NMR in Medicine and Biology

http://en.wikipedia.org/wiki/NMR_Spectroscopy

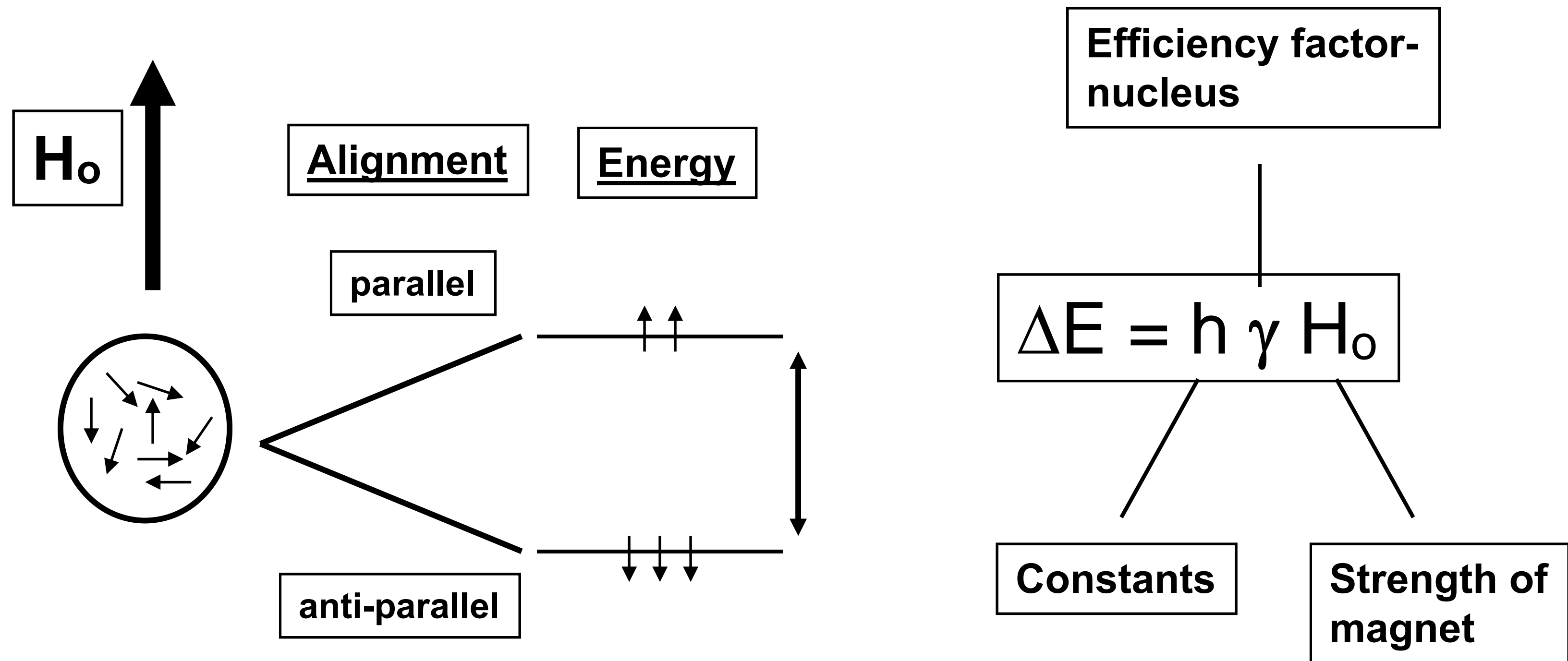
- MRI- Magnetic Resonance Imaging (water)
- In-vivo spectroscopy (metabolites)
- Solid-state NMR (large structures)
- Solution NMR Spectroscopy
  - Analytical biochemistry
  - Comparative analysis
  - Interactions between biomolecules
  - Ligand screening (SAR by NMR)
  - Structure determination
  - Protein Dynamics

Protein NMR
Detection of spin 1/2 nuclei
^{1}H, ^{13}C, ^{15}N, ^{31}P

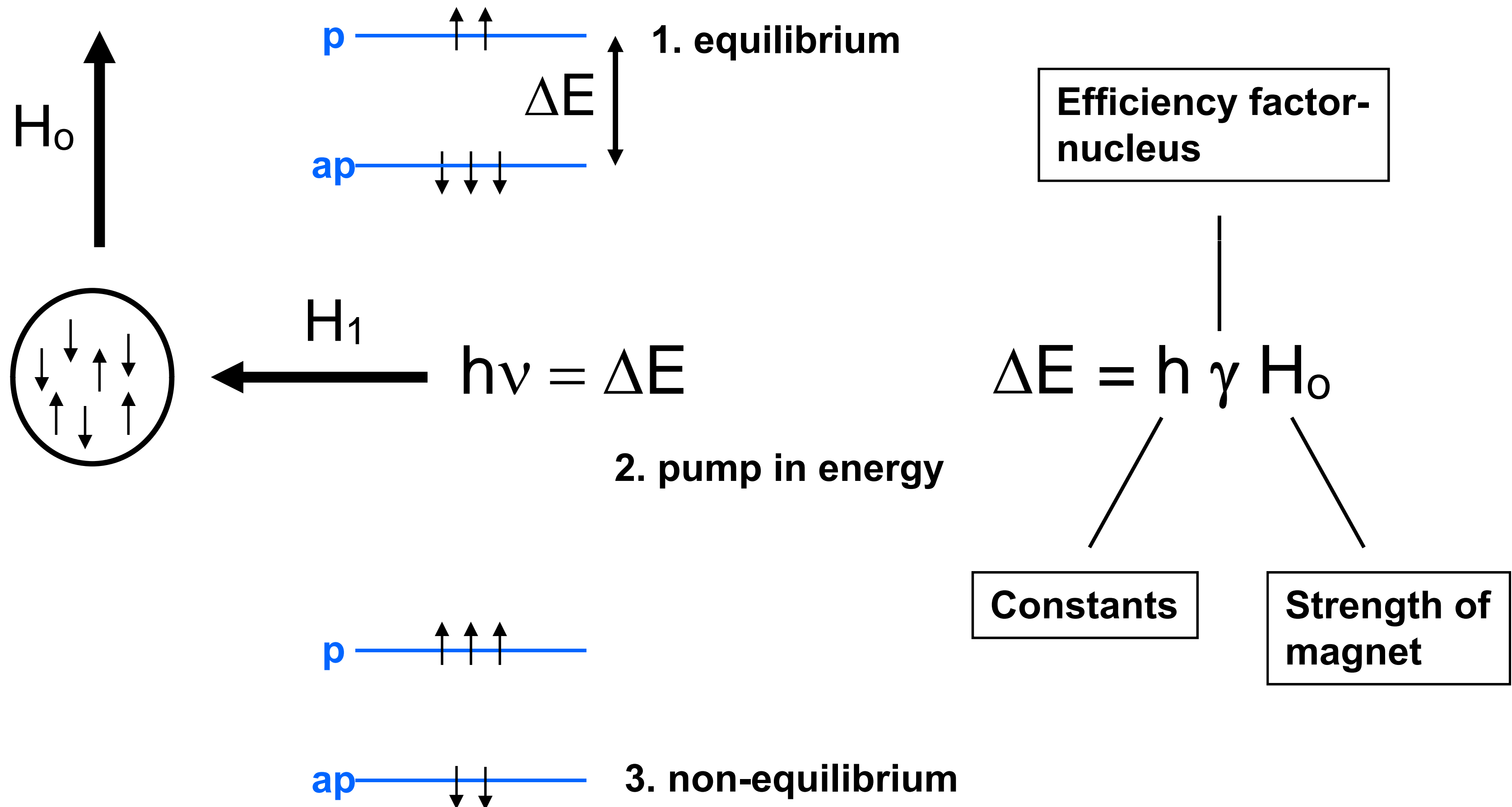
- Only nuclei with ***spin number*** **(I) ≠ 0 can absorb/emit electro-magnetic radiation.**
  - **Even atomic mass & number ⇒ I = 0 (^{12}C, ^{16}O)**
  - **Even atomic mass & odd number ⇒ I = whole integer (^{14}N, ^{2}H, ^{10}B)**
  - **Odd atomic mass ⇒ I = half integer (^{1}H, ^{13}C, ^{15}N, ^{31}P)**

- **Nuclear spin angular momentum is a quantized property of the nucleus in each atom**

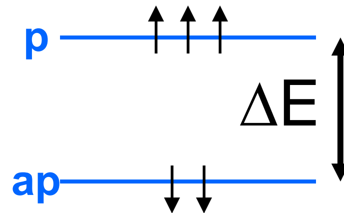
Nuclei With Non-Zero Spin Align in Magnetic Fields



Resonance: Perturb Equilibrium



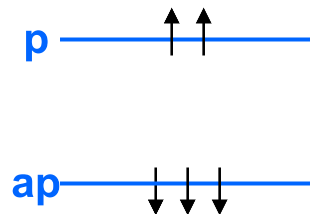
Return to Equilibrium (Relax): Read Out Signals



3. Non-equilibrium

$$h\nu = \Delta E$$

4. release energy (detect)



5. equilibrium

Magnetic Resonance Sensitivity

Sensitivity (S) ~ Δ(population)

$$S \sim \Delta N = \frac{N_p}{N_{ap}} = e^{-\Delta E/kT}$$

$$\Delta E = h\,\gamma\,H_o$$

Efficiency factor- nucleus (γ)

Constants (h)

Strength of magnet (H_o)

ΔE is small

At room temp., ΔN ~ 1:10⁵

Intrinsically low sensitivity→

Need lots of sample

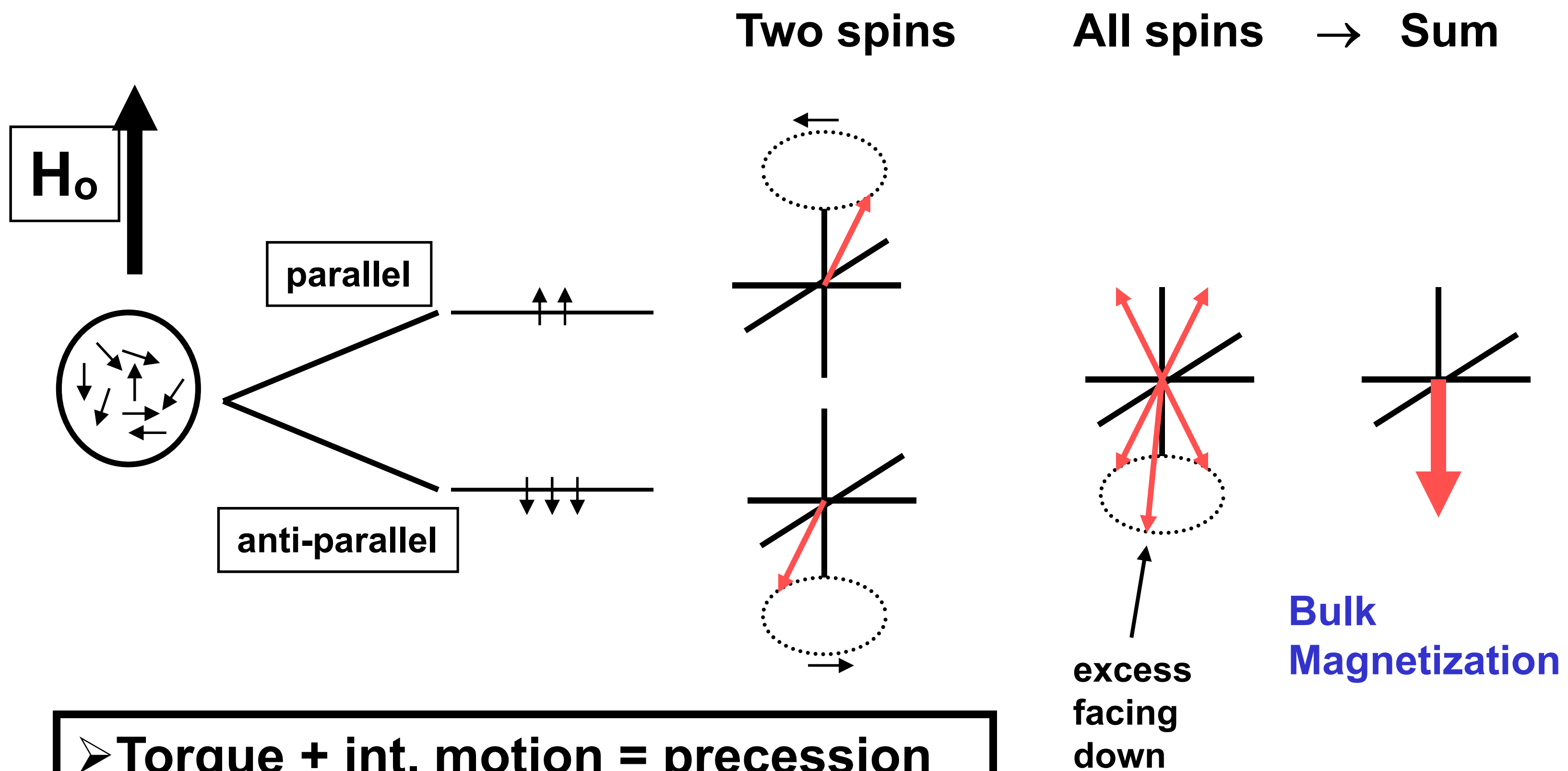
Increase sensitivity by increasing magnetic field strength

And high protein concentration

Intrinsic Sensitivity of Nuclei

Nucleus	γ	% Natural Abundance	Relative Sensitivity
^{1}H	2.7×10^{8}	99.98	1.0
^{13}C	6.7×10^{7}	1.11	0.004
^{15}N	-2.7×10^{7}	0.36	0.0004
^{31}P	1.1×10^{8}	100.	0.5

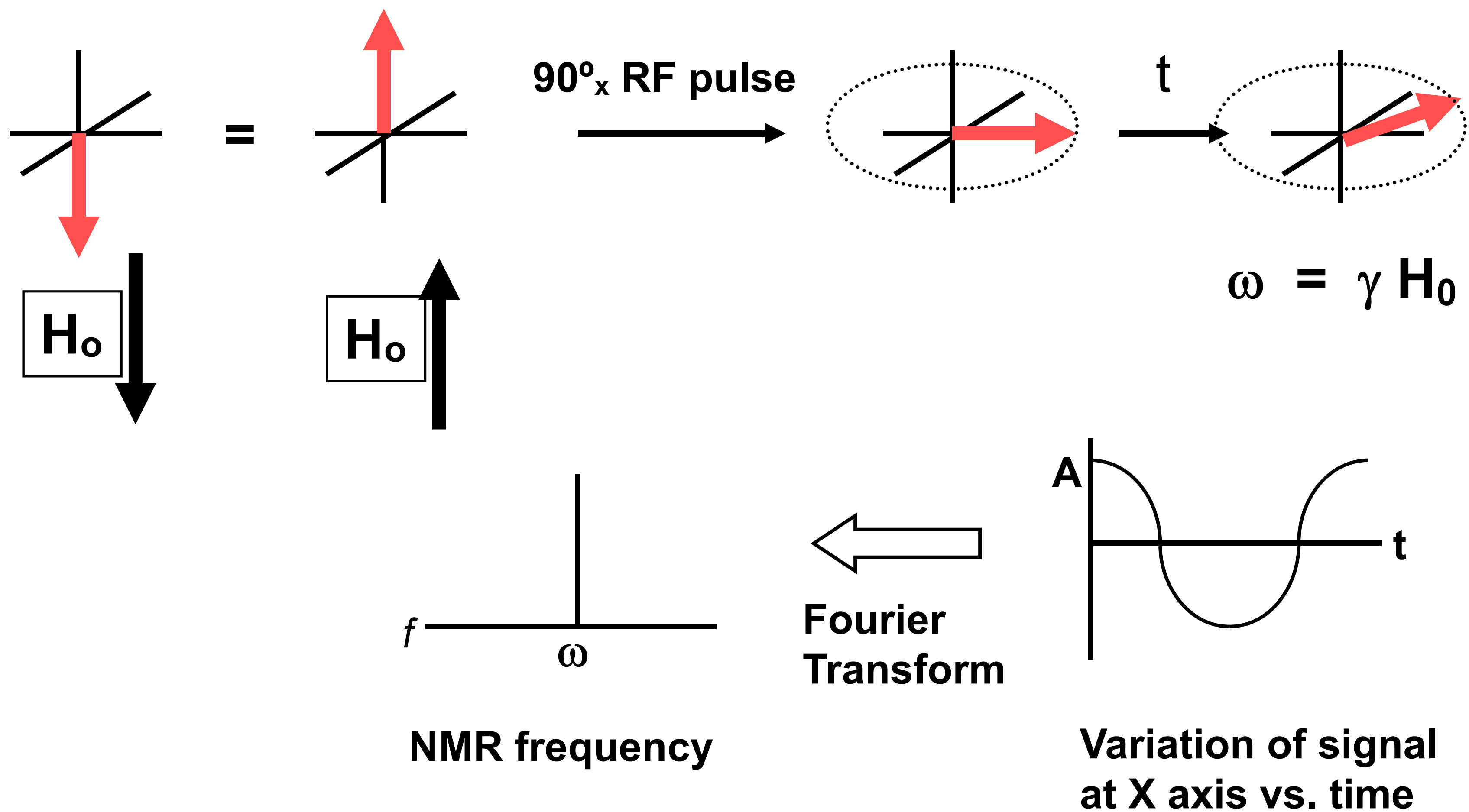
The Classical Treatment: Nuclear Spin Angular Momentum



