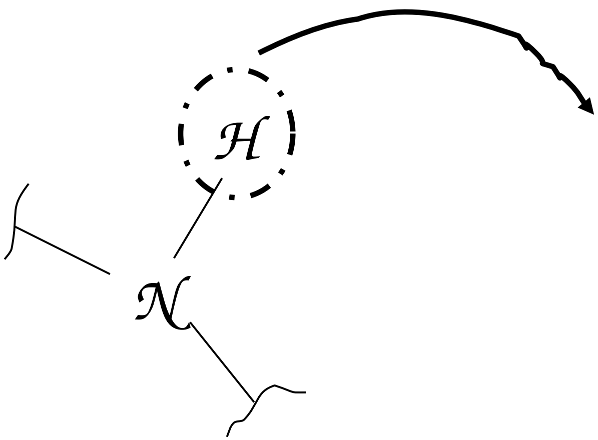
Using NMR to study Macromolecular Interactions

John Gross, BP204A
UCSF

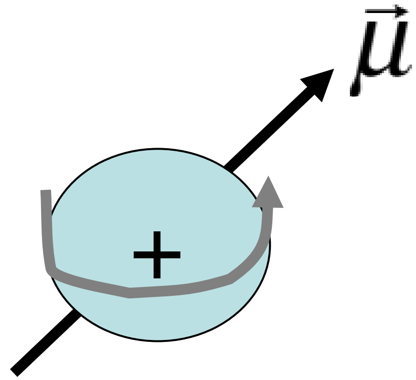
Outline

- Multidimensional NMR
- Macromolecular Interactions
- Dynamics
- Dealing with large complexes
- Structure Determination

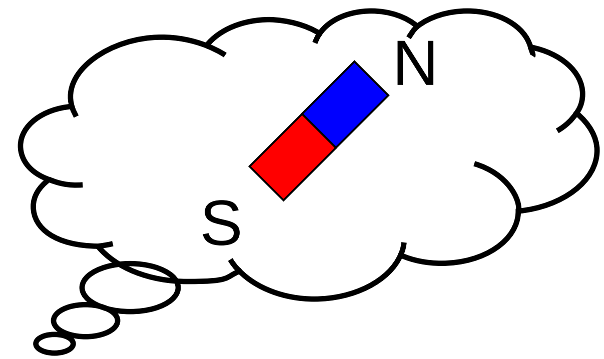
Review: Nuclear Spins are Microscopic Bar Magnets



Protein Fragment



Magnetic Moment



Bar Magnet

Magnetic moment $\vec{\mu} = \gamma \vec{S}$ Angular Momentum

The proportionality constant γ: strength of bar magnet

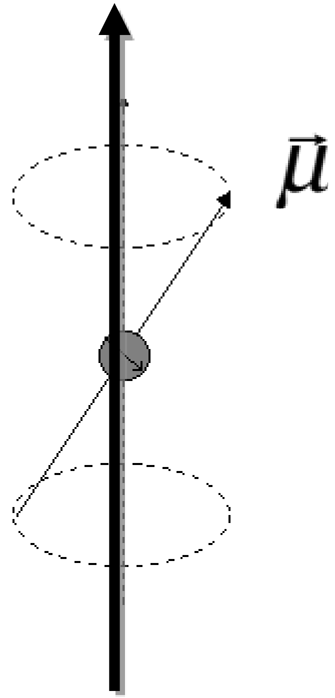
Equation of Motion

$$\frac{d\vec{\mu}}{dt} = \gamma \vec{B} \text{ x } \vec{\mu}$$

Based on magnetic torque:

$$\frac{d\vec{L}}{dt} = \vec{B} \times \vec{L}$$

Spin Precession



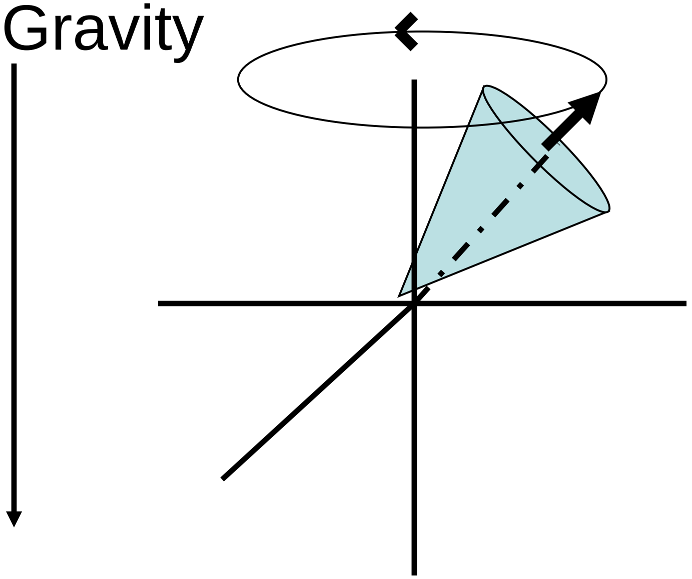
Magnetic Field, Bo

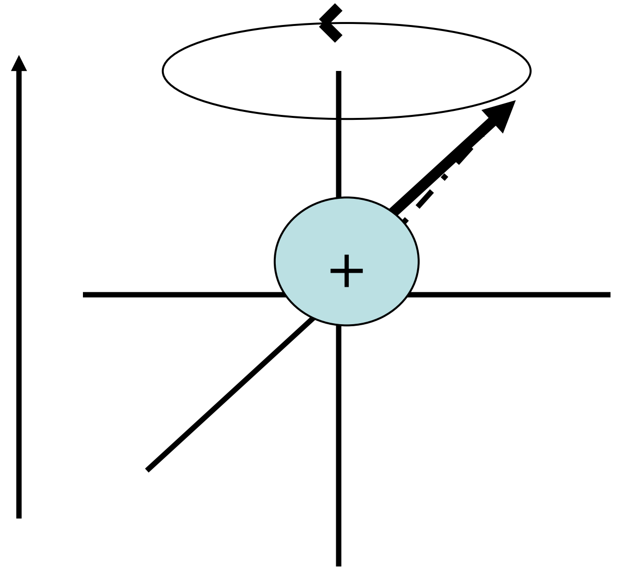
Precession frequency: $\gamma B_0 = \omega_0$

