Nuclear Magnetic Resonance of Proteins

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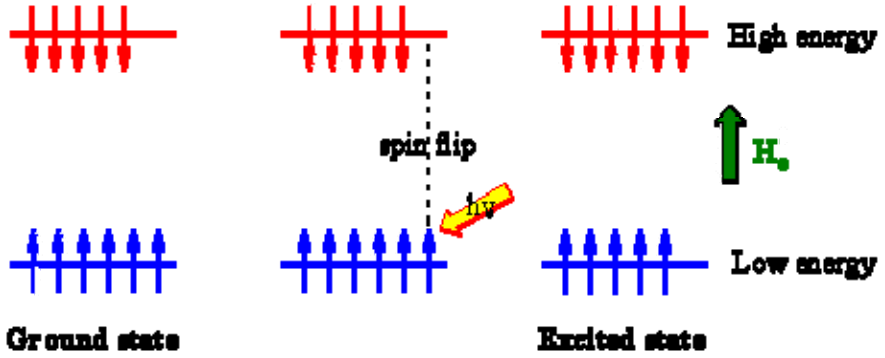
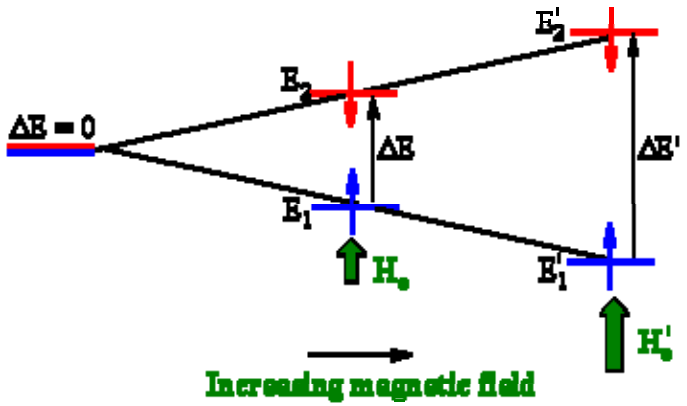
Bioanalytical Chemistry

March 2, 2011

Nuclear Magnetic Resonance

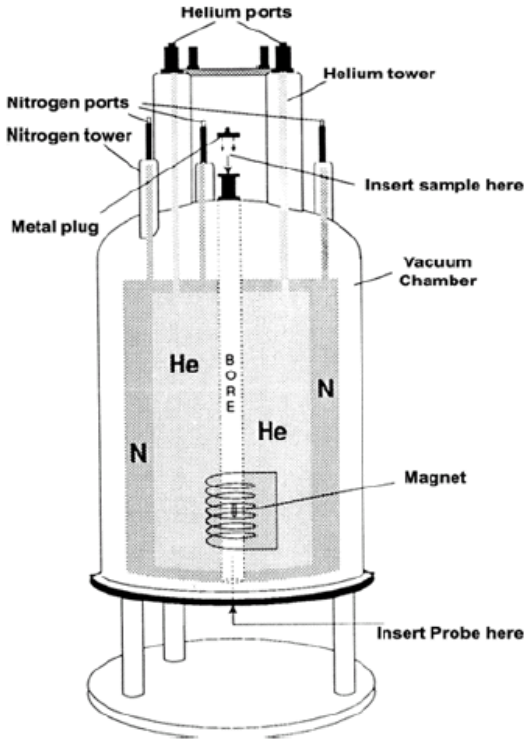
- "NMR"
- Application of a magnetic field causes absorption of EM energy that induces nuclei to resonate in a specific radio frequency (RF) governed by it surrounding electronic environment
- Spin ½ nuclei
  - $E_1 > E_2$ Boltzman distribution
- Absorption of the EM causes a temporary orientation of nuclei with the field
  - Parallel and antiparallel
  - Equilbrium shift with pulses of RF
  - Relaxation causes emission of specific rf

