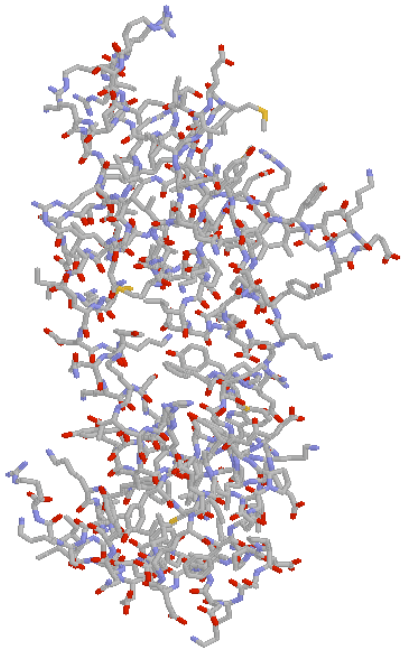
Uses of NMR:

1) NMR is a method of chemical analysis. (Who uses NMR in this way?)

2) NMR is used as a method for medical imaging. (called "MRI")

3) NMR is used as a method for determining of protein, DNA, RNA structure.
(one of only 2 possible methods for doing this)



Plan for today -

1) Basic principles of NMR.

2) NMR as a protein (and DNA and RNA) structure determination method.

① nuclei have mass
nuclei have charge

also, nuclei (some) have "spin" or "intrinsic angular momentum"

② nuclear spin quantum # "I"

"m" = magnetic quantum #

nuclei w/out intrinsic angular momentum have $I = 0$

" with " " " $I > 0$

Examples: ^{1}H $I = \frac{1}{2}$

^{13}C $I = \frac{1}{2}$

^{12}C $I = 0$

^{27}Al $I = \frac{5}{2}$

Allowed values for "m" are $-I, -I+1, -I+2, \ldots, I$

Example: $I = \frac{1}{2}$ nucleus $m = +\frac{1}{2}$ or $-\frac{1}{2}$

Example: $I = \frac{5}{2}$ nucleus $m = -\frac{5}{2}, -\frac{3}{2}, -\frac{1}{2}, \frac{1}{2}, \frac{3}{2}, \frac{5}{2}$

If nucleus has $I \neq 0$, & you put it in a magnetic field, it has an energy of interaction w/ the magnetic field.

This energy of interaction w/ magnetic field is "quantized" (can only have specific values).

$$E_m = \gamma \hbar m H$$

- E_m: energy of nucleus w/ mag. quantum # m
- γ: gyromagnetic ratio (cst.)
- $\hbar$: Planck's cst $\div 2\pi$ $\left(\frac{h}{2\pi}\right)$
- m: magnetic quantum # ($+\frac{1}{2}, -\frac{1}{2}$ for ^1H)
- H: mag. field strength (Tesla)

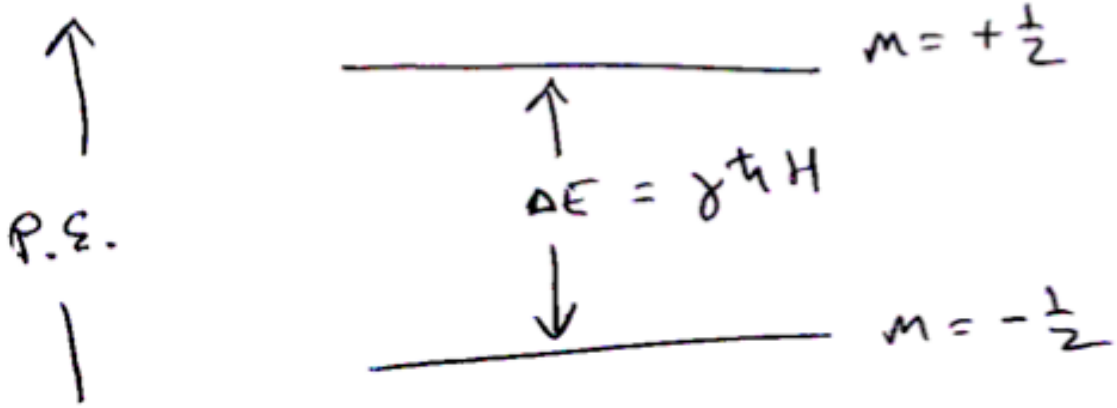
Example: What are the allowed energies of a ^1H in a magnetic field?

$I = \frac{1}{2}$ so $m = \pm \frac{1}{2}$

$E_{+\frac{1}{2}} = \gamma \hbar \left(\frac{1}{2}\right) H$

$E_{-\frac{1}{2}} = \gamma \hbar \left(-\frac{1}{2}\right) H$

} energies of 2 allowed states.



You know $\Delta E = h\nu$ (Planck's law) $\neq \Delta E = \gamma \hbar H$

$\therefore \quad \gamma \hbar H = h\nu$

$(\hbar = h/2\pi)$

$\gamma \frac{h}{2\pi} H = h\nu$

$$\boxed{\nu = \frac{\gamma H}{2\pi}}$$

"Larmor Equation"

frequency $= \frac{\gamma H}{2\pi}$

" frequency of EM radiation with energy equal to diff. in energy between the allowed states of nucleus $= \frac{\gamma H}{2\pi}$