Driving Forces for Precession

Precessional Orbits

Gravity



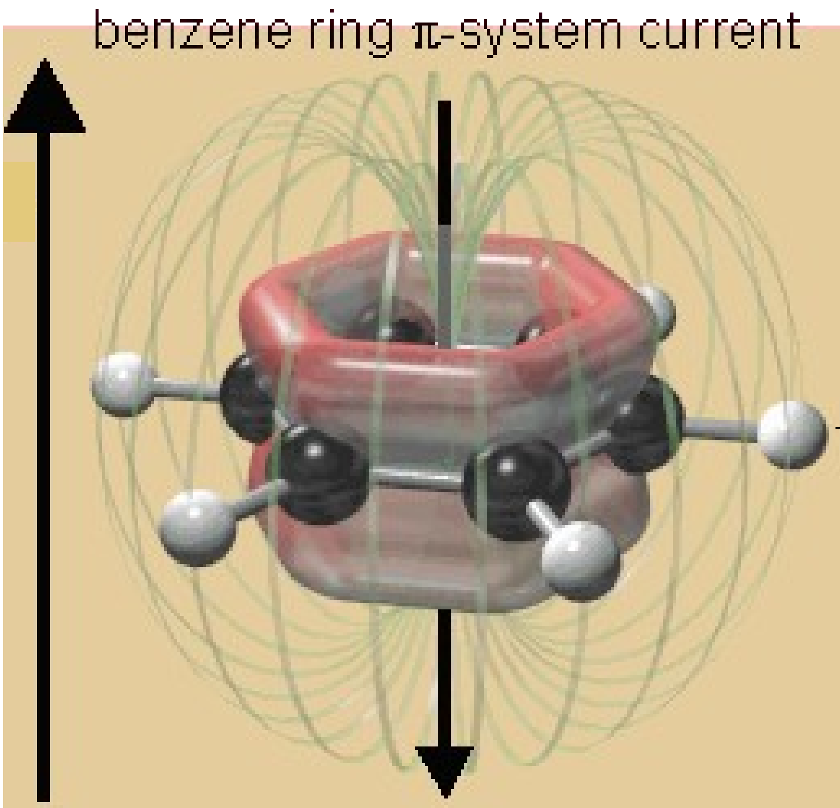


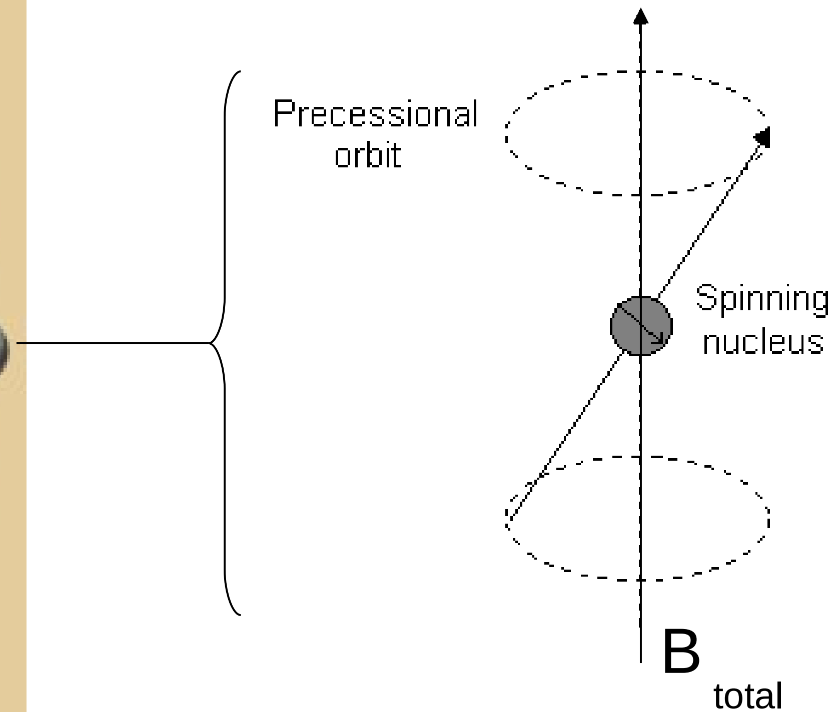
Applied magnetic field, B_0

Spinning Top

Spinning Nucleus

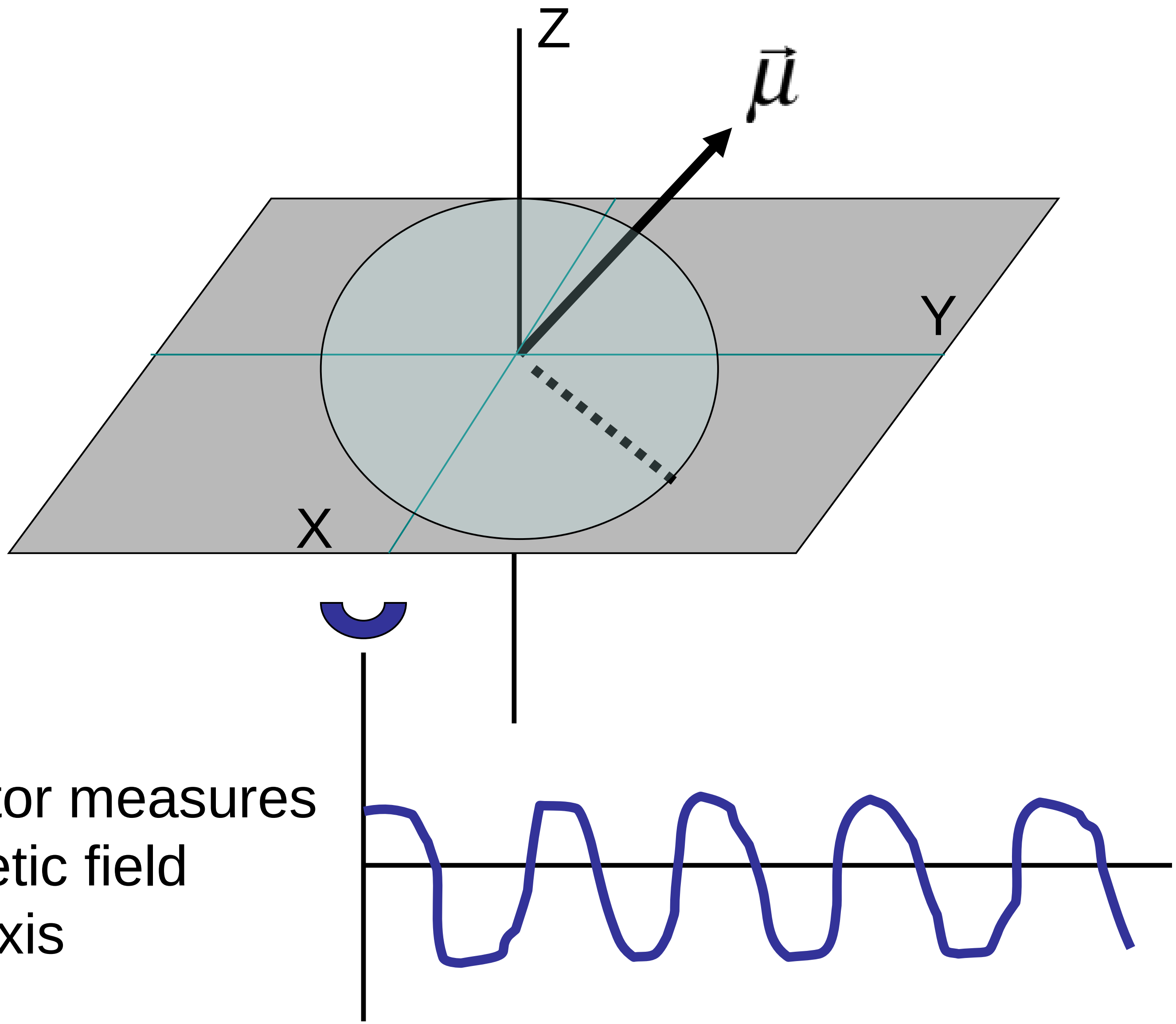
Nuclear Spins Report Local Environment





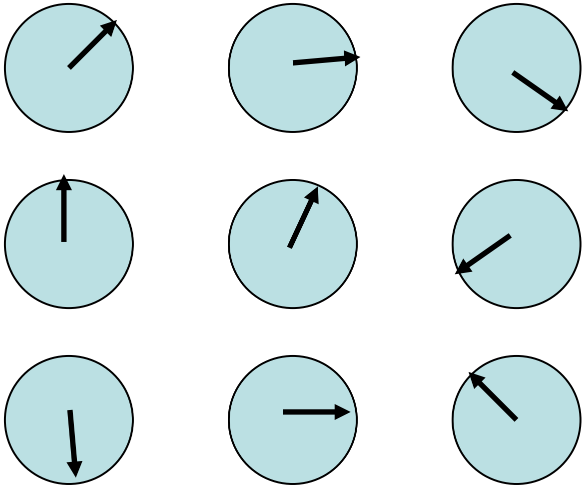
$B_{applied}$ + B_{local} = B_{total} **determines precession**

Detection of Spin Precession



Detector measures magnetic field on x-axis

Net Magnetization



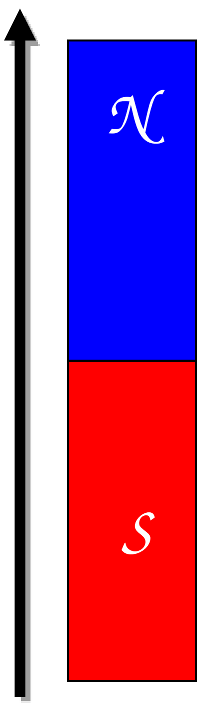
$$M_x = \sum_j \mu_x^j = 0$$

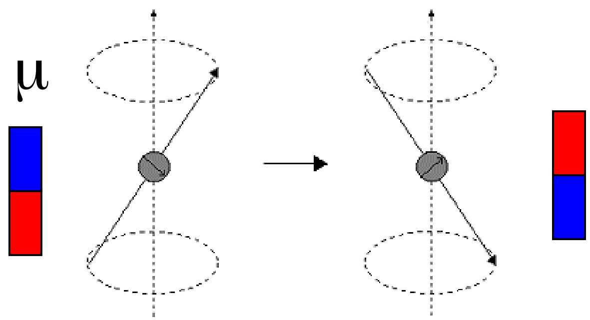
$$M_y = \sum_j \mu_y^j = 0$$

No Transverse Magnetization at equilibrium

Magnetic Energy

$$E = -\vec{\mu} \bullet \vec{B}$$

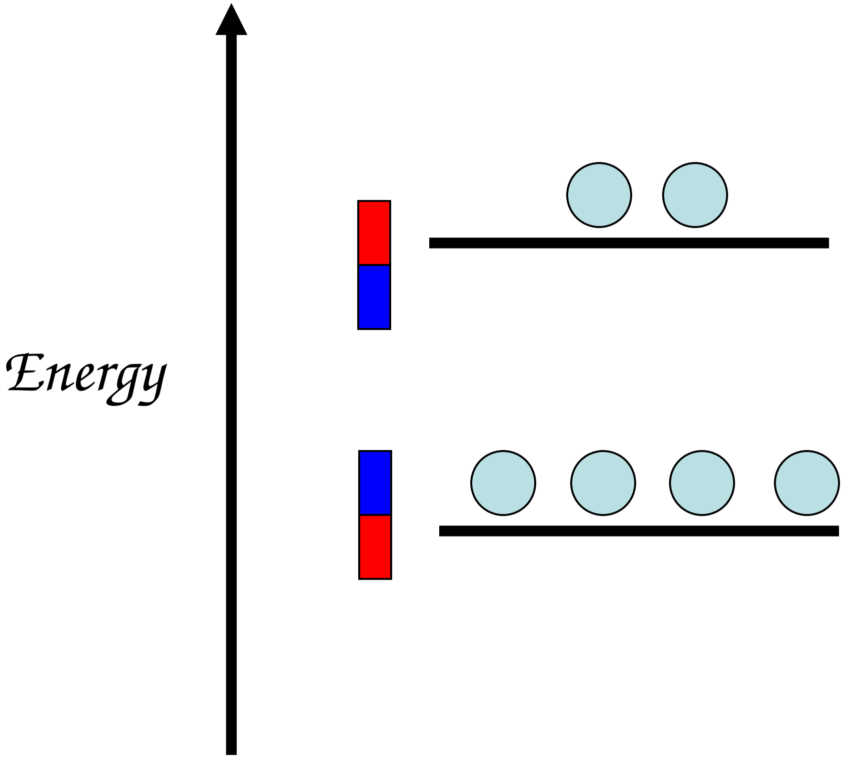




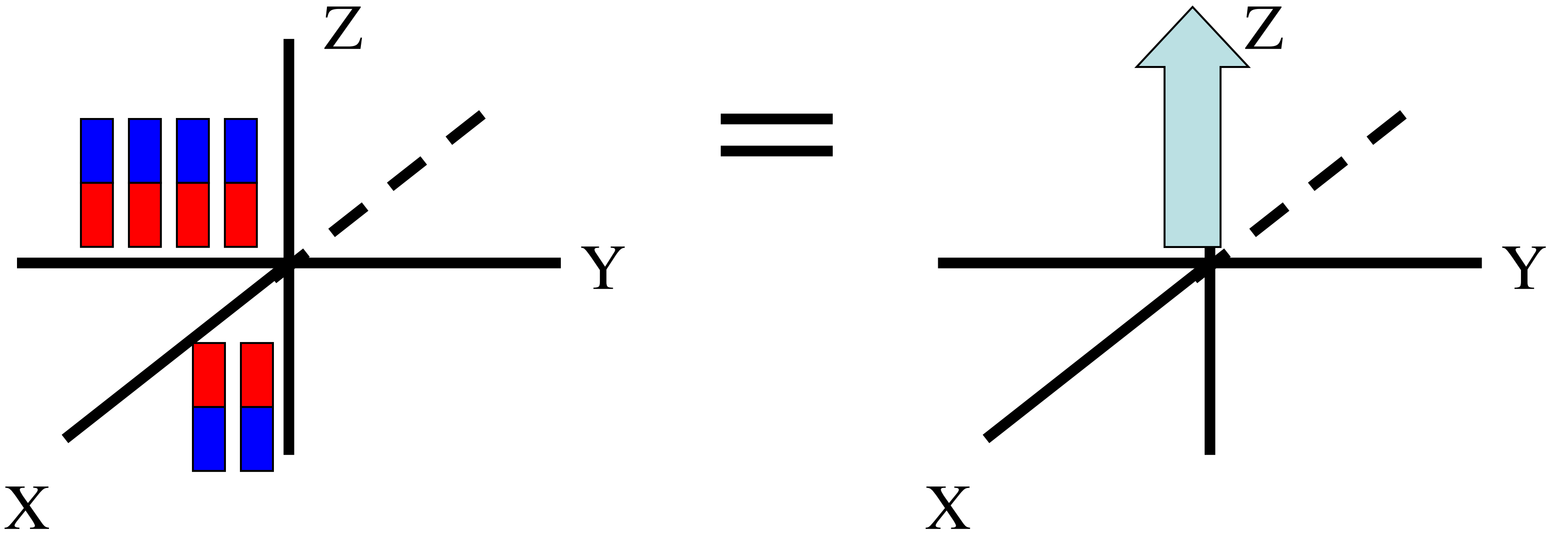
$$E = -\mu_z B_z$$

Static Magnetic Field Oriented Along Z-Axis

Energy States (spin-1/2 nucleus)