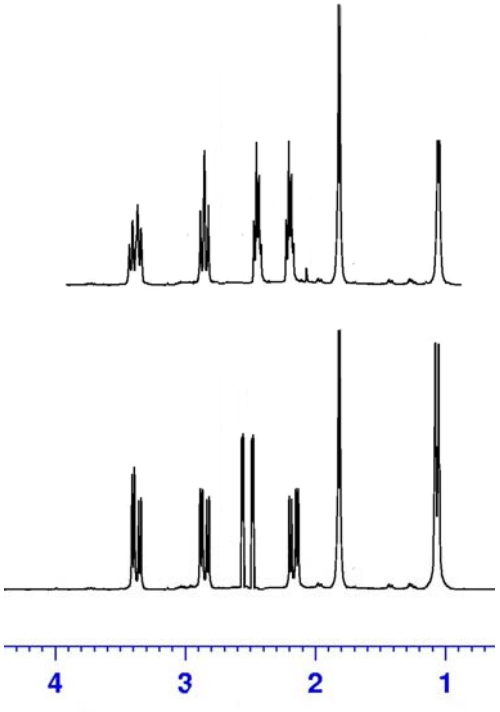




Background

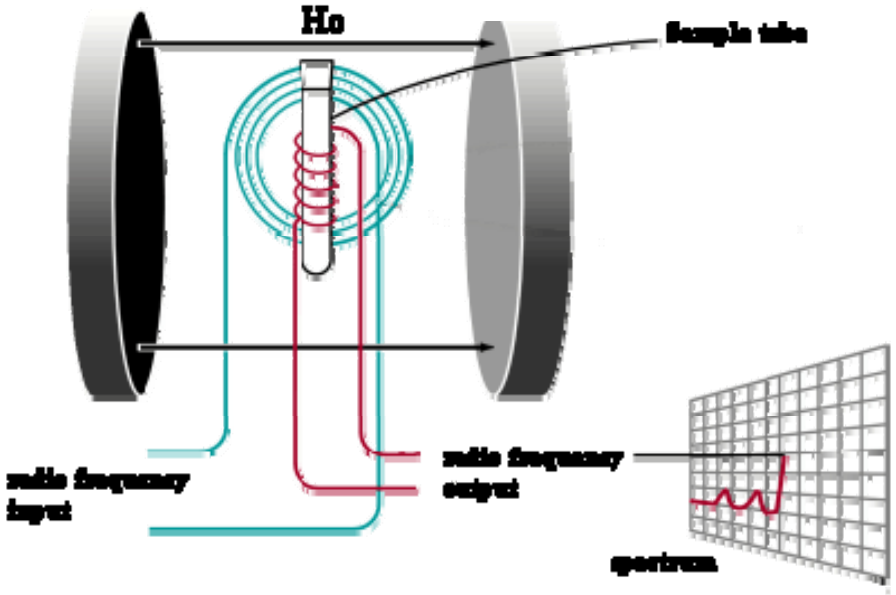
- Original EM source was large Permanent Magnetics
  - 20 Mhz to 60Mhz
- Movement to Superconducting magnets and increased computation power revolutionized NMR's potential
  - Increased computation turnover of complex FID-FT data
  - Exponential increase in peak resolution
  - Great ability to characterization complex molecules



