Why do we care about biomolecules?

Why do we use NMR?

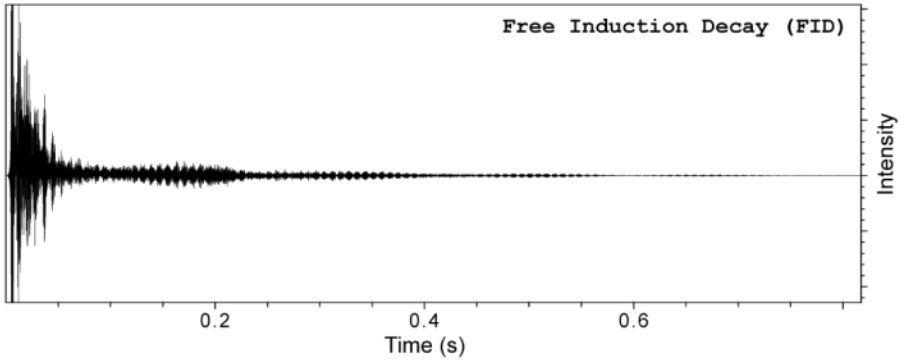
Q: How do we take a spectrum and derive structure and function from it?

A: Take signal features, and relate them back to the originating spin

What is an assignment?

Associate a given signal/frequency back to the originating spin

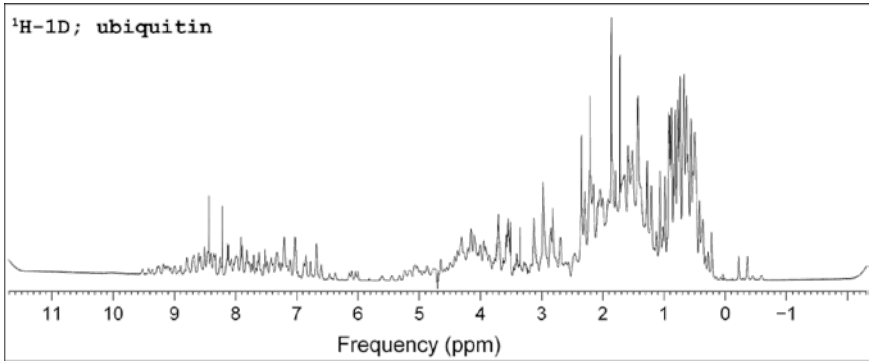
Every atom with spin has a Larmor frequency

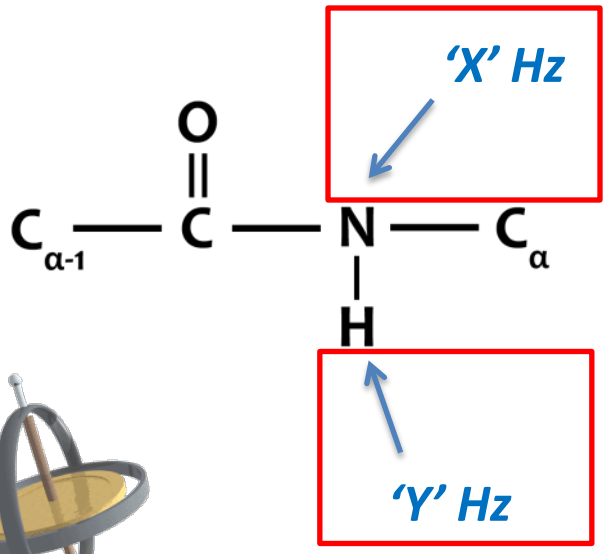


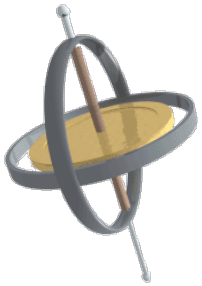
Fourier Transform (FT)

Time → Frequency

$$\delta\ (ppm) = \frac{(\nu_S - \nu_R)}{\nu_R} \times 10^6$$





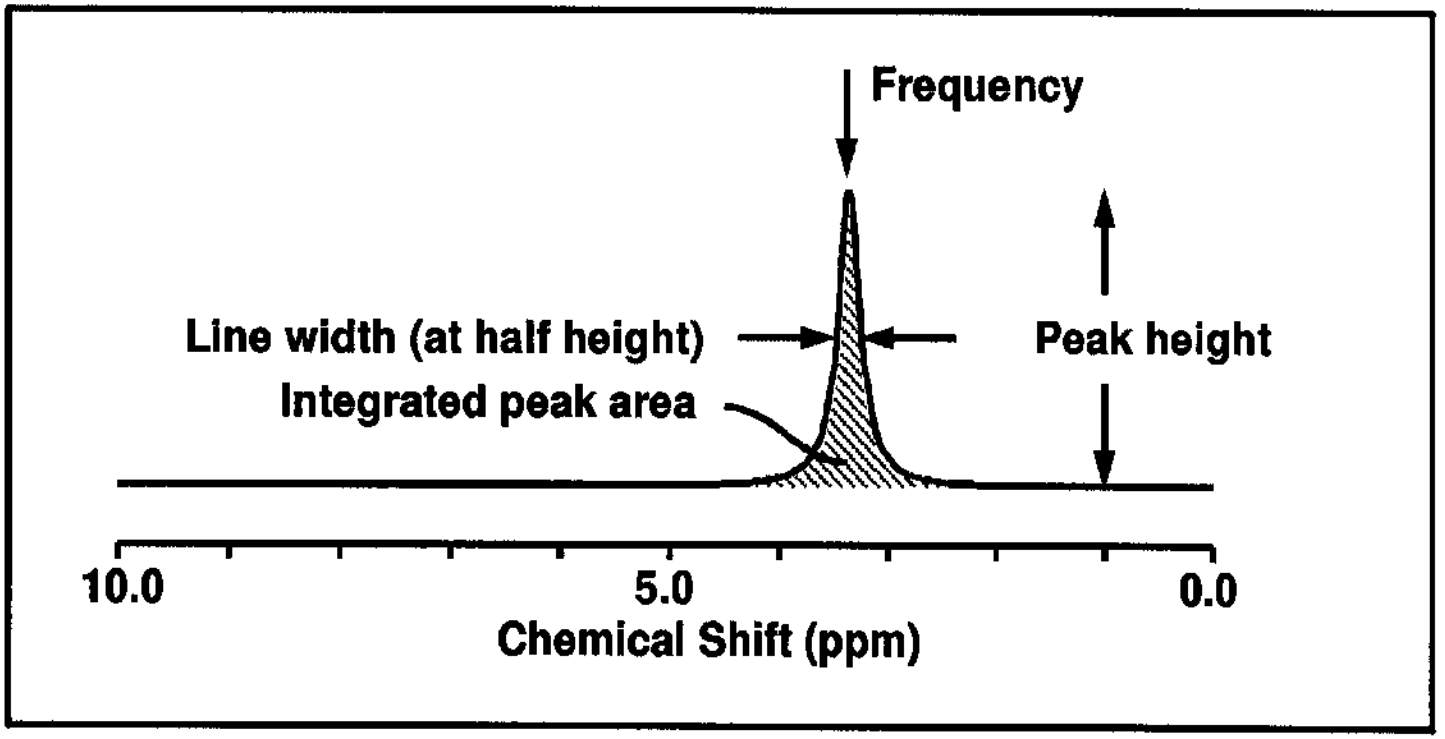


Assignments!

This frequency is a physical property of that atom – it is the same value no matter what experiment is run!

Unique features of each signal

Chemical Shift & Linewidth



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

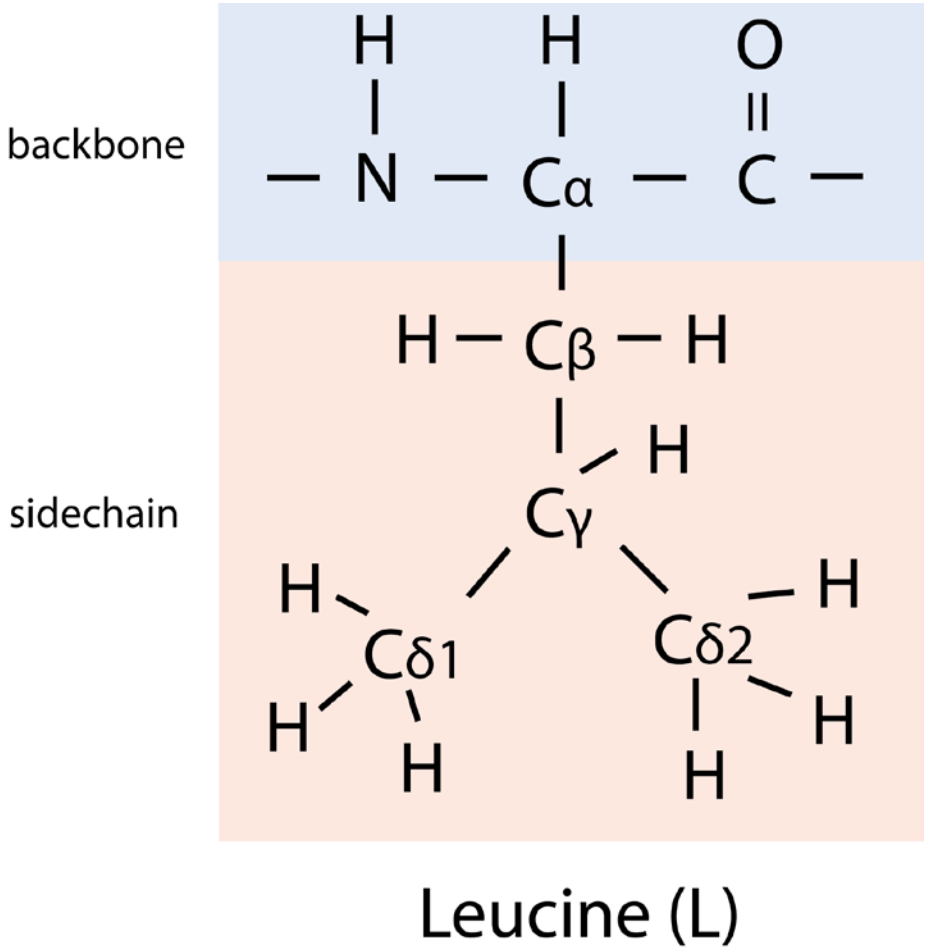
Critical features influence spin frequencies

