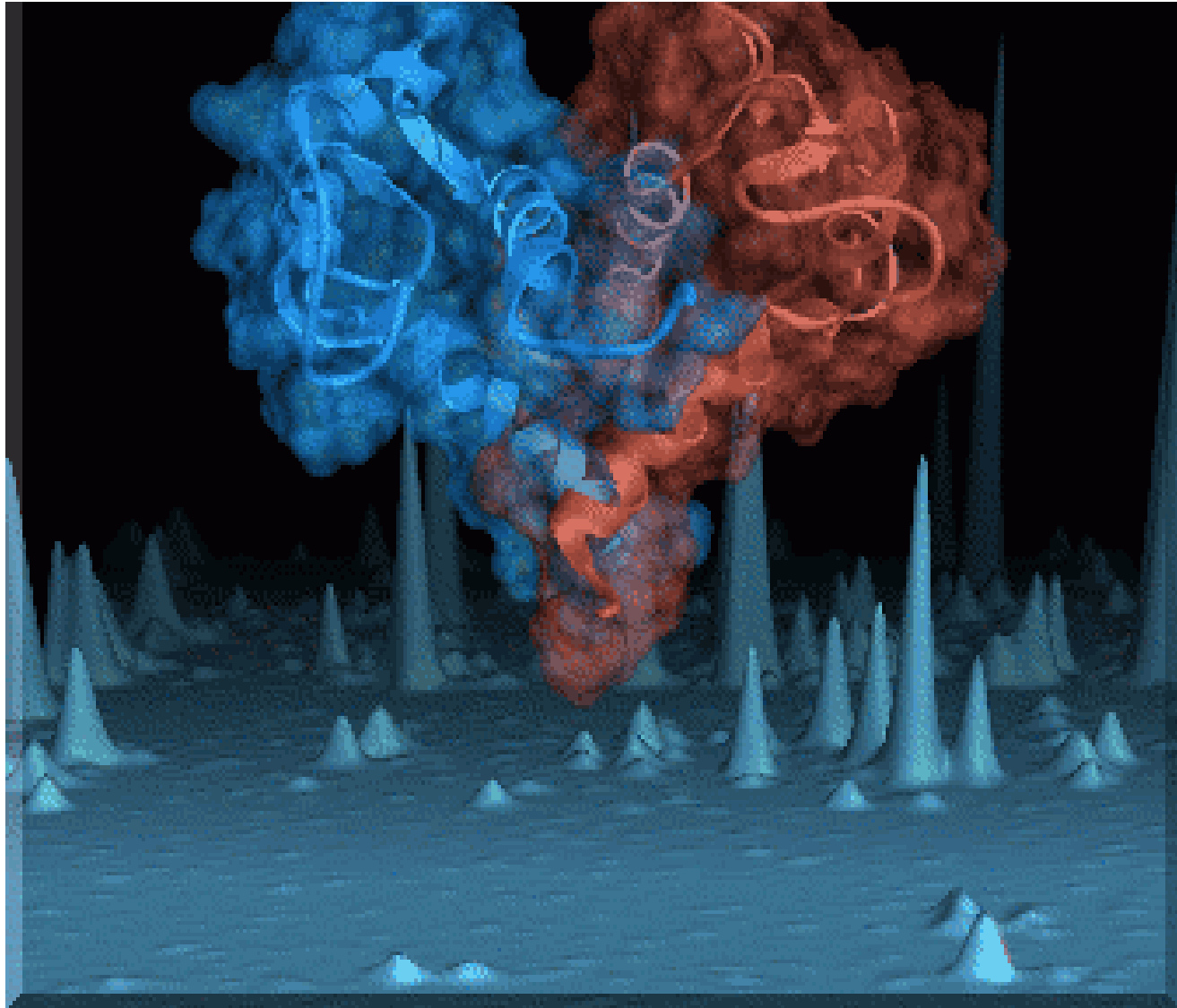


# Nuclear Magnetic Resonance (NMR)



# Presentation Outline

- Introduction – What is NMR Good For?
- Brief Theory – Quantum Chemistry, Magnetization
- NMR Concepts –
  - Frequency, Relaxation, Chemical Shift, Coupling, Integration
- 1-Dimension NMR Experiments
- 2-D NMR – COSY, HMQC/HSQC, NOESY
- \*\*\*\*\*BREAK\*\*\*\*\*
- Biomolecular NMR – 3-D
- NMR Application Examples
  - Dynamic NMR, Solid State NMR, Inorganic, Diffusion
- Spectrometer Description
  - Probes and Gradients
- Structure Determination with NMR

# Introduction – NMR, What is it Good for?

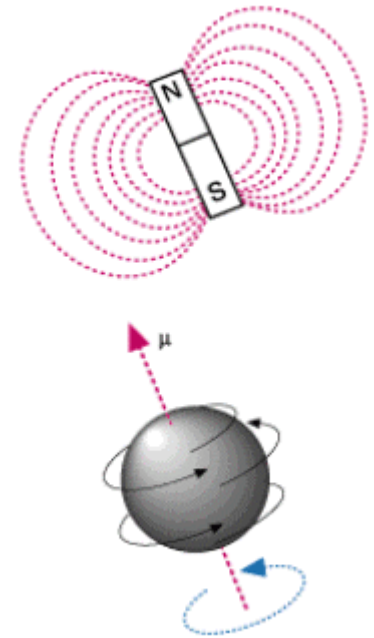
- Determine Solution Structure of Small Molecules
- DNA and Protein Structure Determination
- Molecular Dynamics – Quantifying Motional Properties
  - Exchange Rate/Activation Energy/ $\Delta H$ /  $\Delta S$
- Diffusion Measurements
- Hydrogen Bonding Determination/pKa Measurements
- Drug Screening
- Metabolite Analysis - Metabolomics
- Natural Product Chemistry
- Polymer Chemistry
- Environmental Chemistry

# The Nuclear Magnetic Moment

- All atomic nuclei can be characterized by a nuclear spin quantum number,  $I$ .  $I$  can be  $\geq 0$  and any multiple of  $\frac{1}{2}$ .
- Nuclei with  $I = 0$  do not possess nuclear spin and consequently are termed 'NMR silent'.
- All nuclei with  $I \neq 0$  possess spin, charge, and angular momentum  $P$ , resulting in a nuclear magnetic moment  $\mu$ .

$$\mu = \gamma P$$

Where  $\gamma$  is the magnetogyric ratio of the nucleus.