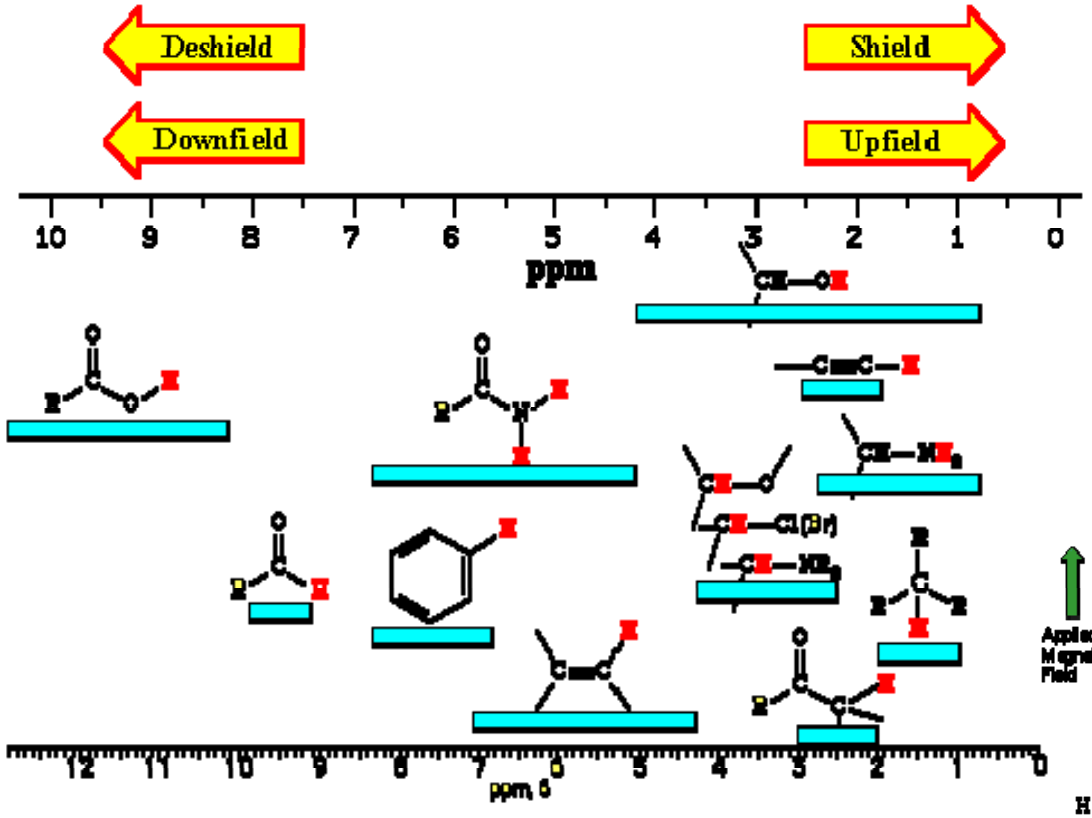


60Mhz

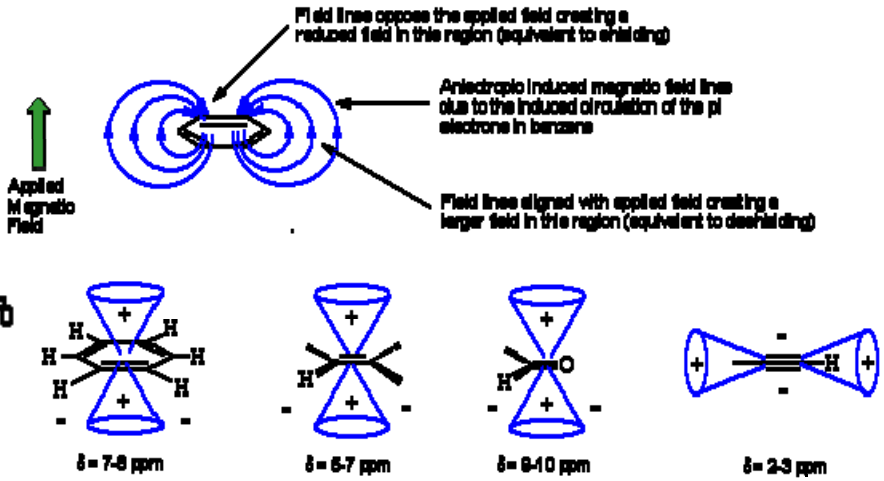
300 Mhz



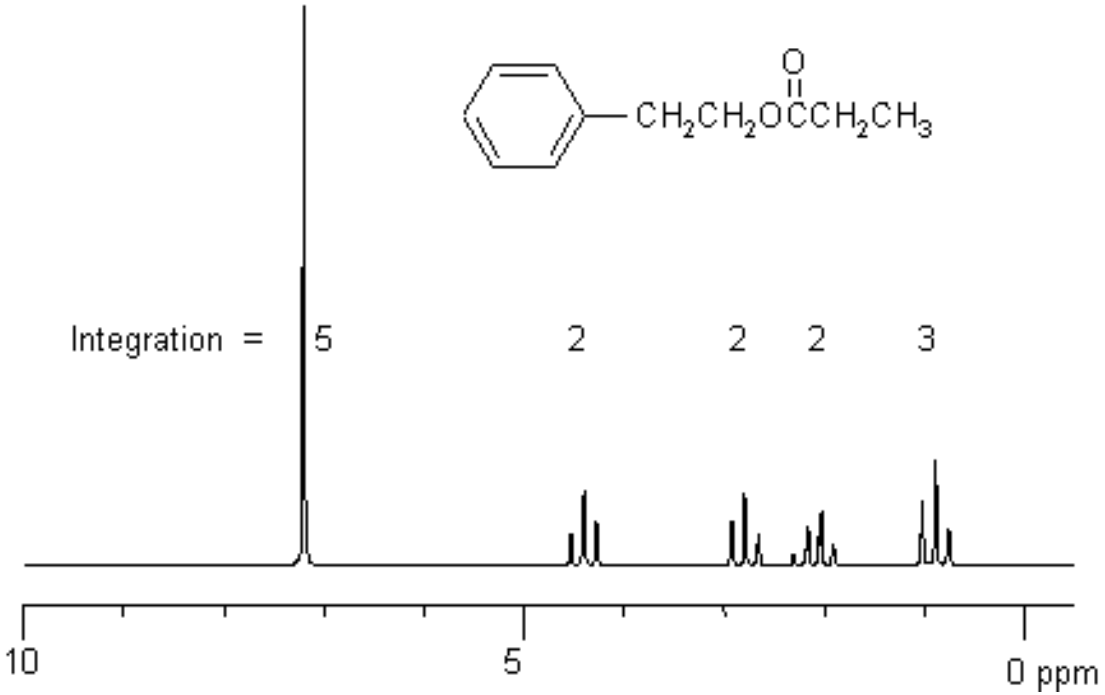
Shielding and Deshielding



- Influences on shifts (ppm):
  - ***Deshielding***: due to reduced electron density (Electronegative atoms)
  - ***Anisotropy***: magnetic field generated by π bonds



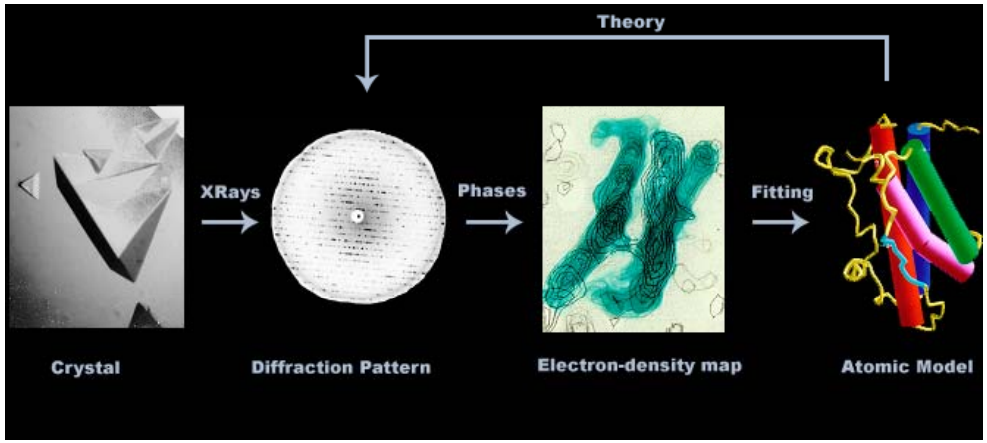
^{1}H-NMR spectra



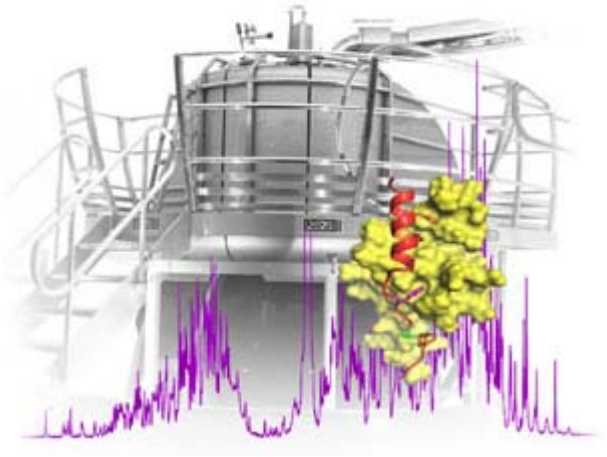
- Sample Prep
  - Dissolve in Deuterated Solvent
  - Concentration dependent
    - $CDCl_3$; DMSO-d_5; CD_3OD; etc
  - Deuteration removes solvent dominance
    - Spin quantum number (I) of 1
      - ½ for H
    - Unique splitting (2In +1)