Spins start at a given frequency based on its gyromagnetic ratio

- **Local chemical bonds influence resonance frequency**
- **Sample solvent conditions influence frequency**
- **Tertiary structure leads to increased dispersion of resonances.**
- **Ligand binding changes spin properties**

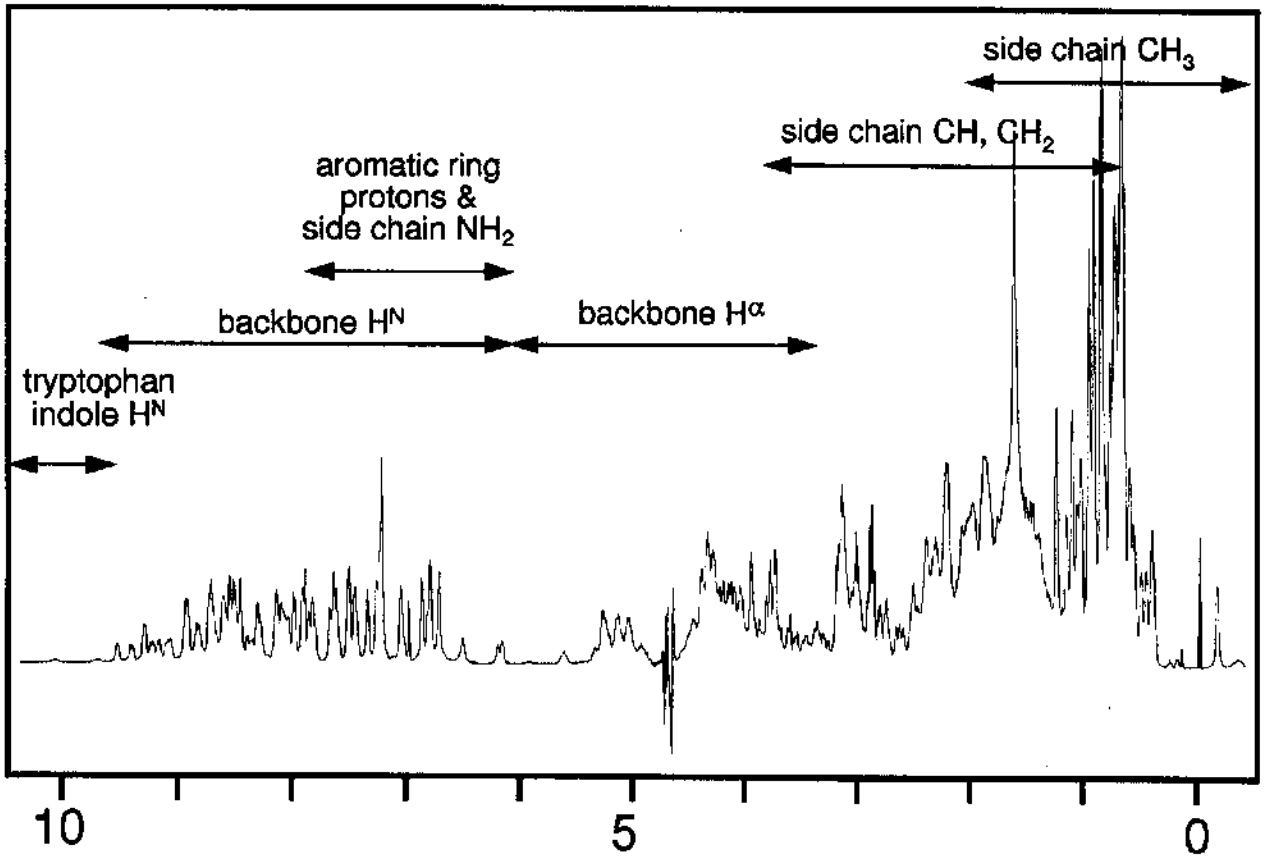
Every atom in a protein is chemically unique, thus every atom has a unique assignment!

Each atom has a unique chemical environment



Leucine (L)

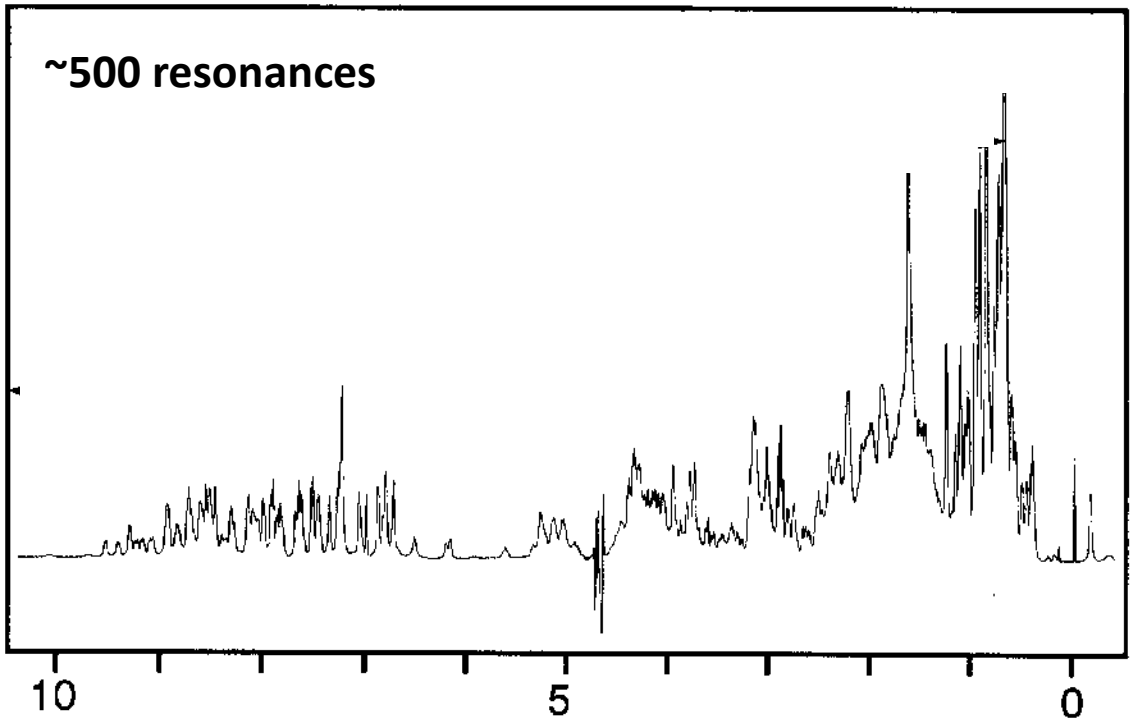
Regions of the ^{1}H NMR Spectrum



What would the unfolded protein look like?

Proteins Have Too Many Signals!

