Nuclear Magnetic Resonance of Proteins

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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 its surrounding electronic environment
- Spin ½ nuclei
  - $E_1 > E_2$ Boltzmann distribution
- Absorption of the EM causes a temporary orientation of nuclei with the field
  - Parallel and antiparallel
  - Equilibrium 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
Shielding and Deshielding

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

![Diagram showing shielding and deshielding with examples of chemical structures and ppm values](Image)
Sample Prep

- Dissolve in Deuterated Solvent
- Concentration dependent
  - CDCl₃; DMSO-d₅; CD₃OD; etc
- Deuteration removes solvent dominance
  - Spin quantum number (l) of 1
    - $\frac{1}{2}$ for H
  - Unique splitting (2ln +1)
**Protein Crystallography vs Protein NMR**

- **X-ray crystallography**
  - Around since early 20th century
  - Accurate, high resolution method
    - 2-3.5 Å
  - 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$_4$Cl
  - 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: N-H, O-H, COO-H
  - Unfold, Fold, Exchange
    - Use: NaOD, D$_2$O; Heat/D$_2$O
- Cross peaks arise from resonances of protons which are within 5Å.
  - Proximity in Space
Cross peaks indicate that a proton at 7 ppm is within 5 Å of the observed H at 3 ppm.
ssMAS NMR

- Solid State Magic Angle Spinning NMR
  - 54.74° from magnetic field
- DOR ssNMR
  - 30° and 54.74°
  - 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
  - X0
    - 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

![2D COSY Diagram](image)

### TOCSY-HSQC

![TOCSY-HSQC Diagram](image)
Problems with NMR-based Protein Structure Determination

- Local Motion of substituents
  - 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 analysis 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.