Protein Crystallography vs Protein NMR

- X-ray crystallography
  - Around since early 20th century
  - Accurate, high resolution method
    - 2-3.5 Aº
  - Requires ability to crystallize protein
    - Salting out, Flash Freeze, etc
      - No set method for this process
    - ***Not all proteins are crystallizable***
      - Partial crystals
- Long time scale, static structure
- Diffraction patterns
- Primary structure must be known

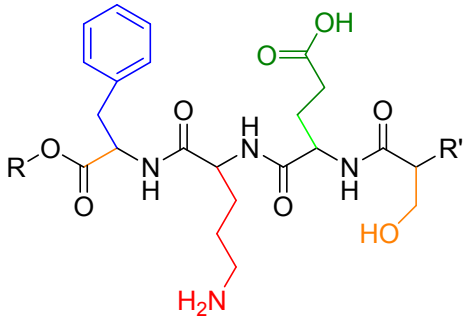


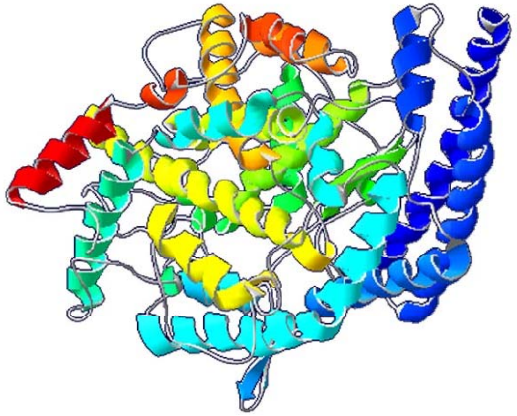
- Same High resolution
- Size limitation
  - 60 kDa monomer, up to 240kDa tetramer
  - 1 Amino acid = 100 Da
- Measures distances between specific atomic nuclei
  - ^{1}H, ^{2}D, ^{13}C, ^{15}N
- Stable solvent system
  - specific pH, salt conc.
  - Solid State
  - Static and Dynamic structure analysis
- Specific preparation of protein
  - Growth within an E.coli plasmid
  - ^{13}C-glucose and $^{15}NH_4Cl$
  - Primary structure must be known

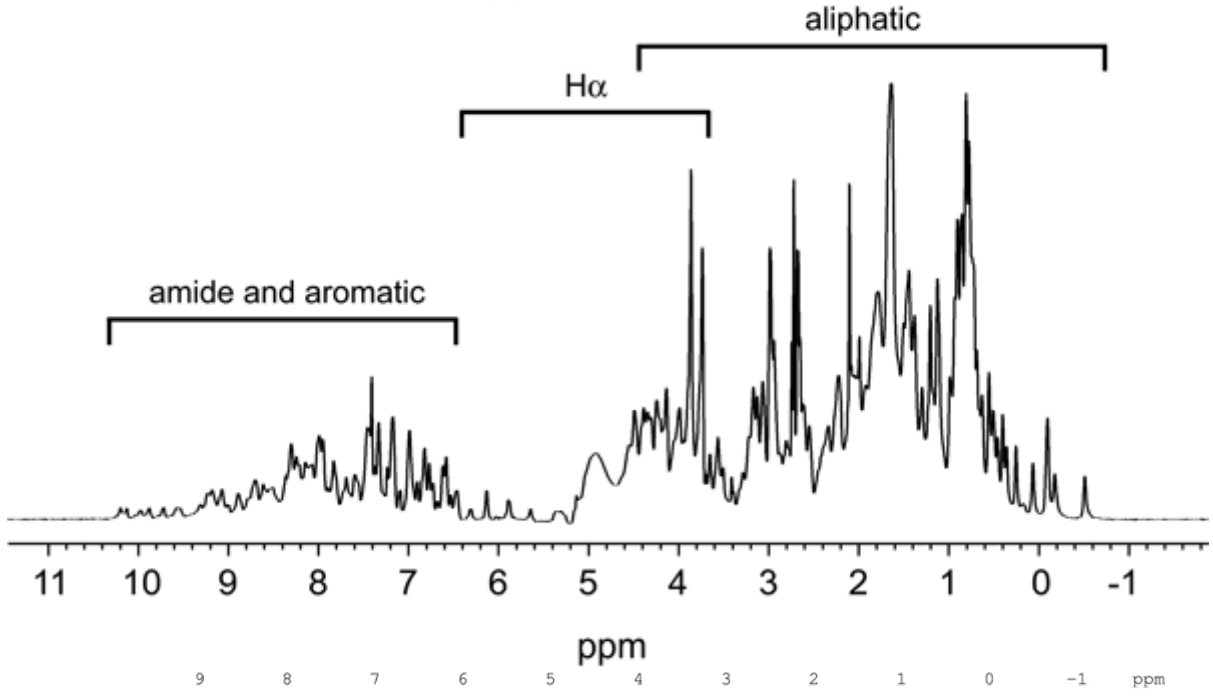


Protein NMR

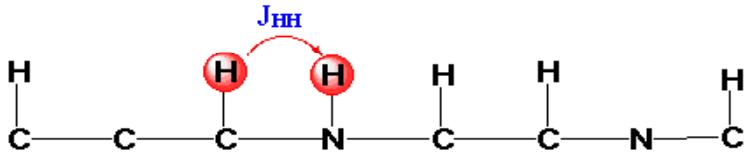
- Highly complex series of spatial experiments
  - 1D NMR identification of small molecules is highly effective
  - Supplies very little information of proteins
    - 2D, 3D, and 4D NMR experiments alleviates these issues
    - NMR strength >300Mhz
    - Computing power allowed this to evolve