# NMR- Quantum Chemistry

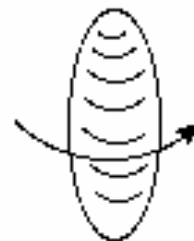
$I$  = the nuclear spin quantum number



No spin  
 $I = 0$



Spinning sphere  
 $I = 1/2$



Spinning ellipsoid  
 $I = 1, 3/2, 2, \dots$

For Nuclei of:

$I =$

Example

Odd Mass

Half Integer

$^1\text{H}$ ,  $^{13}\text{C}$

Even Mass/Even Charge

Zero

$^{12}\text{C}$ ,  $^{16}\text{O}$

Even Mass/Odd Charge

Integer

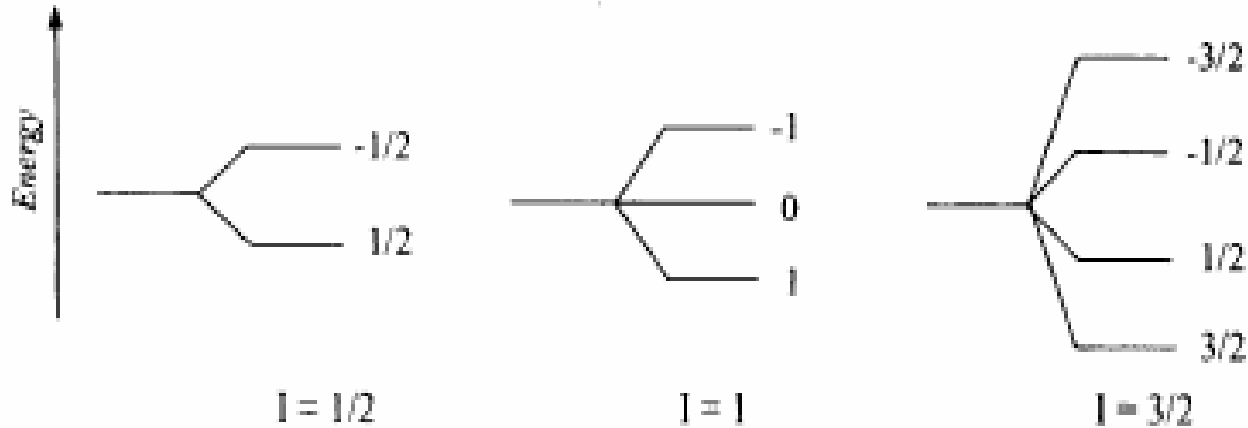
$^2\text{H}$ ,  $^{14}\text{N}$

*If  $I = 0$ , NMR Inactive*

*If  $I \geq 1$ , Quadrupolar (non-spherical nuclear charge distribution)*

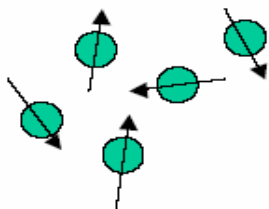
# Magnetic Quantum Numbers

- $I$  is quantized producing  $(2I + 1)$  discrete values of angular momentum,  $m_I$ .
- $m_I = I, I - 1, \dots -I$

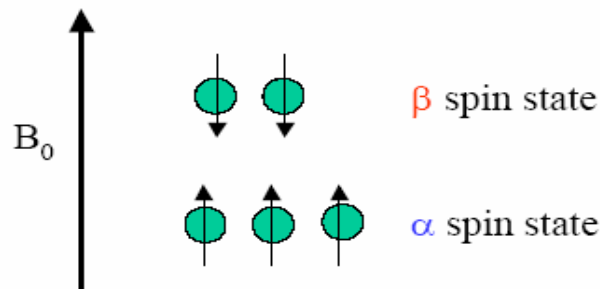


# NMR Concepts – Spin States

A nucleus with spin behaves much like a small magnet. It possesses a magnetic moment which has both magnitude and direction. Consider a collection of  $^1\text{H}$  nuclei with randomly oriented magnetic moments.

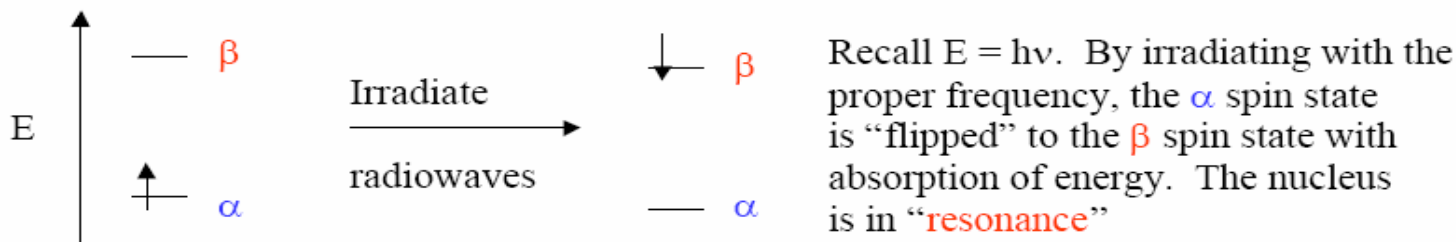


No applied magnetic field  
Randomly oriented



$B_0$  is the applied magnetic field.

The  $\alpha$  spin state is lower energy. There is an excess of  $\alpha$  spin states.



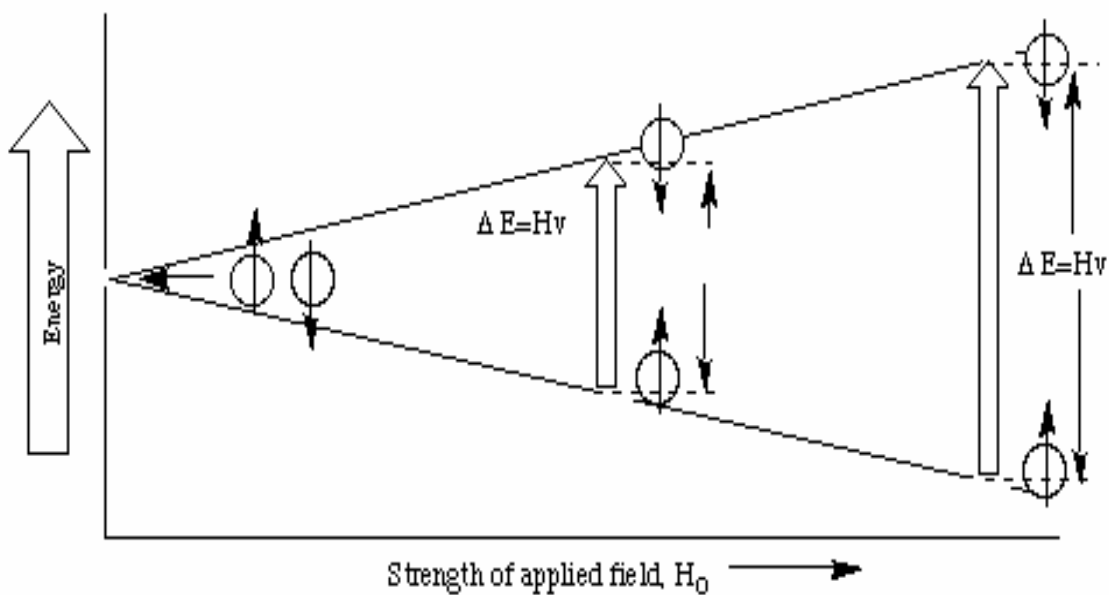
Recall  $E = h\nu$ . By irradiating with the proper frequency, the  $\alpha$  spin state is “flipped” to the  $\beta$  spin state with absorption of energy. The nucleus is in “**resonance**”