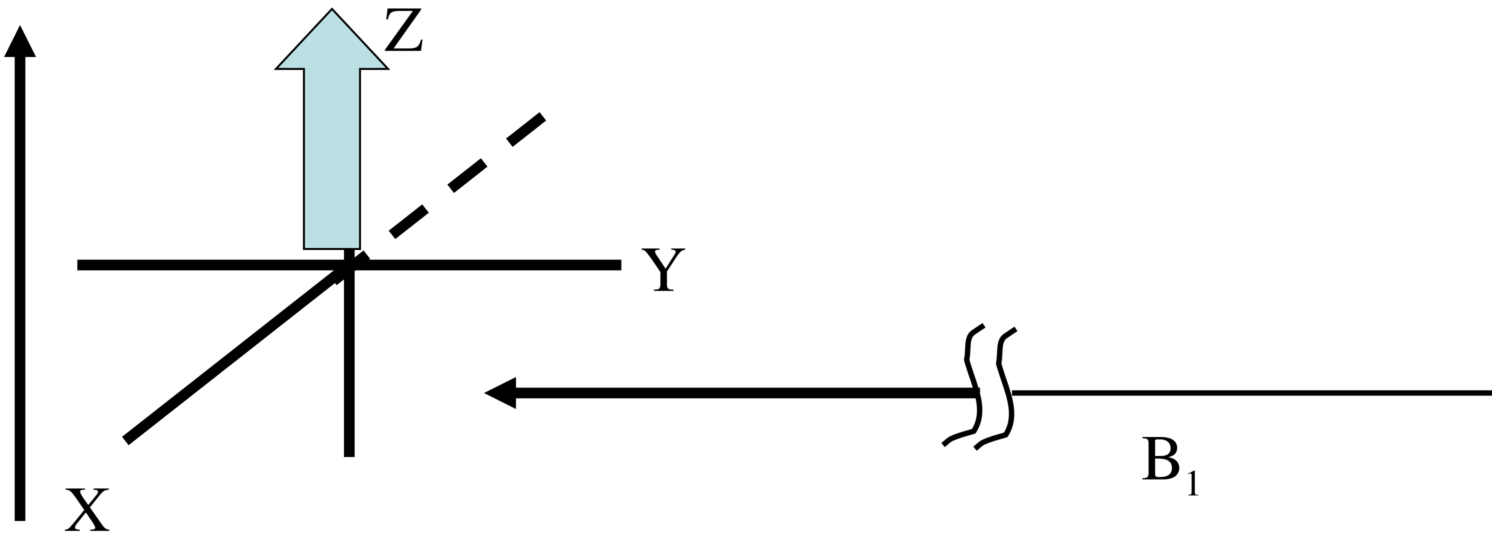


Net Magnetization along Z Axis



$$\sum_{j} \mu_z^j = M_z$$

Thought experiment: apply 2nd field along Y Axis



Bo

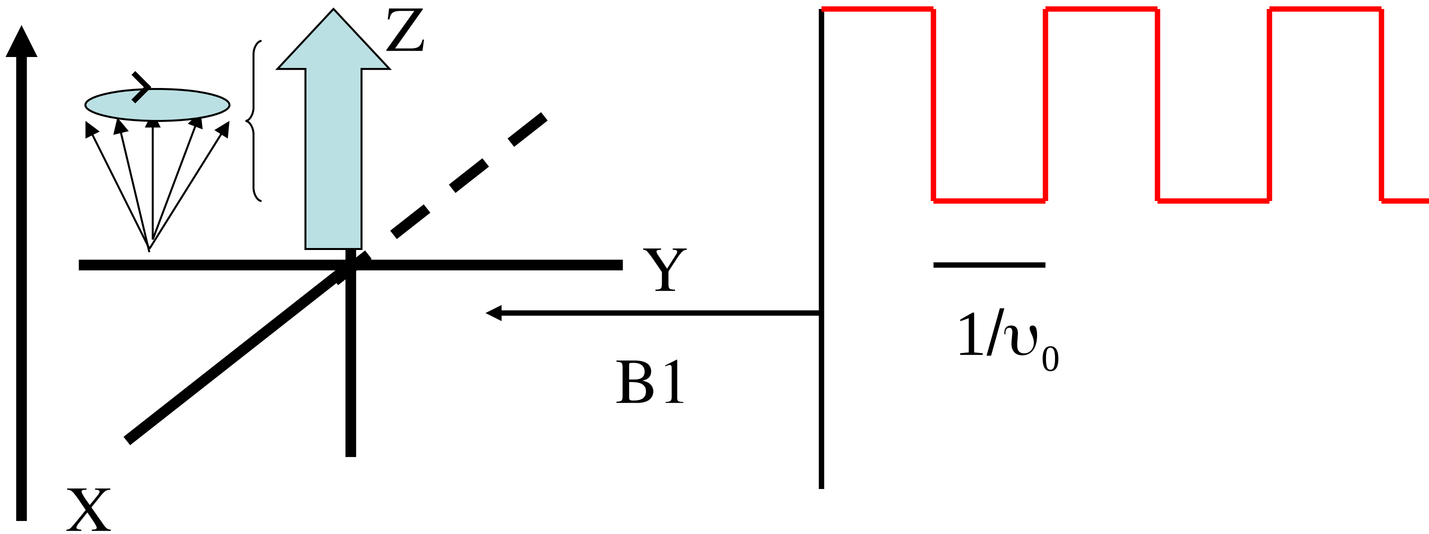
If $B_1 >> B_0$, M_Z would rotate about B_1.

Leave B_1 on until X axis reached ----> transverse magnetization

Approach is not practical.

Same effect achieved with weak, resonant oscillating field

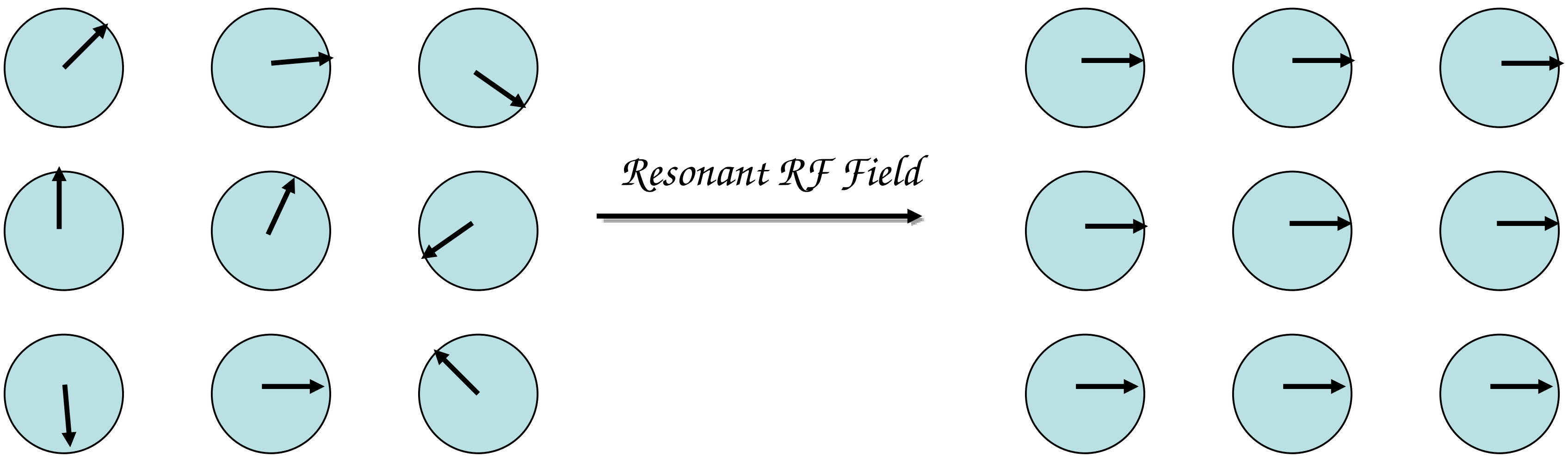
Bo



Turn B_1 on and off with a frequency matching the precessional frequency

Resonance

Ensemble of Nuclear Spins

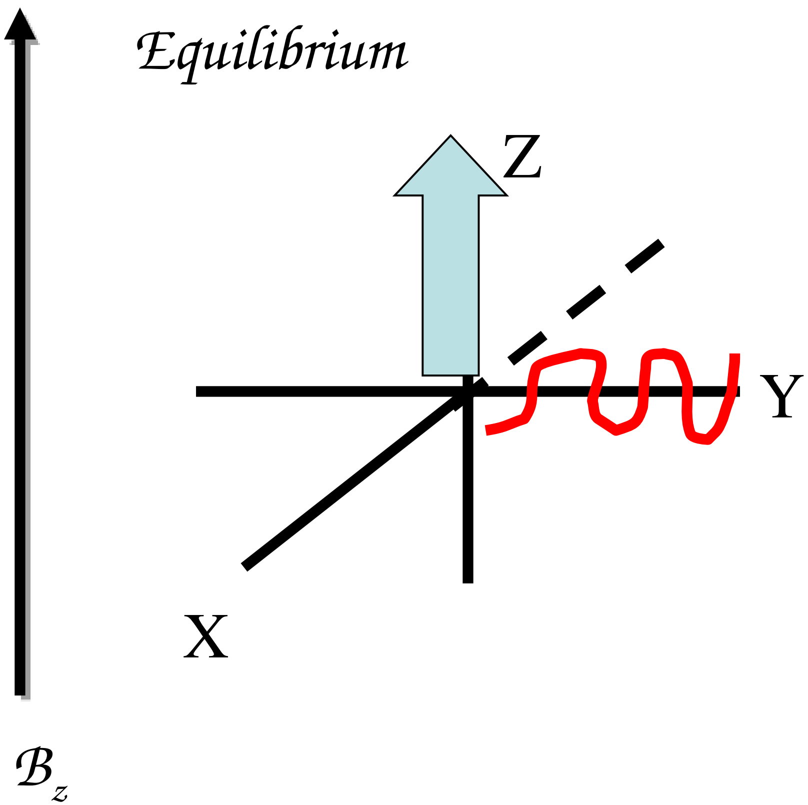


Random Phase
No NMR Signal

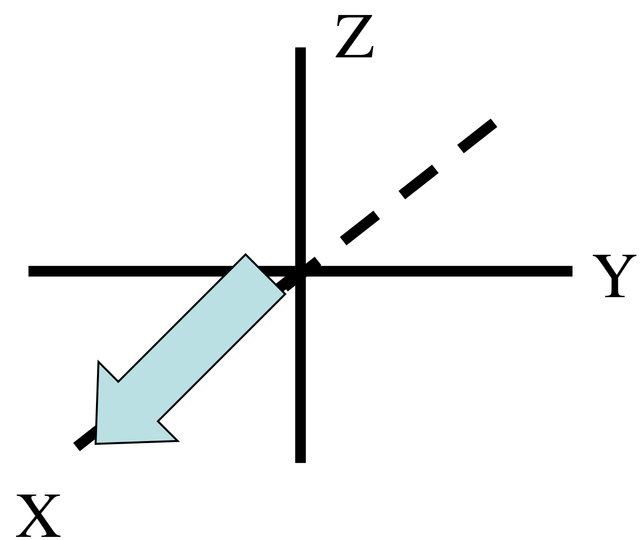
Phase Synchronization
NMR Signal!