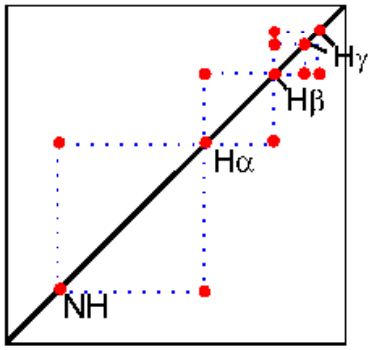




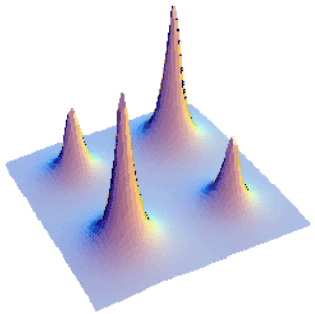


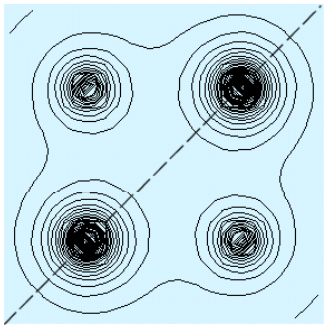
2D experiments

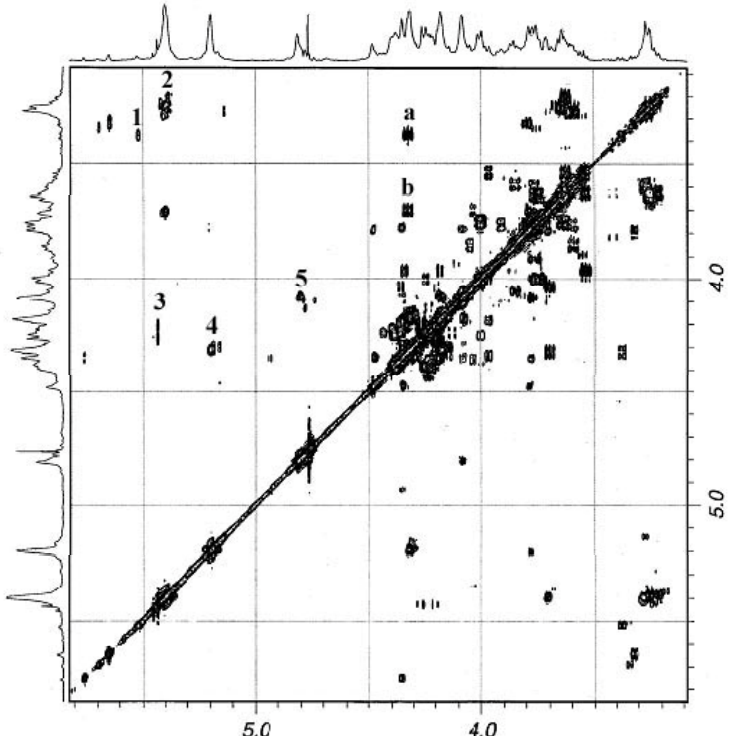




- Revolutionized NMR spectroscopy
  - Provides an ability to analyze the complex structures of highly chiral small molecules and also proteins
  - Essentially a stacking of many 1D spectra taken from different spin-frequency coupling states
    - Topographically representation
- Many different experiments available
  - Simplest is 2D COSY
  - Homonuclear correlation spectroscopy