^{1}H NMR Spectrum of Ubiquitin

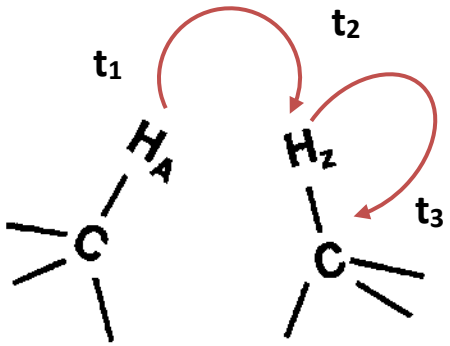


Resolve resonances by multi-dimensional experiments

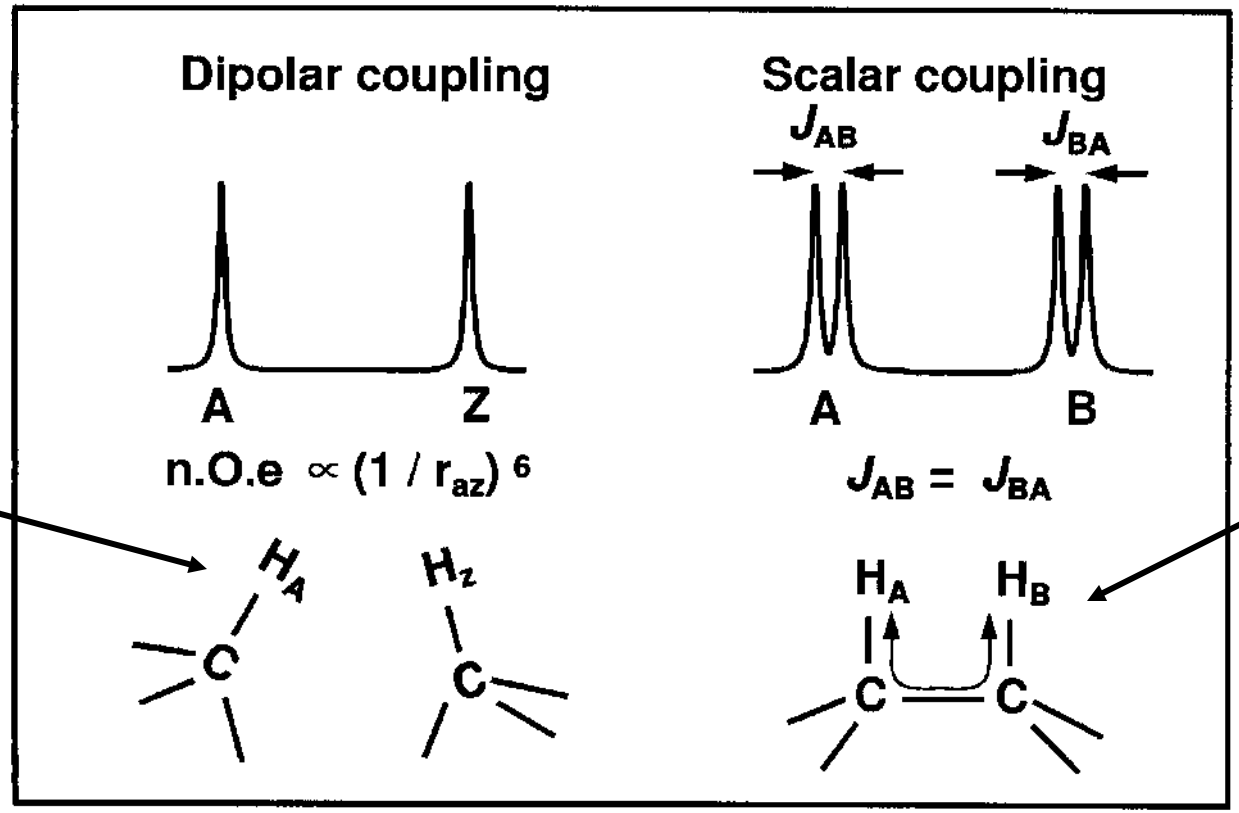
Solutions to the Challenges of Too Many Signals

Increase dimensionality of spectra to better resolve signals: 1→2→3 →4

Higher dimensions link spins to one other

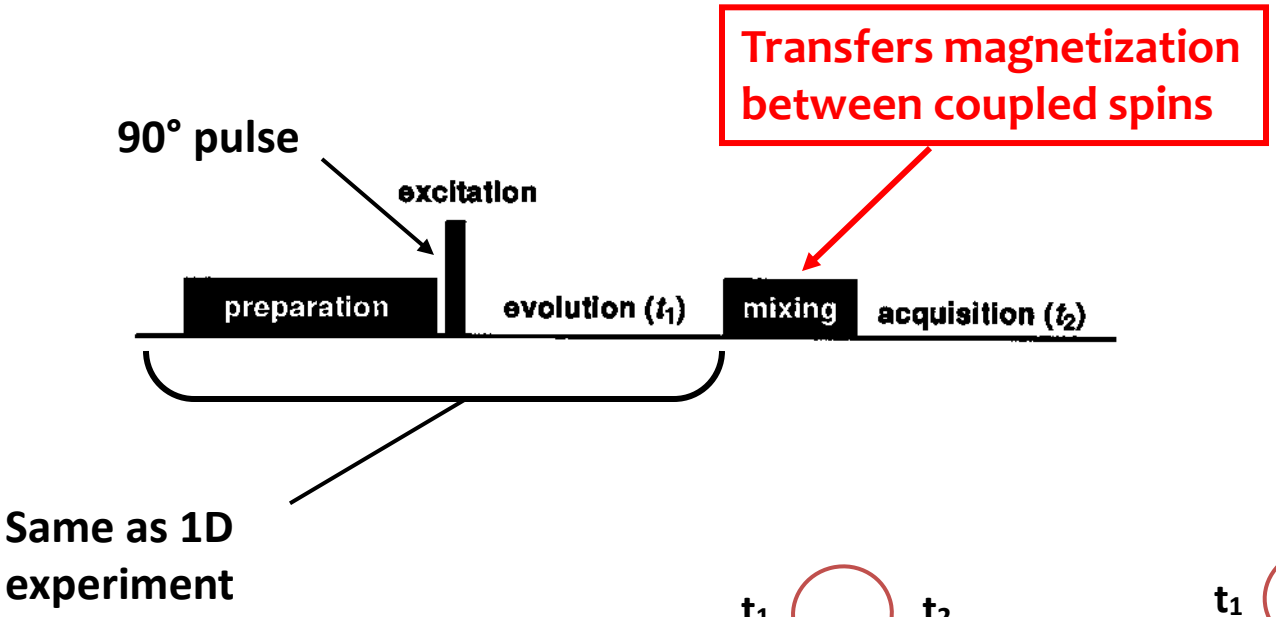


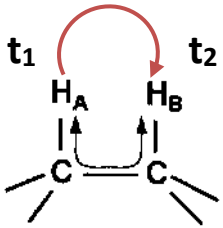
Scalar and Dipolar Coupling

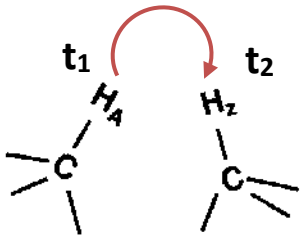


Coupling of nuclei gives information on structure

2D NMR: Coupling is the Key

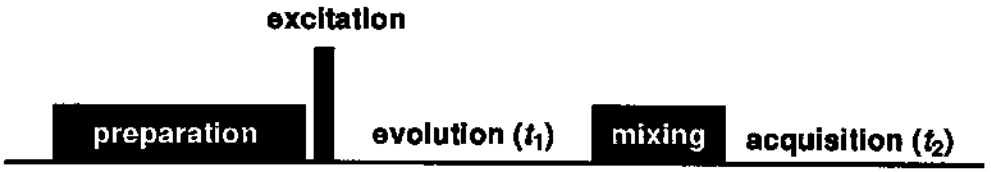




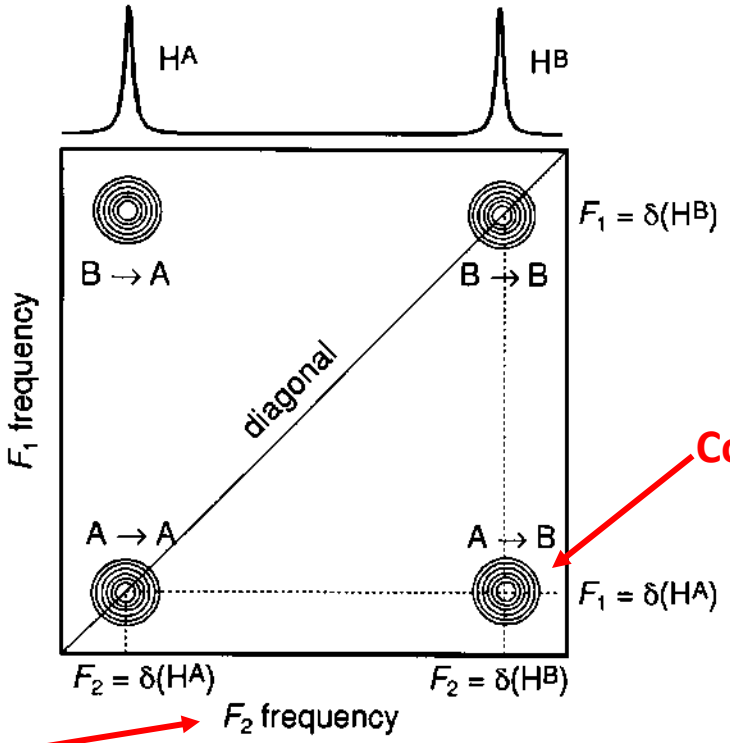


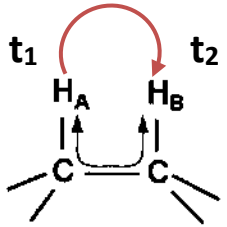
The 2D NMR Spectrum

Pulse Sequence



Spectrum





Before mixing

Coupled spins

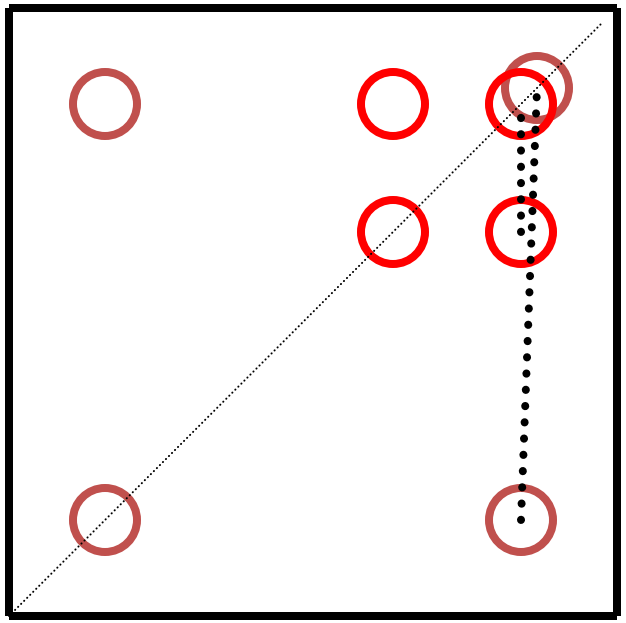
After mixing

The Power of 2D NMR: Resolving Overlapping Signals

1D

2 signals overlapped

2D



2 cross peaks resolved

Basic Strategy to Assign Resonances

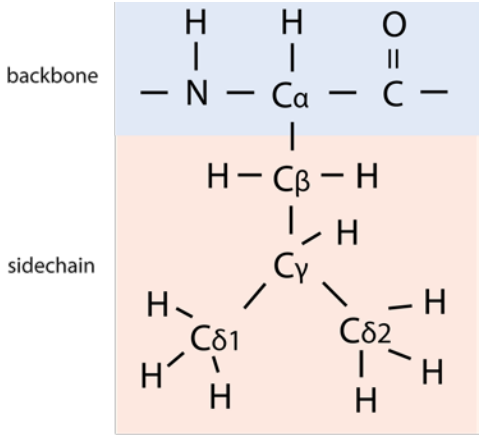
1. **Identify resonances for each residue (scalar)**