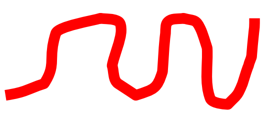
Magnetization Vector Model

Equilibrium



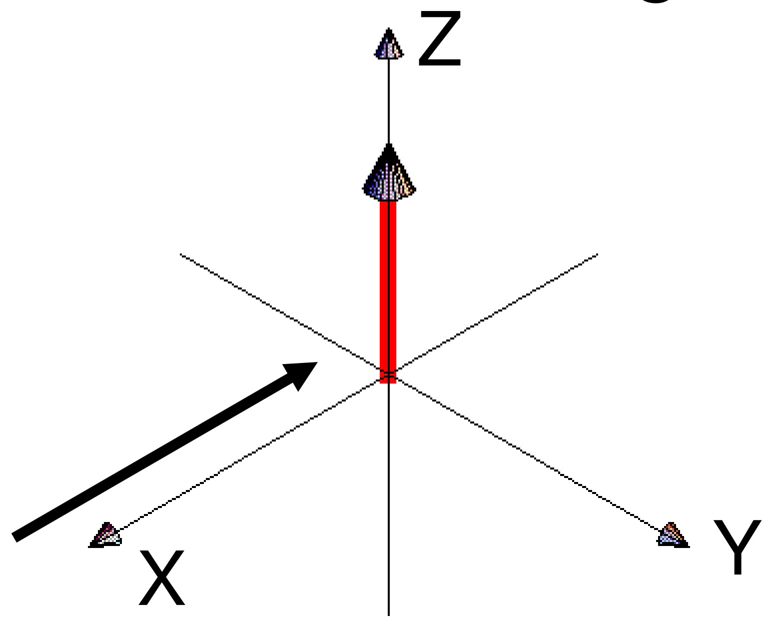
After 90 degree pulse





90y: Resonant 90 Degree Pulse

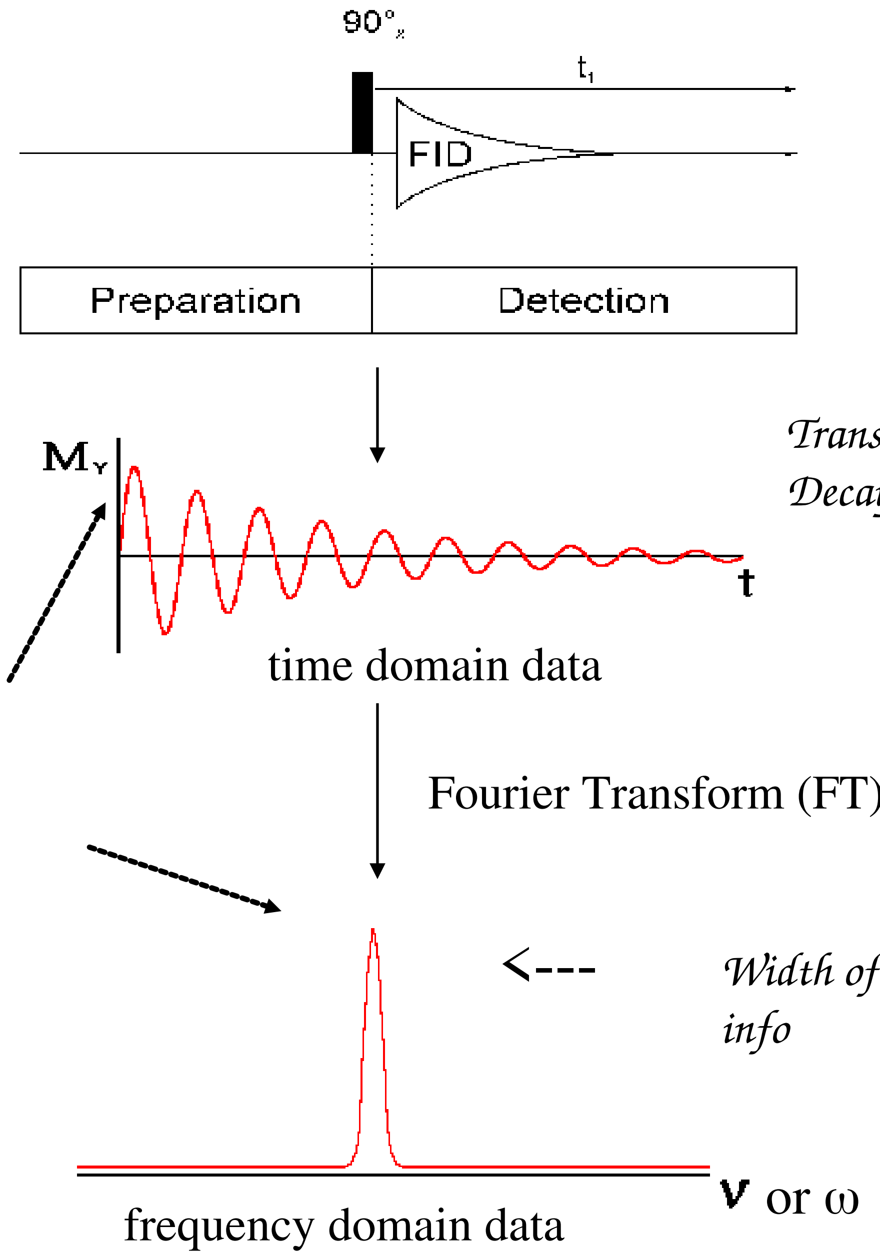
Resonant Pulse in Real Time



R.F. Field (applied at precession frequency)

Net magnetization rotated into transverse plane
Rotates due to static and local fields

Summary of 1D Experiment



Transverse Magnetization Decay constant T2

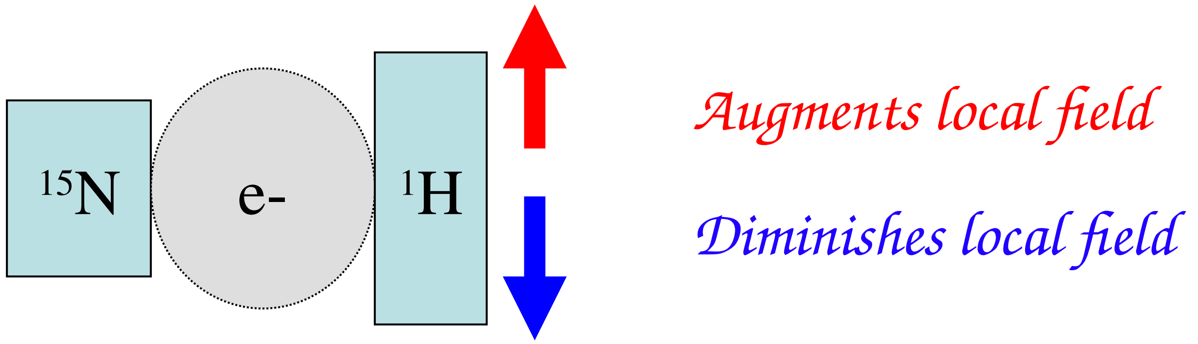
Amplitude propotional to amount magnetization prior to pulse

Width of resonance--dynamical info

Position of resonance ---> local magnetic environment

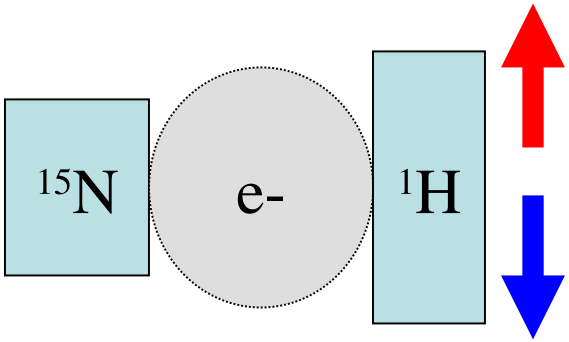
The J Coupling

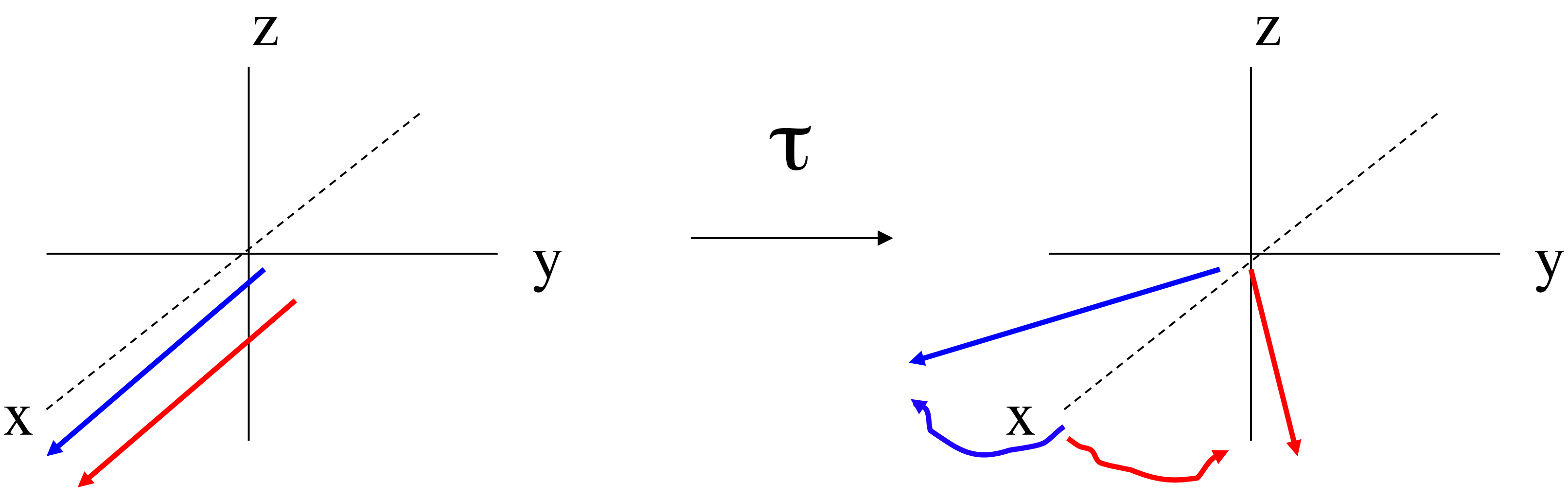
Consider two spin-1/2 nuclei (ie, ^1H and ^{15}N):



Effect transmitted through electrons in intervening bonds

Vector View

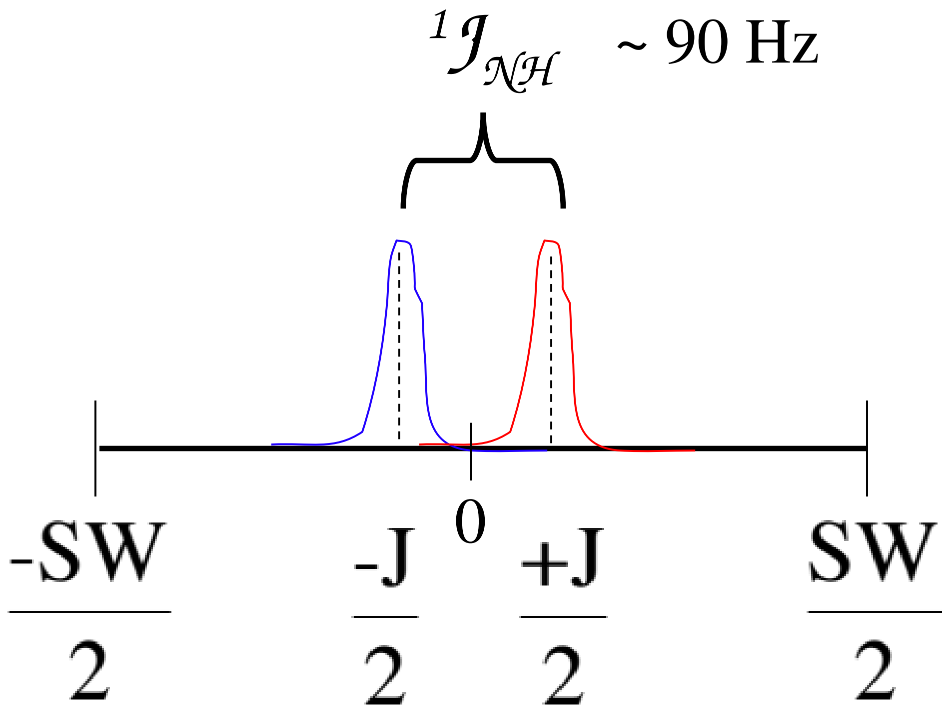




(After 90y pulse)