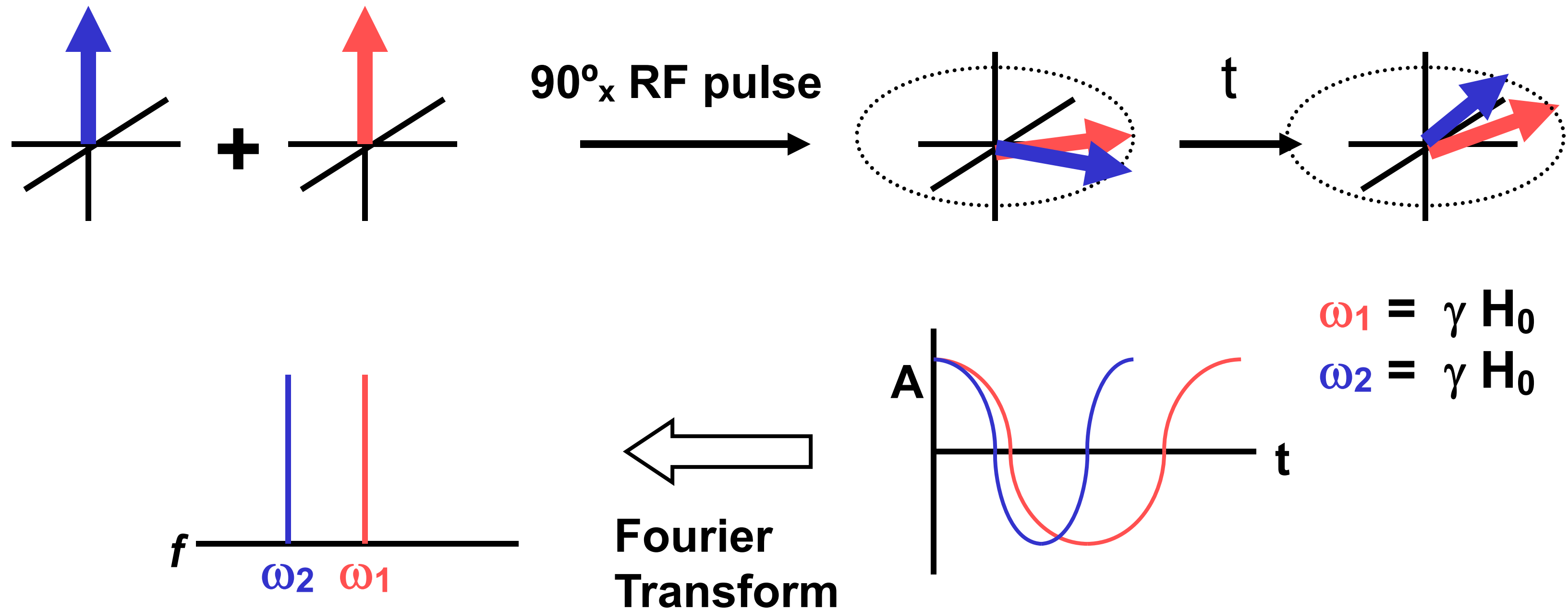
- Torque + int. motion = precession
- Precession around Z axis
- Larmor frequency (ω):

  $\Delta E = h\gamma H_o \rightarrow \Delta E = h\nu \rightarrow \nu = \gamma H_0 = \omega$

Pulse Fourier Transform NMR



The Power of Fourier Transform

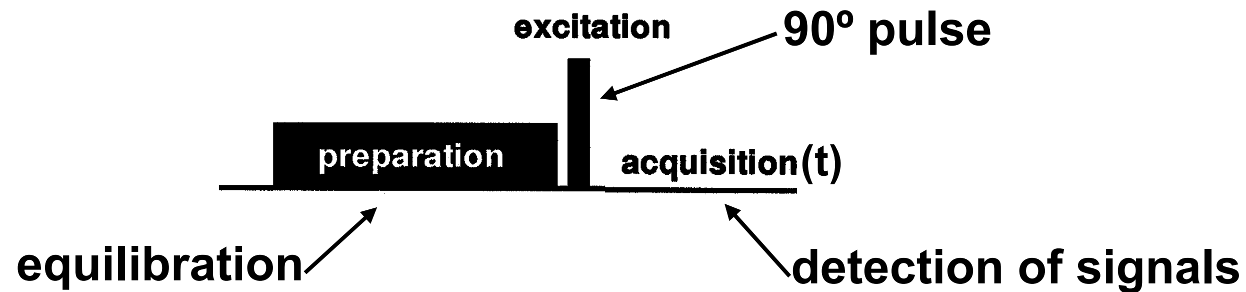


NMR frequency domain
- **Spectrum of frequencies**

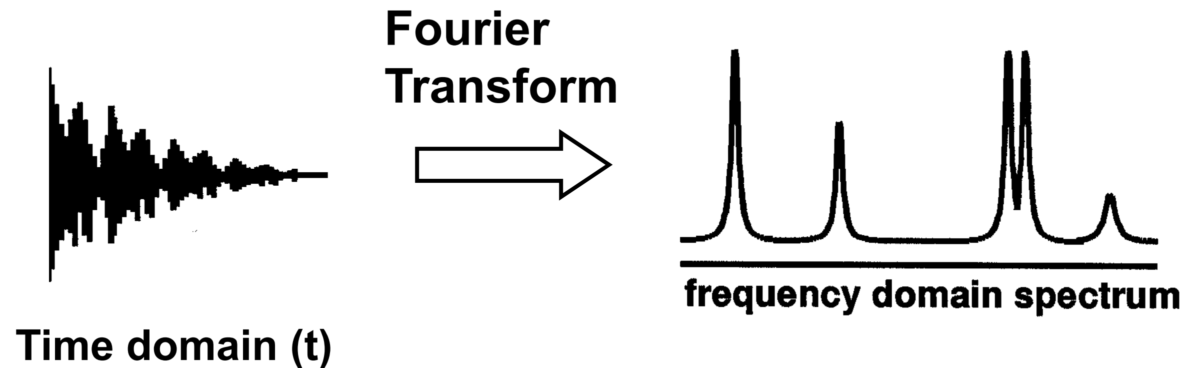
NMR time domain
- **Variation in amplitude vs time**

The Pulse FT NMR Experiment

Experiment



Data Analysis



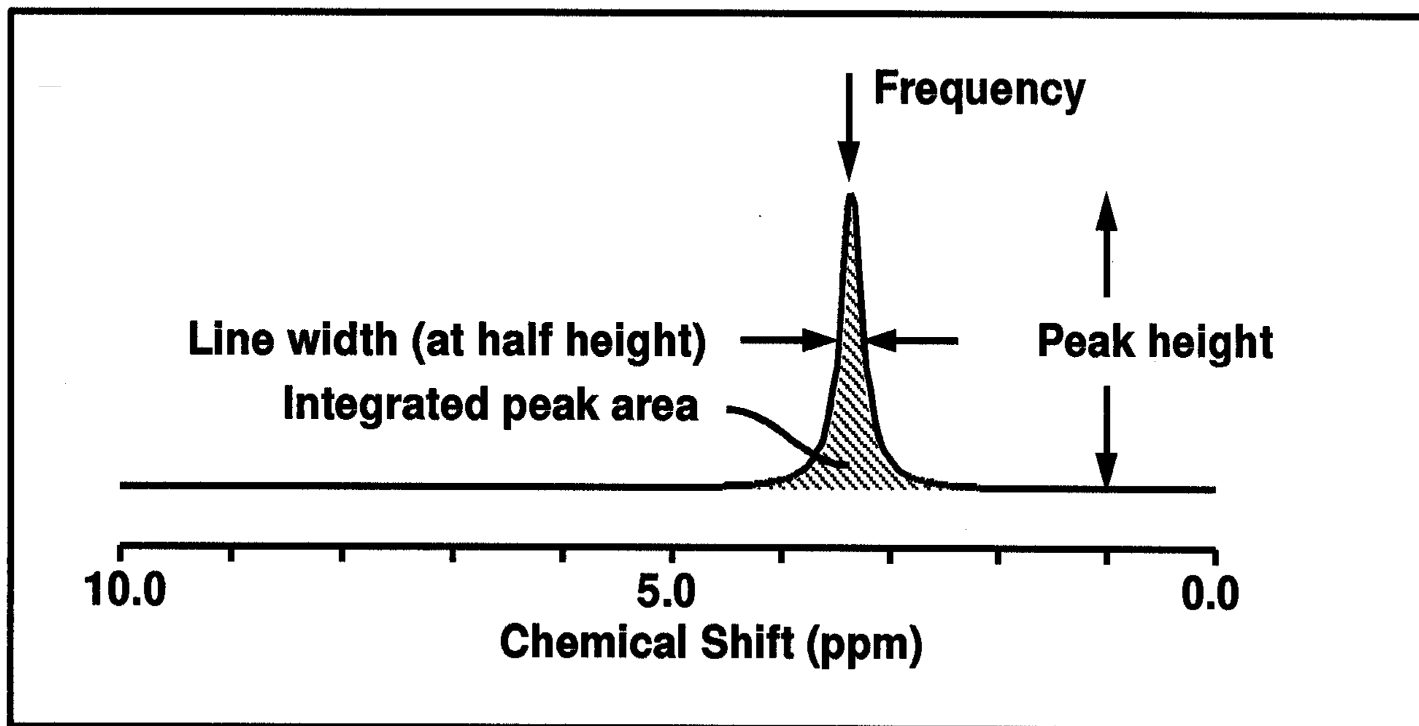
NMR Terminology

$$\delta = \frac{\omega - \omega_{ref}}{\omega_{ref}}$$

ppm (parts per million), Universal Unit

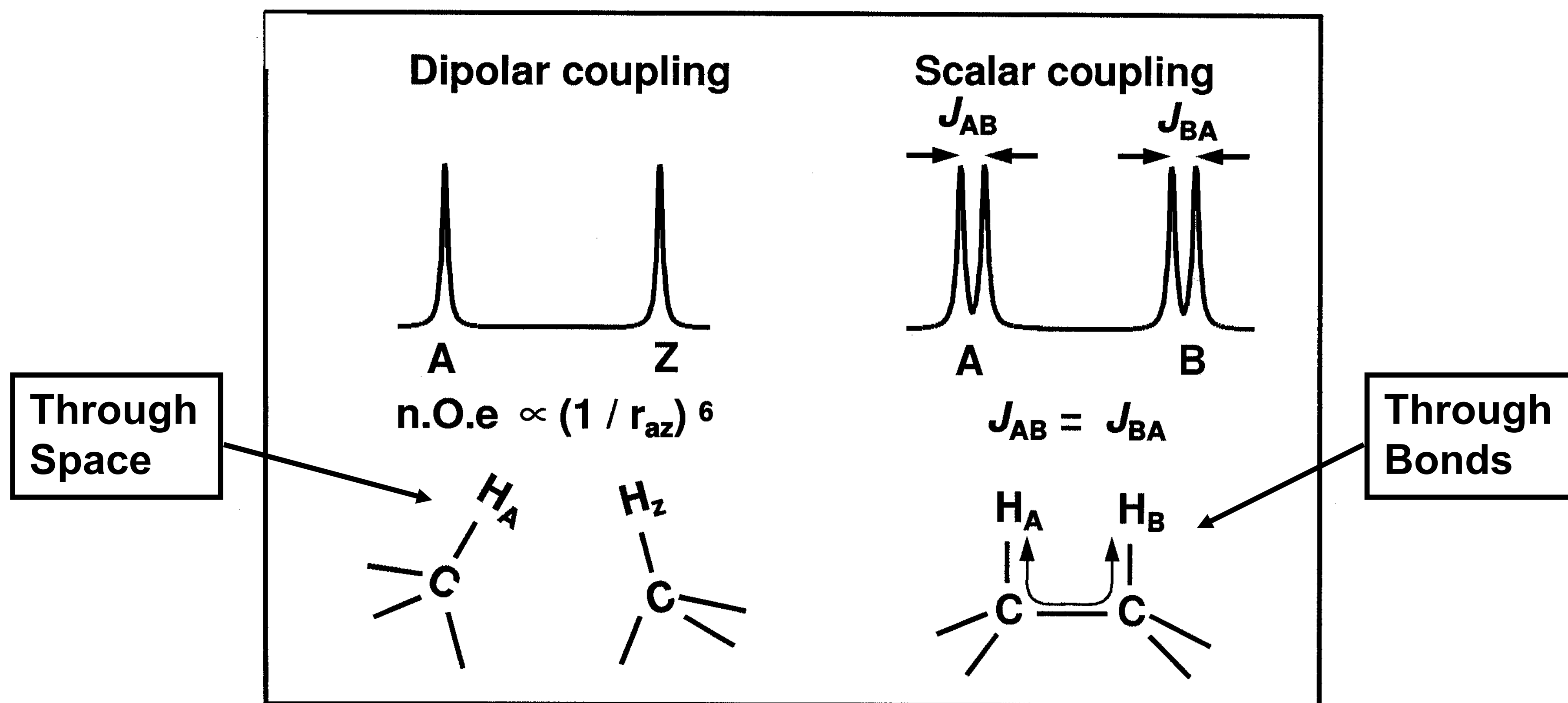
Universal Reference

1. Samples in water
2. Organic compounds



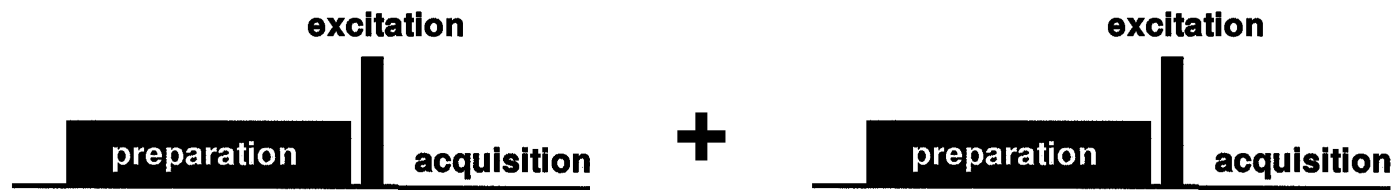
The exact resonance frequency (chemical shift) is determined by the electronic environment of the nucleus

