Protein structure determination using NMR

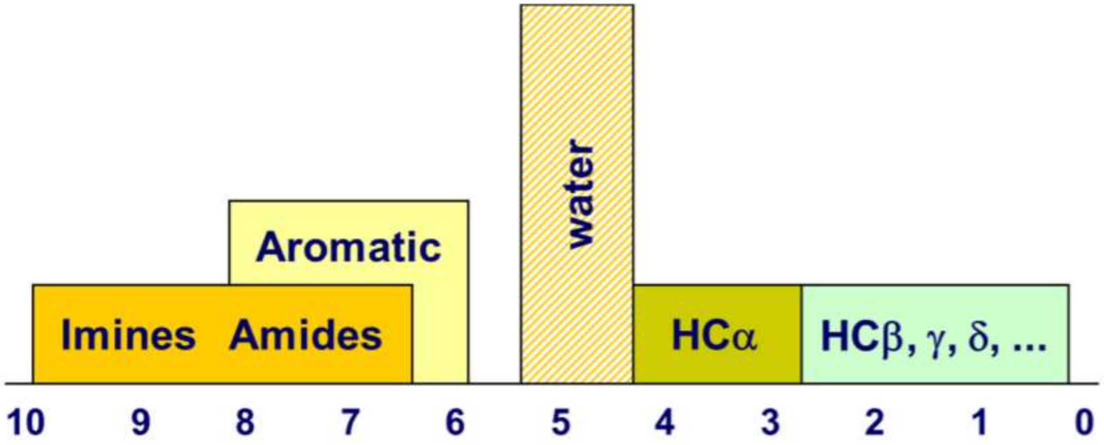
Principles of NMR

Key point:

- Spin of nuclei generates magnetic field.
- When placed in NMR machine's magnetic field, nuclei of atoms align.
- When alignment disrupted by radio waves and disruption of atoms behavior observed. This observation reveals their chemical shift properties.

- Chemical shift depends on their local environment-hence reveal details about the atom's surrounding, thus overall structure
- NMR can help determine structure of proteins with those of mass up to 30kDa
- Isotopically label protein with ^{13}C, ^{15}N or ^{1}H
- Requires a reference signal-given by compound: **TMS or Tetramethylsilane**, $(CH_3)_4Si$

1-dimentional NMR



http://www.dbt.univr.it/documenti/OccorrenzaIns/matdid/matdid943520.pdf

- Each peak corresponds to an H atom
- Can tell you if protein is folded-hence can tell you protein’s functionality
  - Folded: Sharp, narrow peaks + large range of chemical shifts = Folded spectrum contains more protein than unfolded one
  - Unfolded : Broad peaks and range not as wide

- High degree of overlapping: after 3-4 amino acids resolution declines

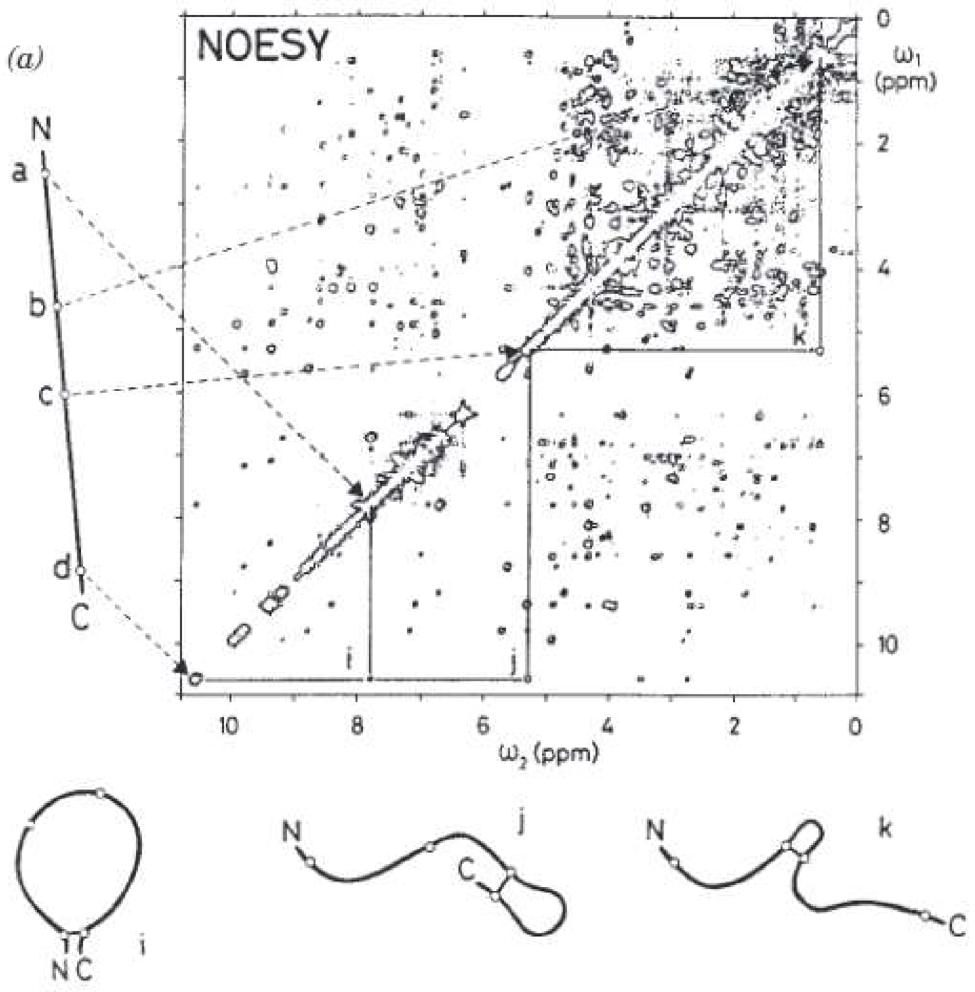
  → 2D NMR spectroscopy used

2-dimentional NMR

- 1D NMR spectrum crowded with overlapping peaks

- 2D NMR spectroscopy yeilds additional peaks arising from intersections of protons that are less than 5Å = 0.5 nm

- NOESY:
  - uses cross peaks arise from NOEs (nuclear Overhauser effect)
  - provides interatomic distances for protons that are close in space



https://www.reasonbio.net/%E5%9F%BA%E4%BA%8E%E6%A0%B8%E7%A3%81%E5%85%B1%E6%8C%AF%E7%9A%84%E8%9B%8B%E7%99%BD%E8%B4%A8%E7%BB%93%E6%9E%84%E8%A7%A3%E6%9E%90

2D NOESY spectrum of a protein

Diagonal:

1D NMR spectrum presented as a counter plot

Line on left:

Extended polypeptide chain with its N- and C-termini, and the positions of 4 protons (a to d)

Structures on bottom:

3 looped structures of polypeptide chain

Advantages of NMR

- Spectra collected quickly
- Investigate what conditions protein is stable at- investigate various buffer conditions. Temperature
- Advantages over X-ray crystallography
  Sample can be in solution and not in crystallized form
  Flexibility + protein interaction can be determined whereas X-ray only reveals rigid structure.