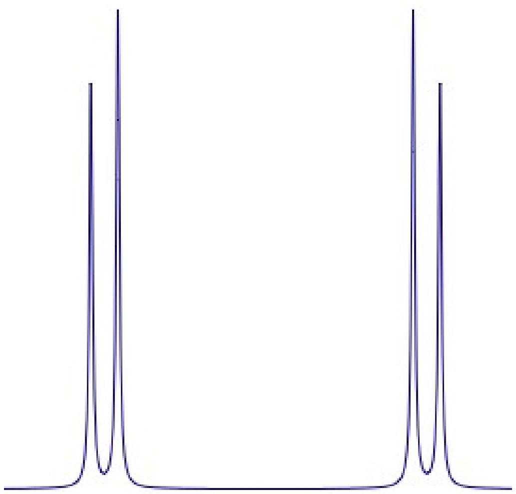
Components rotate faster or slower than rotating frame by +- J/2

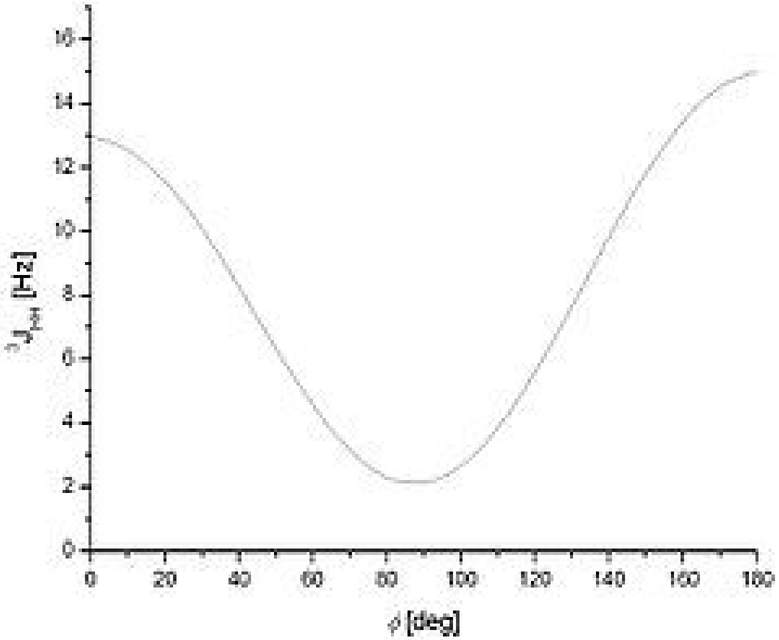
Spectrum with J coupling



^{15}N Detected Spectrum

J couplings contain information on structure





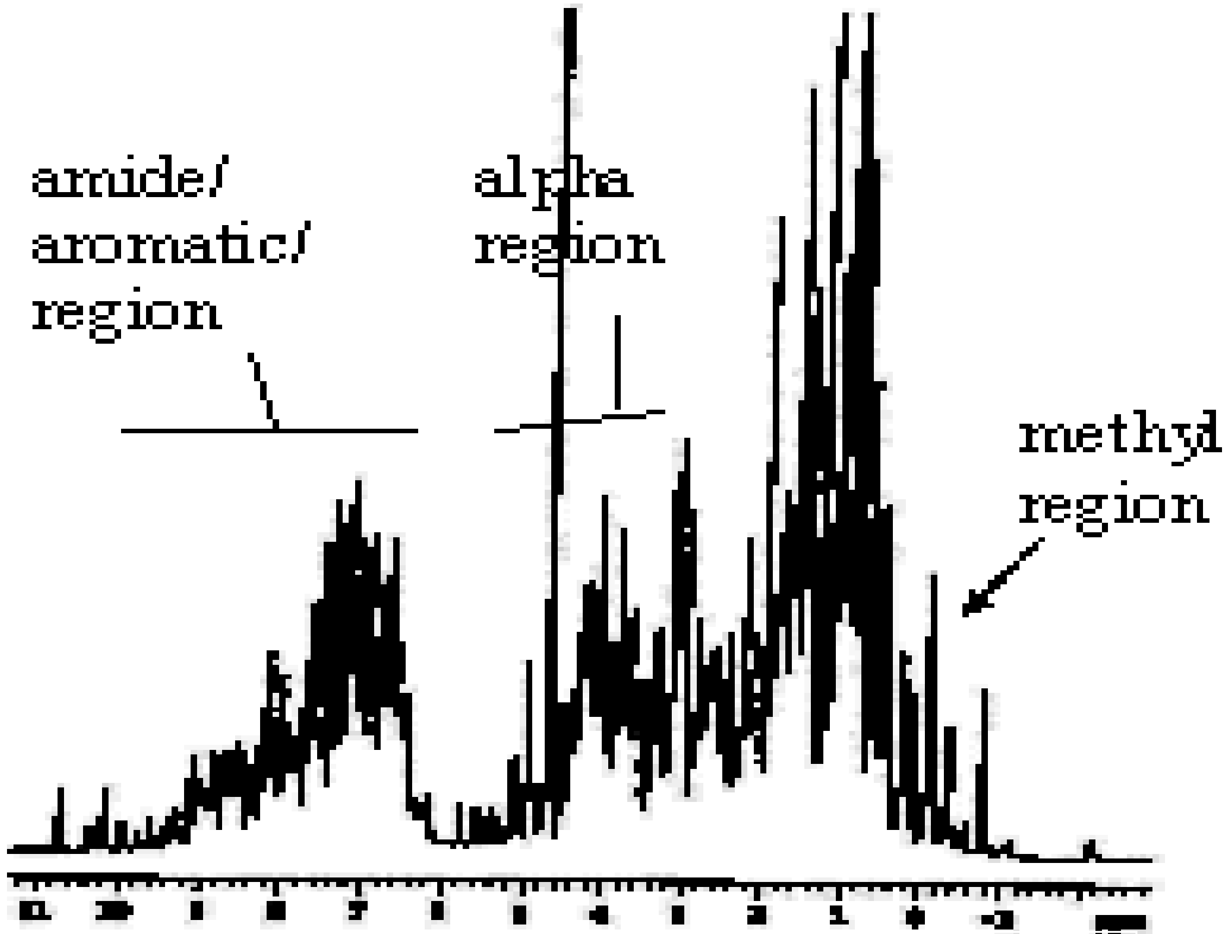
$$J(\phi) = A\cos^2\phi + B\cos\,\phi + C$$

Important Observables

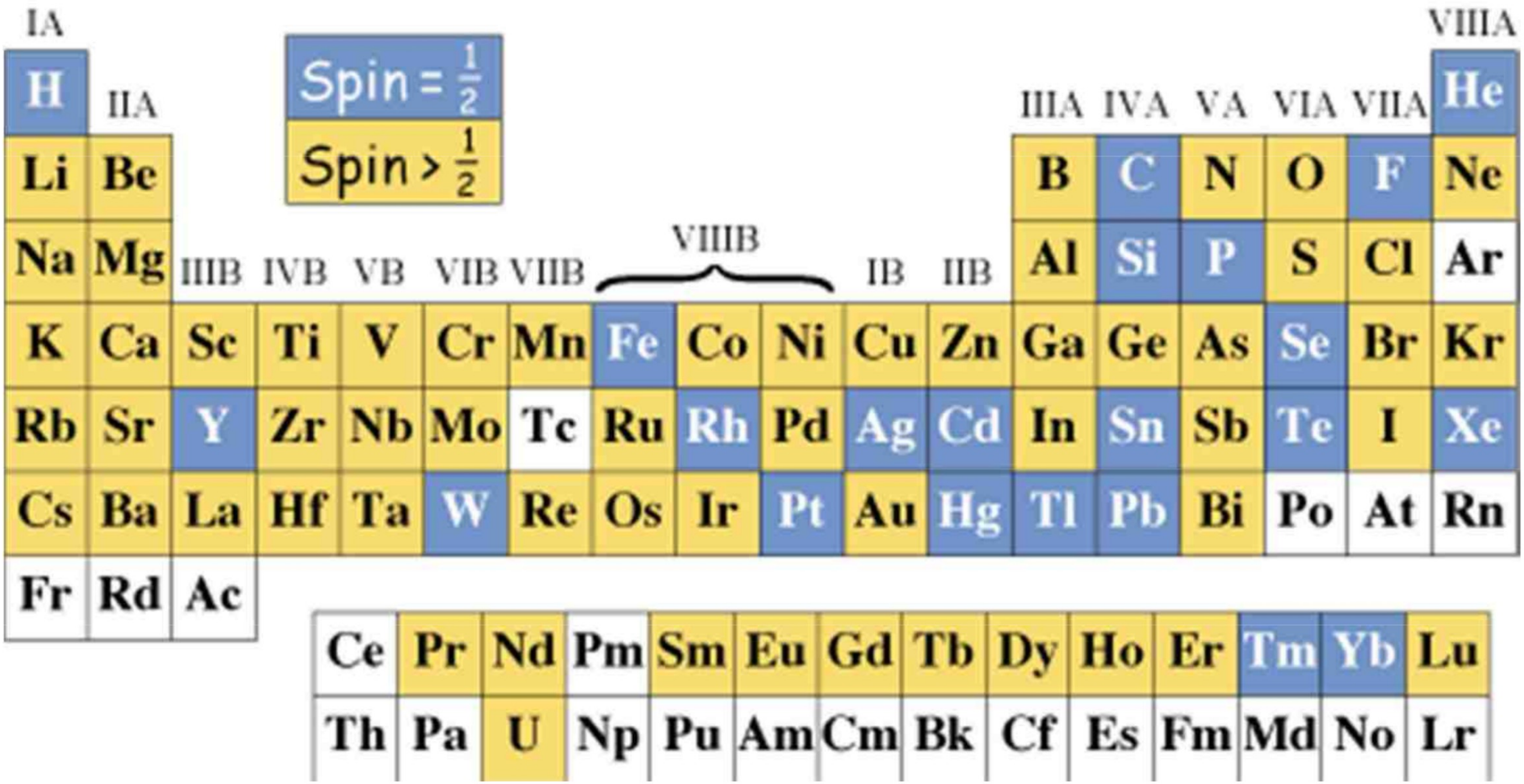
Chemical shift is a reporter of magnetic environment