NMR Scalar and Dipolar Coupling

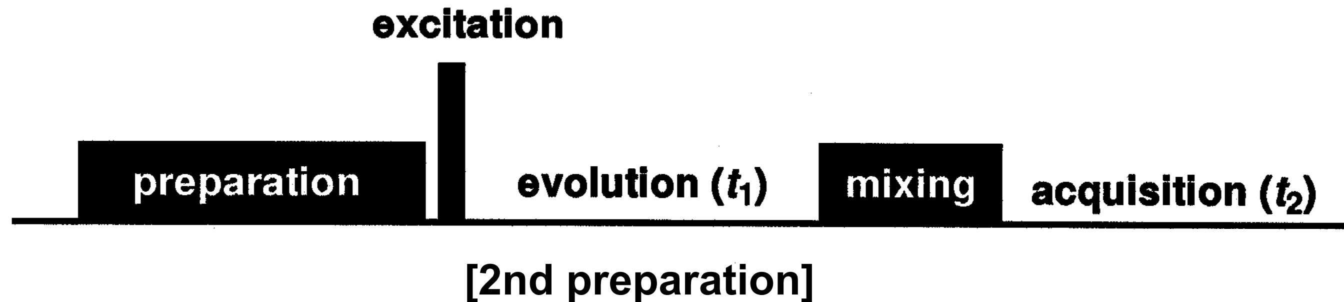


➢ ***Coupling of nuclei gives information on structure***

The 2D NMR Pulse Sequence

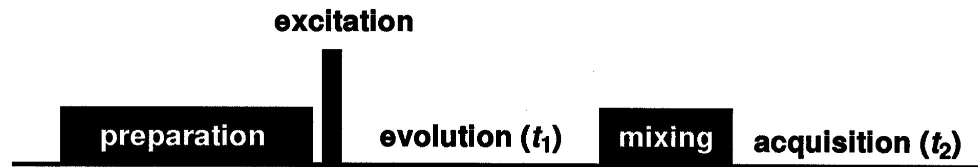


1D + 1D = 2D



The 2D NMR Spectrum

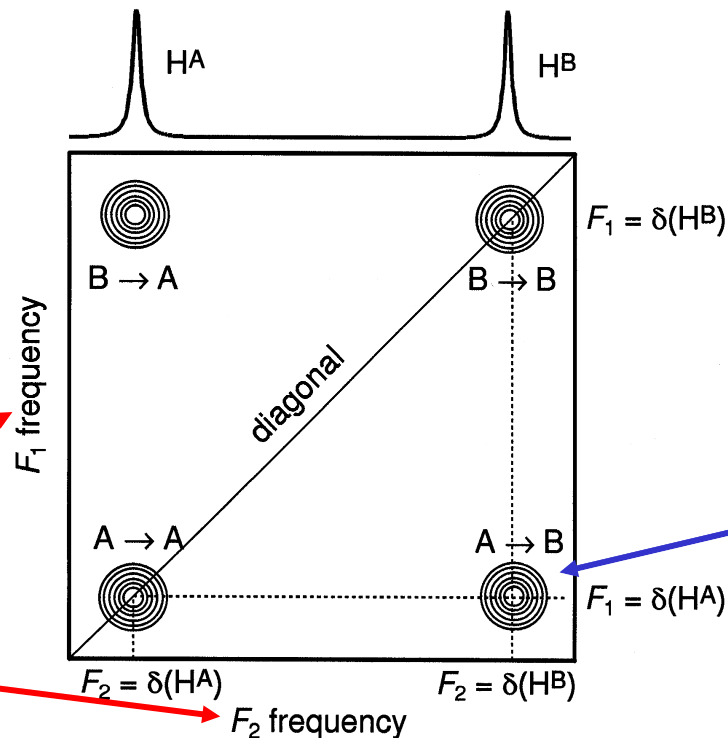
Pulse Sequence

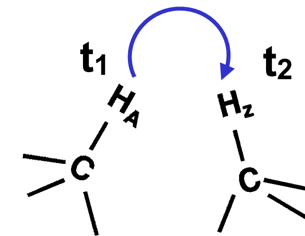


Spectrum

Before mixing

After mixing





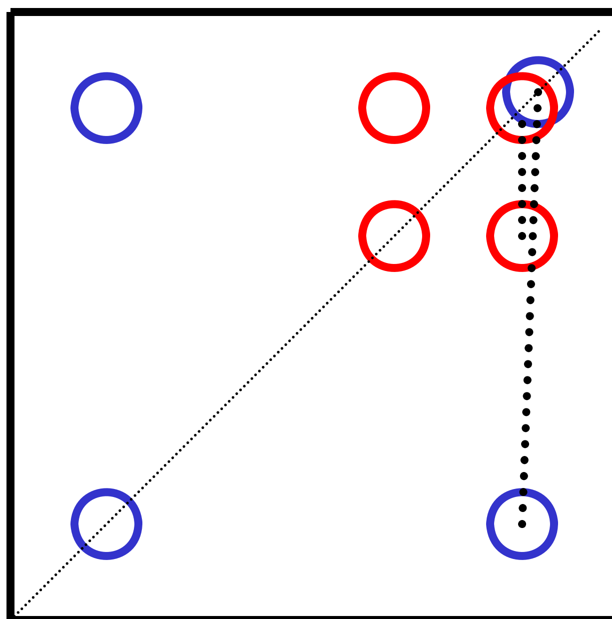
Coupled spins give rise to crosspeaks

The Power of 2D NMR: Resolving Overlapping Signals

1D

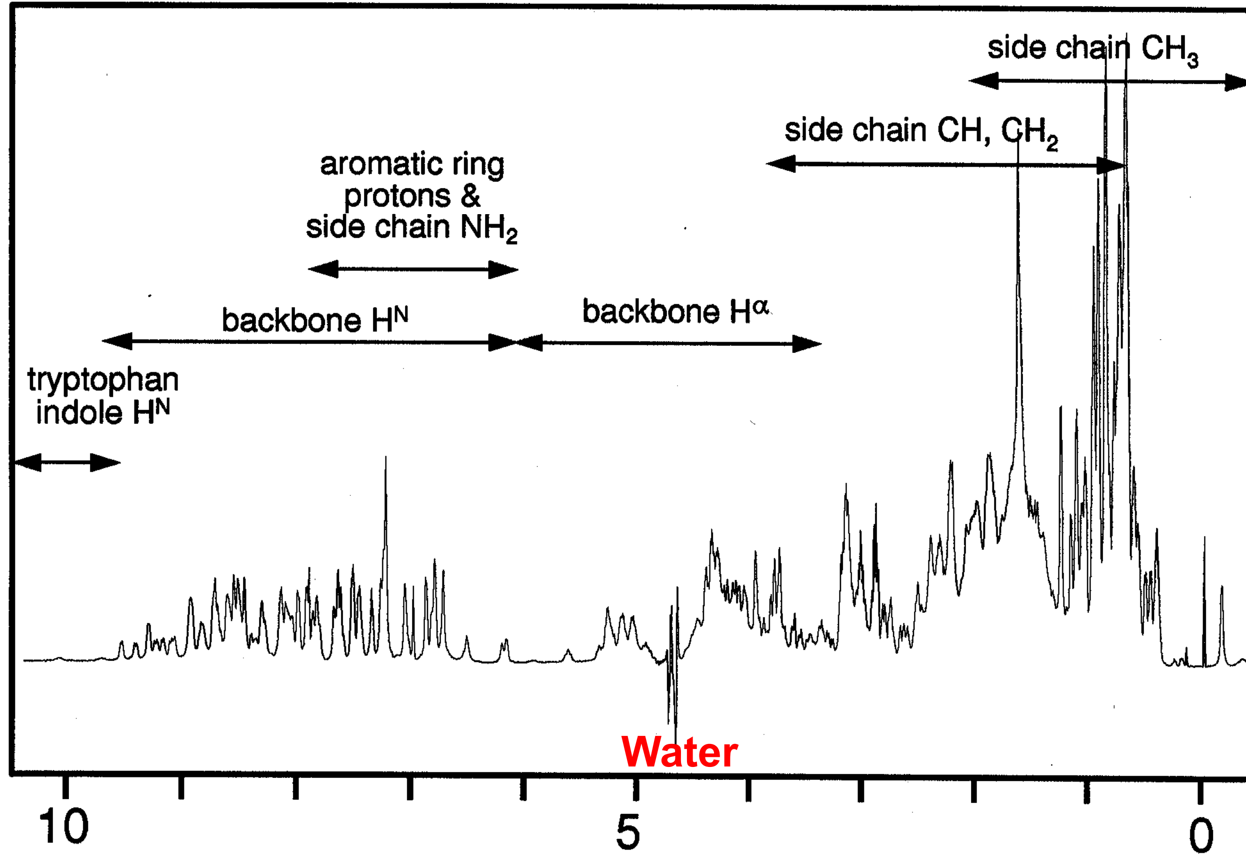
2 signals overlapped

2D



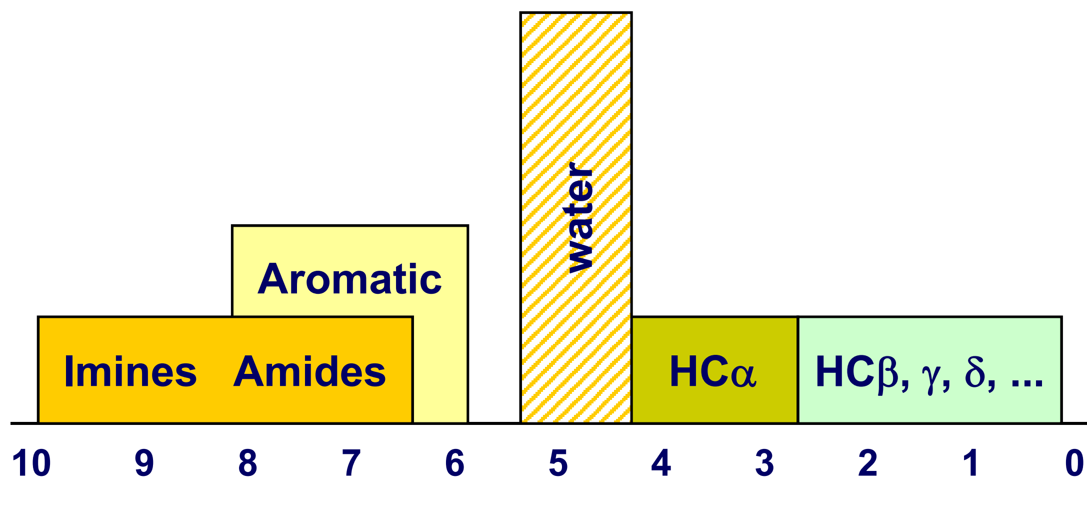
2 cross peaks resolved

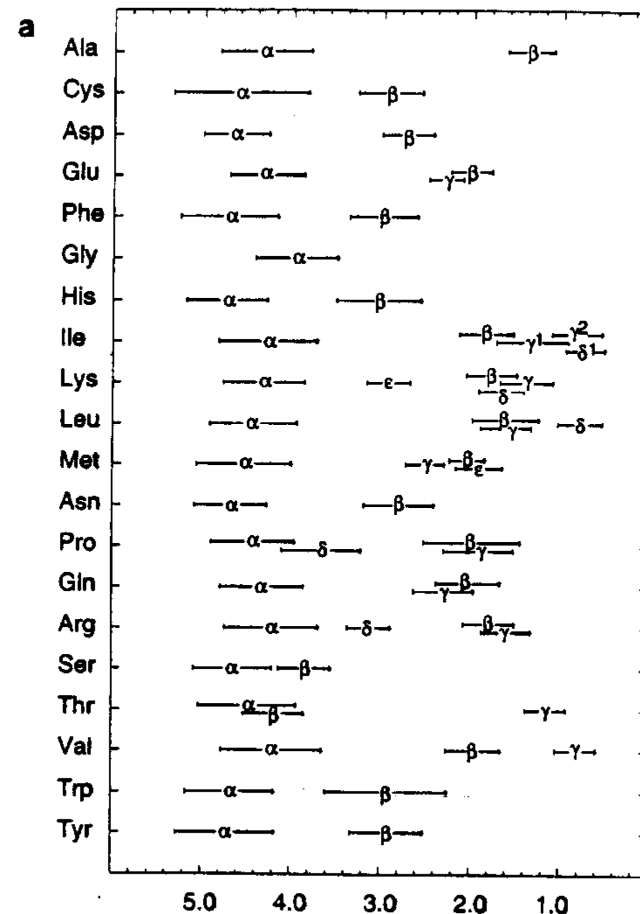
Regions of the ^{1}H NMR Spectrum

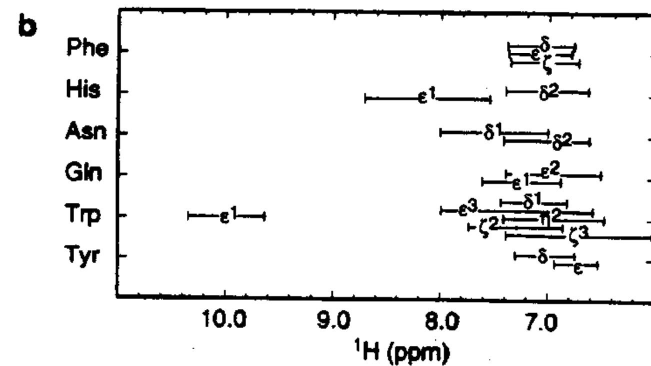


What would the unfolded protein look like?

Each side chain proton has characteristic ppm range of signal on the NMR spectrum.







NMR of Proteins

<u>Challenges</u>

Proteins have hundreds/thousands of signals

Resonance assignment first (who do all these signals belong to?)

Computer programs can be used to get assistance with resonance assignments

<u>Applications</u>

Folded protein?

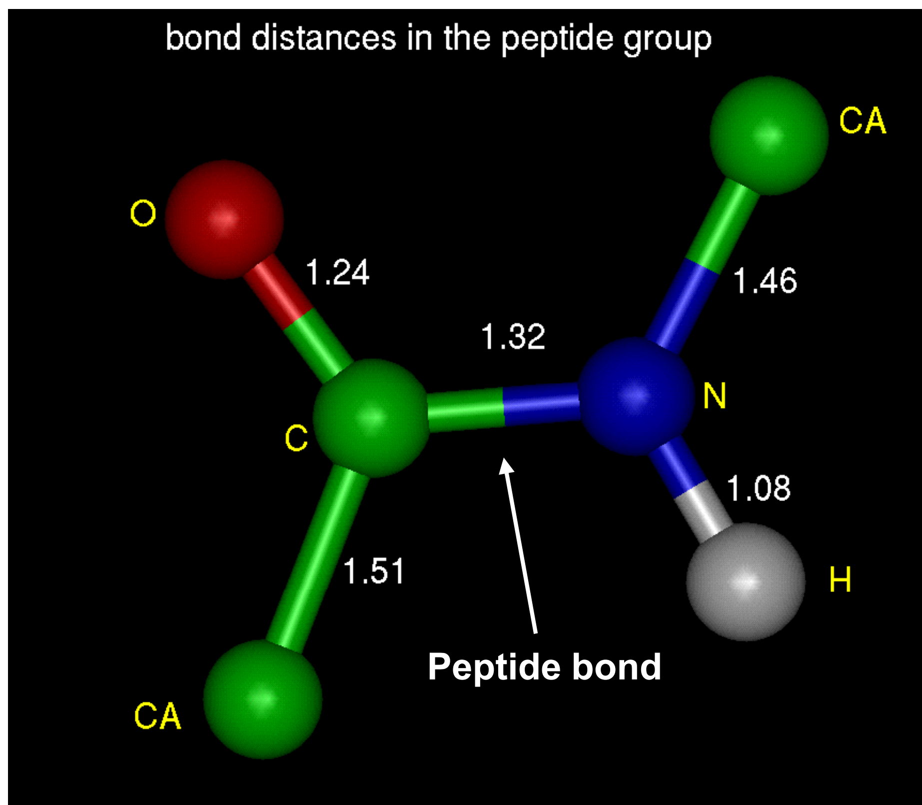
Measure binding constants

Assess structural homology/effect of mutations

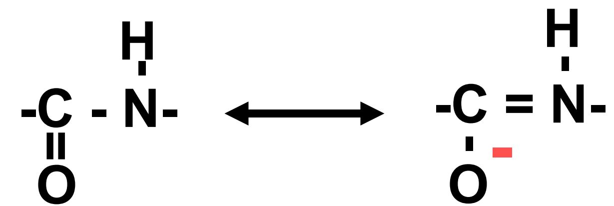
Three-dimensional structure determination

Measure flexibility/dynamics

Beyond Primary Structure: The Peptide Bond

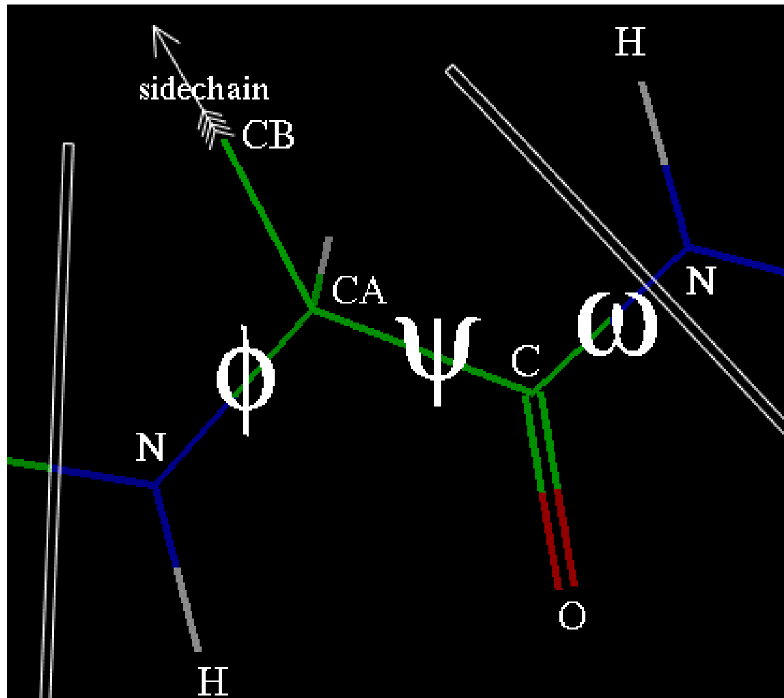


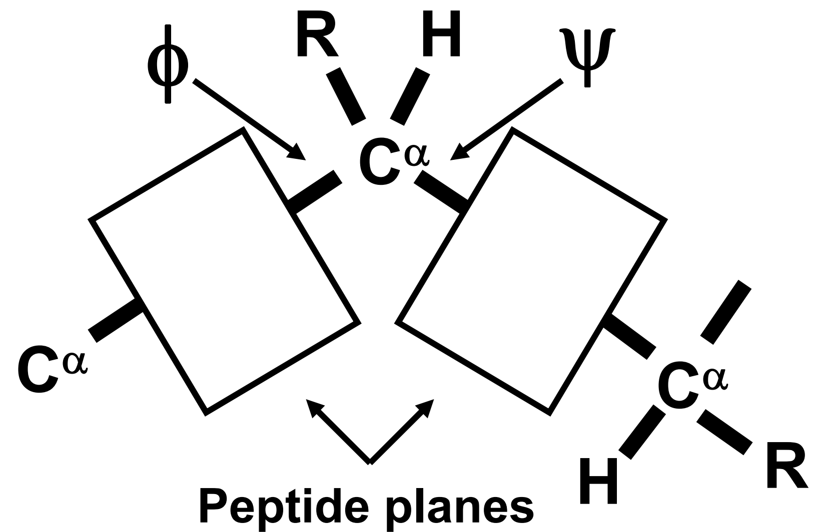
- Peptide plane is flat
  ω angle ~180º
- Partial double-bond:



Resonance structures

Implications of Peptide Planes





- ω angle varies little, ϕ and ψ angles vary alot
- Many ϕ/ψ combinations cause atoms to collide
- Each residue is sandwiched between two planes

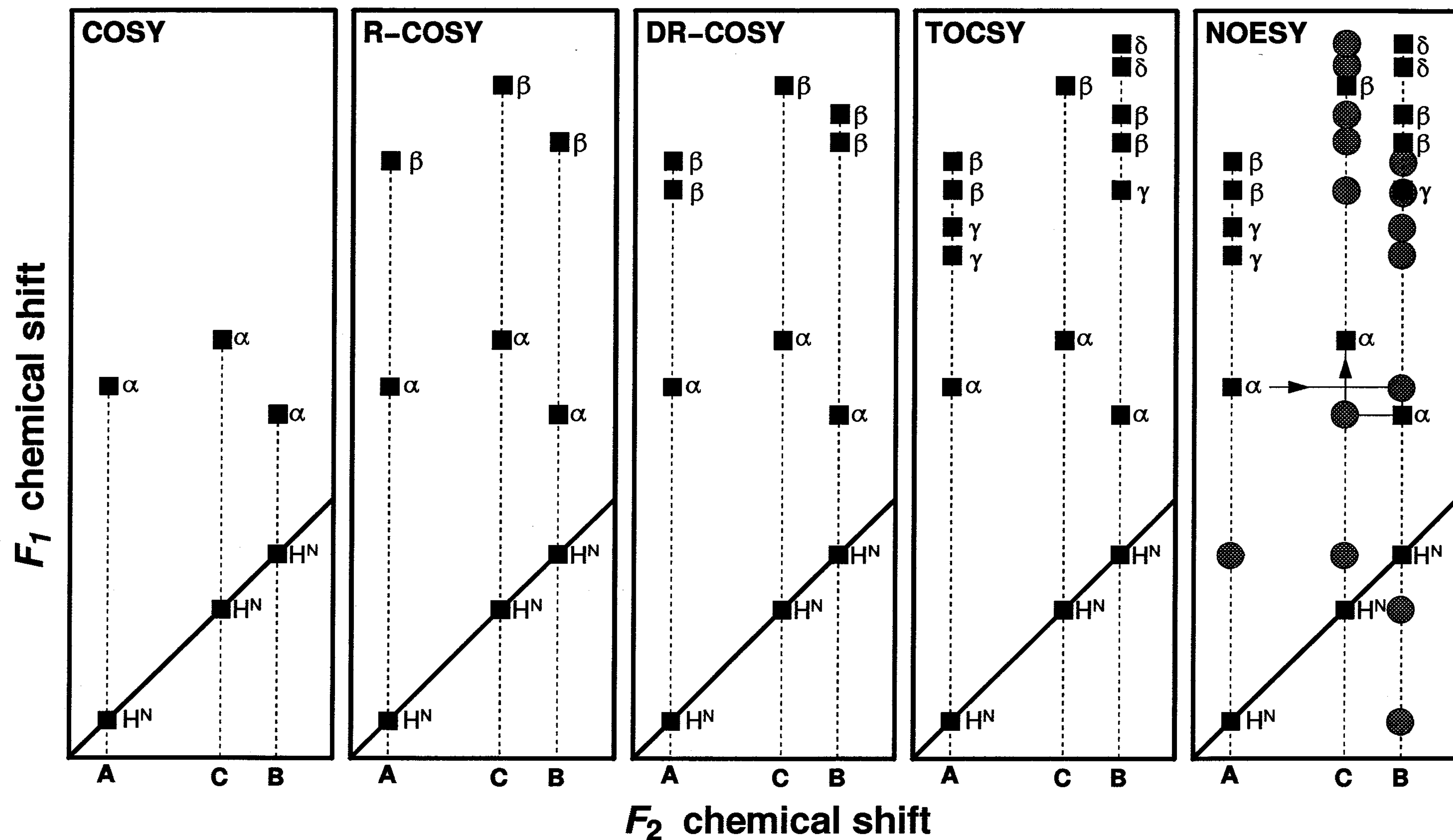
Secondary Structure
Local Conformation of Consecutive Residues

- **Three low energy backbone ϕ/ψ combinations**
  1. **Right-hand helix: α-, 3_{10}helix (-40°, -60°)**
  2. **Extended: antiparallel β-sheet (140°, -140°)**
  3. **Left-hand helix (*rare*): α-helix (45°, 45°)**
     Glycine is special- it has no side chain!

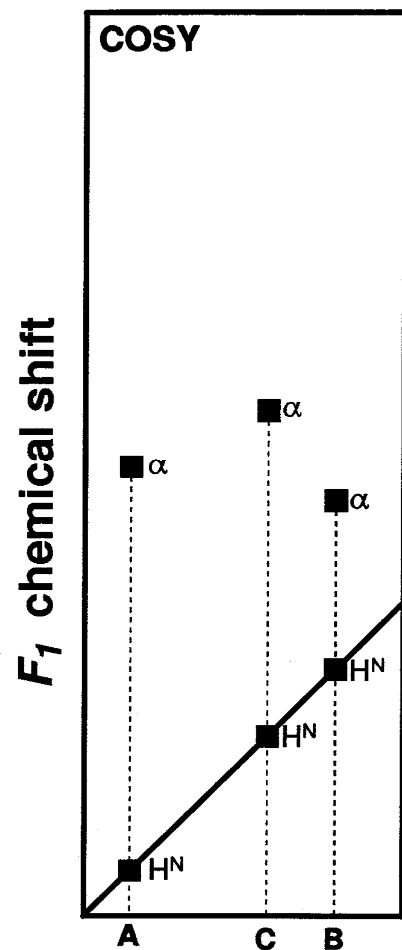
  Secondary structure is readily extracted from chemical shift assignments (Chemical Shift Index- CSI): ψ,ϕ calculation by Talos Software

- **Hydrogen bonds between backbone atoms provides stability to secondary structures, NMR allows direct measurement of these bonds**