$$\Delta E = \frac{\gamma h}{2\pi} B_0$$

$h$  Planck's constant

$\gamma$  Nuclear constant

# NMR Concepts – Energy Levels



$$\Delta E \sim H_0 \text{ \& } \gamma$$

## Magnetic Properties of Selected Nuclei

Nucleus	Abundance %	Spin, $I$	$\gamma_N = \frac{2\pi g_N \mu_N}{h}$ ( $/10^7 \text{Ts}$ )
$^1\text{H}$	99.98	1/2	26.75
$^2\text{H}$ , (D)	0.02	1	4.10
$^{13}\text{C}$	1.1	1/2	6.73
$^{14}\text{N}$	99.64	1	1.93
$^{15}\text{N}$	0.365	1/2	-2.7116
$^{19}\text{F}$	100	1/2	25.1665
$^{35}\text{P}$	100	1/2	10.8289
$^{35}\text{Cl}$	75.4	3/2	2.6210
$^{37}\text{Cl}$	24.6	3/2	2.1718



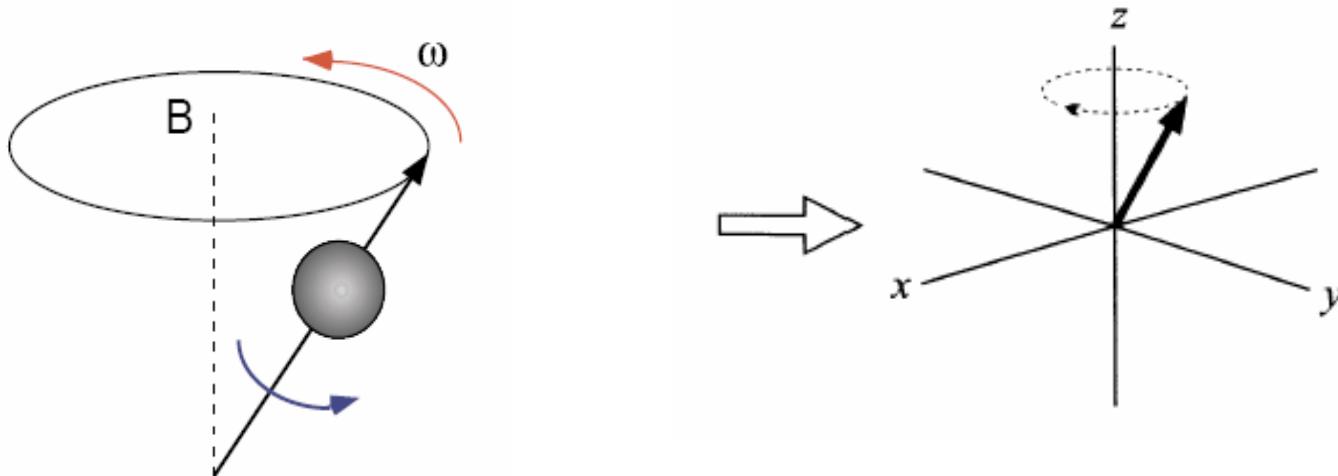
# The Larmor Frequency

A nuclear magnetic moment will precess about the axis of an externally applied field at a frequency proportional to the strength of the applied field,  $B_0$ .

$$\omega = \gamma B_0 \text{ (rad/s)}$$

$$\nu = \gamma B_0 / 2\pi \quad \textit{Larmor Frequency}$$

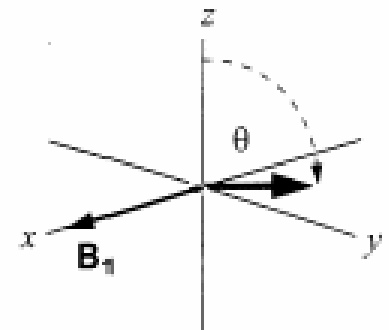
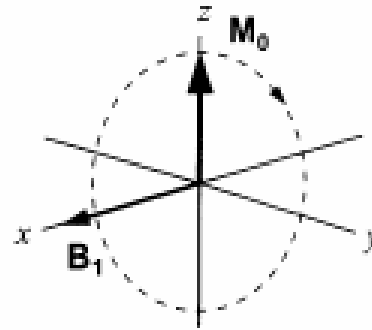
The direction of motion can be clockwise or counterclockwise and is determined by the sign of  $\gamma$ . By convention, the field is applied along the z axis of a Cartesian co-ordinate frame.



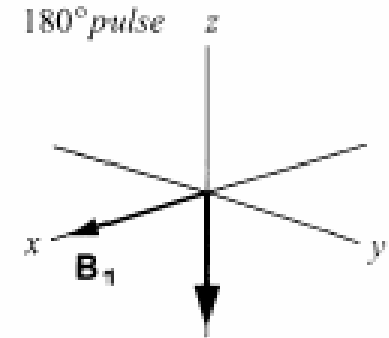
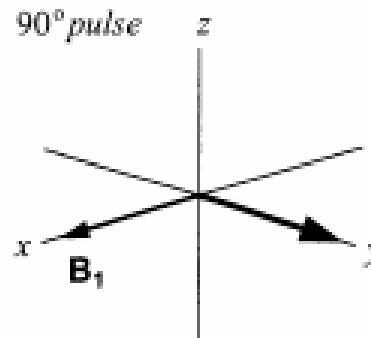
# The RF Pulse

- An rf pulse applies a torque to the bulk magnetization vector,  $M_0$ , which drives it to the x-y plane from equilibrium.

$$\theta = 360\gamma B_1 t_p \text{ degrees}$$

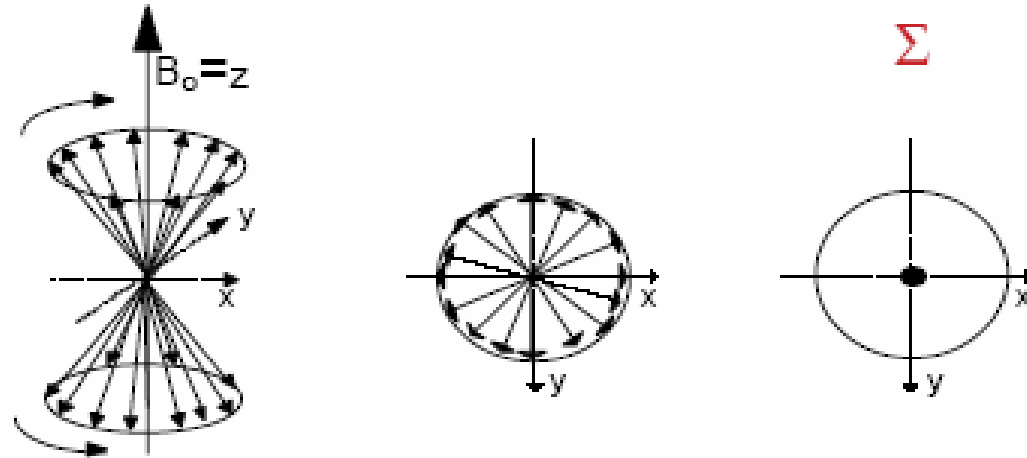


- $90^\circ$  pulse - moves net magnetization from the z-axis to the x-y plane
- $180^\circ$  pulse - changes the net magnetization in the z-axis from the alpha to beta state.