T G L S S R G

2. **Put residues in order**

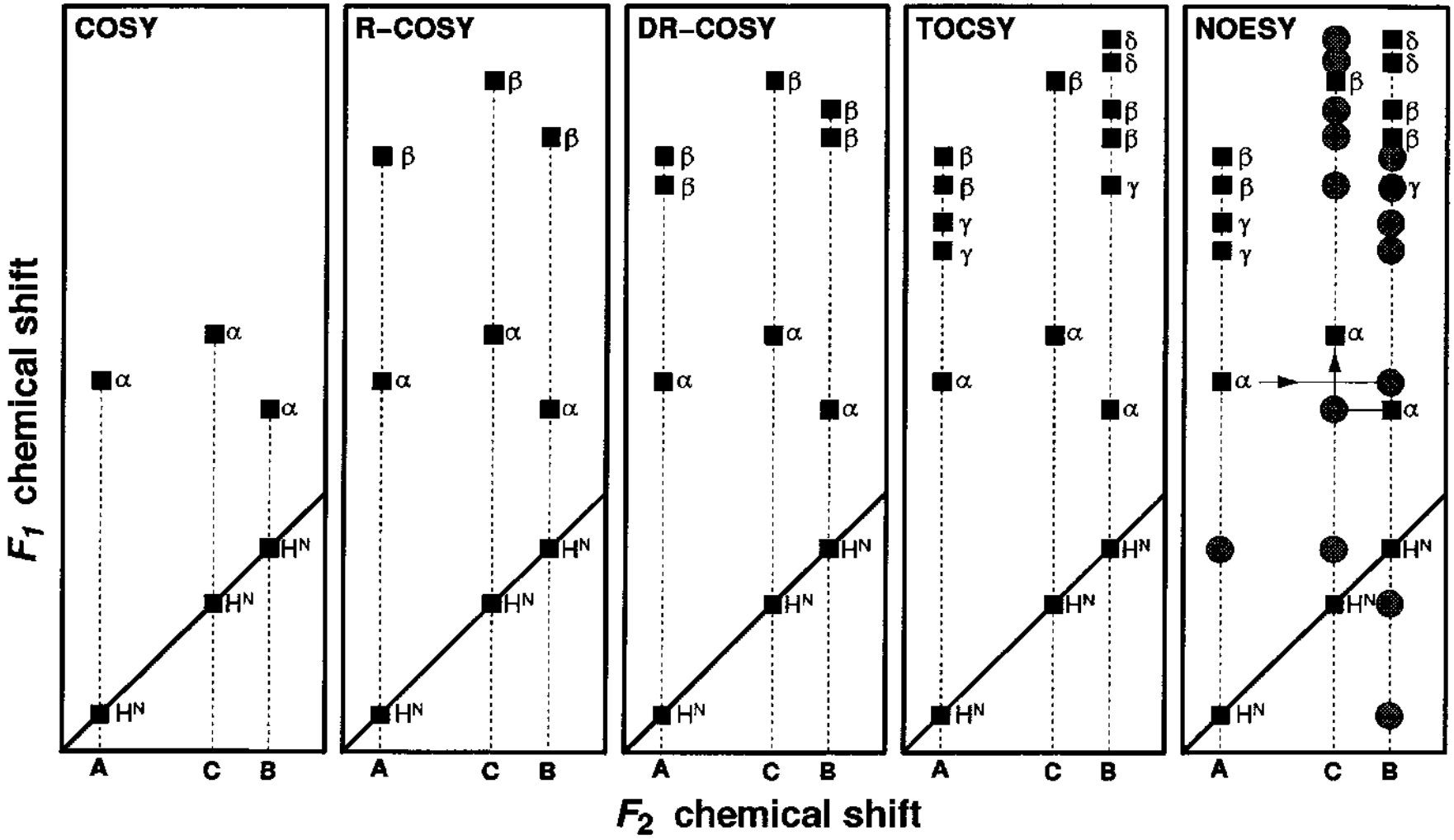
1 2 3 4 5 6 7

R - G - S - T - L - G - S

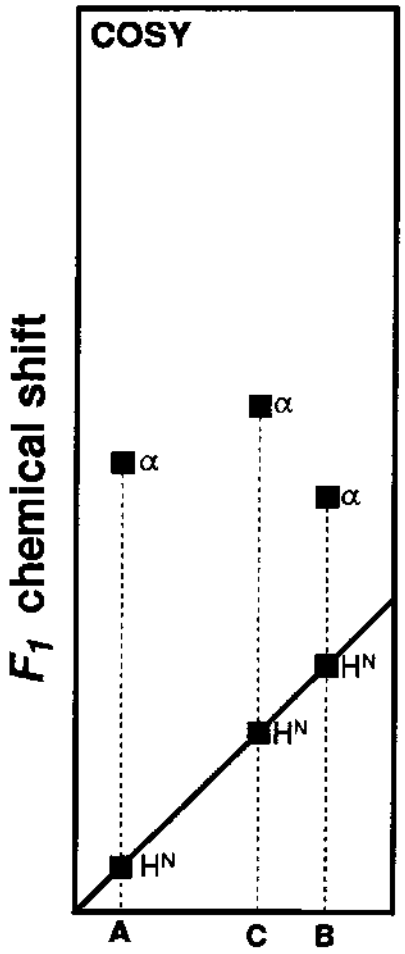


Leucine (L)