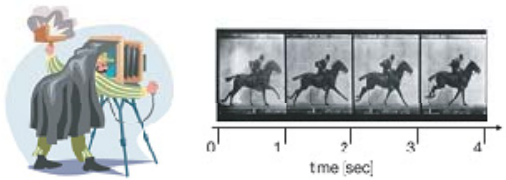


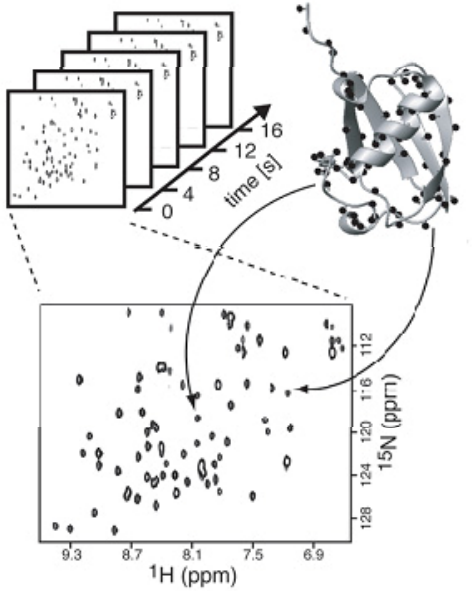


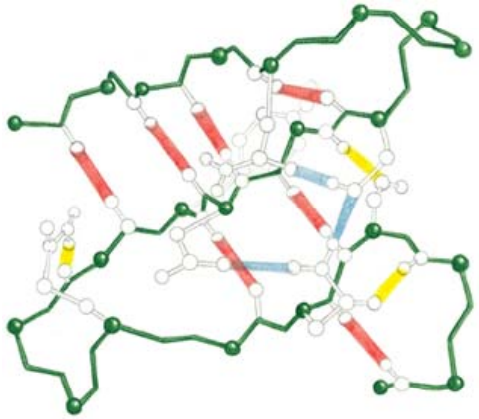


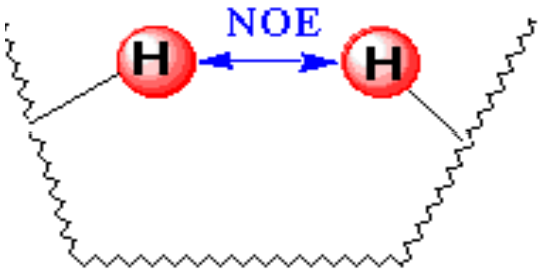
Time-Lapse 2D NOESY

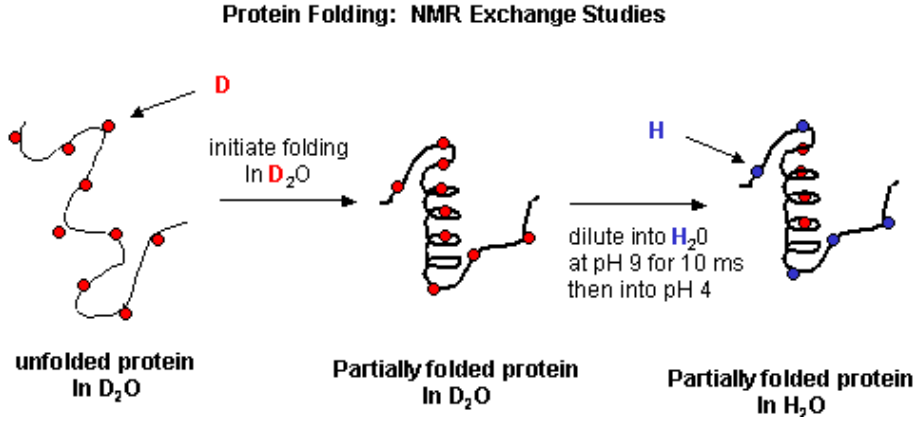
- Deuteration of Protein
- Nuclear Overhauser effect
  - Exchangeable protons ie: N-H, O-H, COO-H
  - Unfold, Fold, Exchange
    - Use: NaOD, D_2O; Heat/D_2O
- Cross peaks arise from resonances of protons which are within 5A^o.
  - Proximity in Space