Summary -

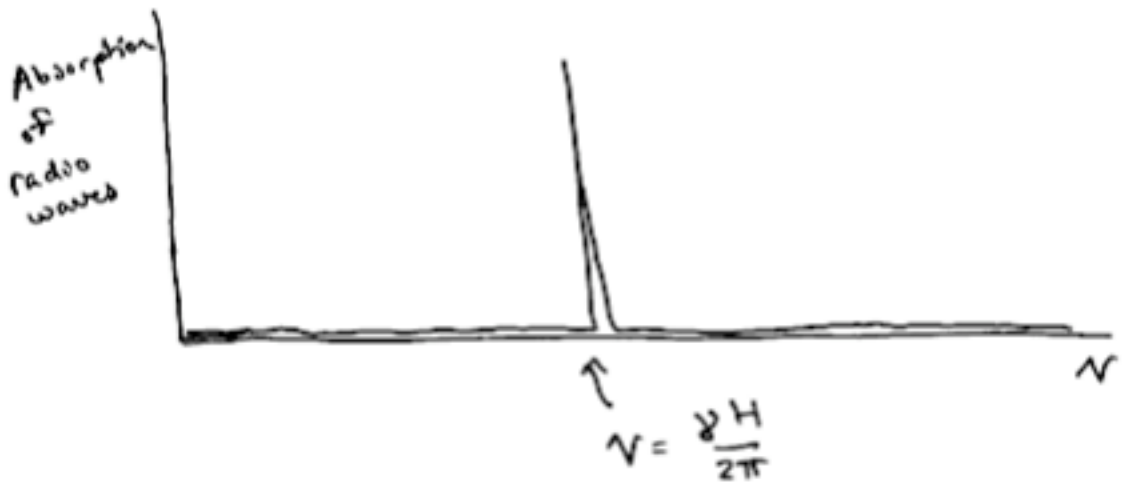
IF you place a nucleus in a mag. field,

* irradiate it w/ E.M. with $\nu = \frac{\gamma H}{2\pi}$, the sample can have transitions from $M = -\frac{1}{2} \rightarrow M = +\frac{1}{2}$ that absorb the E.M.

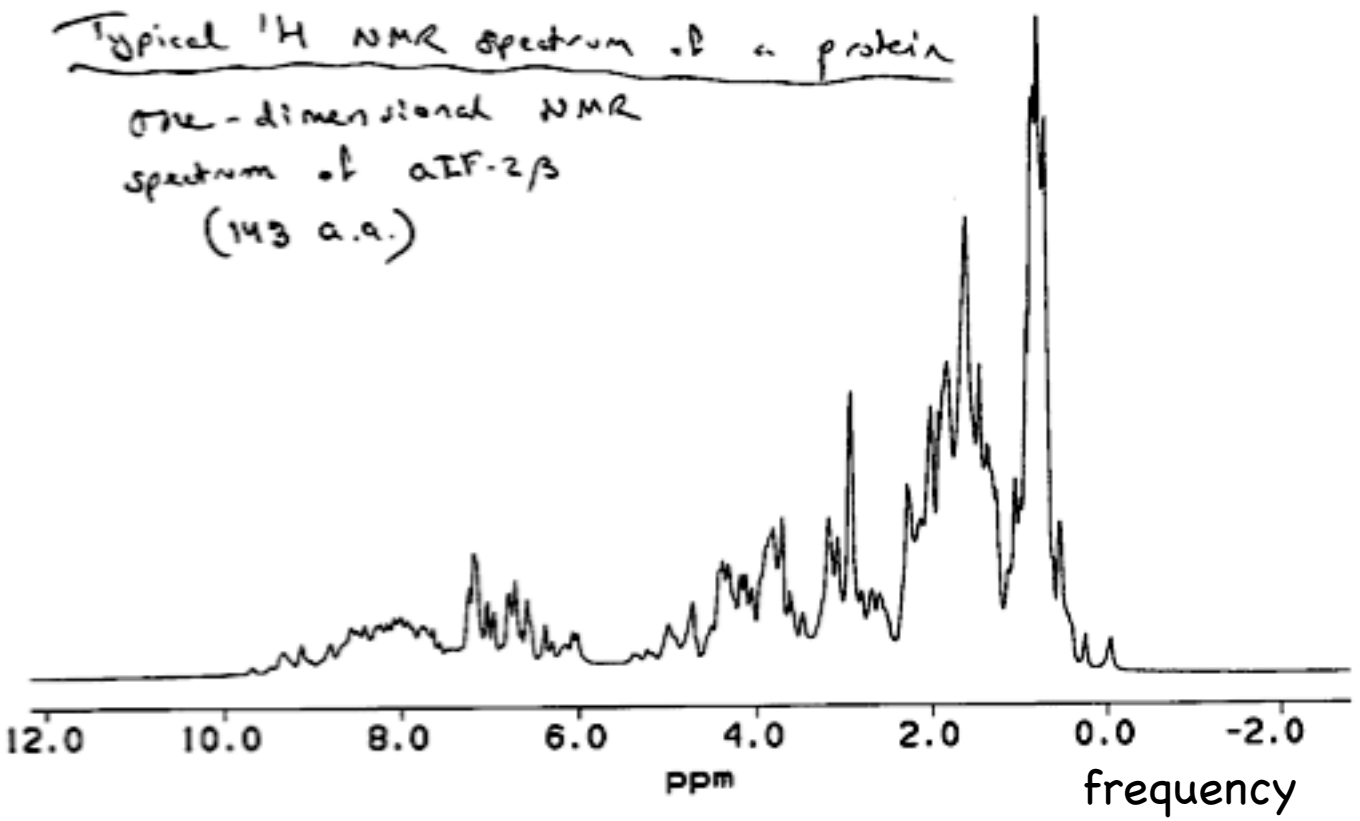
NMR spectrum of H_2O :

Put H_2O in a magnetic field of 11.7 Tesla.