Homonuclear 2D Expts



COSY: One coupling

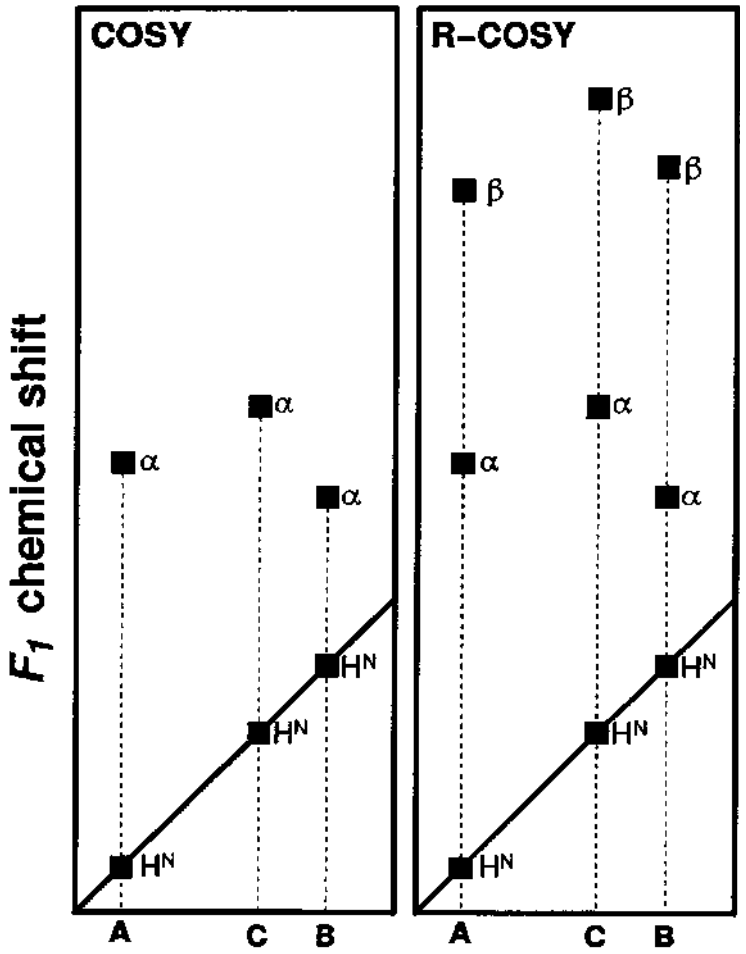


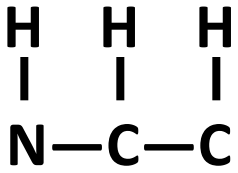
A

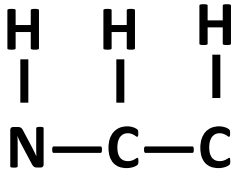
B

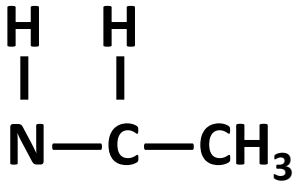
C

R-COSY: Add A 2nd Coupling

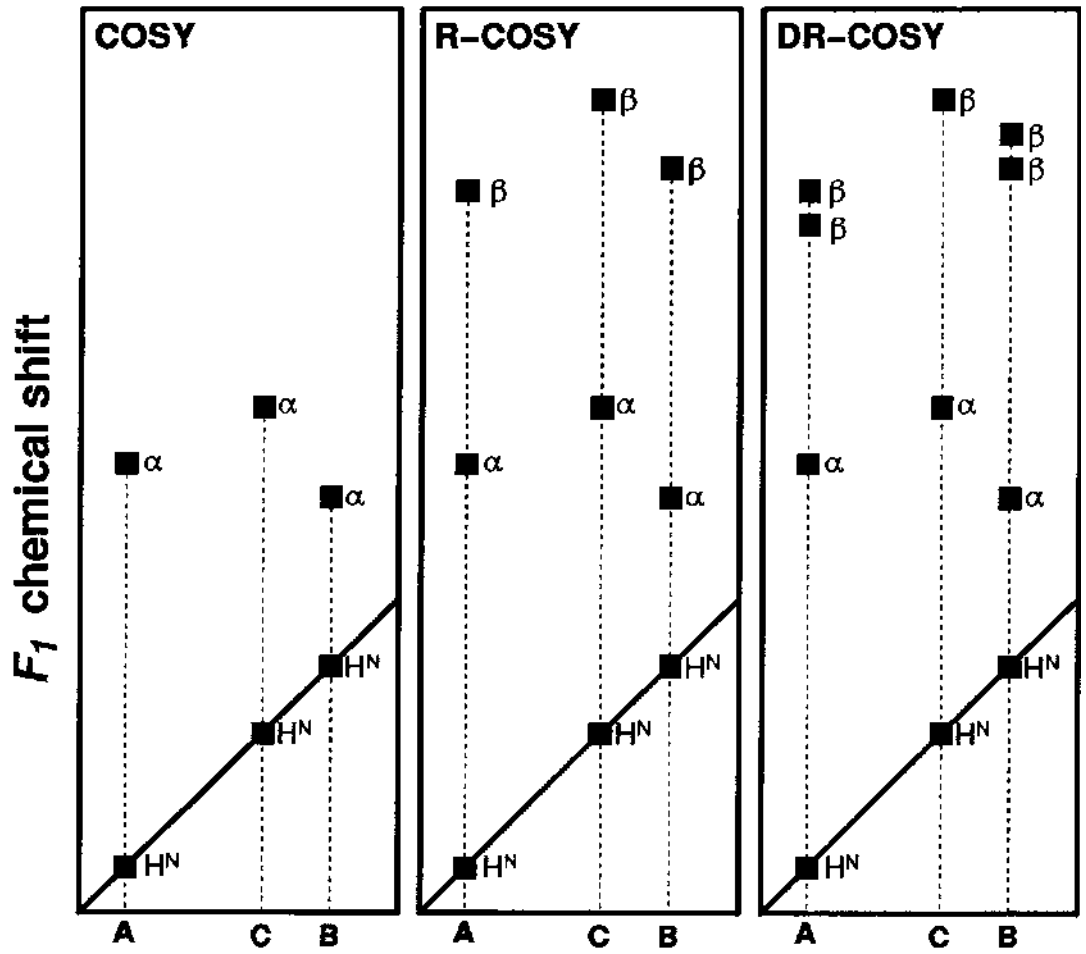


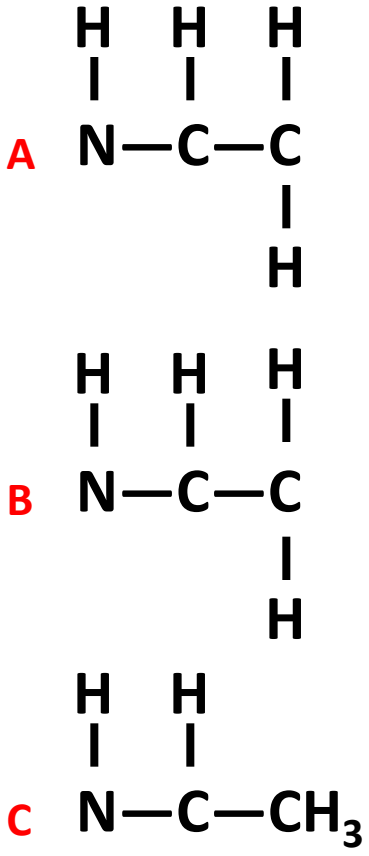
A 

B 

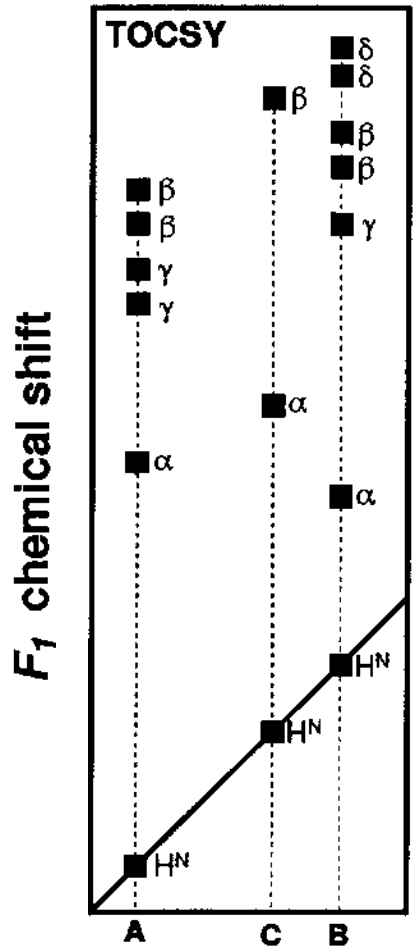
C 

DR-COSY: Add A 3rd Coupling

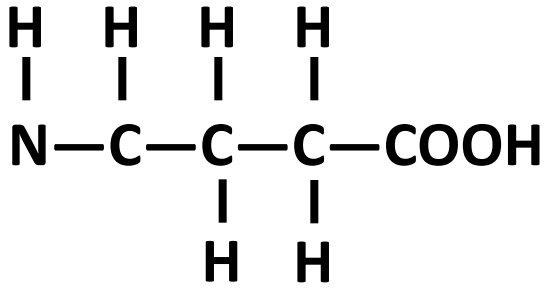




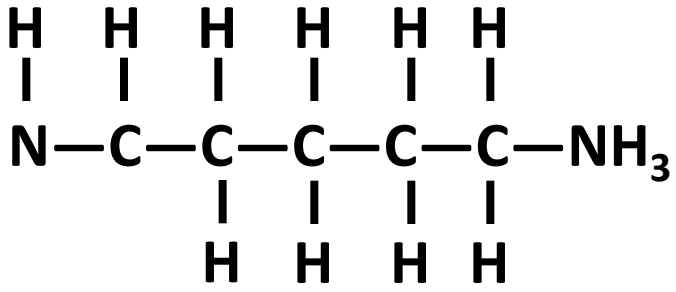
TOCSY: All Coupled Spins



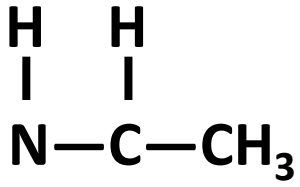
A



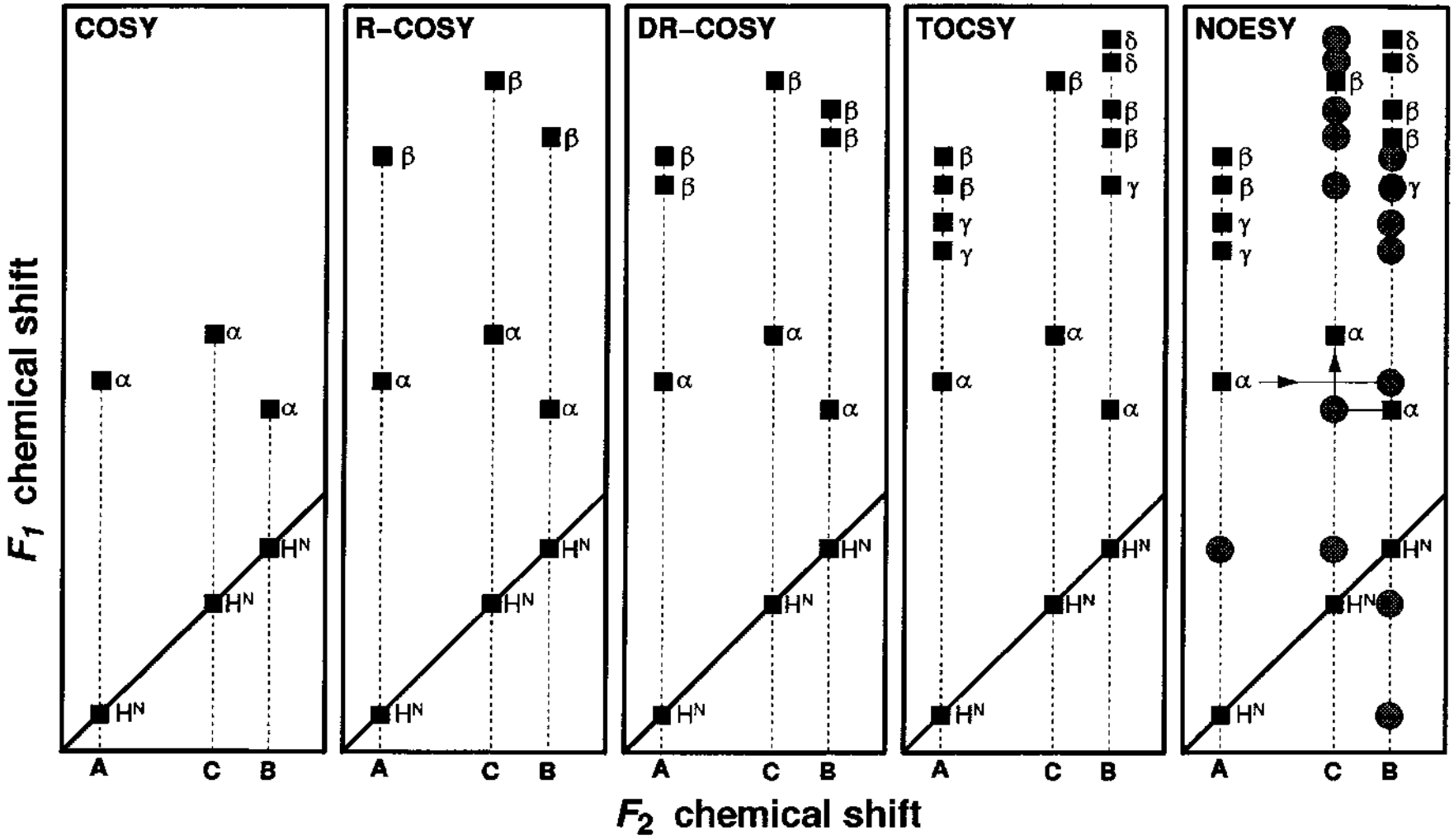
B



C

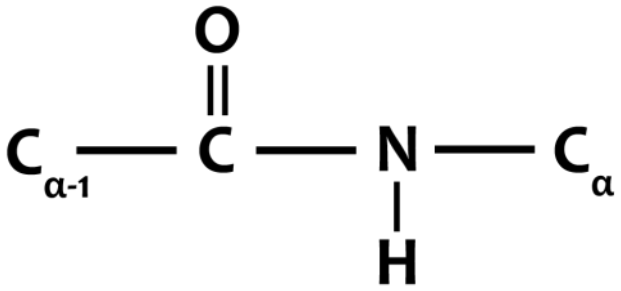


Homonuclear 2D Expts



Limitations of Homonuclear NMR

Nuclei are not all mutually coupled



Each amino acid gives rise to an independent NMR sub-spectrum, which is much simpler than the complete protein spectrum

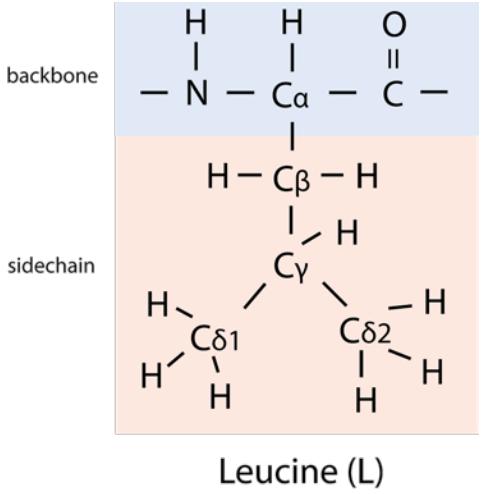