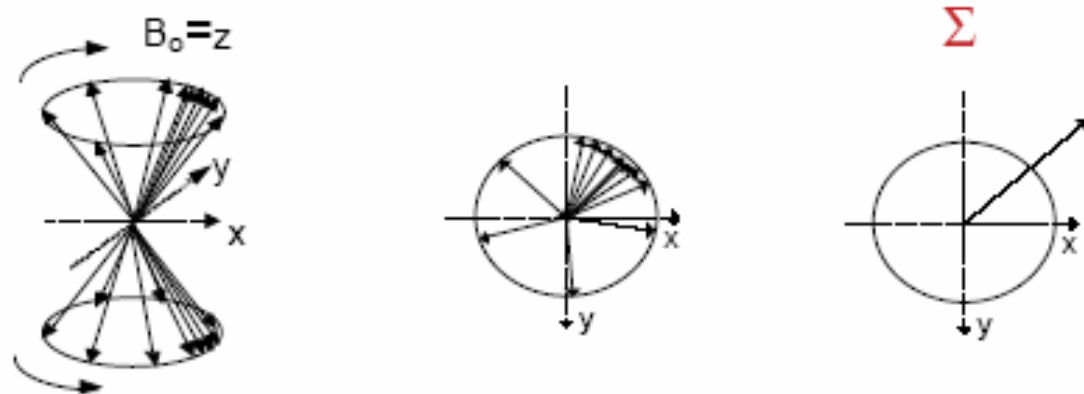


# Visualizing Magnetization Vectors

Static Field



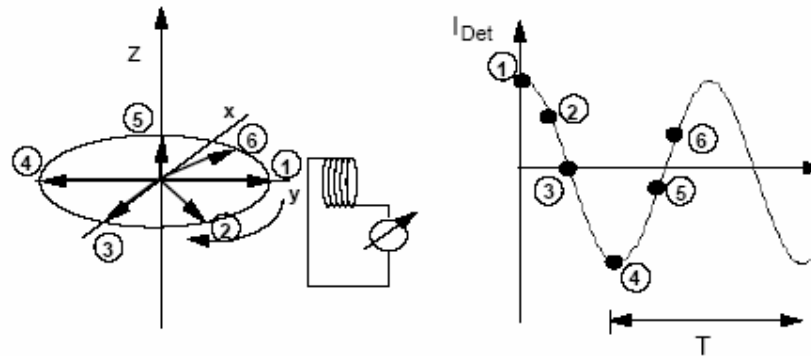
RF Pulse  $\rightarrow$



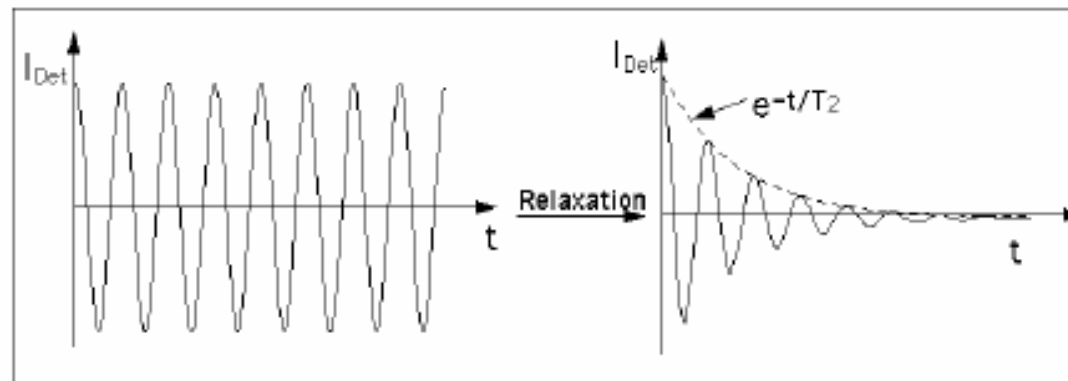
The spin vectors are said to possess spin coherence following a  $90^\circ$  pulse.

# NMR Signal Detection

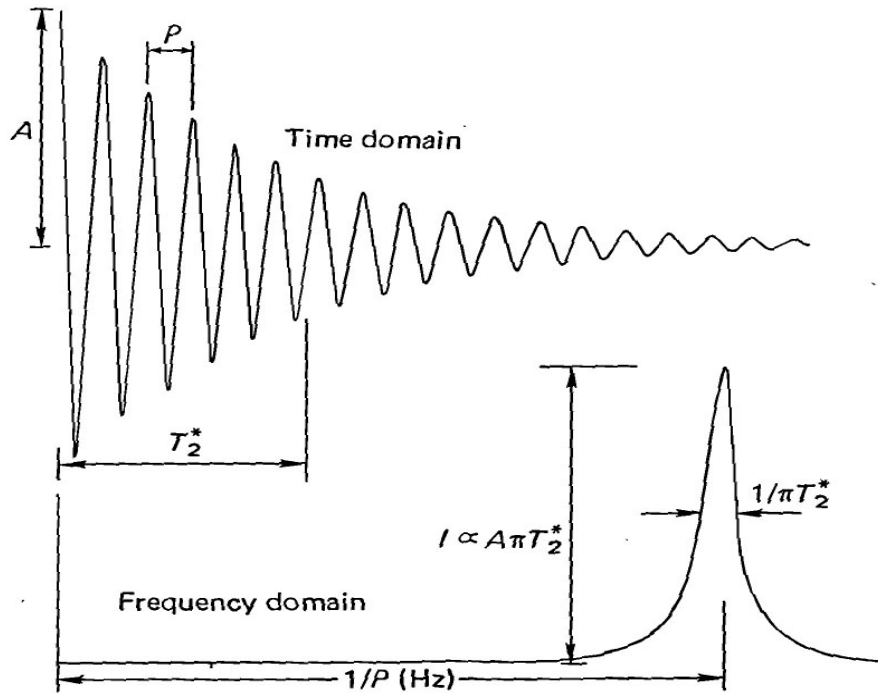
- Signal Detection:
  - The rotating magnetization vector produces a weak oscillating voltage in the coil surrounding the sample giving rise to the NMR signal.