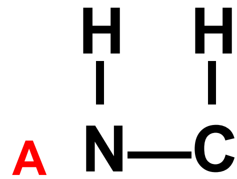
Step 1: Identify Spin System



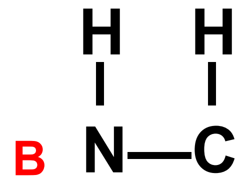
COSY: One coupling



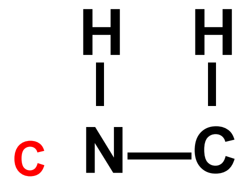
A



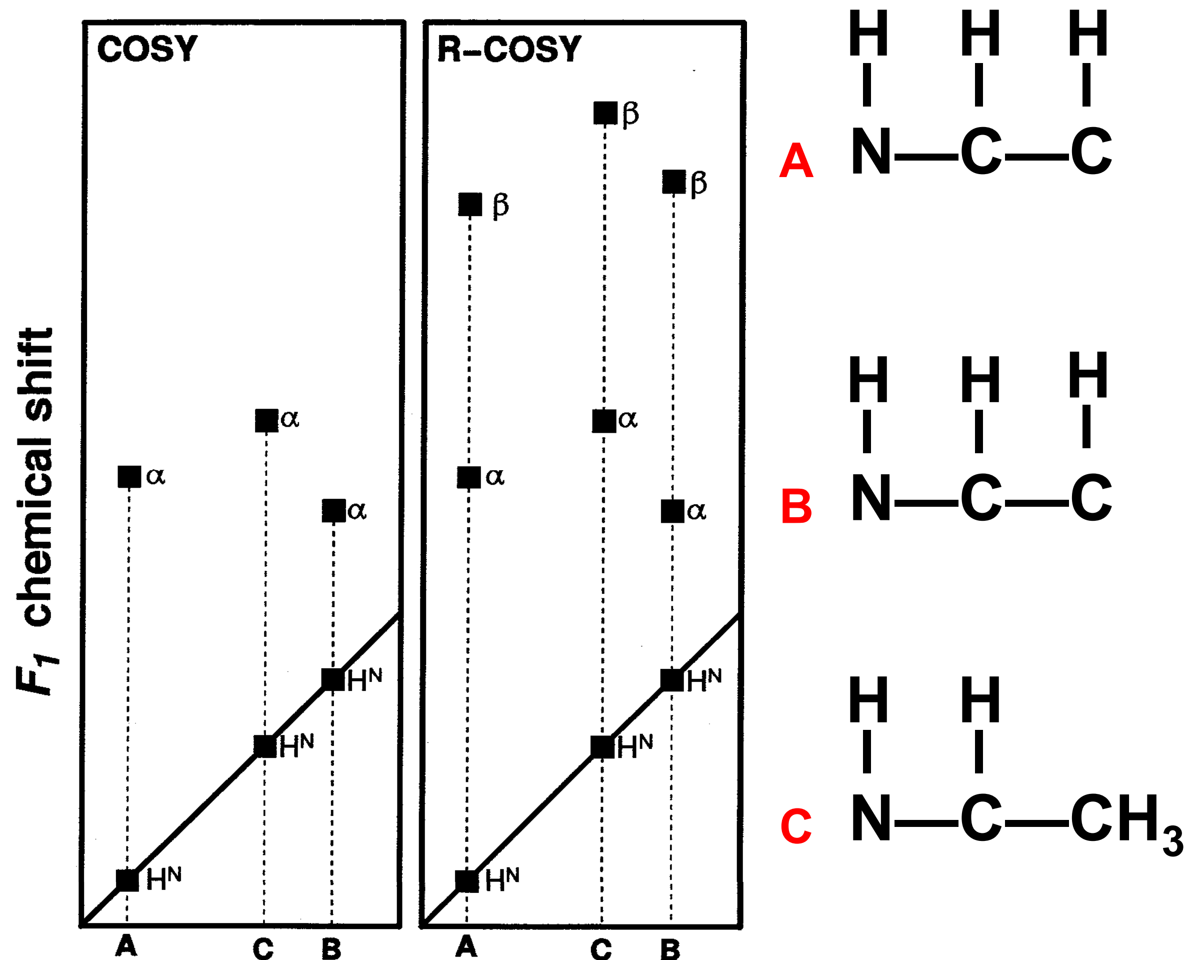
B



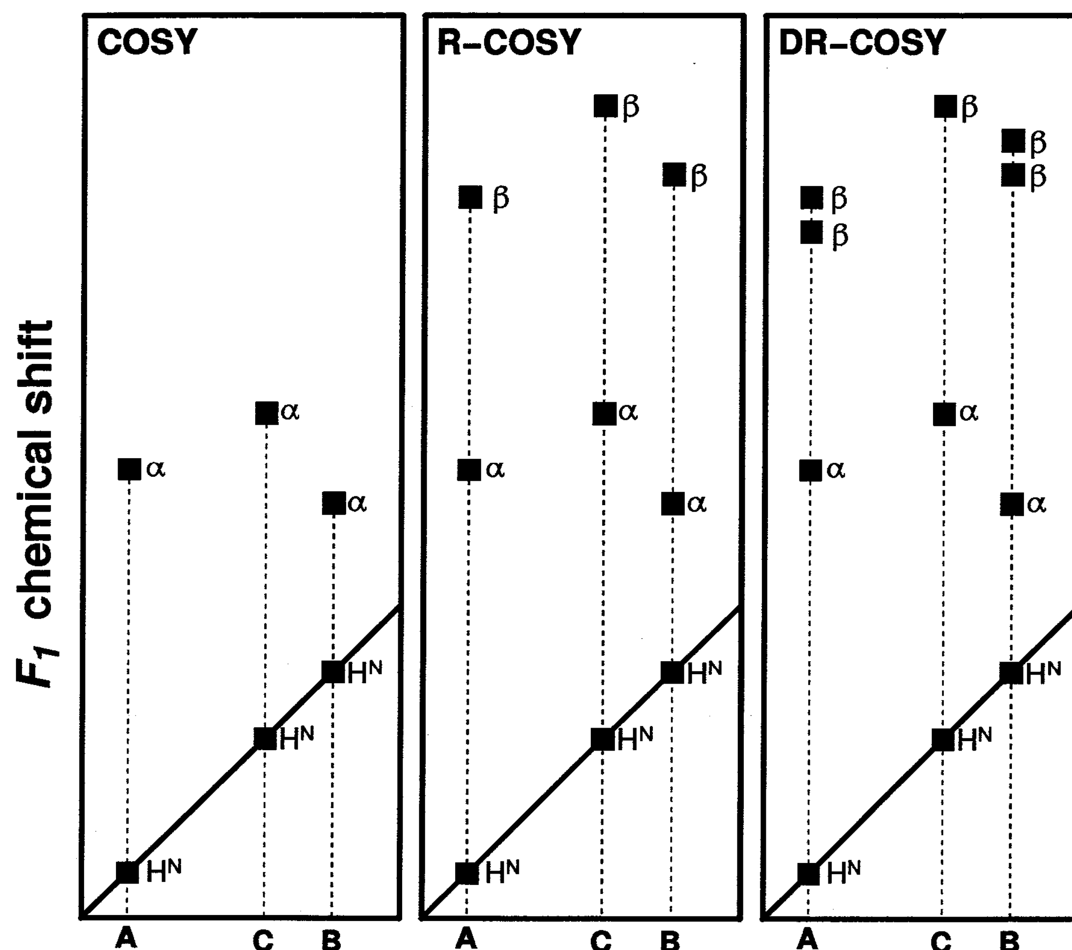
C

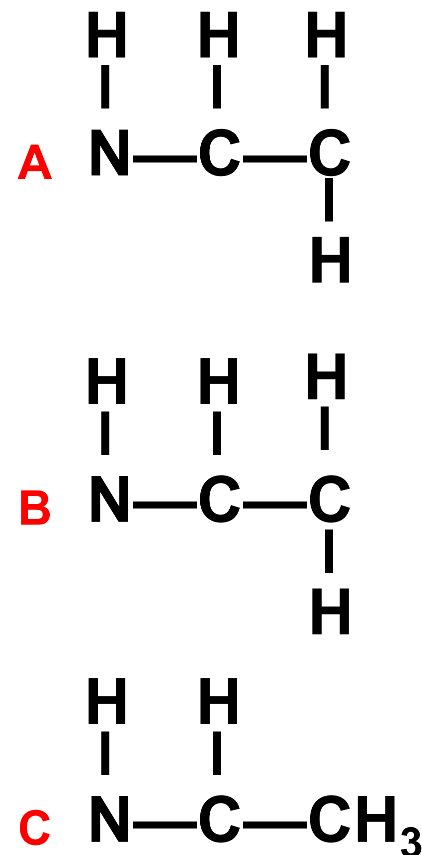


R-COSY: Add A 2nd Coupling

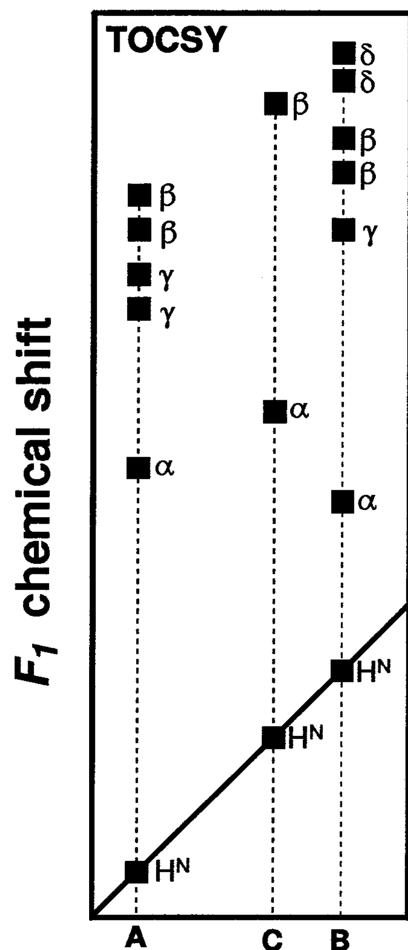


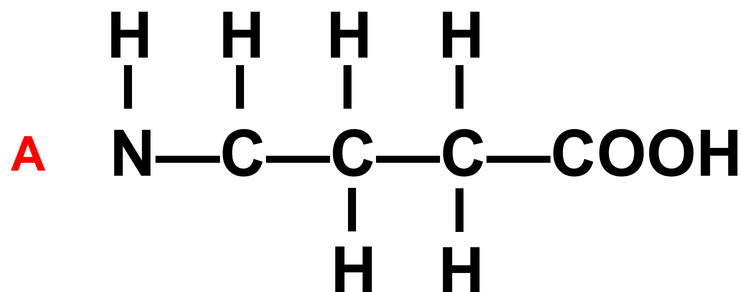
DR-COSY: Add A 3rd Coupling

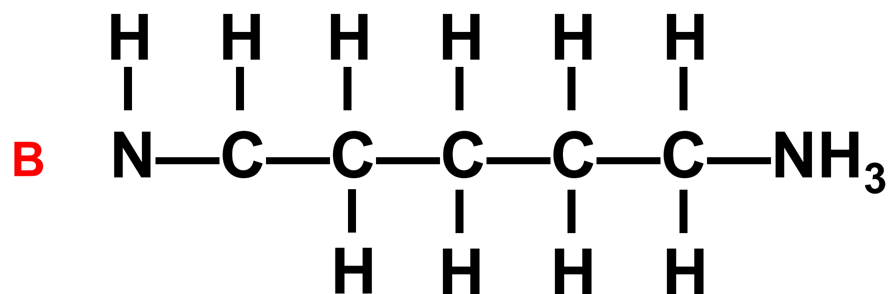


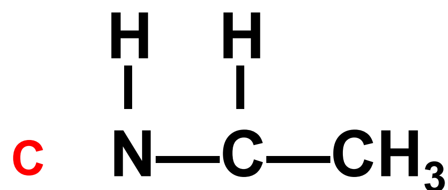


TOCSY: All Coupled Spins

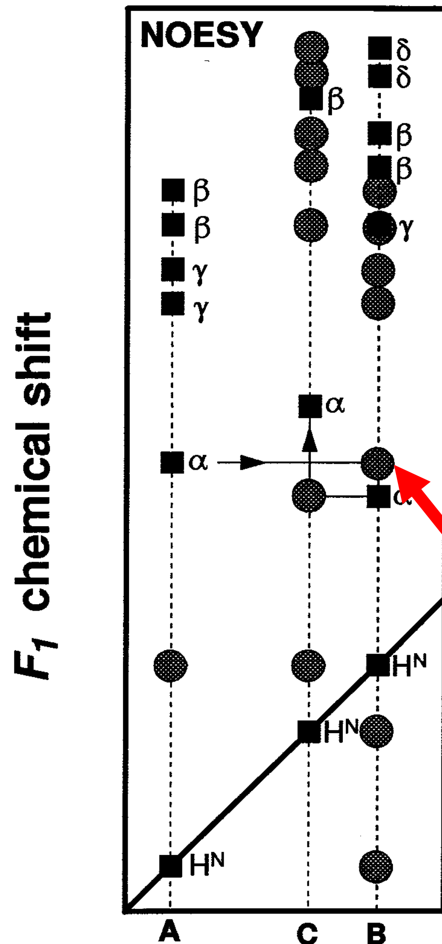








Step 2: Fit Residues in Sequence



A - B - C

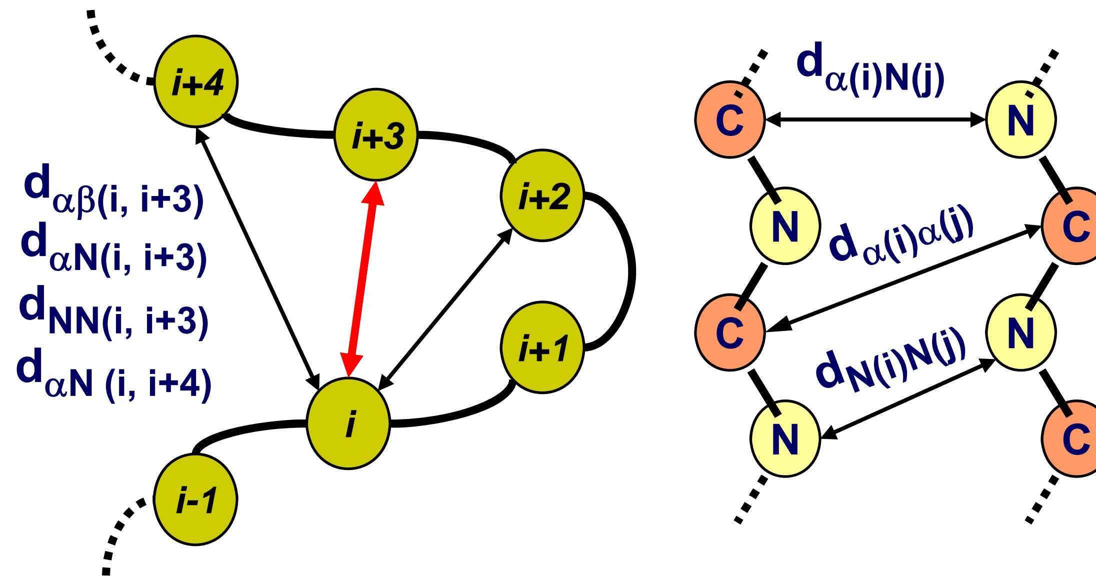
Peaks in NOESY spectra

- ■ Same as scalar coupling peaks
- ● Peaks from residue i to i+1

A → B (B → C)

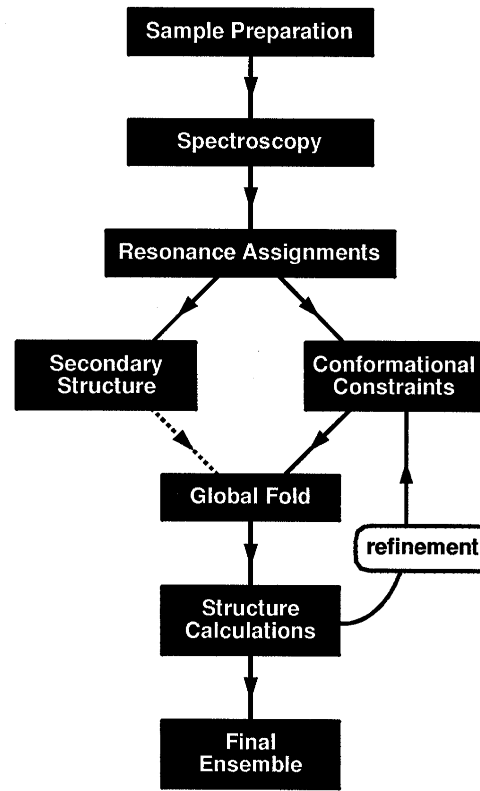
Strong NOE 1.8 - 2.7 Å
Medium NOE 1.8 - 3.3 Å
Weak NOE 1.8 - 5.0 Å

Distance Restraints for helix and sheet

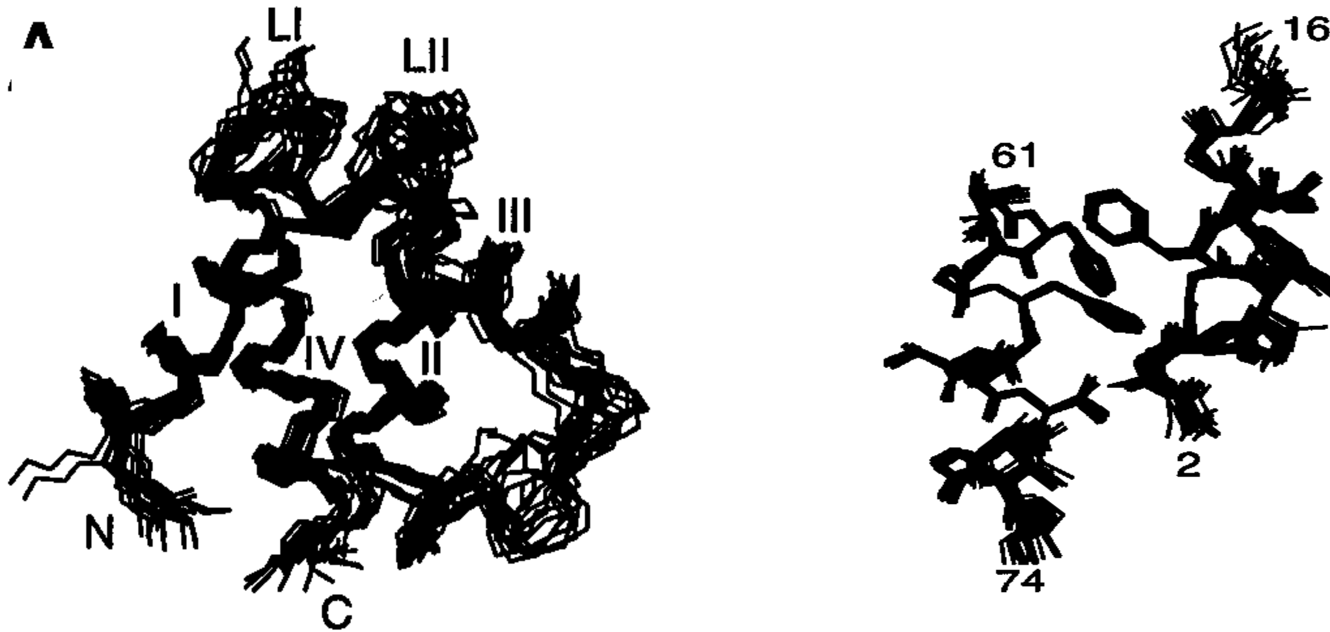


NMR Structure Calculations

- **Objective is to determine all conformations consistent with the experimental data**
- **Programs are available that perform automated assignment.**
- **NMR data are often not perfect: noise, incomplete data $\rightarrow$ multiple solutions (conformational ensemble)**

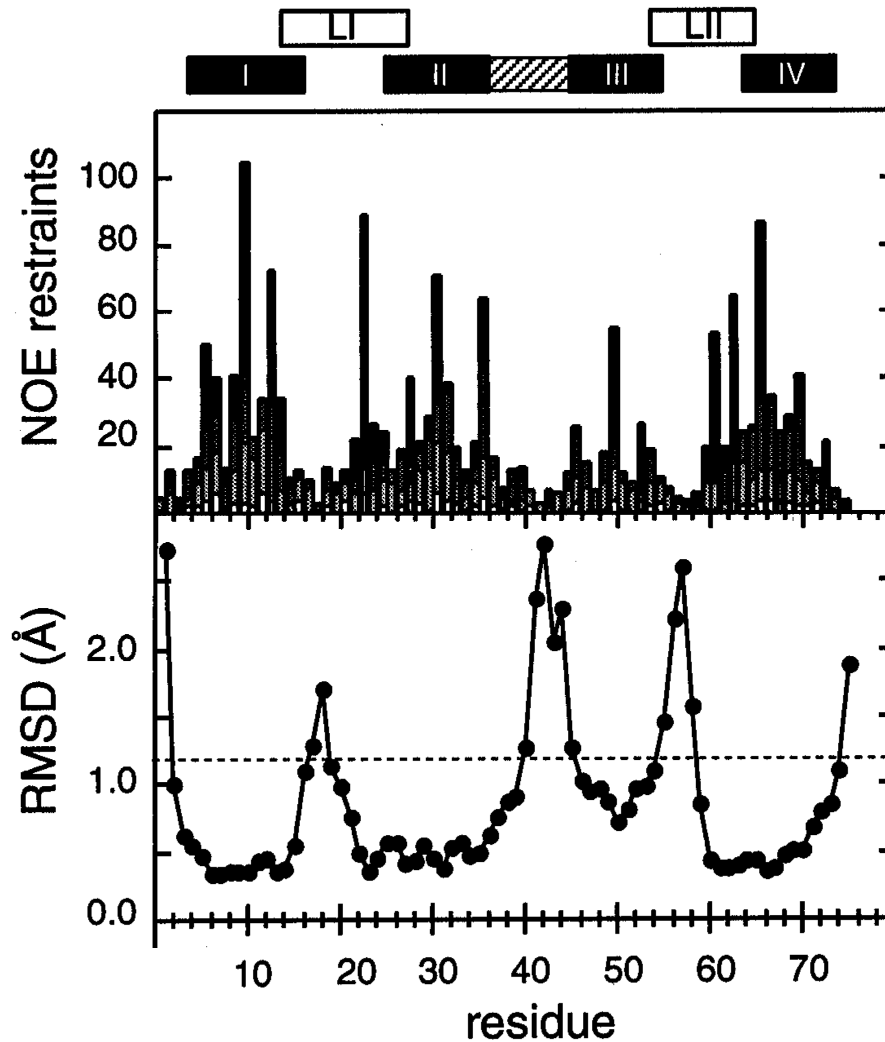


Assessing the Accuracy and Precision
Variable Resolution Structures are reported

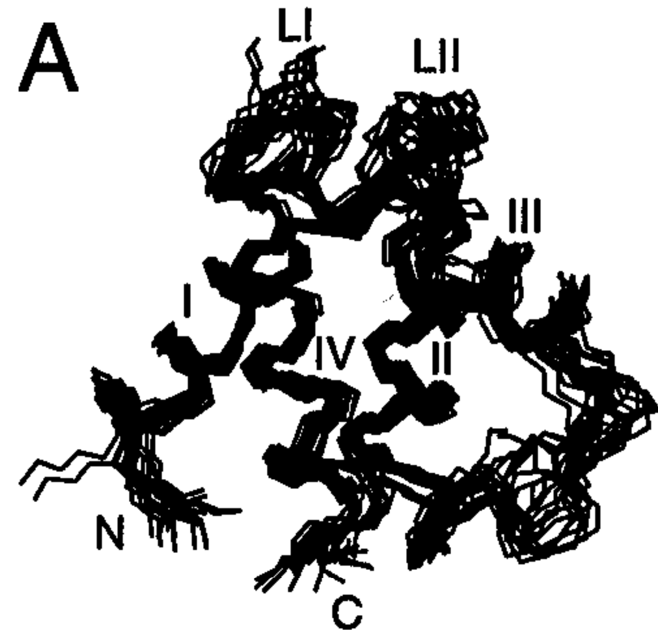


- **Number of experimental restraints (A/P)**
- **Violation of constraints- number, magnitude (A)**
- **Compare model and exptl. parameters (A)**
- **Comparison to known structures: PROCHECK (A)**
- **Molecular energies (A)**
- **RMSD of structural ensemble (P)**

Restraints and Uncertainty

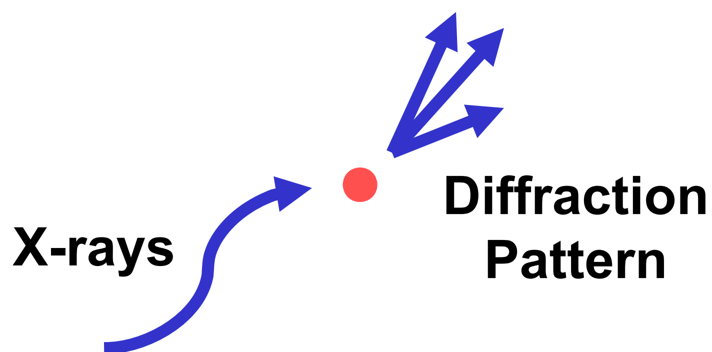


- Large # of restraints = low values of RMSD
- The most important restraints are long-range



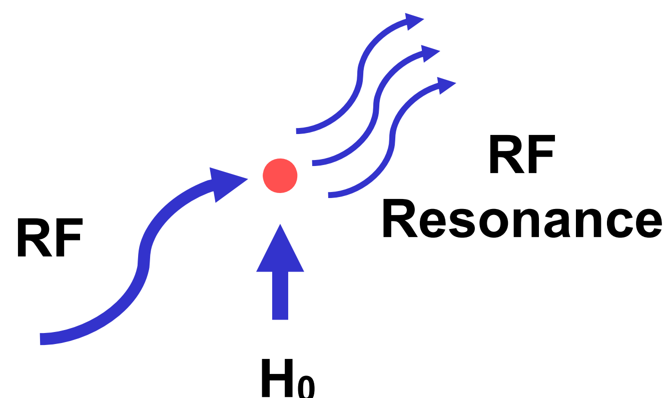
Accuracy/Precision Determined Differently for X-ray and NMR

X-ray



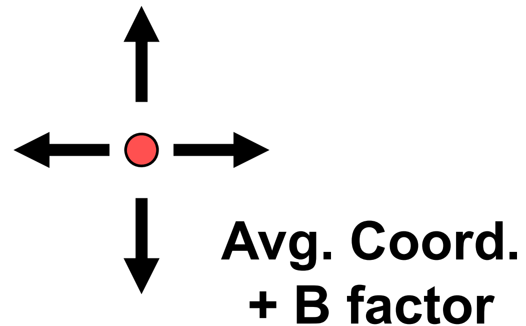
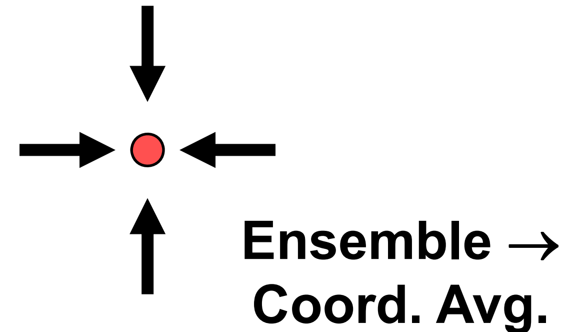
- Direct detection of atom positions
- Crystals