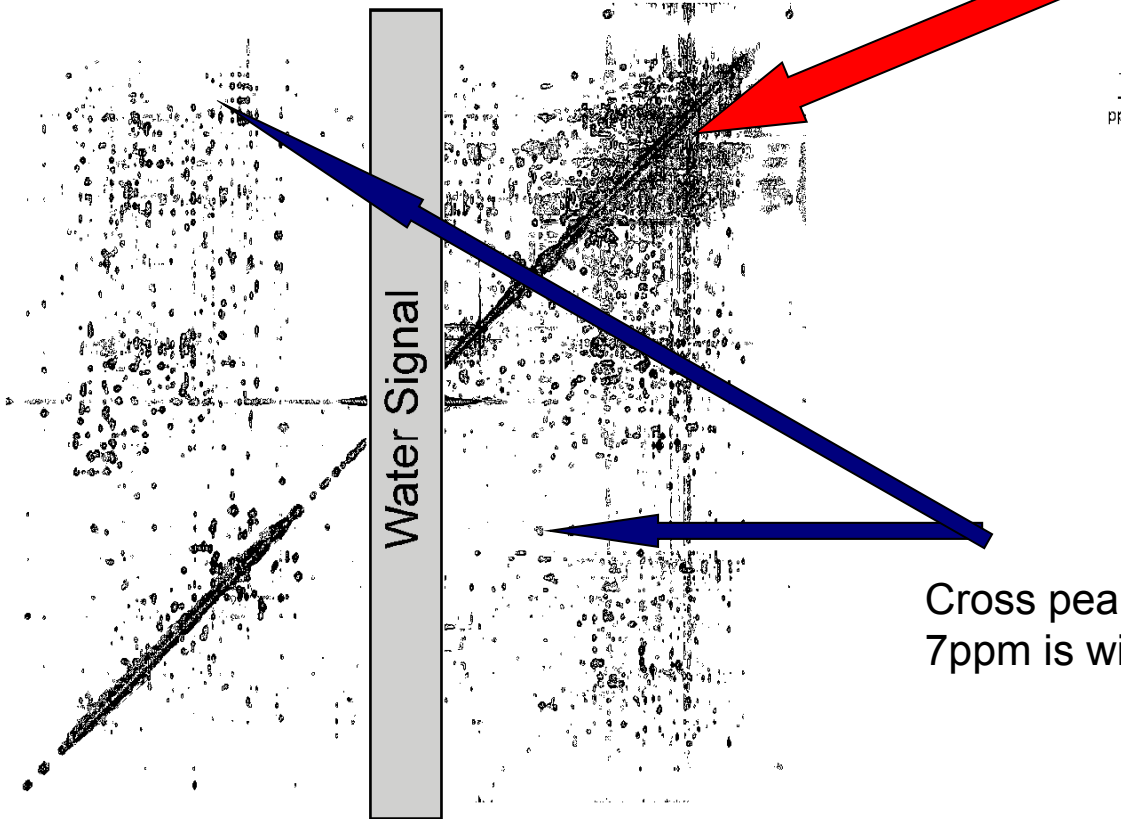


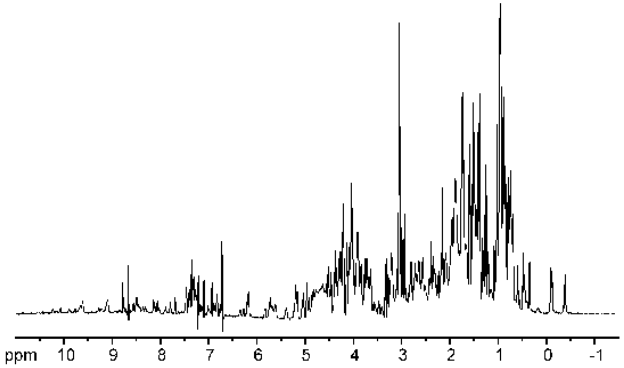






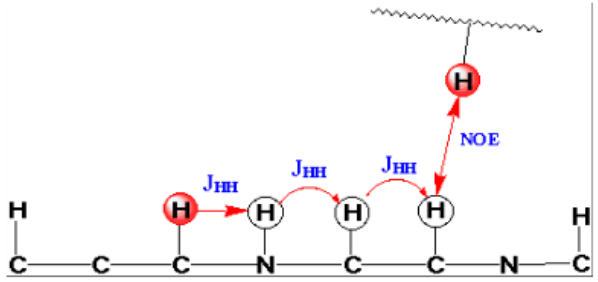




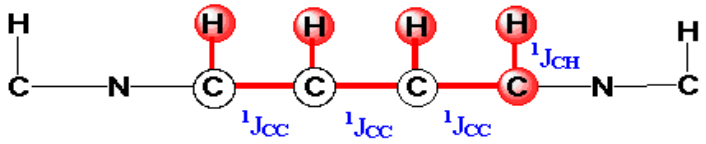


Cross peaks indicate that a proton at 7ppm is within5A^{o} of the observed H at 3ppm

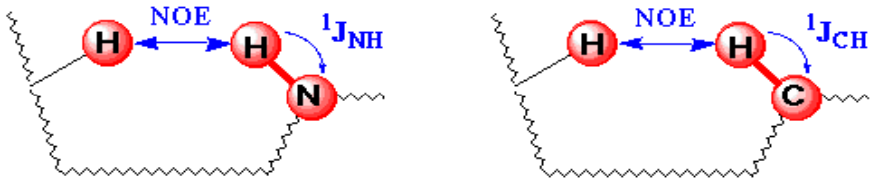
3D TOCSY-NOESY



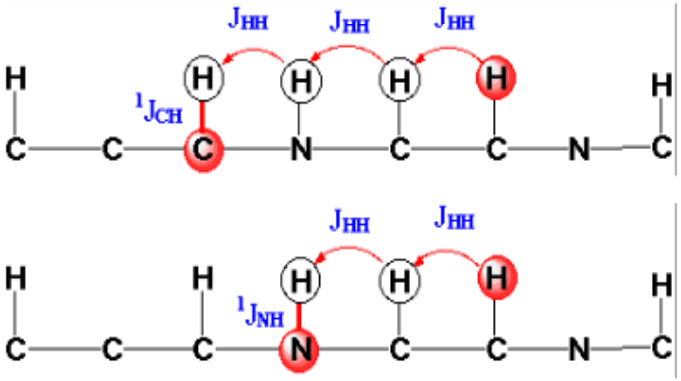
3D HCCH-TOCSY



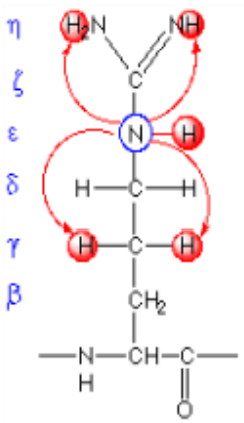
3D-NOESY-HSQC



3D-TOCSY-HSQC



2D-Arg-H(N)H^{a}H^b



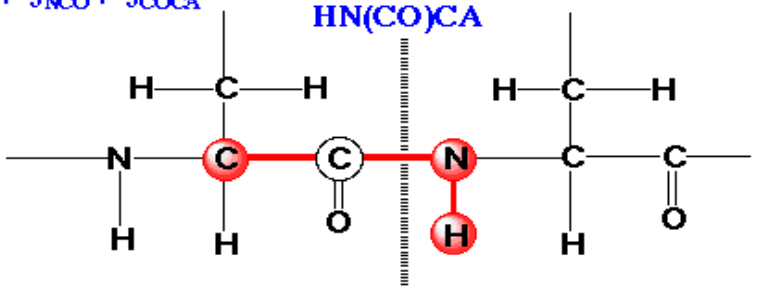
2D HE(NE)GHHH

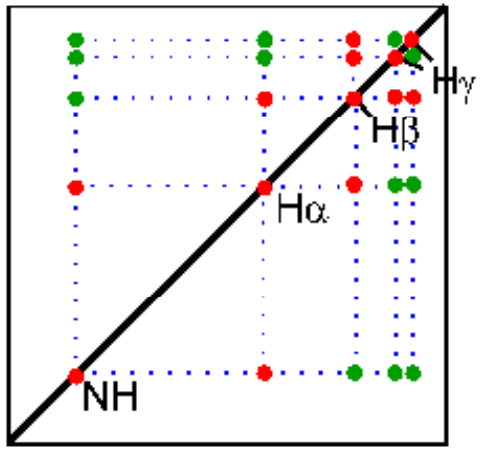
$^1J_{N\varepsilon H\varepsilon} + (^3J_{N\varepsilon H\gamma}$ or $^3J_{N\varepsilon H\eta}$ or $^2J_{N\varepsilon H\delta}$