- Return to equilibrium via relaxation mechanisms:

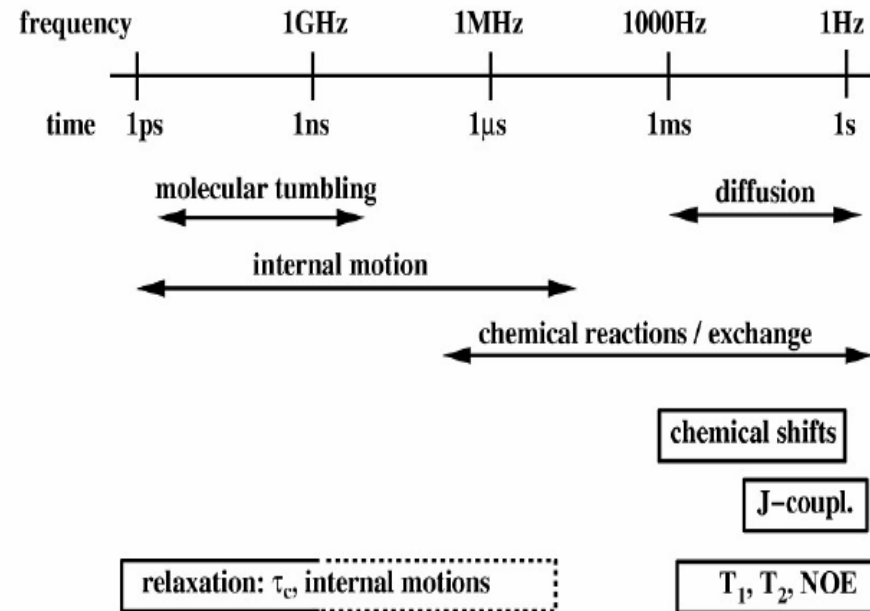


# NMR Concepts – Frequency/Time & FID



The relationships between time and frequency domains. The period  $P$  of the FID gives the position of the line. The rate of decay  $T_2^*$  gives the line-width and the initial amplitude  $A$  gives the line area and therefore its intensity proportional to  $A\pi T_2^*$ .

## Time scales of high-resolution NMR



# NMR Concepts – Relaxation

- Once excited to the higher energy state by an rf pulse, the spins will return to their initial equilibrium condition by means of two relaxation mechanisms,  $T_1$  and  $T_2$ .
  - $T_1$  relaxation (longitudinal): *Spin-lattice relaxation occurs by transfer of energy to the surroundings (heat); dipolar coupling to other spins. Results in recovery of  $M_z$  to 63% of original value.*
  - $T_2$  relaxation (transverse): *Spin-spin relaxation occurs by redistribution of energy among various spins of the system. Results in recovery of  $M_z$  to 37% of original value.*

$$T_2 \leq T_1$$

- $T_1$  and  $T_2$  are routinely equivalent for most NMR experiments.
- NMR Linewidths  $\sim 1/T_2$  for spin  $1/2$  nuclei
- Inorganic/Organometallic Linewidths -

$$W_{1/2} \sim \frac{(2I + 3) Q^2 q_{zz}^2 \tau_c}{I^2 (2I - 1)}$$

$Q$  = quadrupole moment  
 $q_{zz}$  = electric field gradient  
 $\tau_c$  = correlation time  
 $I$  = spin quantum number

# Longitudinal Relaxation Mechanisms

- Dipole-Dipole interaction "through space"-
  - Most significant for high natural abundance nuclei with a large magnetogyric ratio ( $^1\text{H}$ )
  - Depends highly on the gyromagnetic ratio and the distance between the two nuclei

$$R_1 = k \cdot \gamma_I^2 \cdot \gamma_S^2 \cdot (r_{IS})^{-6} \cdot \tau_c$$

- Electric Quadrupolar Relaxation – nuclei of spin  $>1/2$  possess a non-spherical distribution of electrical charge and consequently, an electric magnetic moment. The quadrupolar coupling constant is large – MHz range and dominates over the other types of relaxation and depends on:
  - Quadrupole moment of the nucleus (eQ) – eg.  $2\text{H}$  - eQ = 0.003;  $^{55}\text{Mn}$  – eQ = 0.55
  - Electric Field gradient (eq) – dependent on the symmetry of the molecule
    - The Quadrupole coupling vanishes in a symmetrical environment.  
e.g. for symmetrical  $[\text{NH}_4]^+$  : eq \* eQ = 0 and therefore has very long  $T_1 = 50$  sec.  
whereas  $\text{CH}_3\text{CN}$  : eq \* eQ about 4 MHz and  $T_1 = 22$  msec.

# Longitudinal Relaxation Mechanisms

- Paramagnetic Relaxation –
  - Molecular motion, electron spin relaxation, and chemical exchange randomly modulate the interaction between the nucleus and unpaired electrons in solution.
  - There is dipole relaxation by the electron magnetic moment (magnetic moment is 600X that of a 1H so it is very efficient – oxygen in the nmr solvent can cause enhanced relaxation).
  - There is also a transfer of unpaired electron density to the relaxing nucleus.
- Chemical Shift Anisotropy – (anisotropic = unsymmetrical) –
  - Due to the inherently unsymmetrical distribution of electrons in chemical bonds, the magnetic field experienced by a nucleus will depend on the orientation of its bonds with respect to the magnetic field.
  - This effect – chemical shift anisotropy- is averaged out by rapid molecular tumbling in solution but the fluctuating field can still enhance relaxation depending its magnitude. This effect is more pronounced for nuclei exhibiting a large chemical shift range (most metals, <sup>19</sup>F, <sup>31</sup>P).

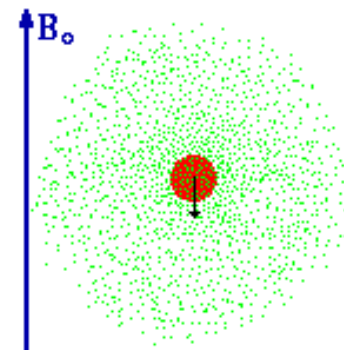


# NMR Concepts – Chemical Shift

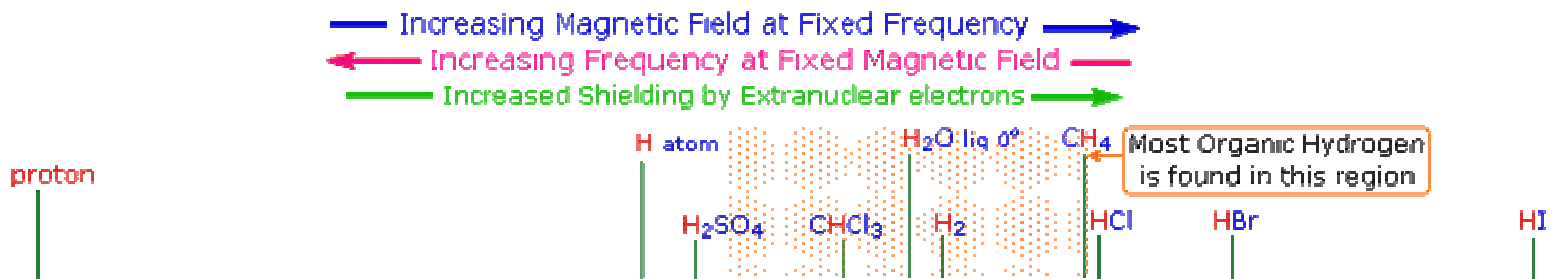
- When placed in a magnetic field, the electrons surrounding the nucleus begin to precess in the direction of the applied magnetic field, thereby creating an opposing magnetic field which shields the nucleus. The effective field  $B_{\text{eff}}$  experienced by the nucleus is therefore lessened by a factor  $\sigma$ .