Basic Strategy to Assign Resonances

1. **Identify resonances for each residue (scalar)**

T G L S S R G

2. **Put residues in order**

1 2 3 4 5 6 7
R - G - S - T - L - G - S



Solutions to the Challenges

- **Most abundant nitrogens, carbons, and oxygens do not provide NMR-viable signal**
- **Detect signals from heteronuclei (non-hydrogens)**
- **Isotopically enrich protein sample with ^{13}C, ^{15}N, or ^{2}H to overcome low natural abundance**

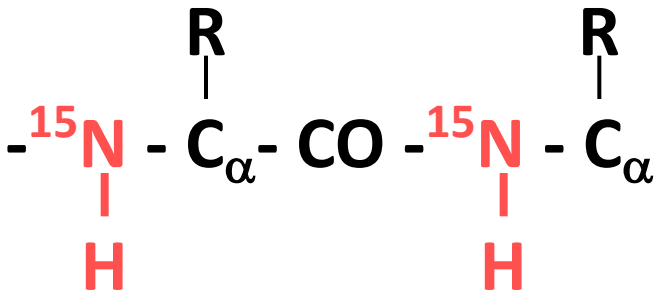
Intrinsic Sensitivity of Nuclei

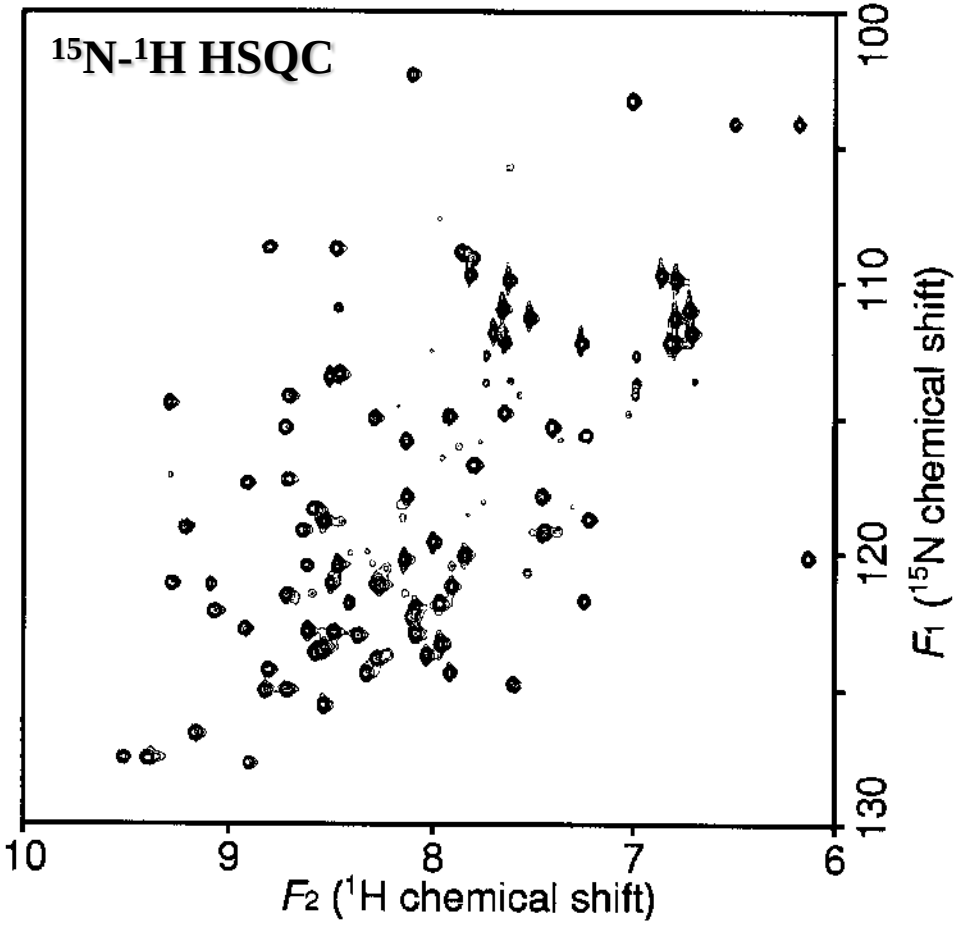
Nucleus	γ	% Natural Abundance	Relative Sensitivity
^{1}H	2.7×10^{8}	99.98	1.0
^{13}C	6.7×10^{7}	1.11	0.004
^{15}N	-2.7×10^{7}	0.36	0.0004
^{31}P	1.1×10^{8}	100	0.5

Prepare samples enriched in these nuclei

Double-Resonance Experiments

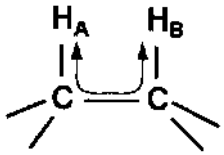
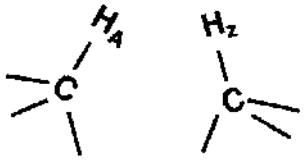
Increases Resolution/Information Content