3D-C^{b}C^{a}CON(H) ***Torsion Angle***

$^1J_{NH} + {}^1J_{NCO} + {}^1J_{CCOCA}$

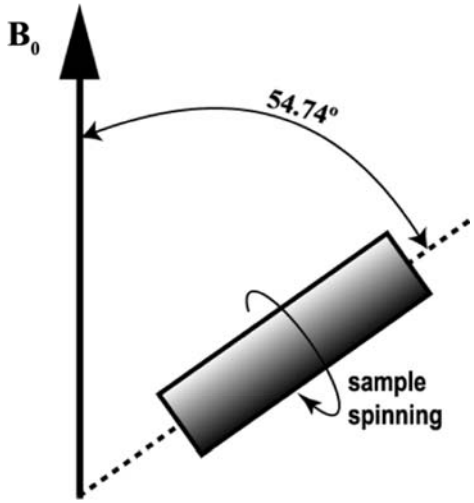
HN(CO)CA

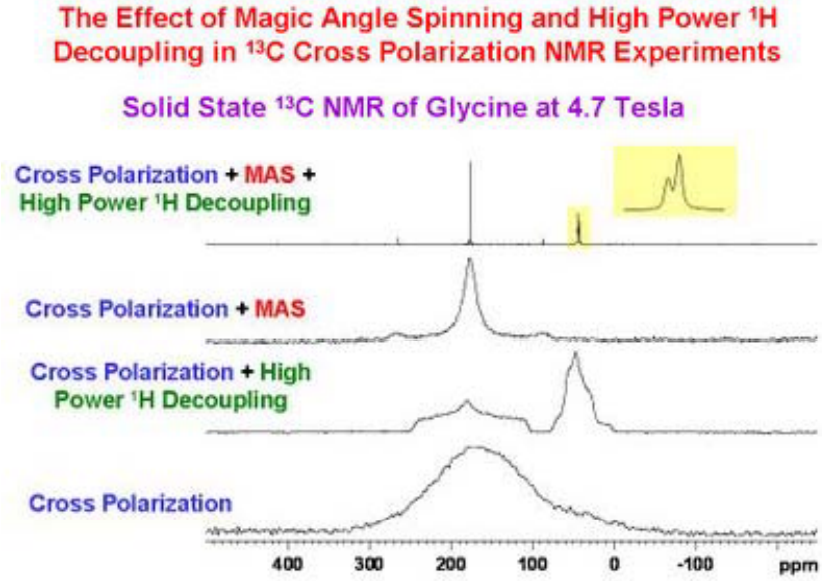




ssMAS NMR

- Solid State Magic Angle Spinning NMR
  - 54.74^0 from magnetic field
- DOR ssNMR
  - 30^o and 54.74^o
  - Bisection of both *d* and *f*-orbital
- Solvent Free
- Samples that cannot dissolve in solution NMR must be analyzed via solid-state NMR
  - Membrane/ Transport proteins, aggregates or proteins which cannot be crystallized or dissolved in a solvent
  - Similar experiments done to solution-protein NMR

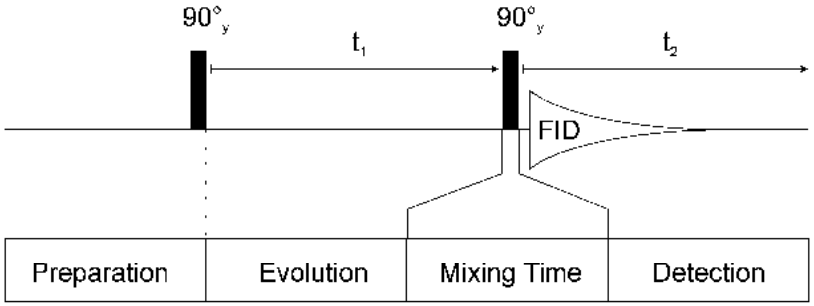




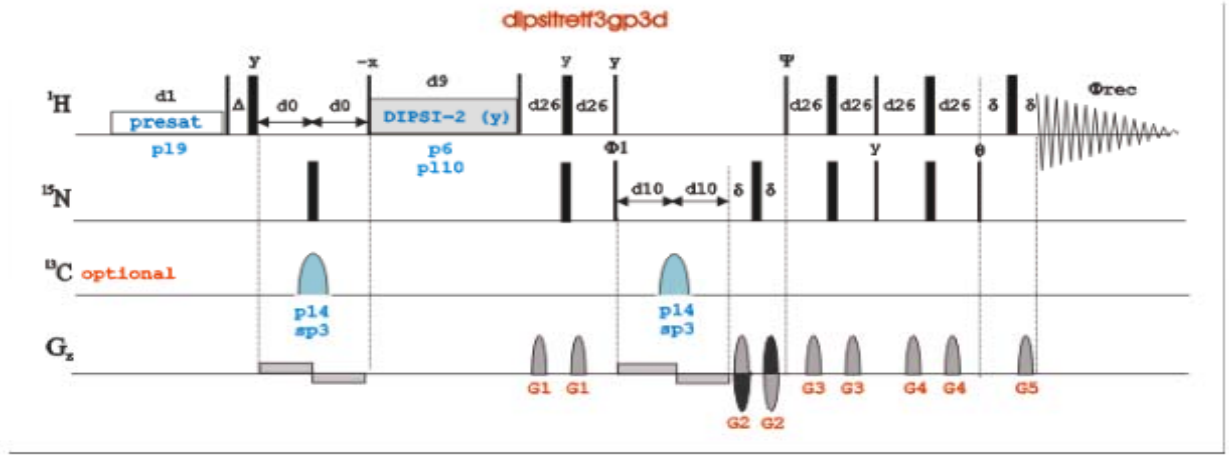
Computation Involvement

- Simple 2D COSY
- 1st Pulse system
  - X^0
    - 45,90,180
    - X, Y, Z plane
- Detect Signal
- 2nd pulse
  - Opposite angle
- Detect signal
- ***Complexity increases exponentially***
- ***Most new work to optimize NMR of proteins is with formulation of new and more specific pulse sequences to optimize signal to noise ratios***

2D COSY



TOCSY-HSQC



Problems with NMR-based Protein Structure Determination

- Local Motion of substiuents
  - Methyl rotation, ring flipping, etc
- Spin diffusion
  - Improper relaxation times can give erroneous data

Conclusion

- NMR is an extremely robust and powerful tool to analyze not only small molecules but also macromolecules
- Largest Current NMR spectrometer is 900Mhz
  - Allows for analyst of monomeric proteins as large as 60kDa
- Solvent-NMR and ssMAS NMR provide multiple avenues to acquire structural data on all forms of protein
  - Catalytic, Transport, etc.