$$B_{\text{eff}} = B_0 (1 - \sigma)$$

- Variations in electron density surrounding each non-equivalent nucleus in a molecule will therefore cause each nucleus to experience a different  $B_{\text{eff}}$ . The differences in  $B_{\text{eff}}$  for non-equivalent nuclei define the chemical shift phenomenon.
- Chemical shift,  $\delta$  is measured in frequency versus a reference, usually TMS (tetramethyl silane). It is presented in units of parts per million or PPM.



$$\delta = (\nu - \nu_{\text{ref}}) / \nu_{\text{ref}} \times 10^6$$



# The Chemical Shift

- Other factors affecting chemical shift:
  - **Paramagnetic** contribution arises from non-spherical electron distribution (nuclei with non-s orbitals). It is the dominating factor of chemical shift for all nuclei other than protons.
  - **Magnetic anisotropy of neighboring bonds and ring currents** –  $\pi$  electrons of triple bonds and aromatic rings are forced to rotate about the bond axis creating a magnetic field which counteracts the static field.
  - **Electric field gradients** are the result of strongly polar substituents. The distortion of the electron density alters the chemical shift.
  - **Hydrogen bonding** can lead to a decrease in electron density at the proton site resulting in a chemical shift to higher frequency. Hydrogen bonded protons exhibit shifts that are highly dependent on temperature, solvent, and concentration.
  - **Solvent effects** are often exploited to separate overlapping signals of interest in a spectrum. Large changes in chemical shift can be observed for solvents that can selectively interact with one portion of a molecule (acetone for its carbonyl group, and benzene for its ring currents)

# NMR Concepts – Chemical Shift

In molecules, the nuclei are screened by the electrons.

So the effective field at the nucleus is:

$$B_{\text{eff}} = B_0(1 - \sigma)$$

Where  $\sigma$  is the shielding constant.

The shielding constant has 2 terms:  $\sigma_a$  (diamagnetic) and  $\sigma_p$  (paramagnetic)

$\sigma_a$  - depends on **electron distribution in the ground state**

$\sigma_p$  - depends on **excited state** as well. It is zero for electrons in s-orbital.

This is why the **proton shift is dominated by the diamagnetic term.**

But **heavier nuclei are dominated by the paramagnetic term.**

# Spin-Spin Coupling

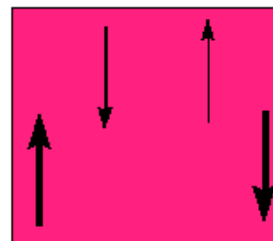
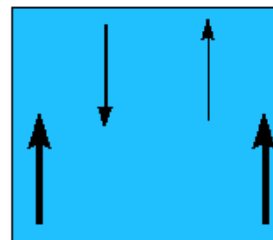
- Spin-spin or scalar coupling is the result of Fermi contact interaction between electrons in the s orbital of one nucleus and the nuclear spin of a bonded nucleus.
- The magnitude of coupling depends on the degree of electron orbital overlap. The s-character of the orbitals relies heavily on the hybridization of the nuclei involved.

$\nu_{sp^3}$ : 140 Hz

$\nu_{sp^2}$ : 160 Hz

$\nu_{sp}$ : 250 Hz

**Bold face arrows are nuclear spins  
light face arrows are electron spins.  
Pauli's exclusion principle dictates that electrons in one orbital be antiparallel in spin**

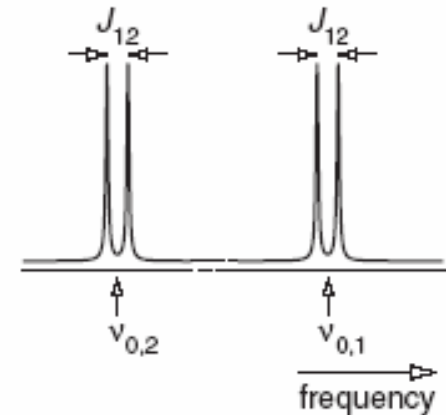
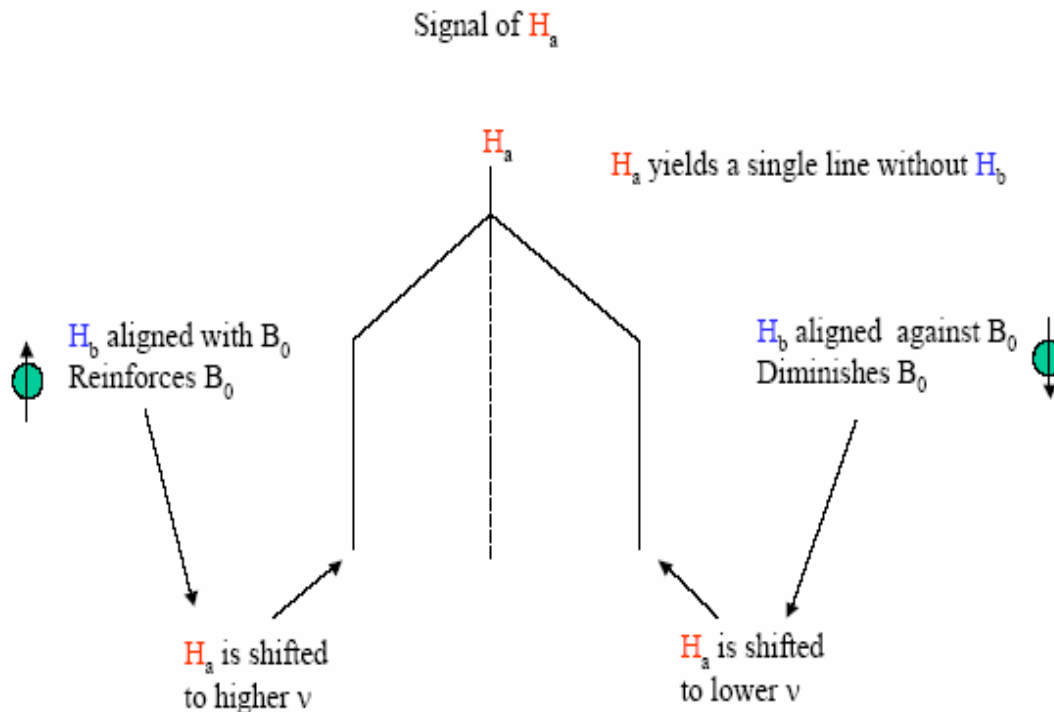
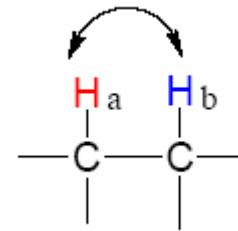