(H_2O molecule protons, chemically equivalent)



$$\nu = \frac{\left(2.67 \times 10^{+8} \frac{\text{radians/sec}}{\text{Tesla}}\right)(11.7 \text{ Tesla})}{2\pi}$$



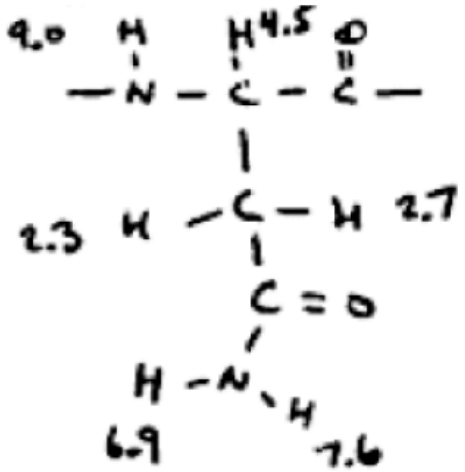
Each H in the protein is in a unique magnetic environment, and can absorb (and emit) radio waves of a unique frequency.

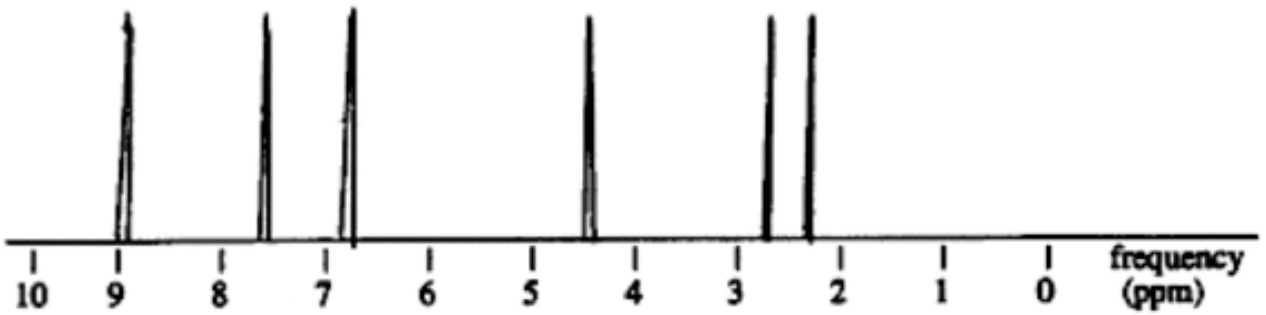
Protein structure information is derived from NMR data (primarily) through measurement of the "Nuclear Overhauser Effect" or "NOE" involving ^{1}H NMR signals.

NOE = the change in the intensity of the NMR signal of one ^{1}H nucleus when the sample is irradiated with radiowaves at the NMR absorption frequency of another nearby ^{1}H nucleus.

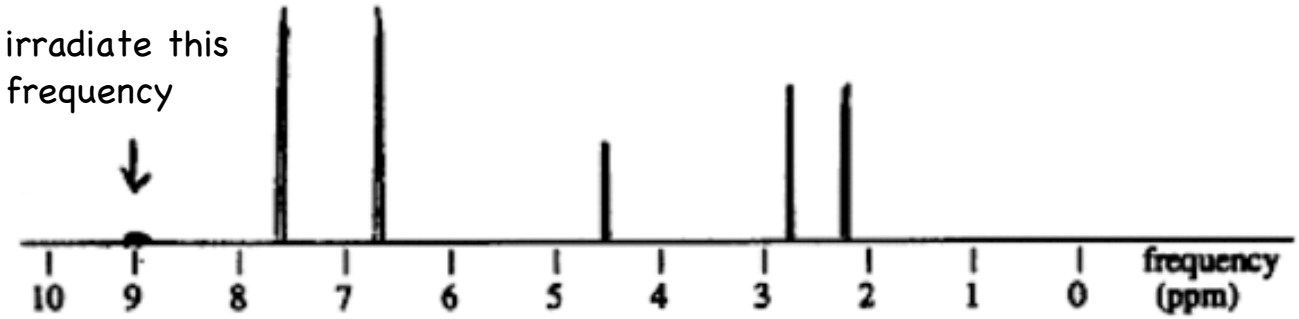
The NOE can be illustrated by example. Consider the NMR spectrum of asparagine, as part of a protein:

asparagine



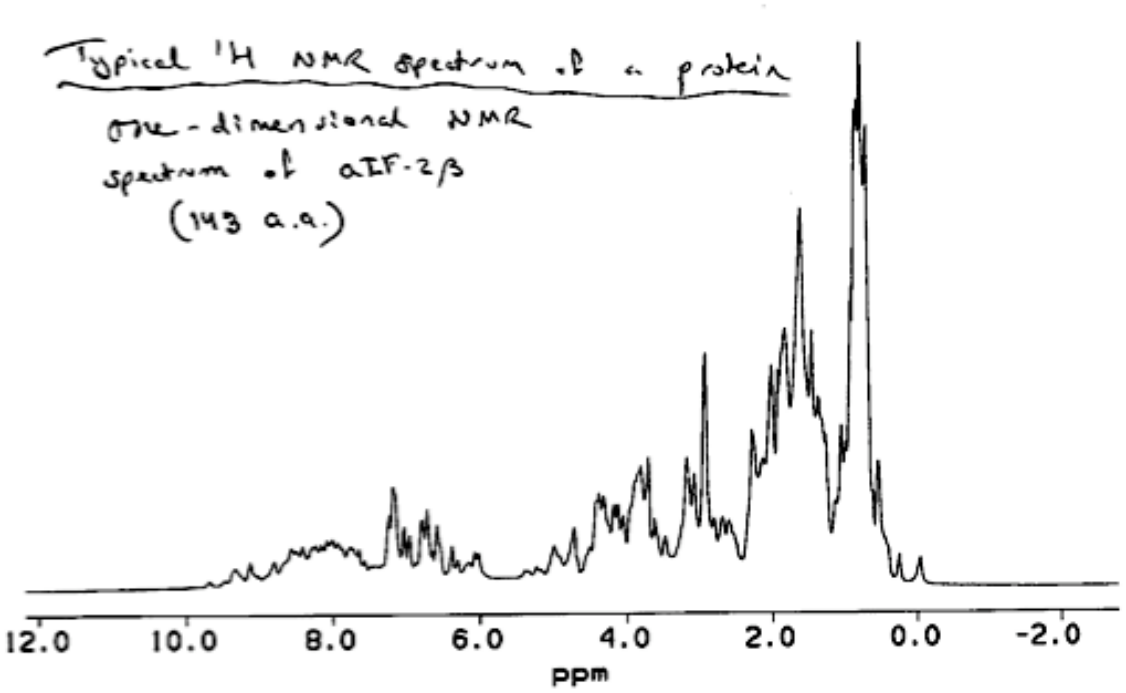


To see the NOE effect, the sample can be irradiate with RF waves at frequency 9 ppm:



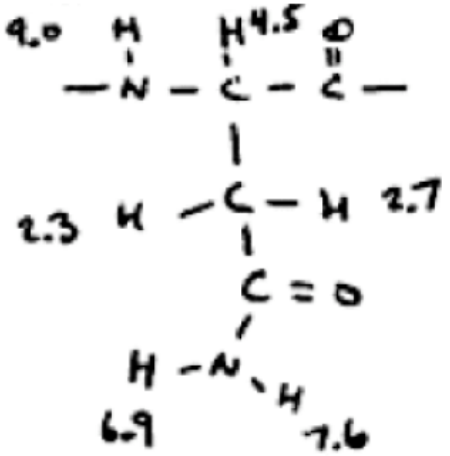
Observe intensity changes in nearby protons. This is the NOE.

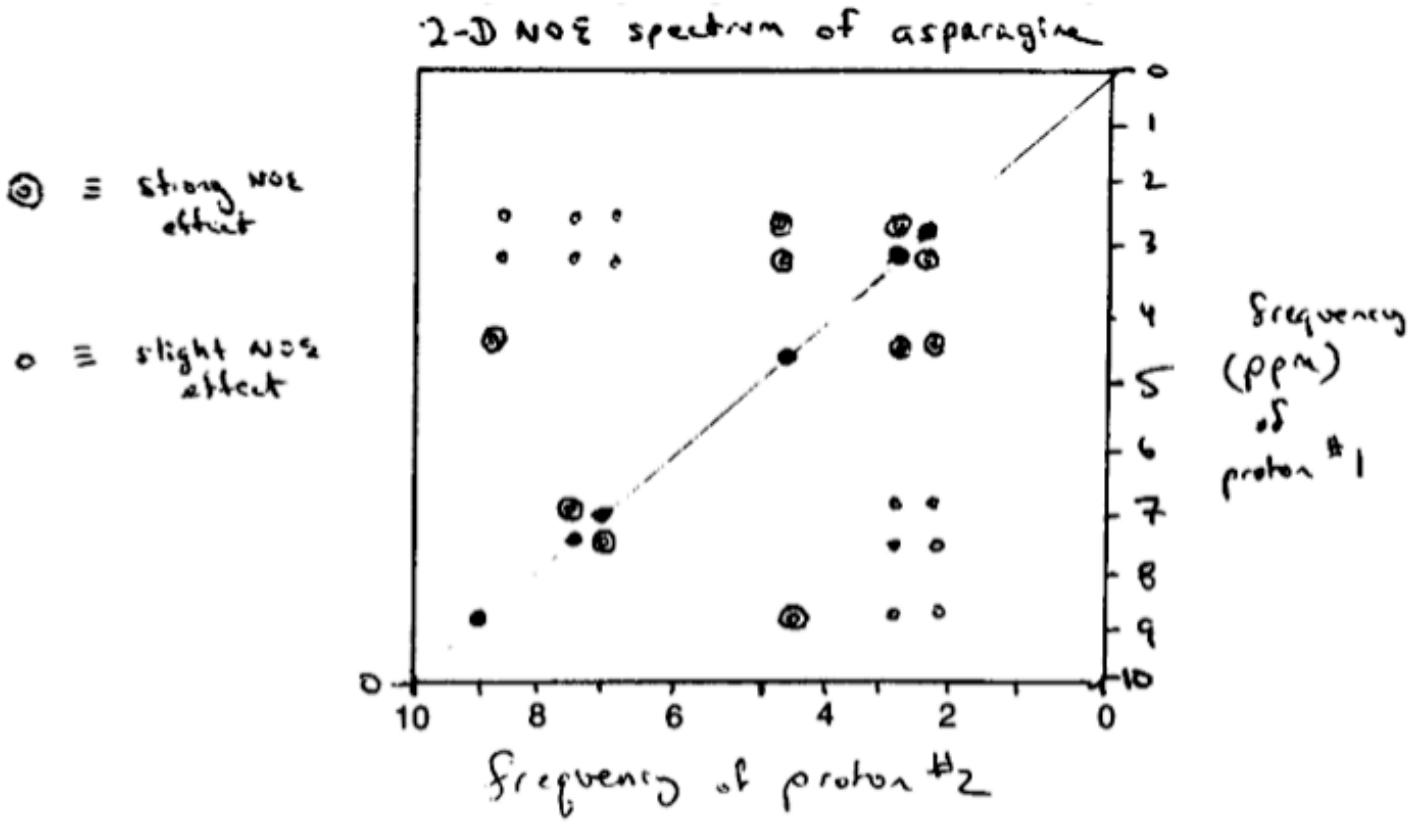
One-dimensional NMR spectra are not useful for measuring the NOE in proteins, due to overlap in the ^1H spectra.