The J coupling can inform torsion angles

Protein NMR Spectroscopy



Periodic Table of NMR active Nuclei



Isotopic Labeling Proteins for NMR

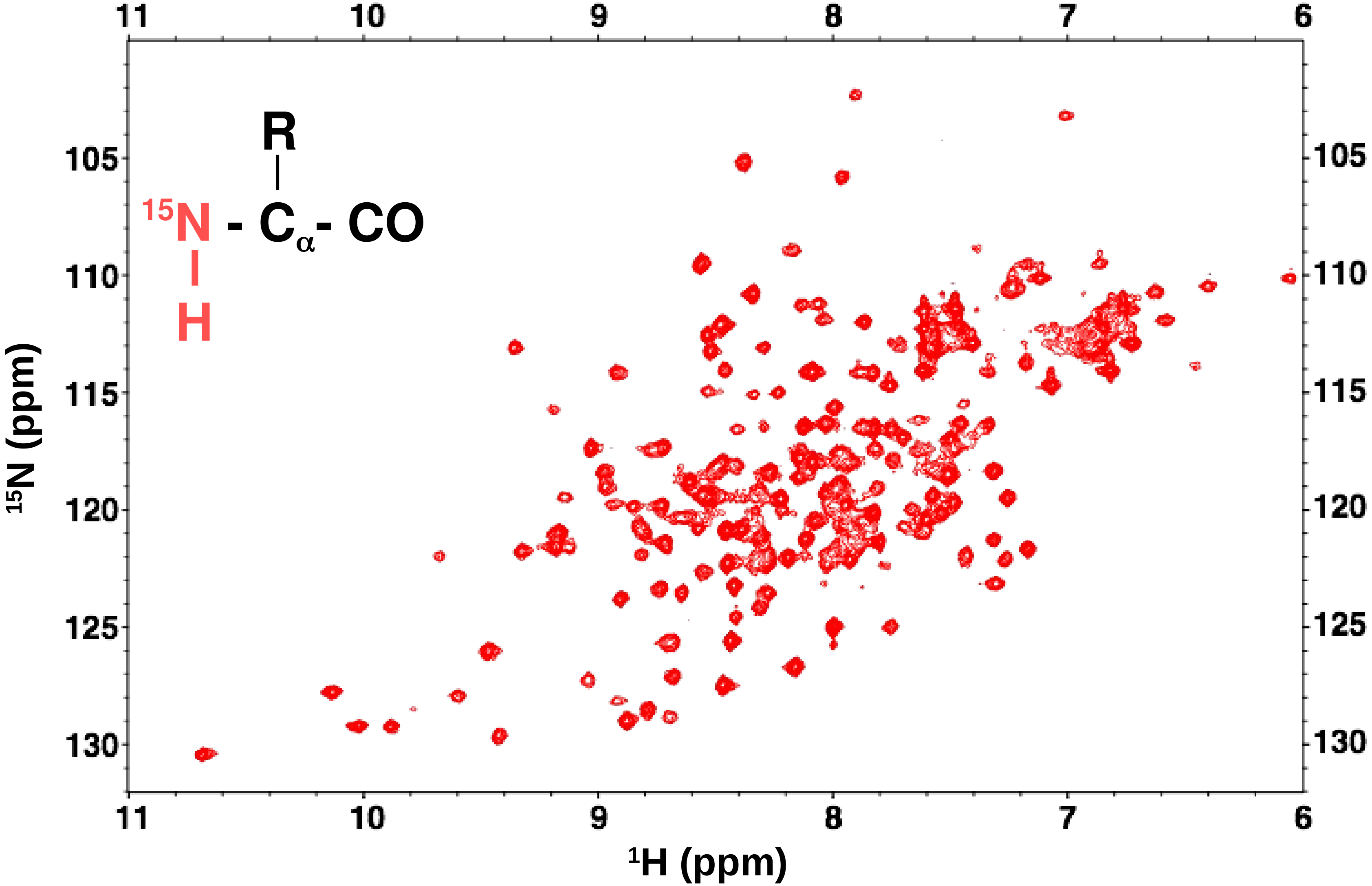
Bacterial expression:
Minimal media, ^{15}N NH_4Cl or ^{13}C glucose as sole nitrogen and carbon source

Amino acid-type labeling
Auxotrophic or standard strains (ei, BL21(DE3) depending on scheme

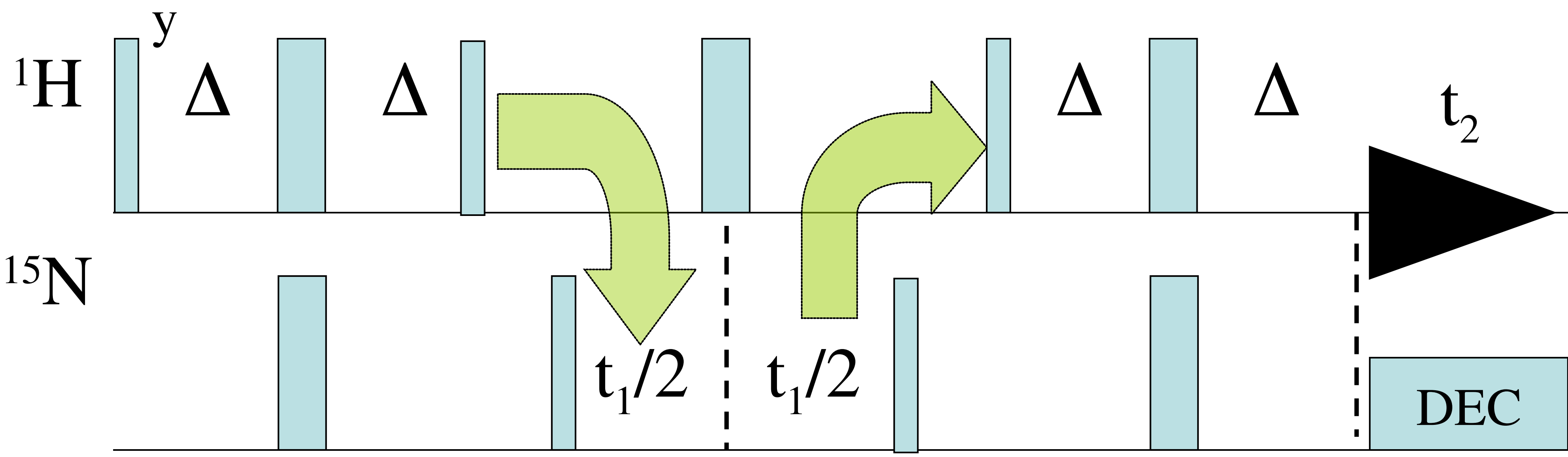
Labeling post purification ; reductive methylation of lysines