**It should be straightforward to see that the two spin systems shown will differ in energy.**

**Origin of electron-coupled spin-spin interaction**

# NMR Concepts – Spin-Spin Coupling

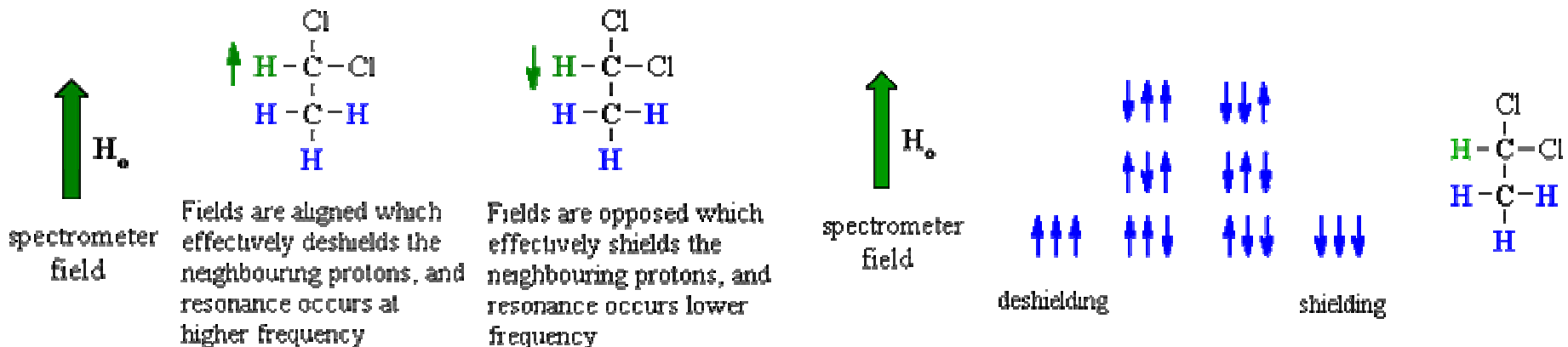
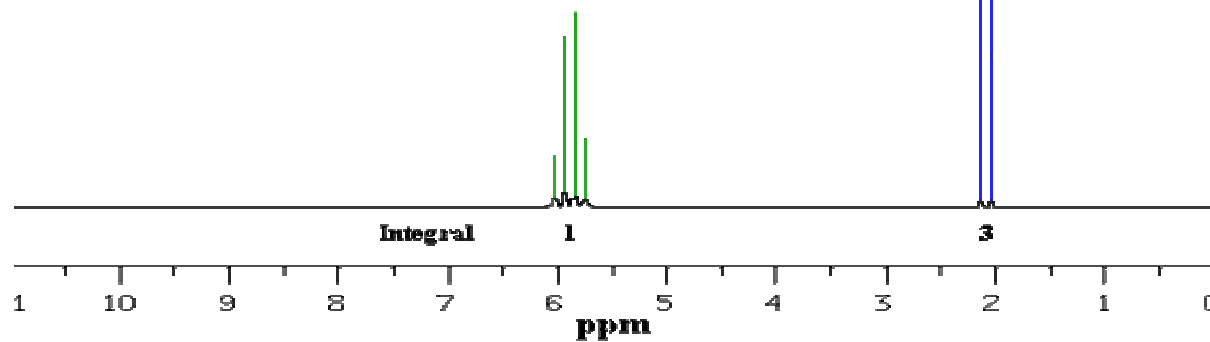
- Nuclei in a molecule are affected by the spins systems of neighboring nuclei. This effect is observed for non-equivalent nuclei up to 3 bond lengths away and is termed spin-spin coupling or J coupling.



# Spin-Spin Coupling



1,1-dichloroethane contains two types of hydrogen atoms, so we see two sets of peaks, a doublet at 2.1 ppm and a quartet at 5.9 ppm.



# NMR Concepts – Spin-Spin Coupling

**N + 1 Rule:** a hydrogen nucleus with N adjacent equivalent hydrogen nuclei will be split into N + 1 lines.

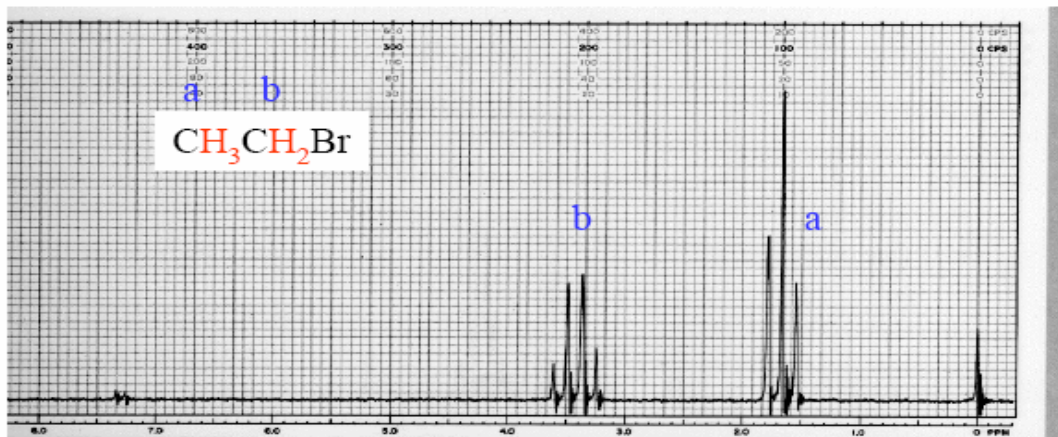
Consider the  $^1\text{H}$  NMR spectrum of ethyl bromide.