NMR

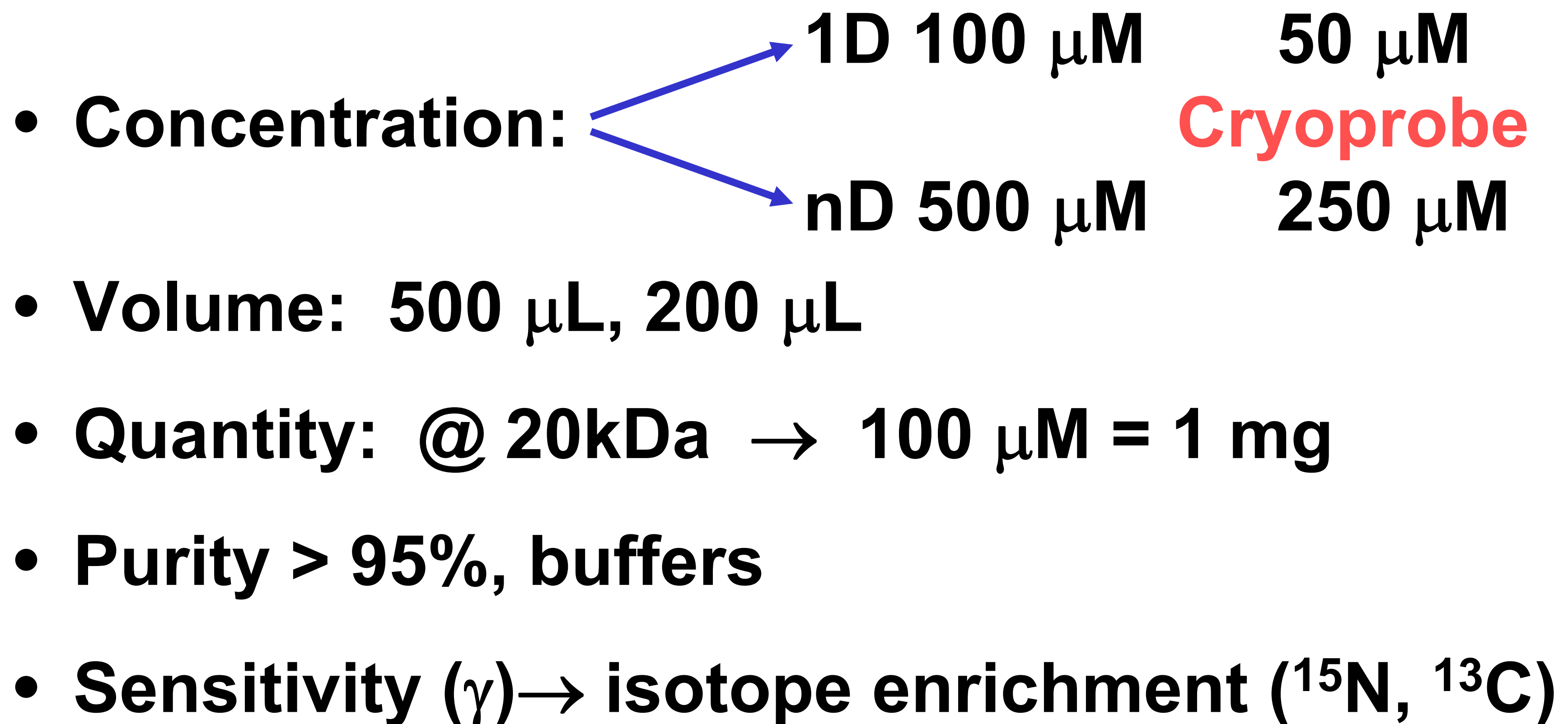


- Indirect detection of H-H distances
- In solution

Variability: Uncertainty and Flexibility in Experimental Structures

	<u>X-ray</u>	<u>NMR</u>
•Uncertainty		
•Flexibility	Mix static + dynamic	Measure motions

Practical Issues:
Sample Preparation

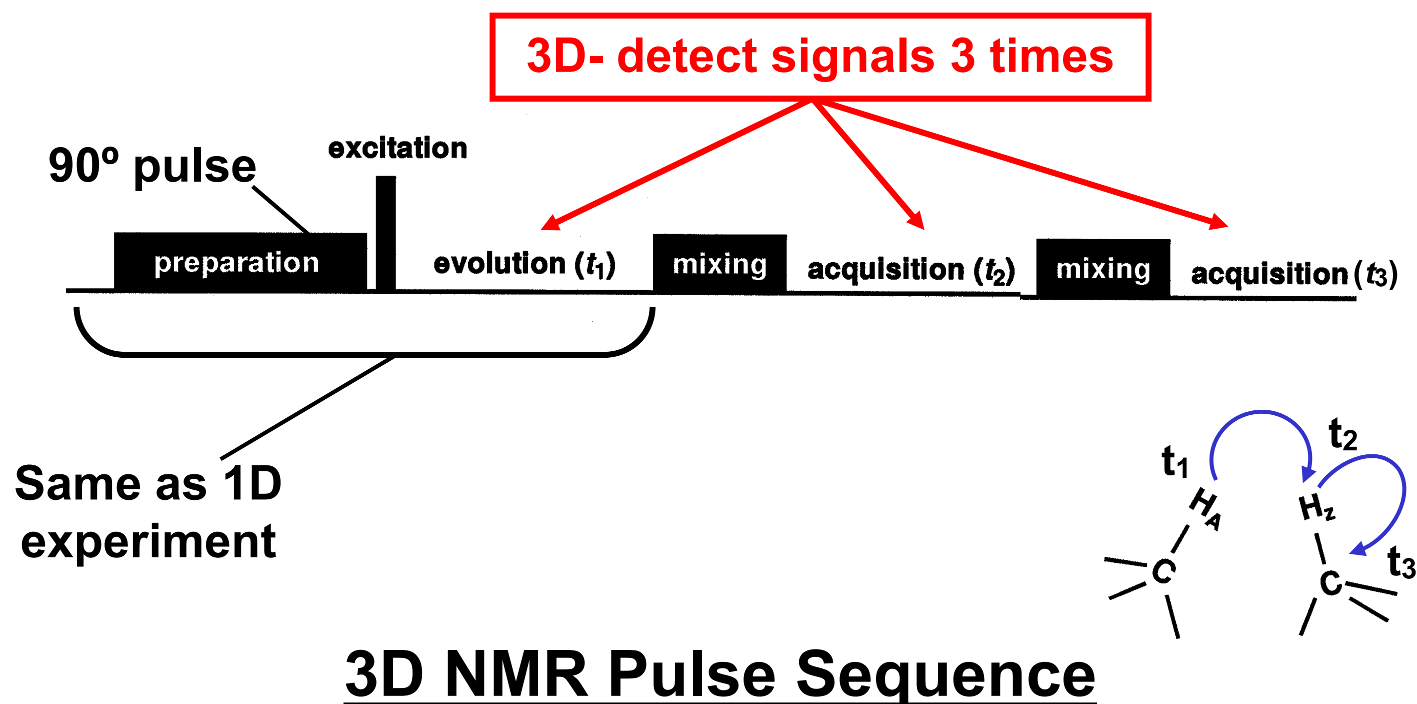


Practical Issues: Molecular Weight

Symmetry reduces complexity
8 x 10 kDa ≠ 80 kDa

- **<u>30-40 kDa</u> for 3D structure → domains**
- **<u>40-100 kDa</u>: residue-, site-, and atom-specific labeling, uniform deuteration**
- **TROSY (Transverse Relaxation Optimized [NMR] Spectroscopy)**

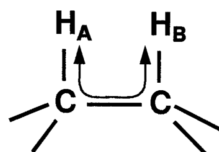
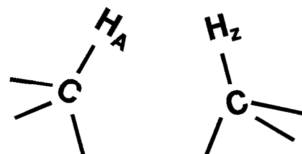
Multi-Dimensional NMR: Built on the 2D Principle



3D NMR Pulse Sequence

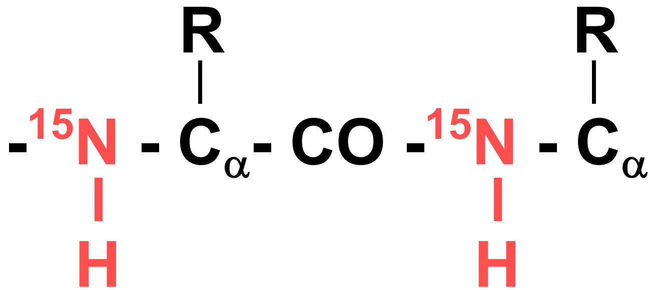
➤*Experiments are composites;* **Increase dimensionality of spectra to better resolve signals: 1→2→3 →4**

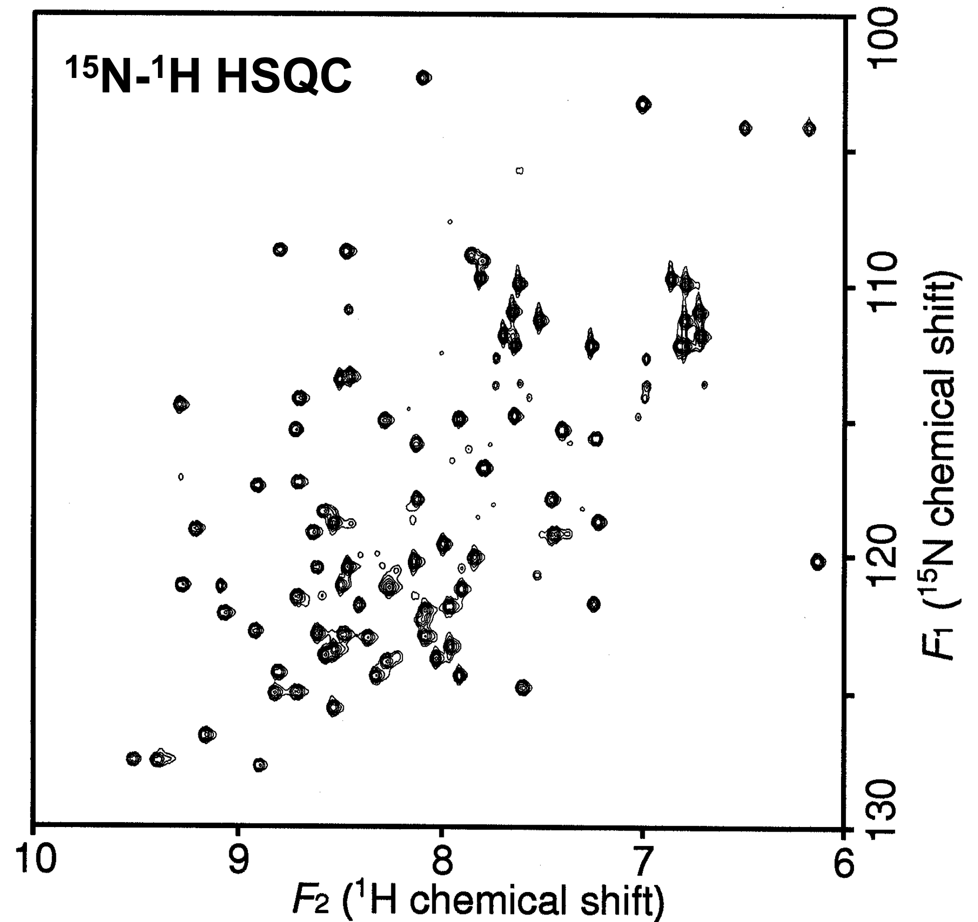
Acronyms For Basic Experiments *Differ Only By The Nature Of Mixing*

	Homonuclear	Heteronuclear
Scalar Coupling 	COSY TOCSY Multiple Quantum	HSQC Hetero-TOCSY HMQC
Dipolar Coupling 	NOESY	NOESY-HSQC NOESY-HMQC

Double-Resonance Experiments

Increases Resolution/Information Content





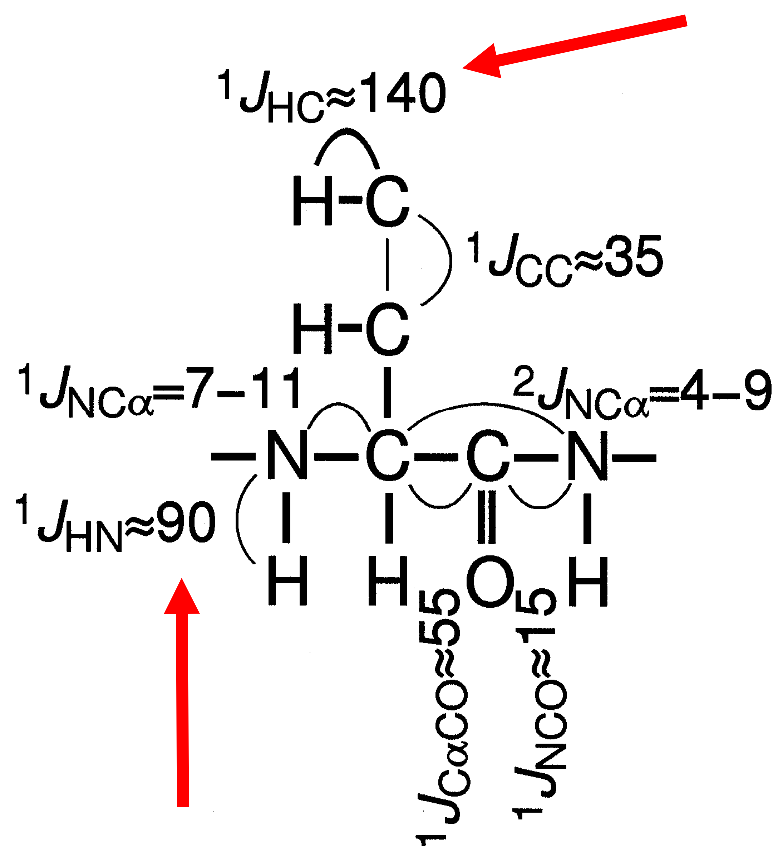
Detect signals from heteronuclei (^{13}C or ^{15}N)

Better resolution of signals/chemical shifts not correlated between nuclei

More information to identify signals

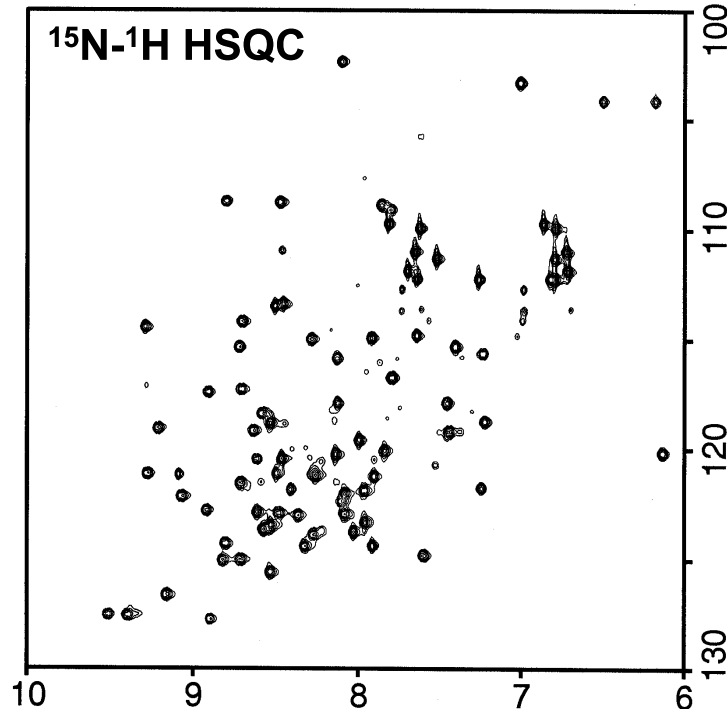
Lower sensitivity to MW of protein

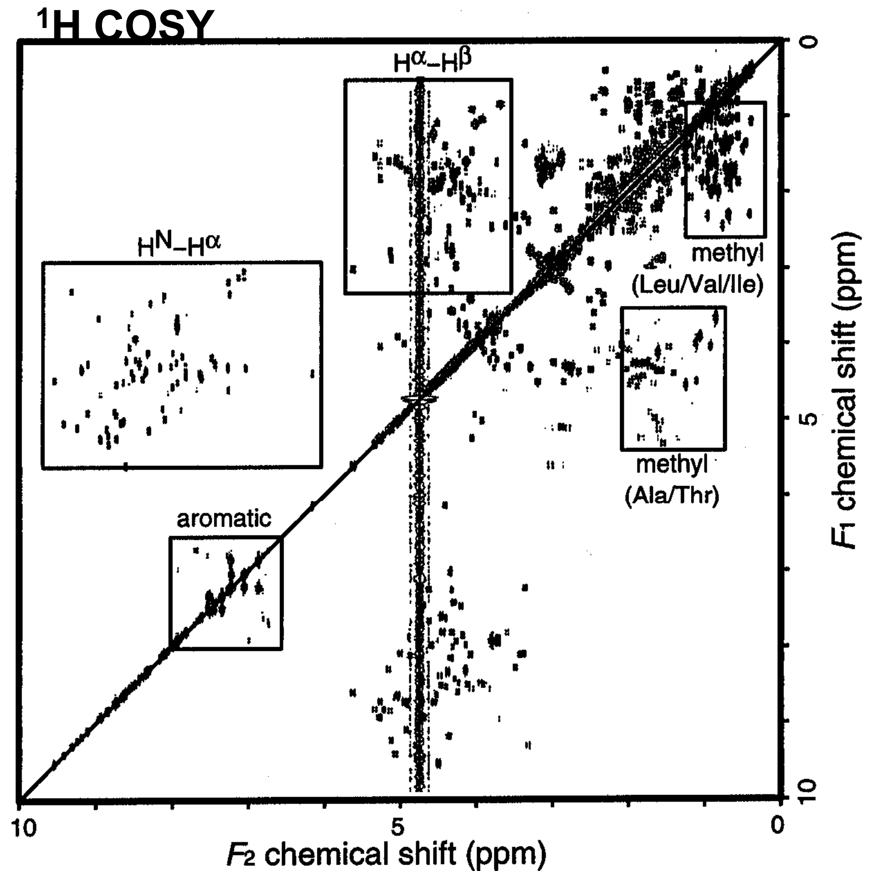
Large Scalar Couplings → Less Sensitive to MW of the Protein



