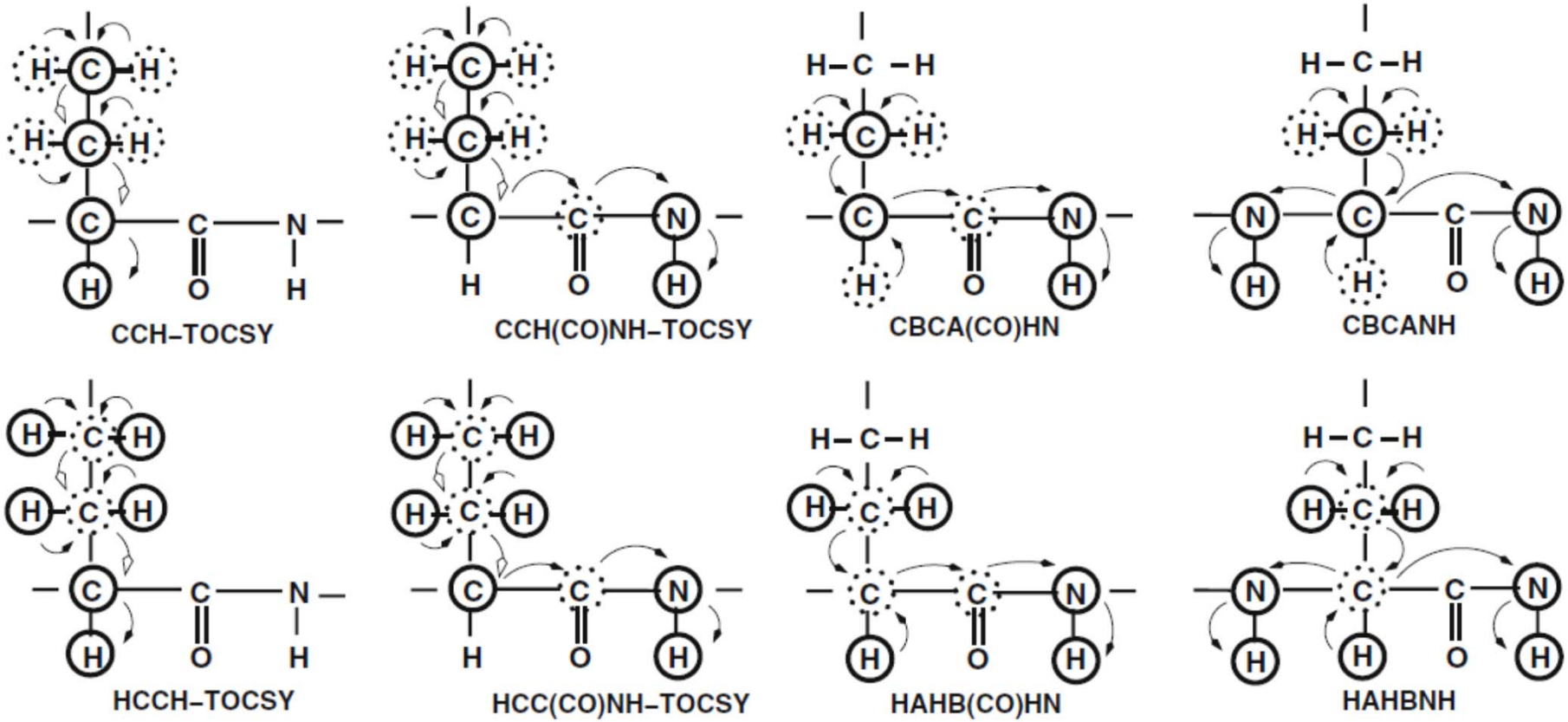
- See peaks at H_N, N, and C'_{i-1} (i.e. the C' of the prior residue)
- Requires ^{13}C and ^{15}N labeled protein

Backbone Assignment



From *Fundamentals of Protein NMR Spectroscopy*
Rule & Hitchens, Chapt. 14, p. 281

Sidechain Assignment



From *Fundamentals of Protein NMR Spectroscopy*
Rule & Hitchens, Chapt. 14, p. 302

Summary

- NMR spectra are Fourier transforms of time domain signals (FIDs)
- In a magnetic field, individual spins feel a torque, and $\frac{d\vec{\mu}}{dt} = \gamma\vec{\mu} \times \vec{B}$
- The frequency of absorption ($\omega = \gamma B_0$) is **identical** to the Larmor precession frequency ($|\Omega| = \gamma B_0$)
- NMR spins will relax over time
- Magnetization transfer → multidimensional NMR