It was necessary to invent two-dimensional (2-D) NMR to measure the NOE (and hence ^1H-^1H distances) in proteins.

Illustrate the 2-D NOE spectrum using asparagine as an example.



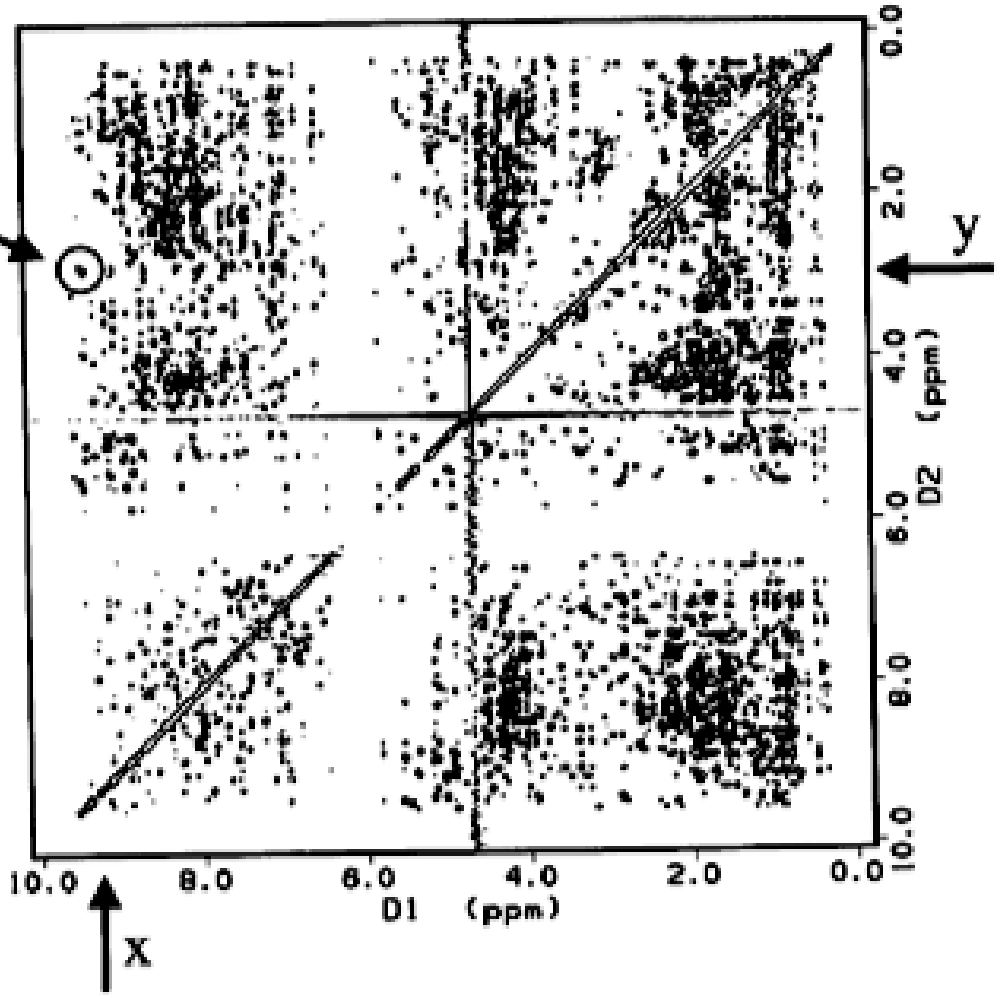


The 2-D NOE spectrum of a 140 a.a. protein.

Peak has 2 coordinates (9.5, 3.0)

The proton with NMR frequency of 9.5 ppm is near the proton with NMR frequency 3.0 ppm.

"Near" means < 6 Å



In summary:

The 2-D NOE spectrum provides information from which the distance between protons can be derived.

How are NOE intensities converted to distances?

Most commonly, a range of distances is used, something like:

large NOE effect: $d = 1.8 \rightarrow 3.0$ Å

medium NOE: $d = 2 \rightarrow 4.0$ Å

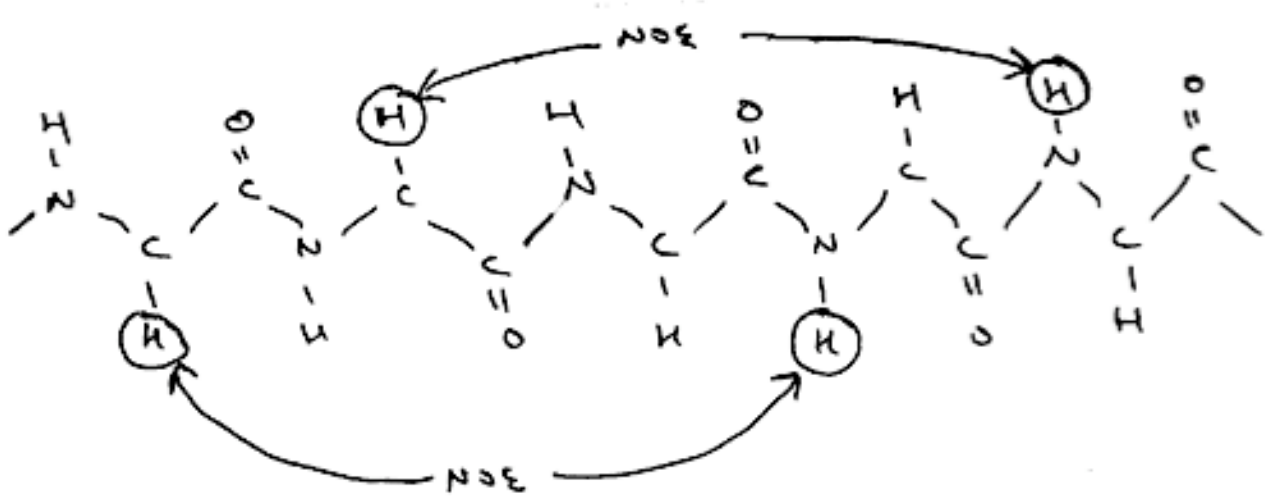
slight NOE: $d = 2.5 \rightarrow 5.0$ Å

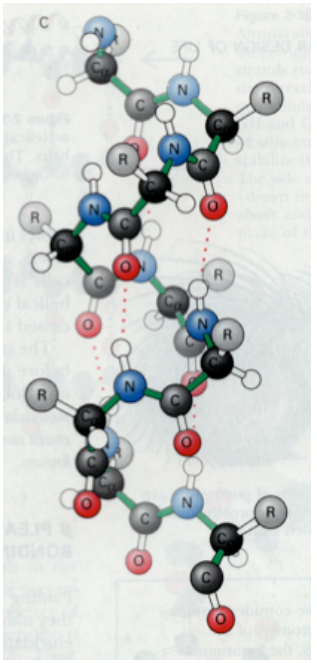
very small NOE: $d = 3.0 \rightarrow 6.0$ Å

How are these distances calibrated?

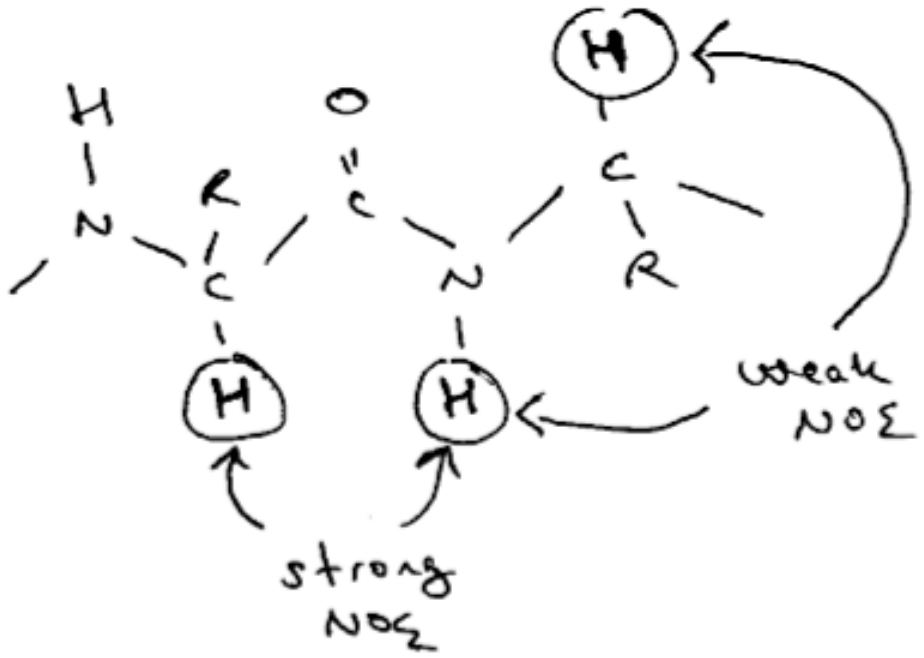
Usually by looking at NOE intensities in regular secondary structure, such as helix, where the distances are well known.

Observed NOEs in α-helix: (3.6 a.a. / turn)