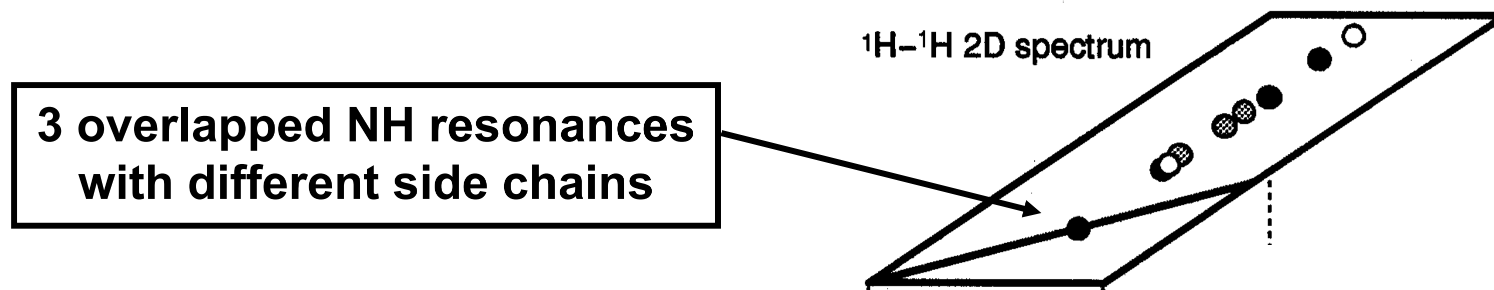
- Superior to ^1H homonuclear NMR: all $J_{H\text{-}H}$ < 20 Hz
- Mixing is faster so less time for signal to relax

Protein Folding and Fingerprints



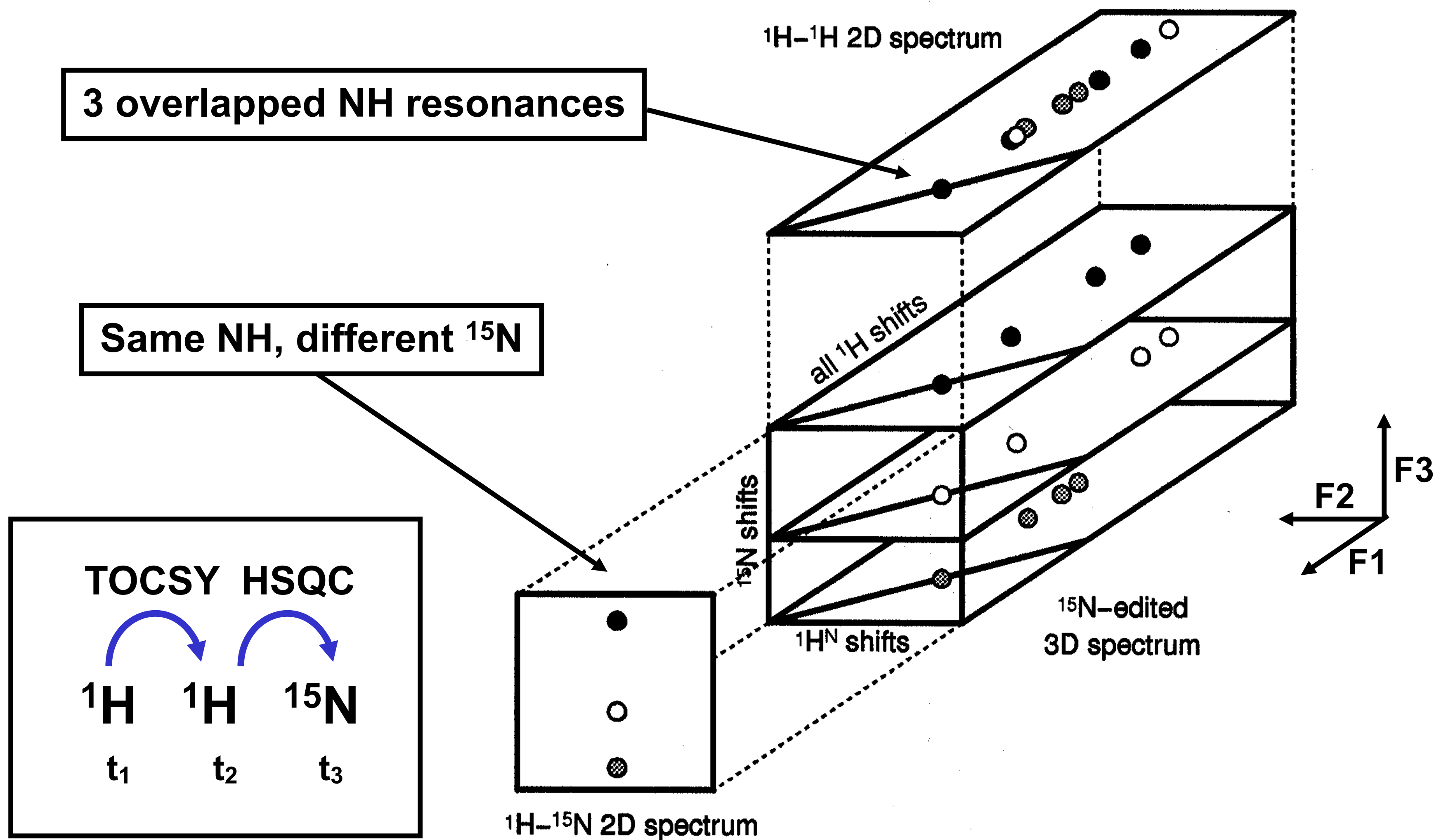


^{15}N Dispersed ^{1}H-^{1}H TOCSY



Add a 3rd dimension separating out H^N overlaps by their ^{15}N frequency

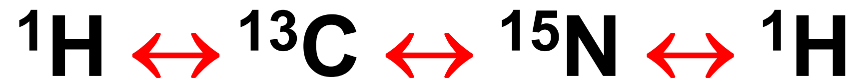
^{15}N Dispersed ^{1}H-^{1}H TOCSY



Heteronuclear (^{1}H,^{13}C,^{15}N) Strategy

- One bond at a time - all atoms (except O)
- Even handles backbone $^{15}N^{1}H$ overlaps
  → disperse with backbone

- Het. 3D/4D increases signal resolution

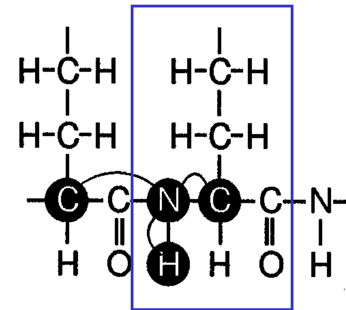


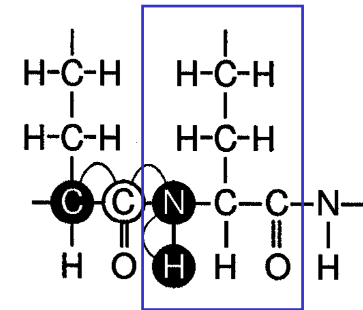
- Works on bigger proteins because one bond scalar couplings are larger

Heteronuclear Assignments: Backbone Experiments

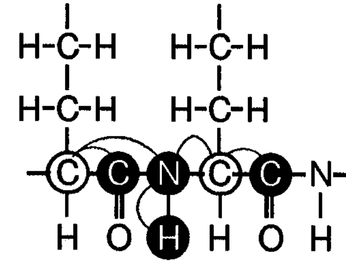
Names of scalar experiments based on atoms detected

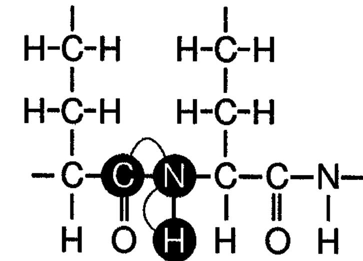
HNCA / HN(CO)CA



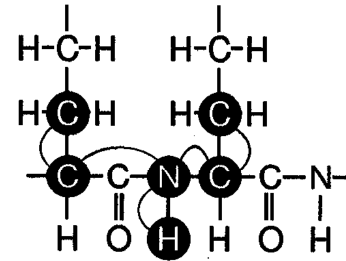


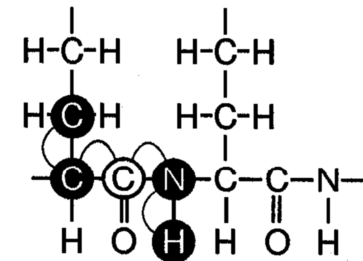
HN(CA)CO / HNCO





HNCACB / HN(CO)CACB

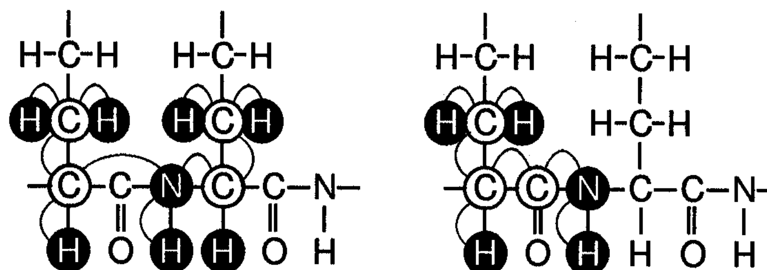




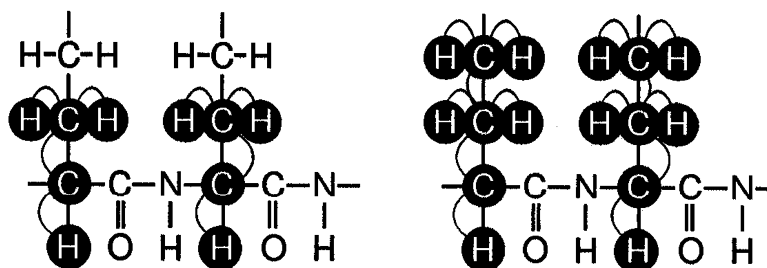
Direct assignment of Consecutive residues (without NOESY)

Heteronuclear Assignments: Side Chain Experiments

HBHA(CBCA)NH /
HBHA(CBCACO)NH



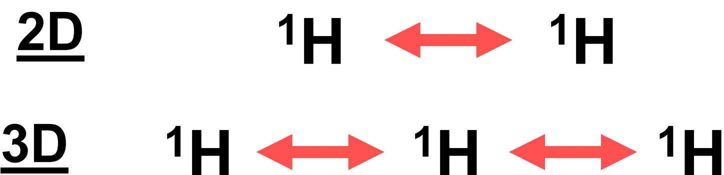
HCCH–COSY /
HCCH–TOCSY



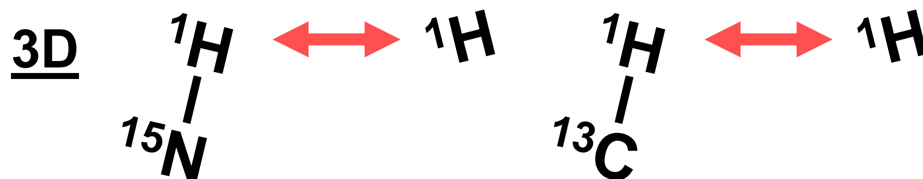
Multiple redundancies increase reliability

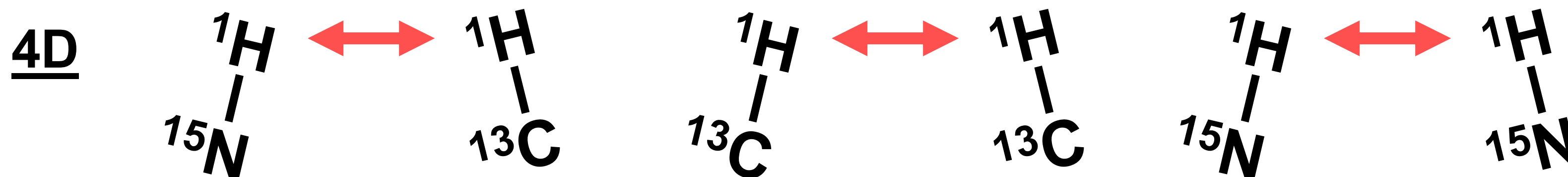
Approaches to Identifying NOEs

- ^{1}H-^{1}H NOESY



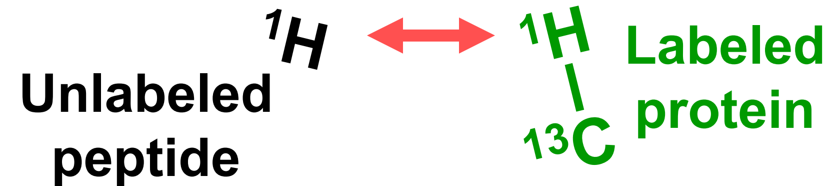
- ^{15}N- or ^{13}C-dispersed ^{1}H-^{1}H NOESY





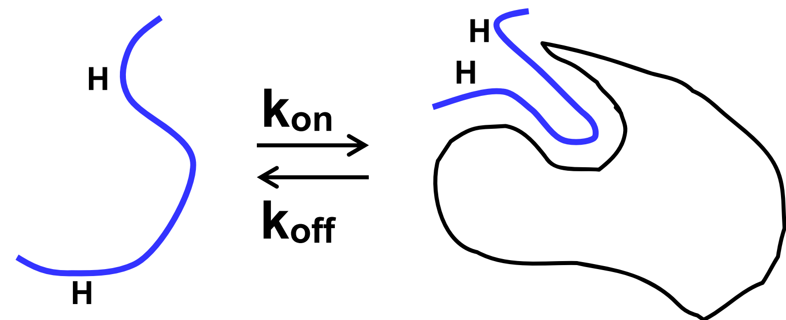
Special NOESY Experiments

- **Filtered, edited NOE:** ***based on selection of NOEs from two molecules with unique labeling patterns.***



Only NOEs at the interface

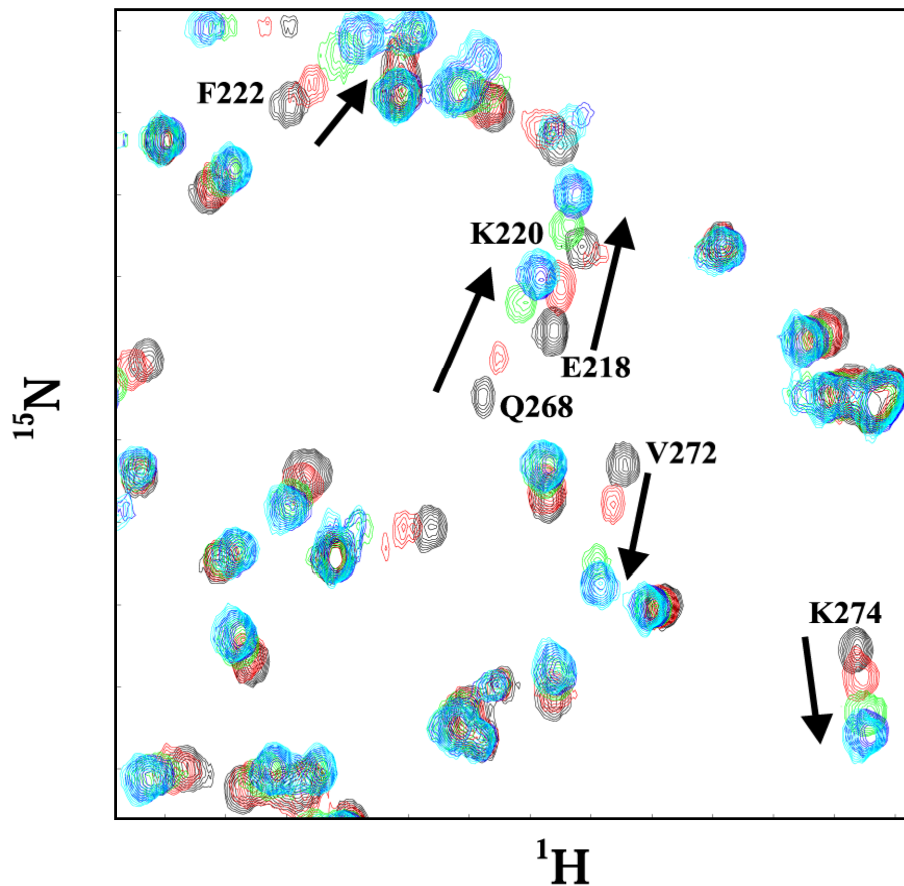
- **Transferred NOE:** ***based on 1) faster build-up of NOEs in large versus small molecules; 2) Fast exchange 3) NOEs of bound state detected at resonance frequencies of free state***