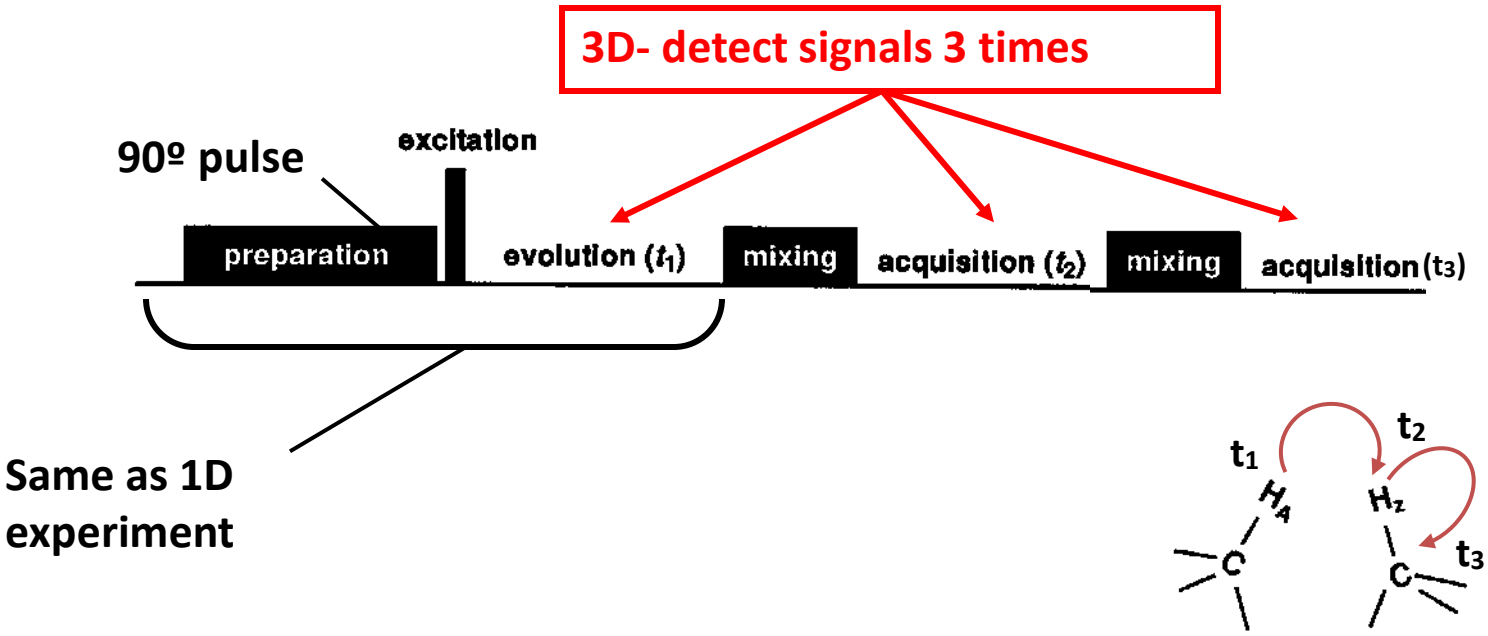


Acronyms For Basic Experiments

Differ Only By The Nature Of Mixing

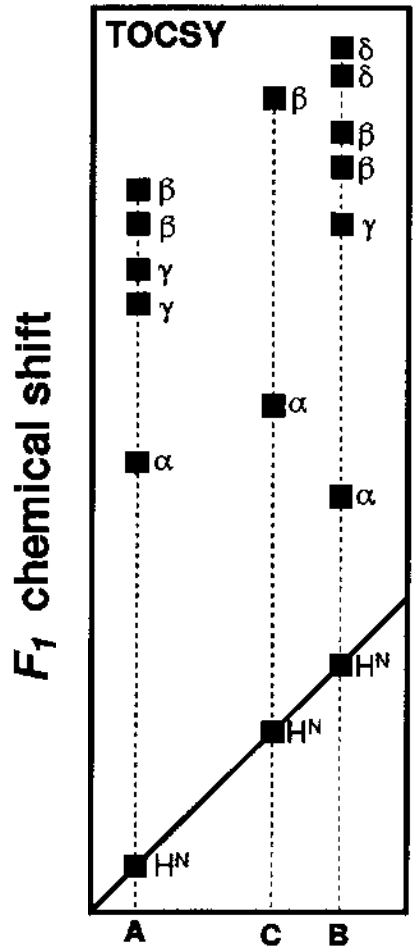
	Homonuclear	Heteronuclear
Scalar Coupling	**COSY**	**HSQC**
	**TOCSY**	**Hetero-TOCSY**
	Multiple Quantum	**HMQC**
Dipolar Coupling	**NOESY**	**NOESY-HSQC**
		**NOESY-HMQC**