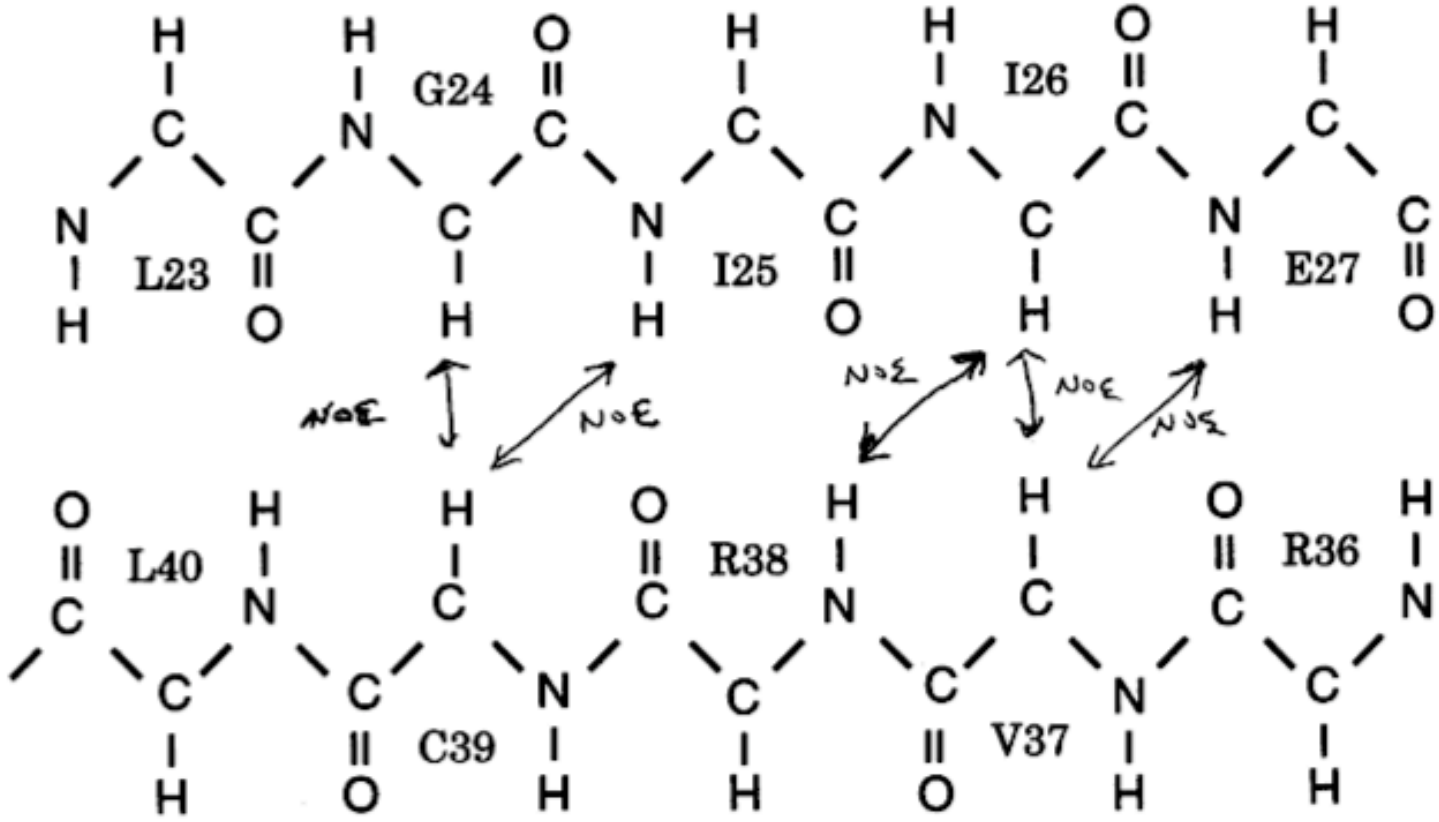




Observed NOE's in extended β-strand:



Some observed NOE's in an H parallel β-strand structures :



What goes into determining a protein structure from NMR data?

A medium sized protein may contain 150 amino acids, and > 2000 atoms.

NMR data may provide the distances between 1000 pairs of ^{1}H nuclei (from measured NOE peaks).

Are 1000 ^{1}H-^{1}H distances enough to determine the structure of this protein?

Nope.

What to do?

What else do we know about the protein structure, in addition to the 1000 ^{1}H-^{1}H distances?

Bond lengths can be assumed known.

Many bond angles are known (tetrahedral carbons, planar rings).

Van der Waals radii of atoms are known.

How can these different types of information be combined to yield a structure?

An energy function is defined:

$$E = \text{Sum of} (E_{bond} + E_{angle} + E_{vdw} + E_{noe})$$

where:

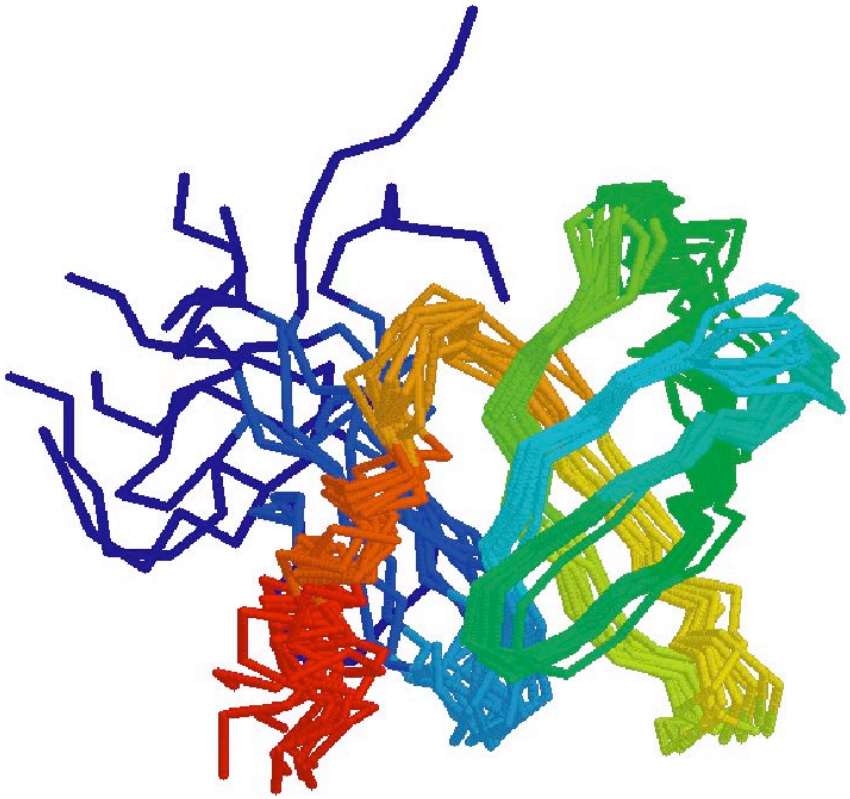
E_{bond} is a penalty for any bond lengths that deviate from accepted values.
E_{angle} is a penalty for any bond angles that deviate from accepted values.
E_{vdw} is a penalty for van der Waals violations.
E_{noe} is a penalty for H-H distances that deviate from NOE-derived bounds.