Only NOEs from bound state

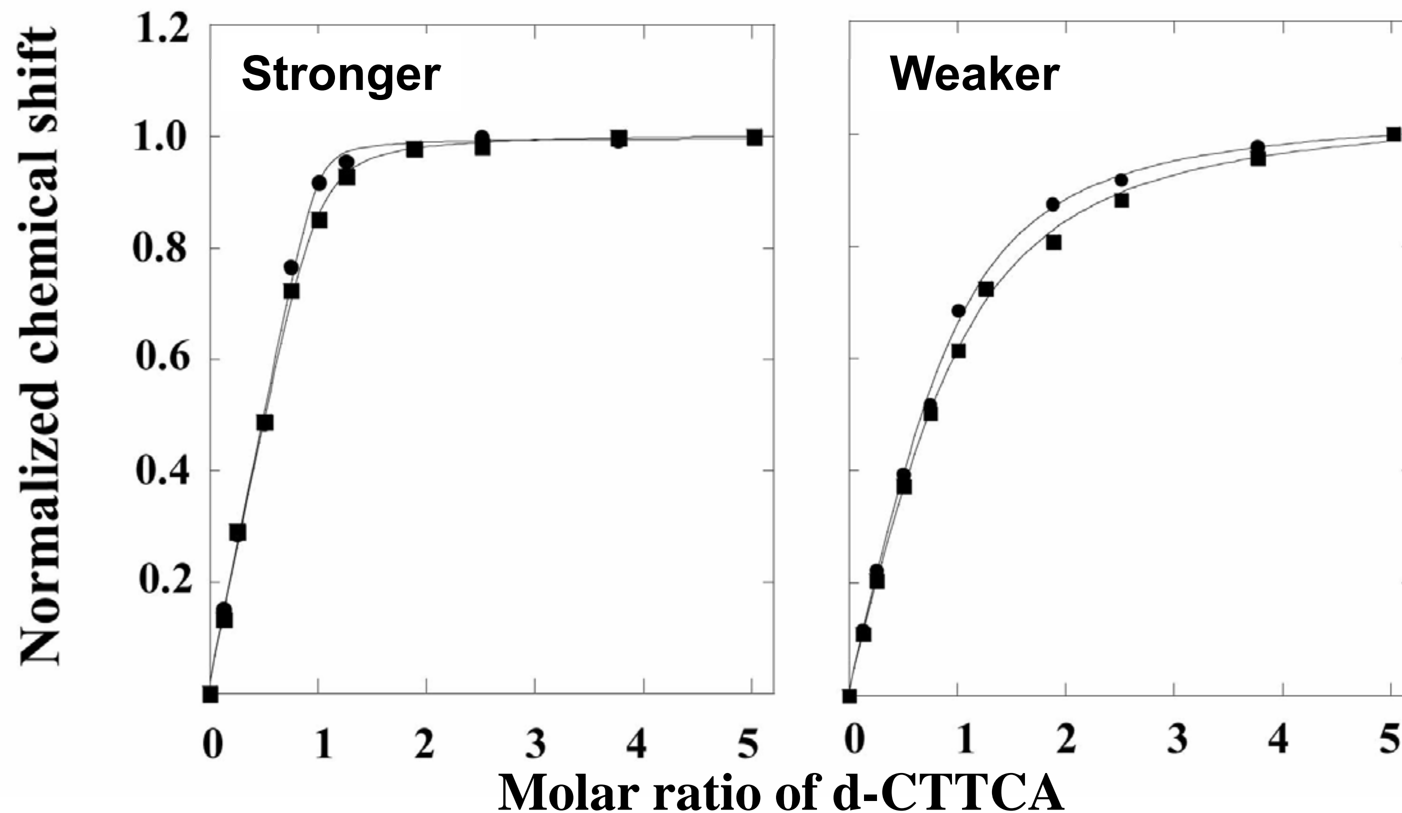
NMR to Study Interactions

Titration monitored by ^{15}N-^{1}H HSQC



- **Monitor the binding of molecules**
- **Determine binding constants (discrete off rates, on rates)**
- **Sequence and 3D structural mapping of binding interfaces**

Binding Constants From Chemical Shift Changes



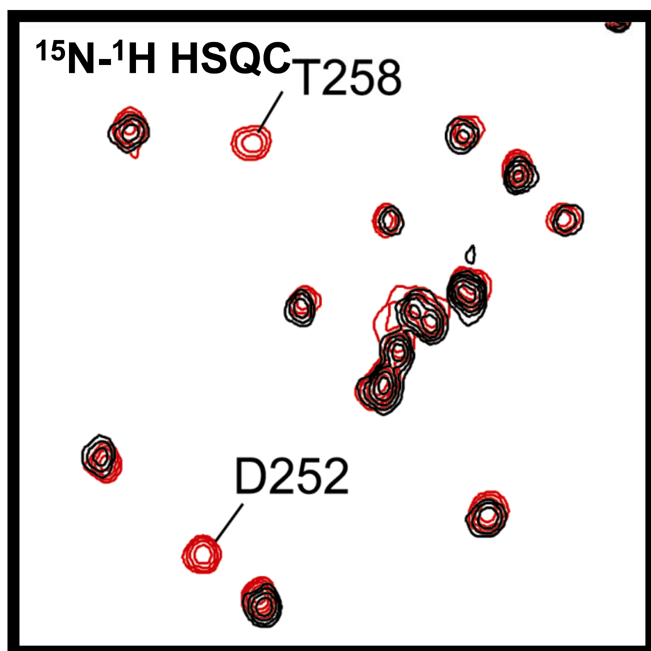
- ***Fit change in chemical shift to binding equation***

Arunkumar et al., JBC (2003)

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12881520

Probe Binding Events by NMR

^{15}N-RPA32C + Unlabeled XPA$_{1-98}$

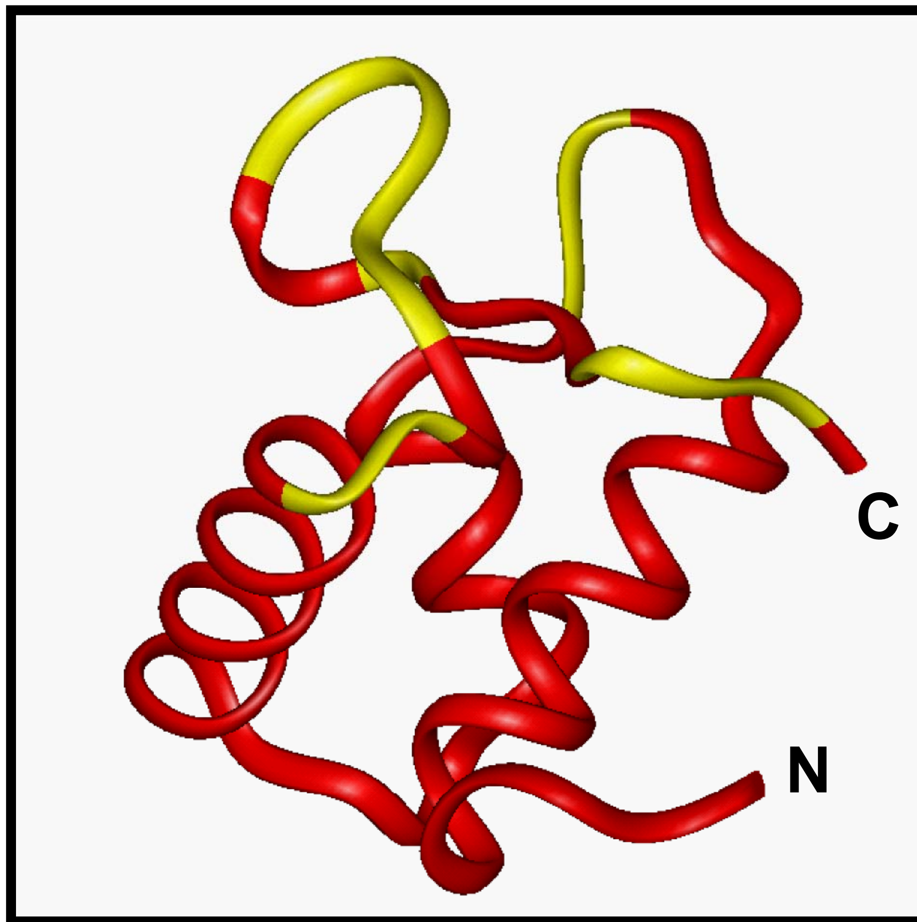


RPA32C
RPA32C + XPA$_{1-98}$

- 19 residues affected
  - ➢ *Discrete binding site*
- Signal broadening → exchange between the bound and un-bound state
  - ➢ *Kd ~ 1 μM*

Mer et al., Cell (2000)
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11081631

Map XPA Binding Site on RPA32C Using NMR

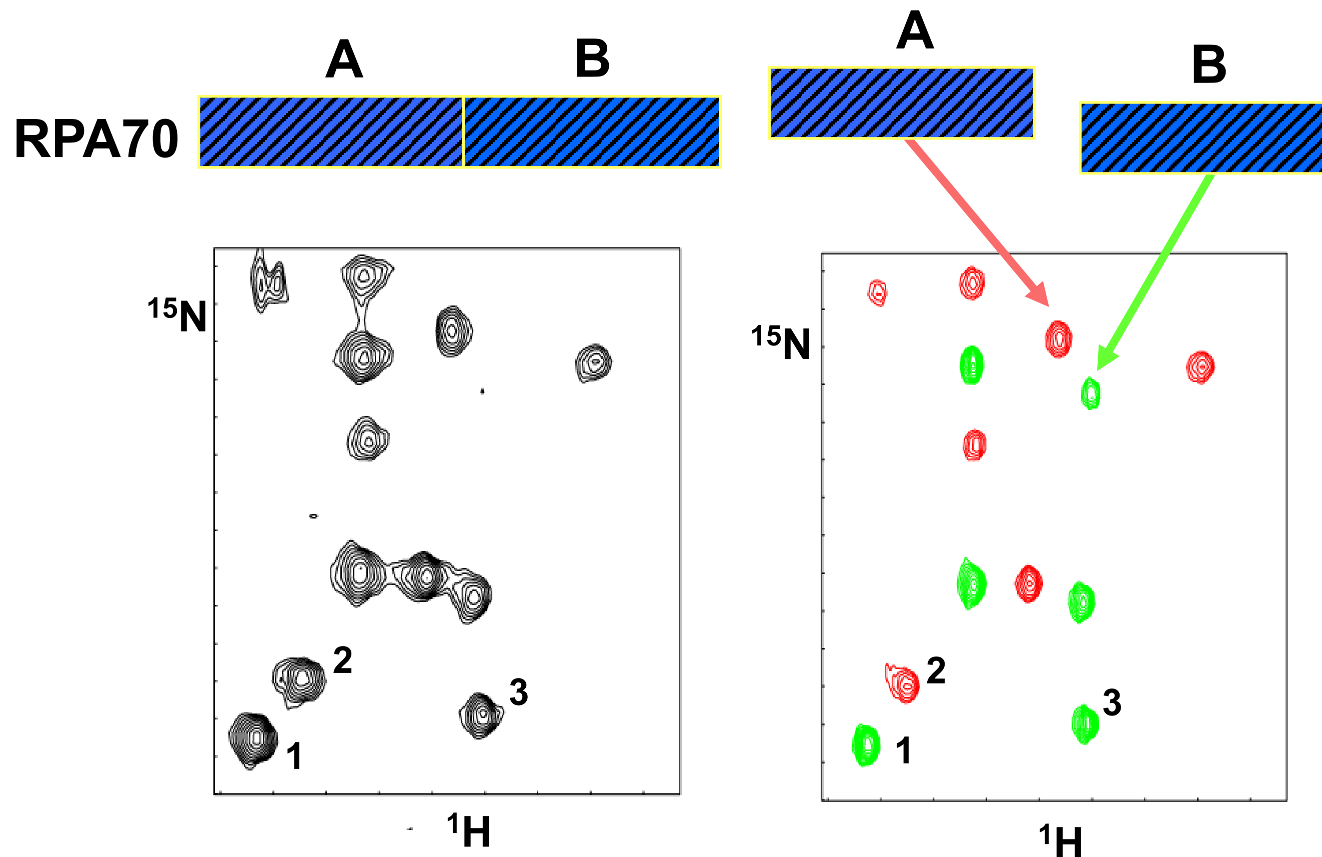


- Map chemical shift perturbations on the structure of RPA32C
- Can even map directly on to sequence with no structure

Mer et al., Cell (2000)

Folding and Domain Structure

Are domains packed together or independent?

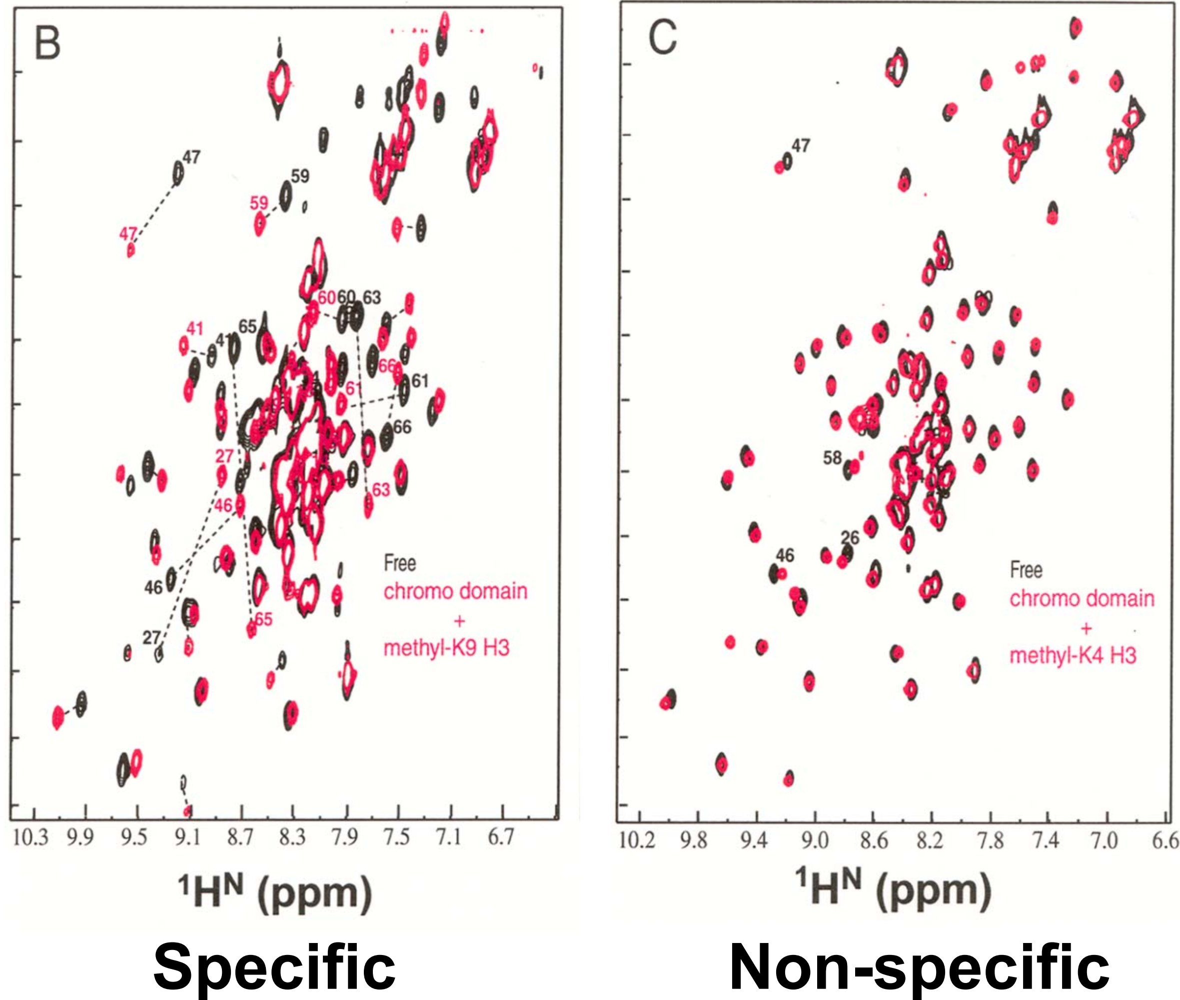


Chemical shift is extremely sensitive

- If peaks are the same, structure is the same
- If peaks are different, the structure is different but we don't know how much

Arunkumar et al., JBC (2003)

Binding of histone H3 peptide to HP1 chromodomain requires H3K9 methylation



Jacobs et al (2001) EMBO J.

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11566886