Results in additional spin-1/2 nuclei which can be used as probes

The HSQC is an NH chemical shift correlation map



An overview of the HSQC

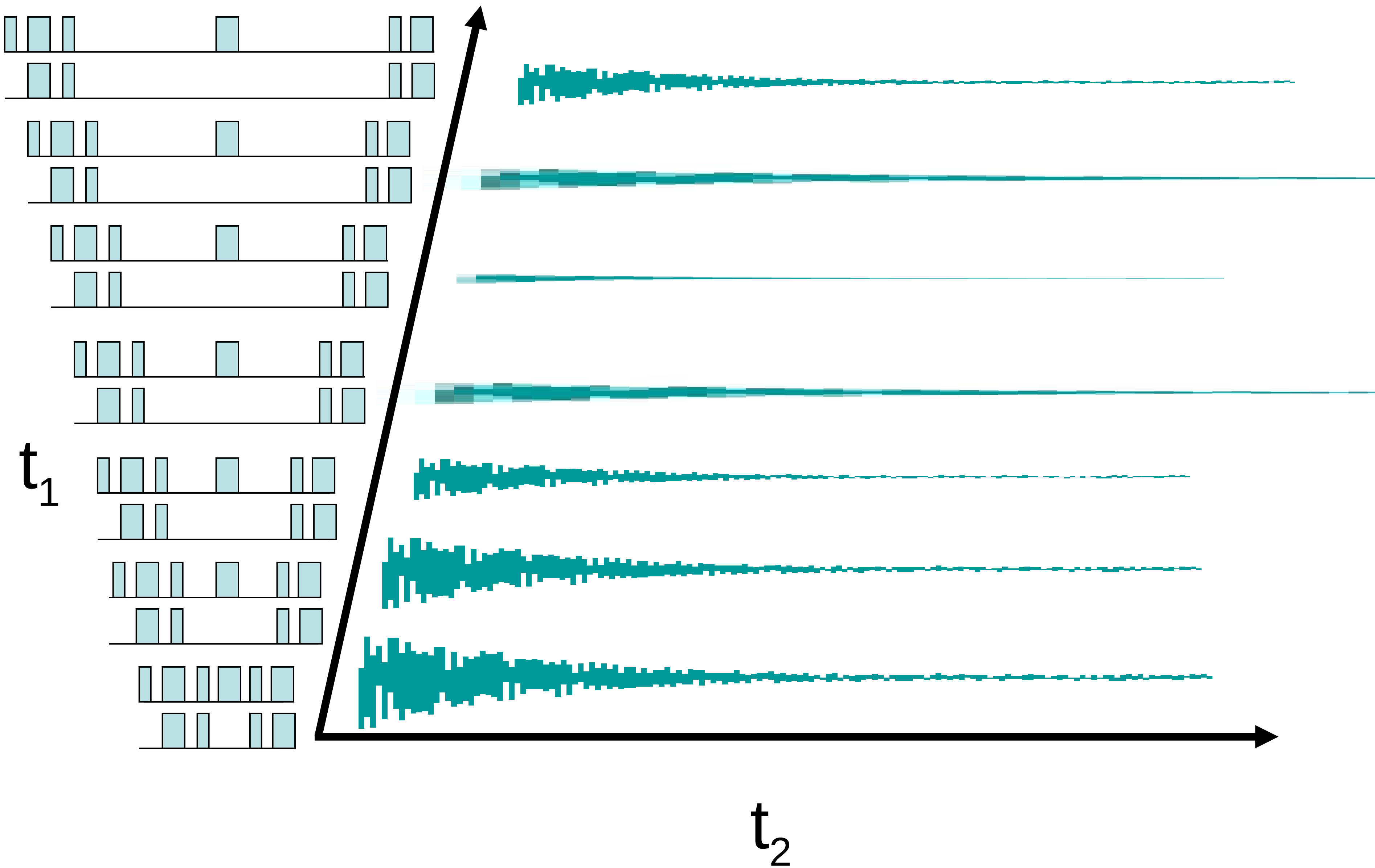


Transfer to ^{15}N → Transfer back to ^{1}H

Encode ^{15}N chemical shift for time t_1

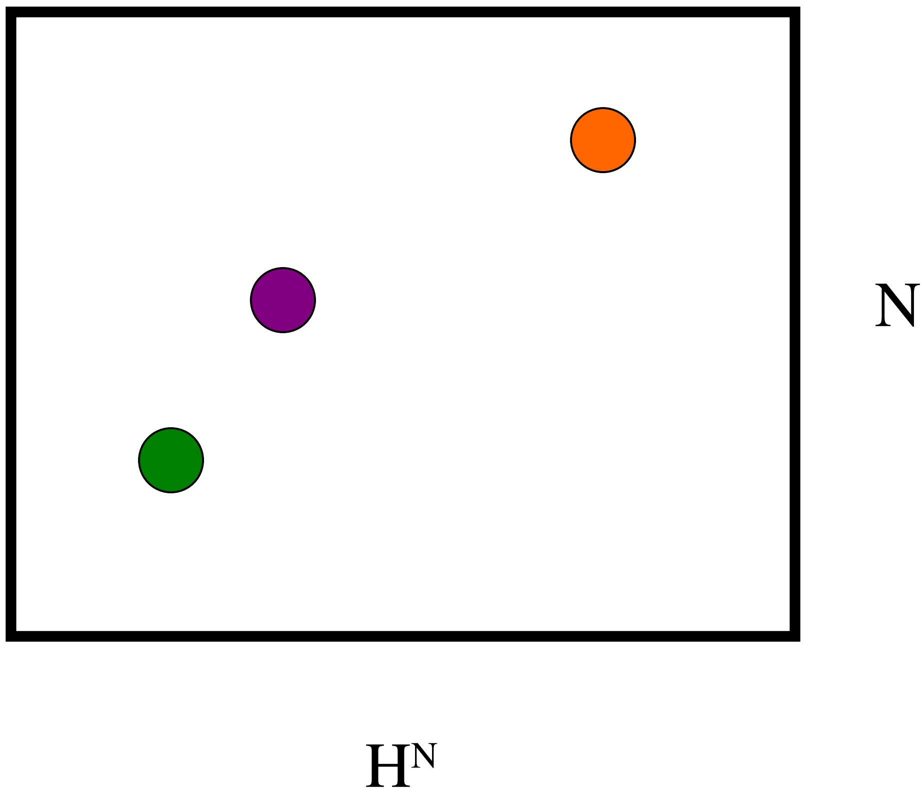
Bodenhausen & Ruben

2D Time-Domain Data

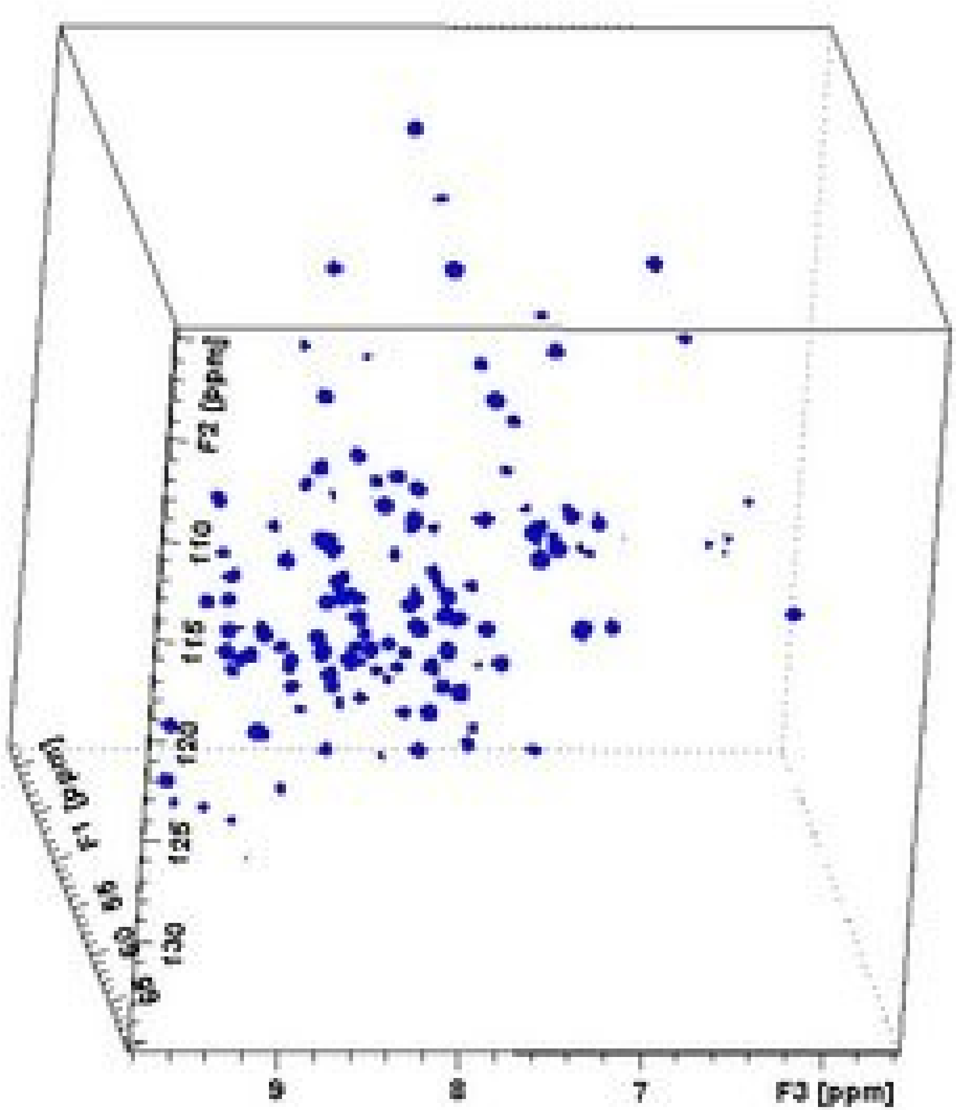


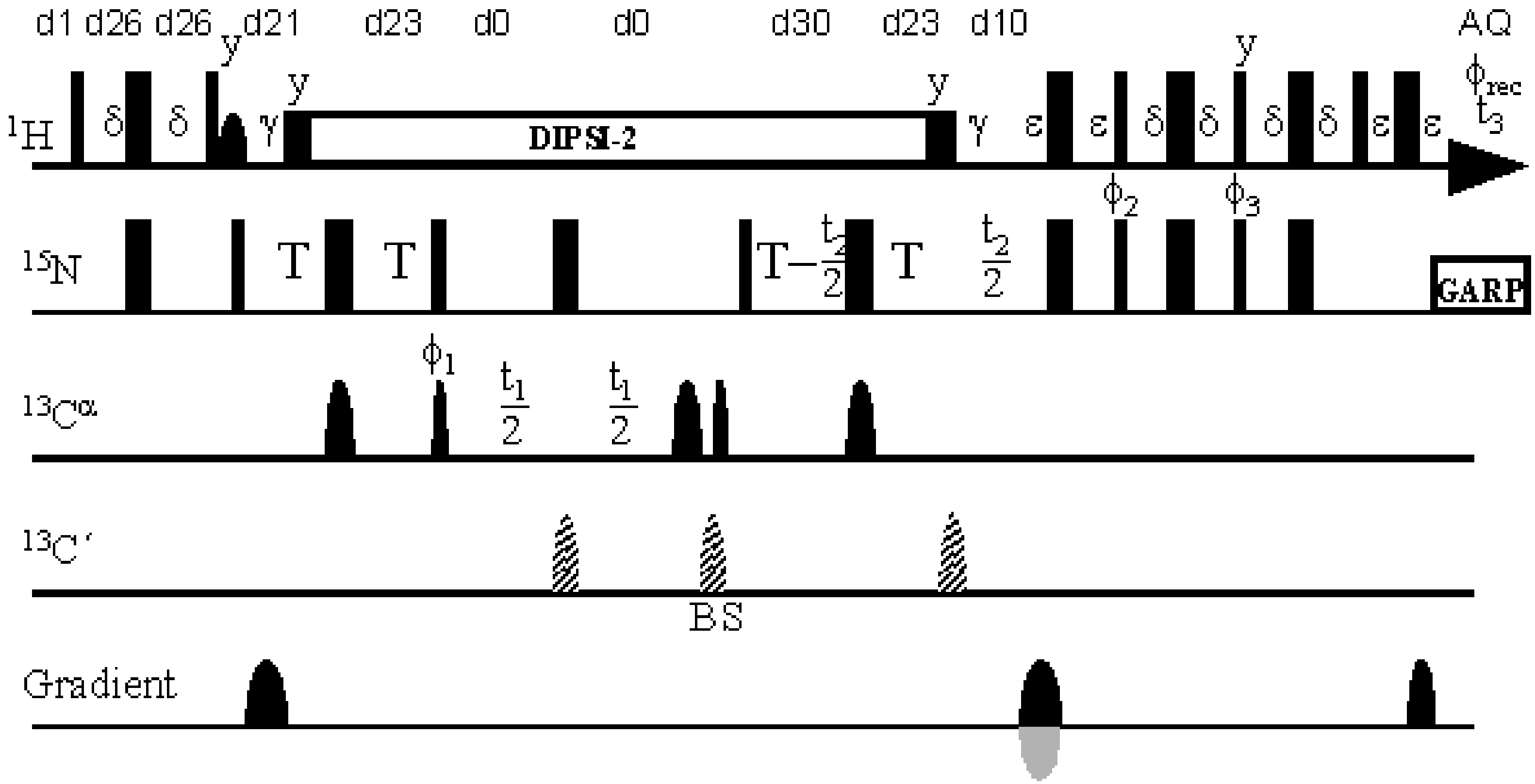
Some data shuffling then 2D FT =the HSQC Spectrum