Structure determination is accomplished by "simulated annealing":

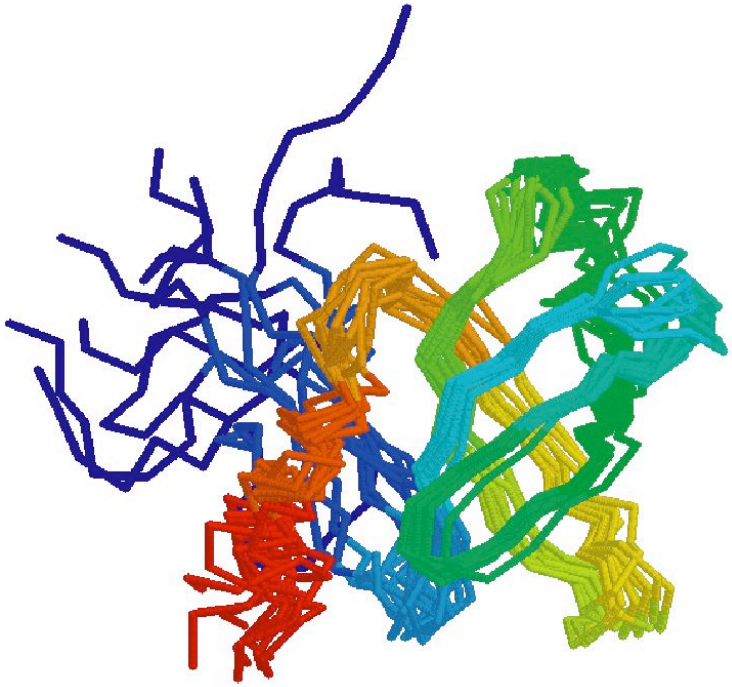
"Simulated annealing" means varying the **x**, **y** and **z** coordinates of all atoms in a protein, in order to minimize the value of the energy function E.

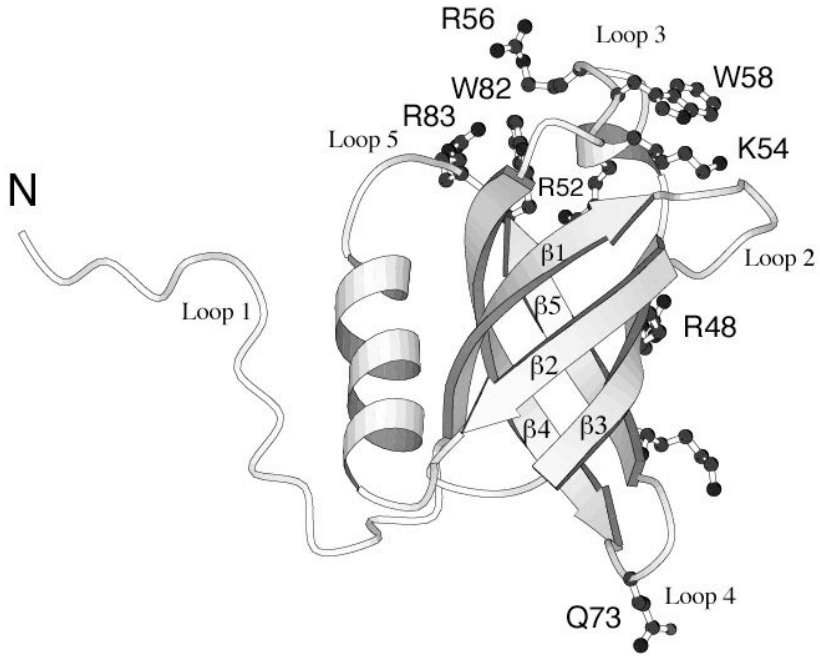
Typical result of the "simulated annealing" process used for protein structure determination:

This is a set of 12 super-imposed structures, all of which fit the NMR-derived distance restraints equally well. In other words, these 12 structures all have the same value of "E" after the simulated annealing process.



Visualizing the protein structure.





Comparing NMR and x-ray: Some things to think about.

a) Limitations.

X-ray: Need crystals
NMR: MW limit (assignment problem, resonance linewidth problem)

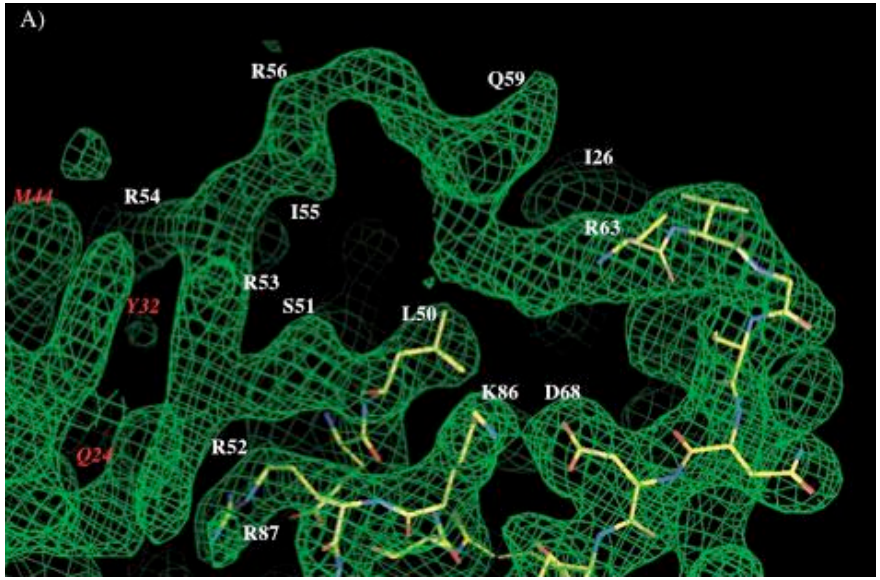
b) Ease of structure determination.

c) Quality of structural information obtained.

X-ray: Usually has the advantage, especially with high-resolution structures, due to direct visualization of the molecule.

NMR: Very good quality structures are also obtained, though usually not as detailed as the best x-ray structures. Quality of the NMR structure depends on the # of distance and angle constraints obtainable from the data.

With x-ray crystallography, the structure can be directly visualized.



Compare this with NMR, where a family of possible structures is derived from a set of inter-proton distances, consistent with the observed NOE data.