Higher Dimensional NMR: Built on the 2D Principle

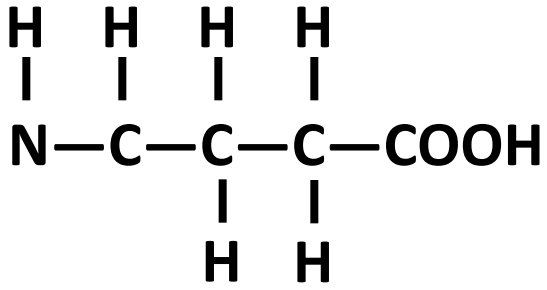


3D NMR Pulse Sequence

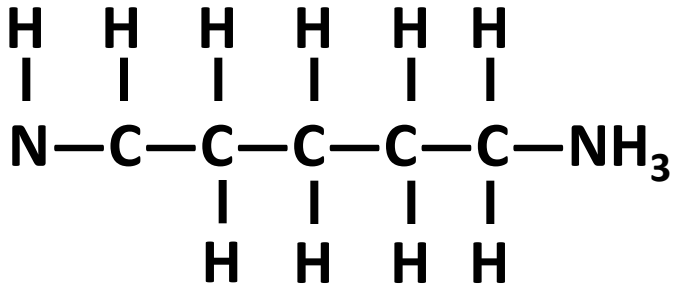
TOCSY: All Coupled Spins



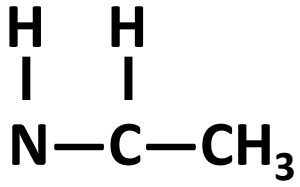
A



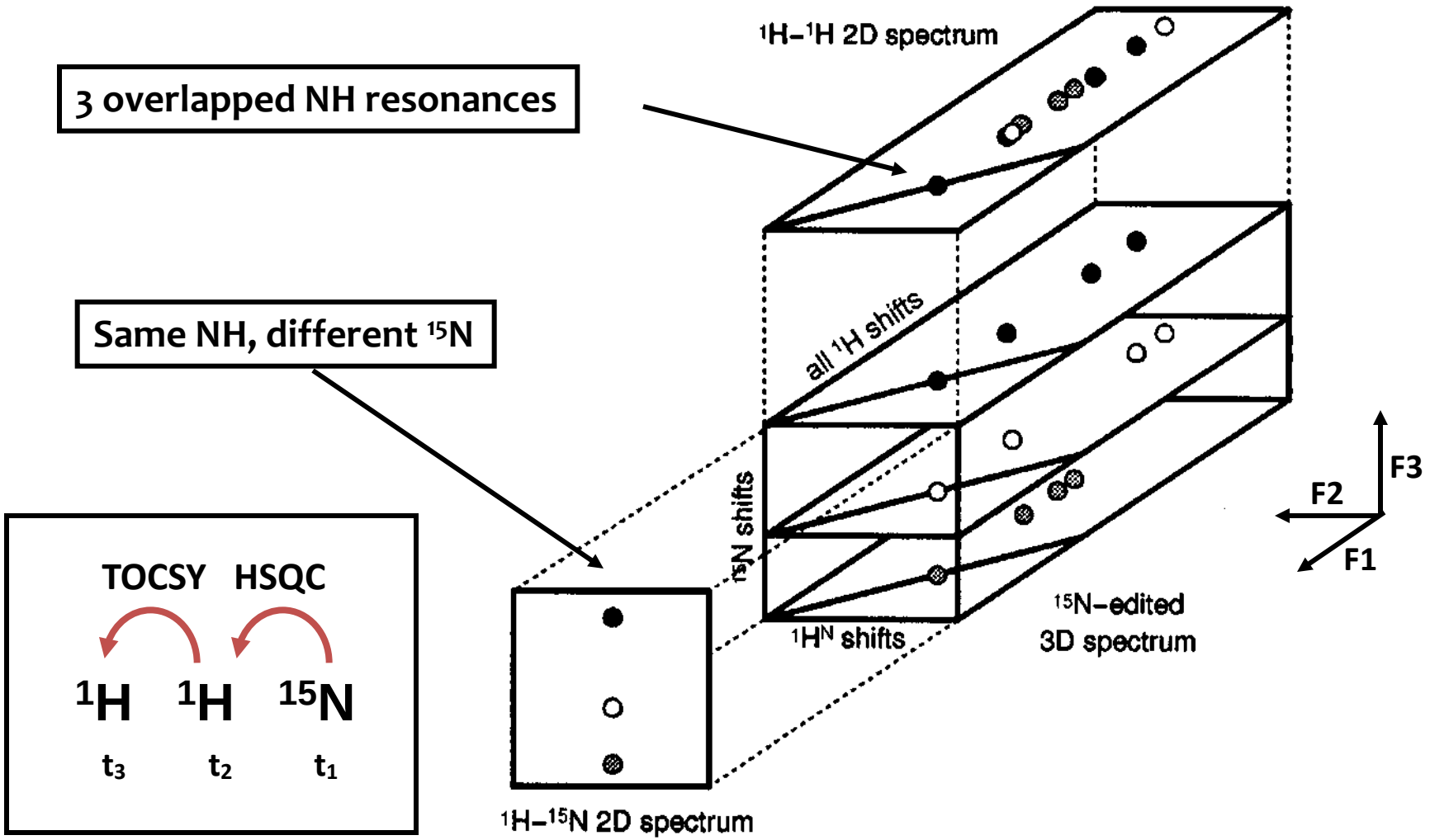
B



C

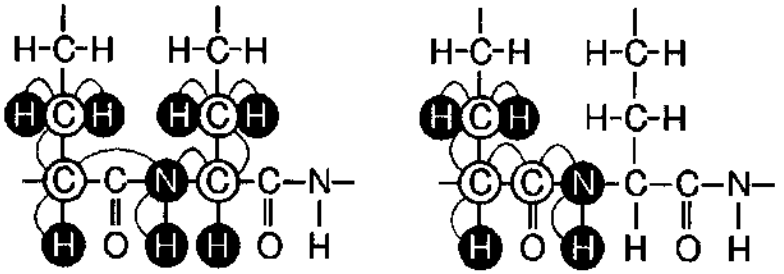


^{15}N Dispersed ^{1}H-^{1}H TOCSY

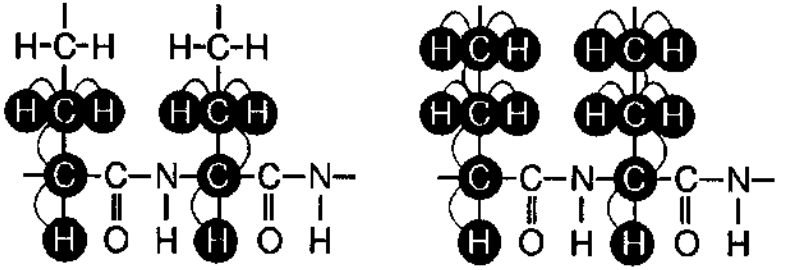


Heteronuclear Side Chain Experiments

HBHA(CBCA)NH /
HBHA(CBCACO)NH



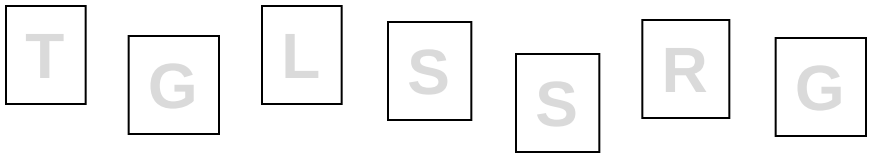
HCCH–COSY /
HCCH–TOCSY



Multiple redundancies increase reliability

Basic Strategy to Assign Resonances in a Protein

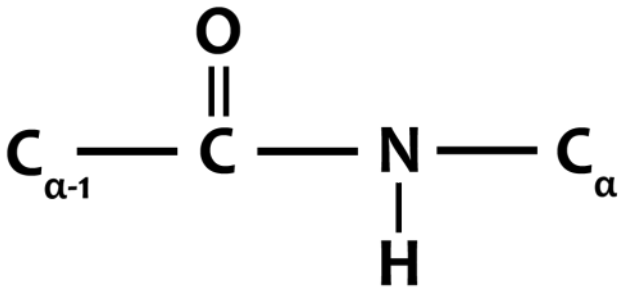
1. Identify resonances for each residue (scalar)



2. **Put residues in order**

1 2 3 4 5 6 7

R - G - S - T - L - G - S



Need a coupling to cross the carbonyl!

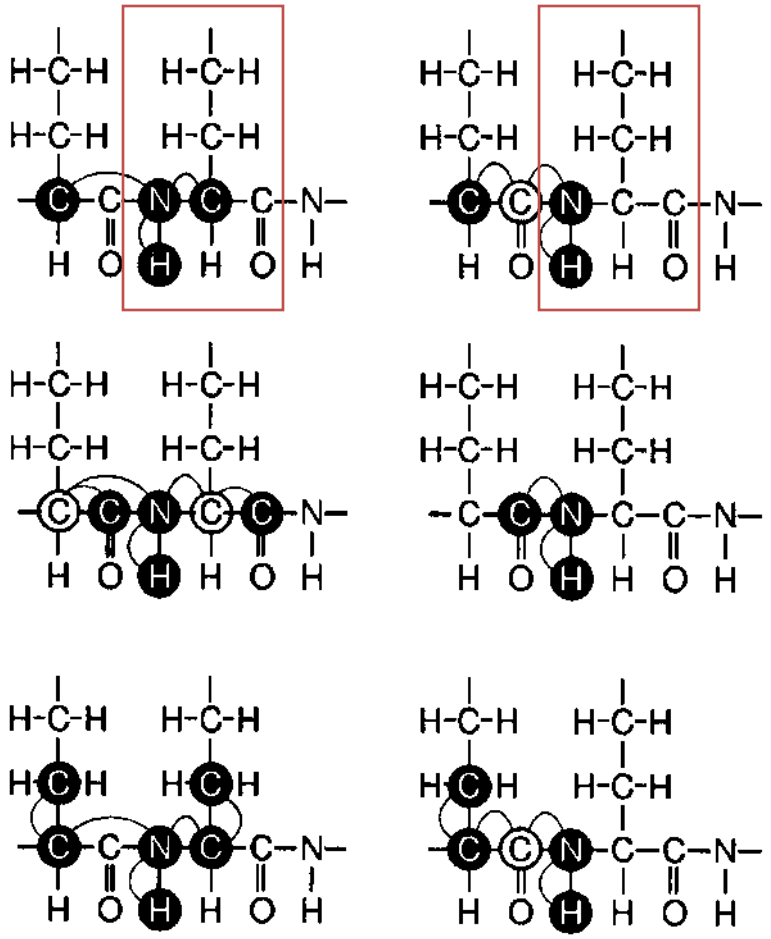
Heteronuclear Triple-Resonance Backbone Experiments

Names of scalar experiments based on atoms detected

HNCA / HN(CO)CA

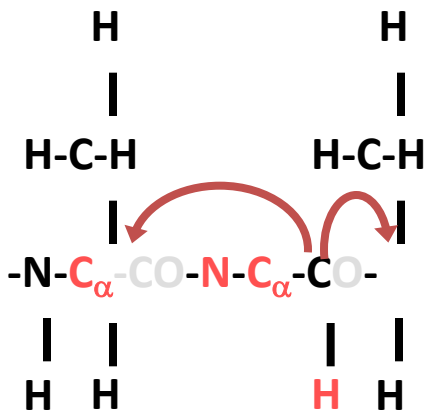
HN(CA)CO / HNCO

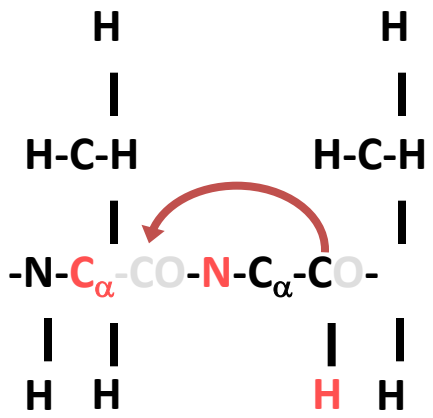
HNCACB / HN(CO)CACB



Pairs of Experiments

Distinguishes Intra-residue from Inter-residue





HNCA

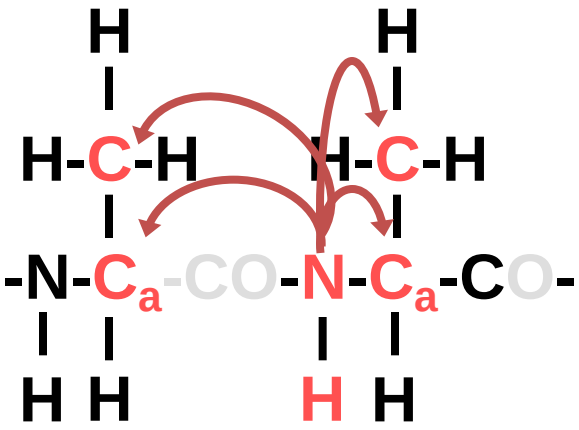
- $H(t_3)$, $N(t_2)$, $CA(t_1)$
- 2 peaks- Intra + Inter
- Mixing from N→Ca occurs over 1 bond to same Ca and over 2 bonds to adjacent Ca

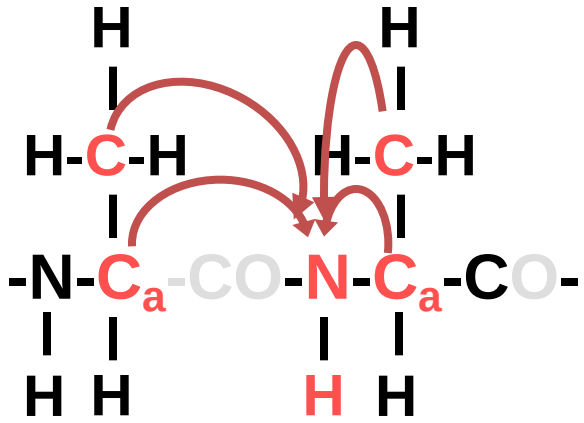
HN(CO)CA

- $H(t_3)$, $N(t_2)$, $CA(t_1)$
- 1 peak- Inter only
- Mixing from N→Ca occurs ONLY over 2 bonds to adjacent Ca

Equivalent Experiments

Can be run in either direction





HNCACB

- H(t_3), N(t_2), CA and CB(t_1)
- Mixing from N→Ca occurs over 1 bond to same Ca and over 2 bonds to adjacent Ca

CBCANH

- CB and CA(t_1), N(t_2), H(t_3)
- Mixing from N→Ca occurs over 1 bond to same Ca and over 2 bonds to adjacent Ca