$$\mathrm{Re}\,[S'(\nu_1, \nu_2)] = A_1^N A_2^H$$



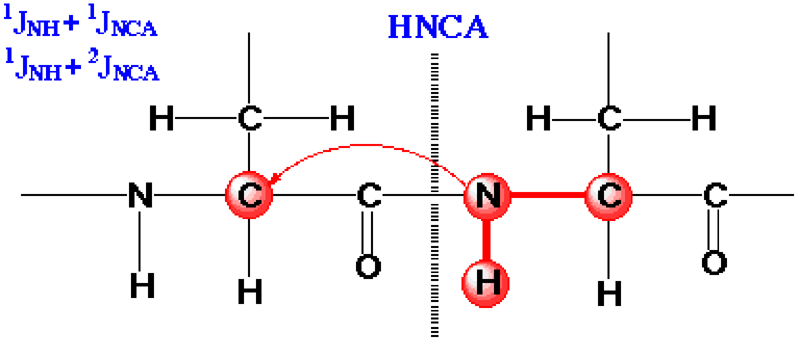
3D Dimensional NMR

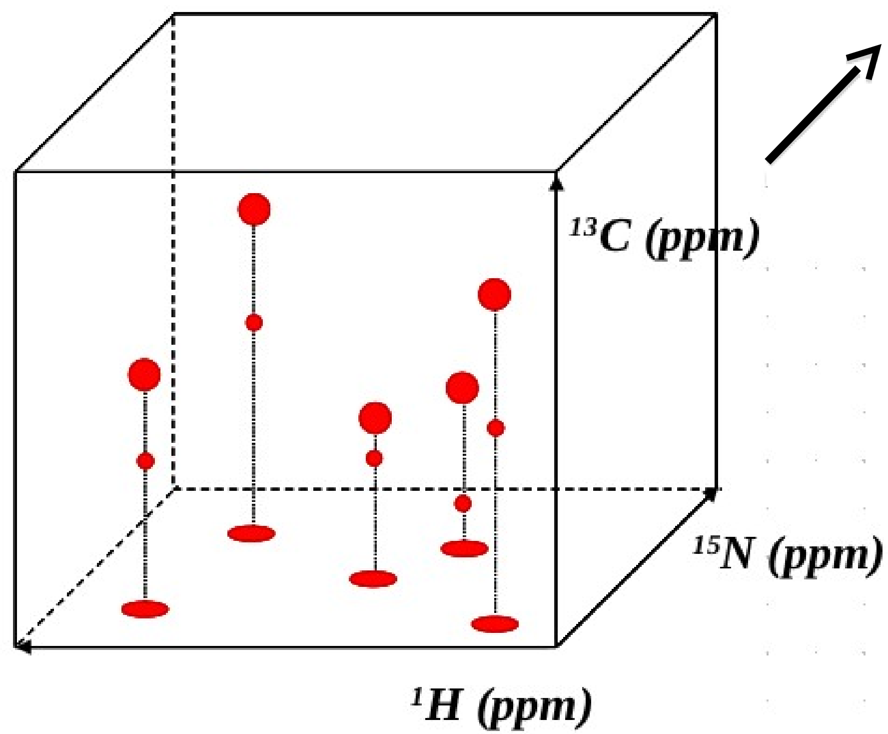


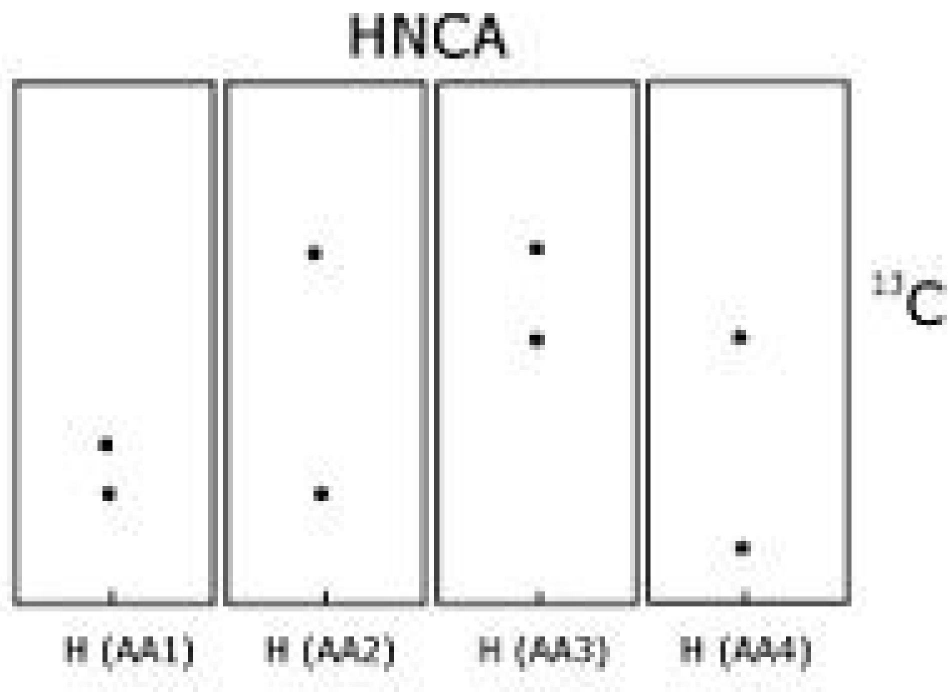


The 3D HNCA Experiment

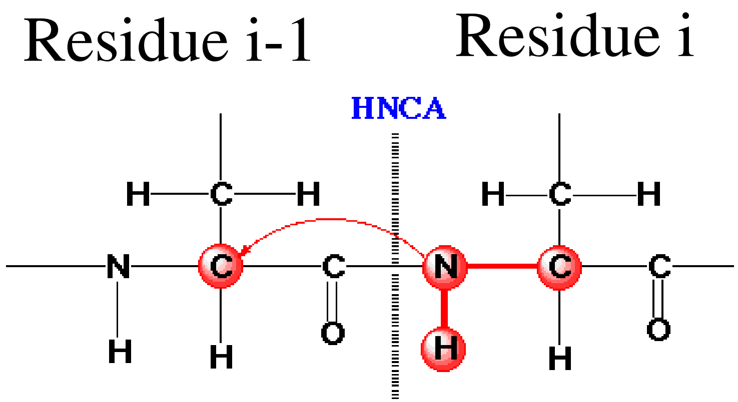
Backbone Resonance Assignments from HNCA

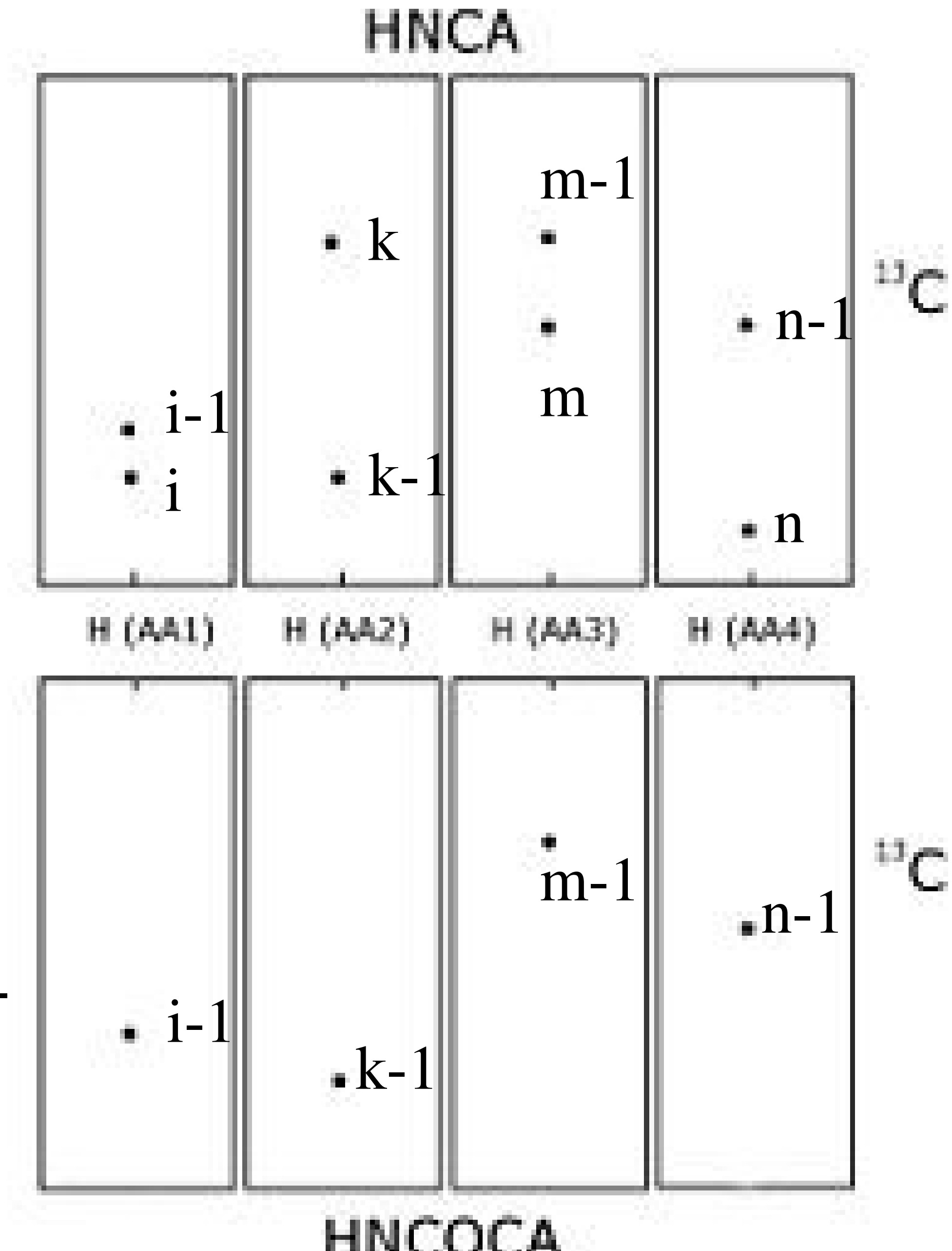




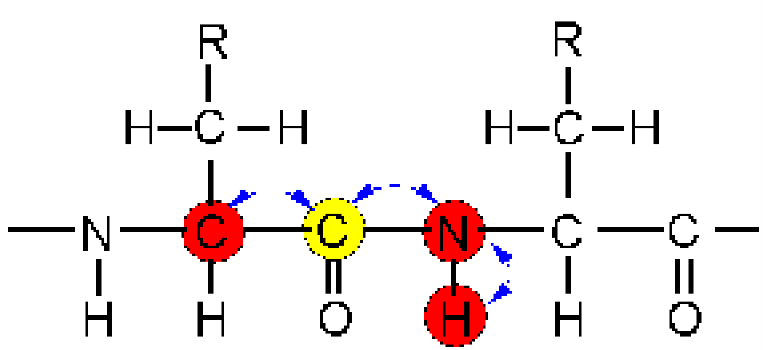


Triple Resonance Pairs



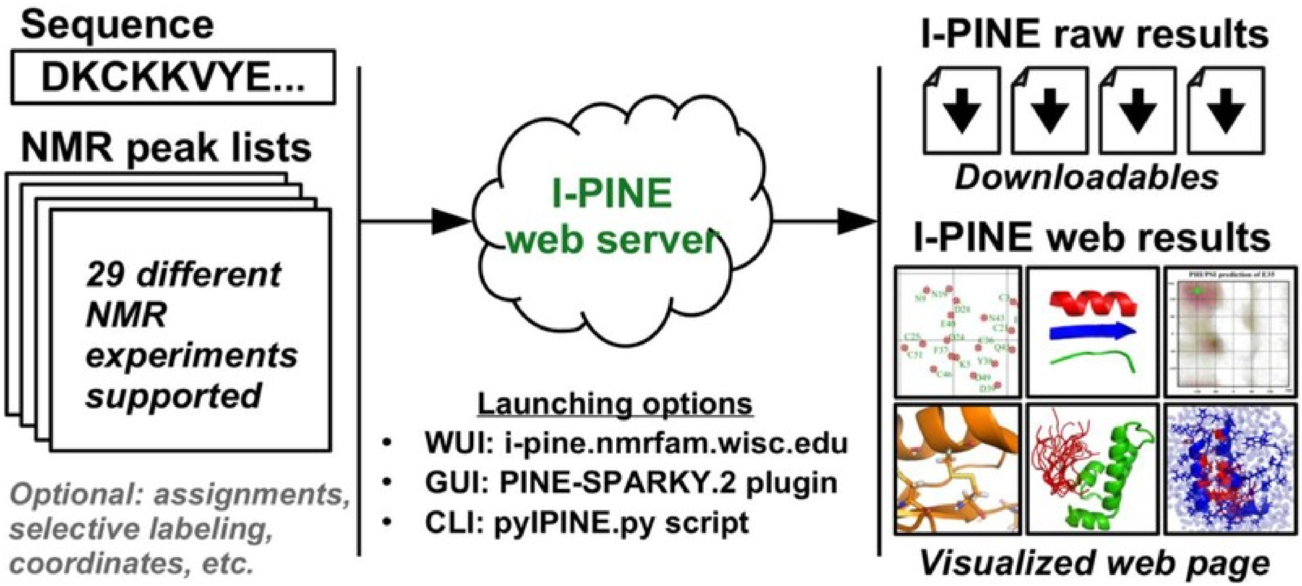


HN(CO)CA



sequential correlation only

Automated Resonance Assignment using PINE



http://i-pine.nmrfam.wisc.edu/

Applications for NMR

- Mapping protein interactions
- Fragment based drug discovery, SAR-by-NMR
- Protein folding , allostery and dynamics
- TROSY: deuteration and Methyl labeling to do this on large assemblies (~1 MDa)
- Structure Determination (<40 kDa)