The  $\text{CH}_2$  hydrogens are split into  $3 + 1 = 4$  lines

The  $\text{CH}_3$  hydrogens are split into  $2 + 1 = 3$  lines

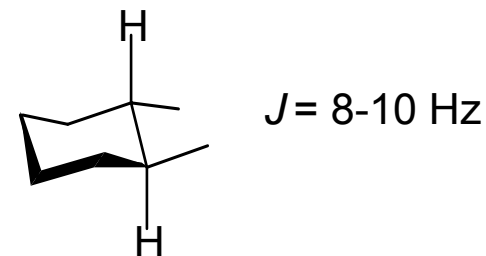
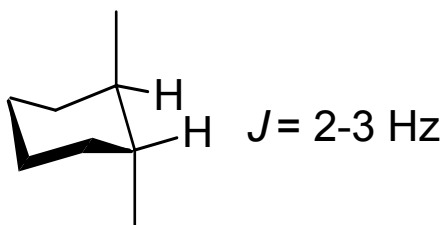
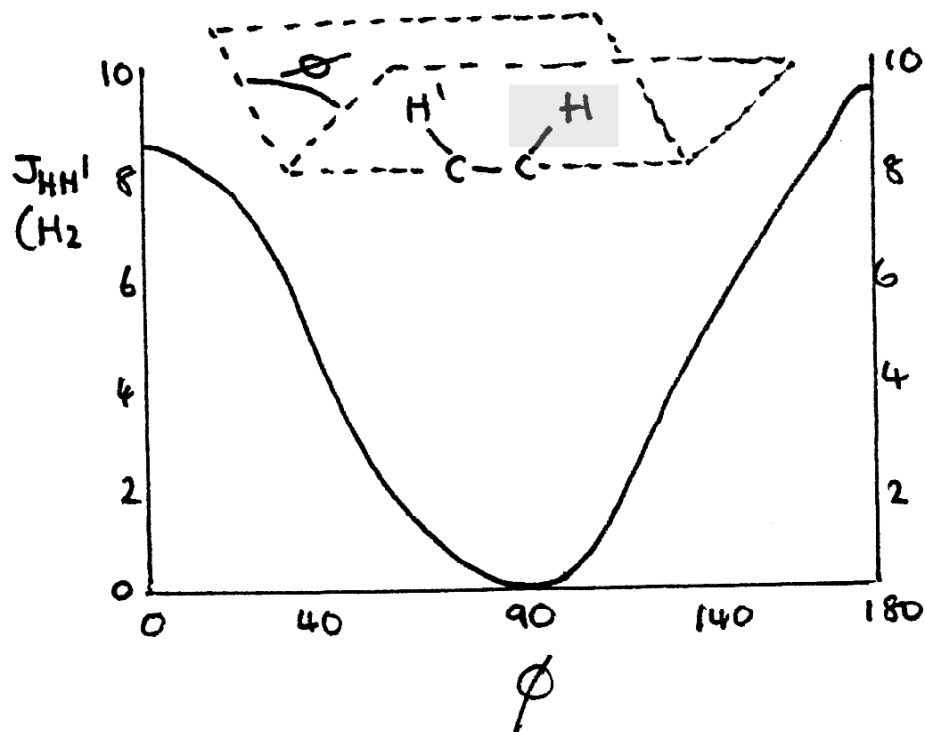
Coupling with 0 protons	1
Coupling with 1 proton	1 1
Coupling with 2 protons	1 2 1
Coupling with 3 protons	1 3 3 1
Coupling with 4 protons	1 4 6 4 1



# NMR Concepts – Spin-Spin Coupling

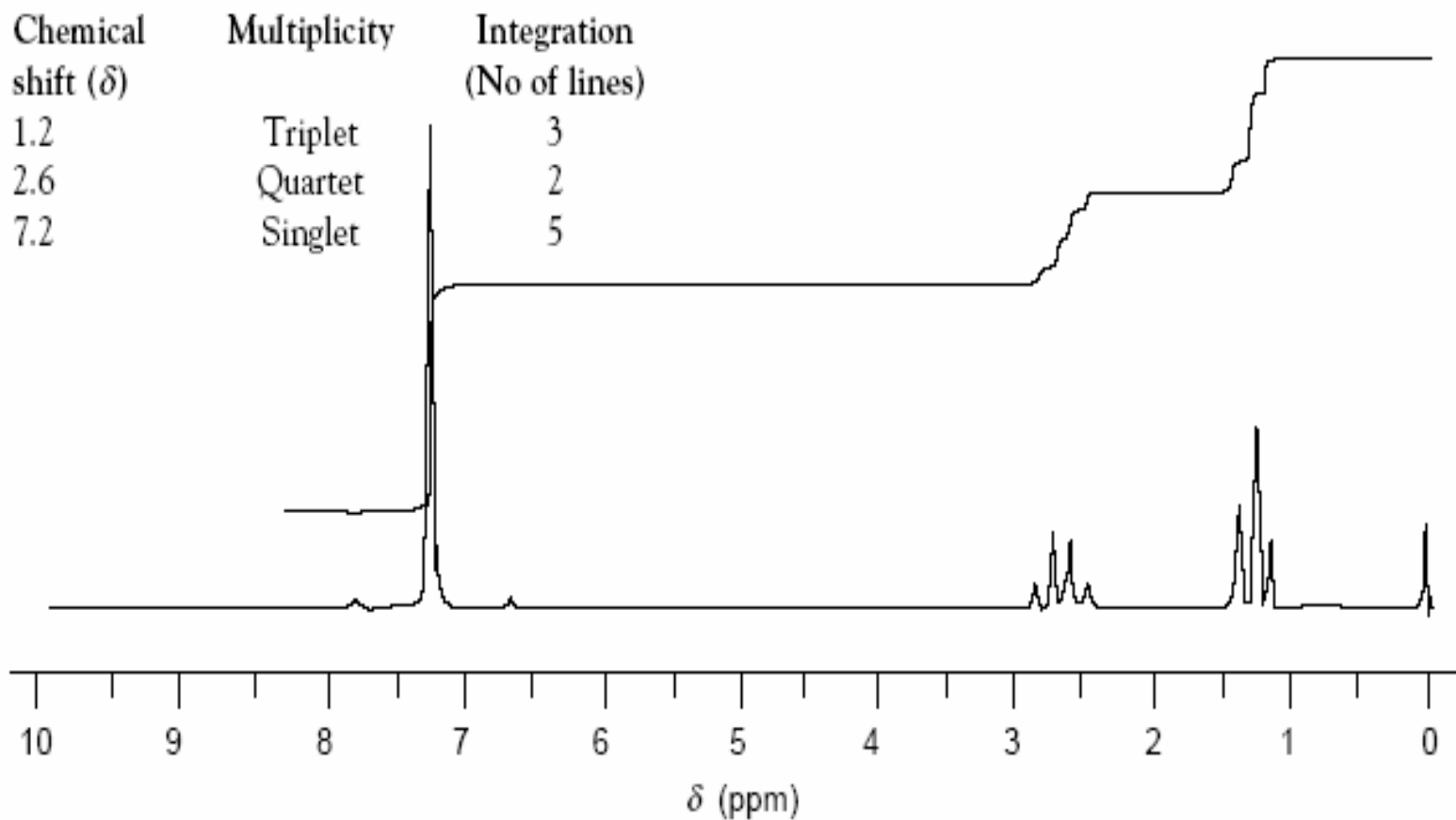
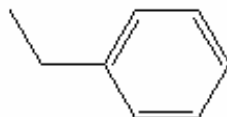
Karplus Equation:

$$J_{HH'}(\phi) = A + B \cdot \cos(\phi) + C \cdot \cos(2 \cdot \phi)$$



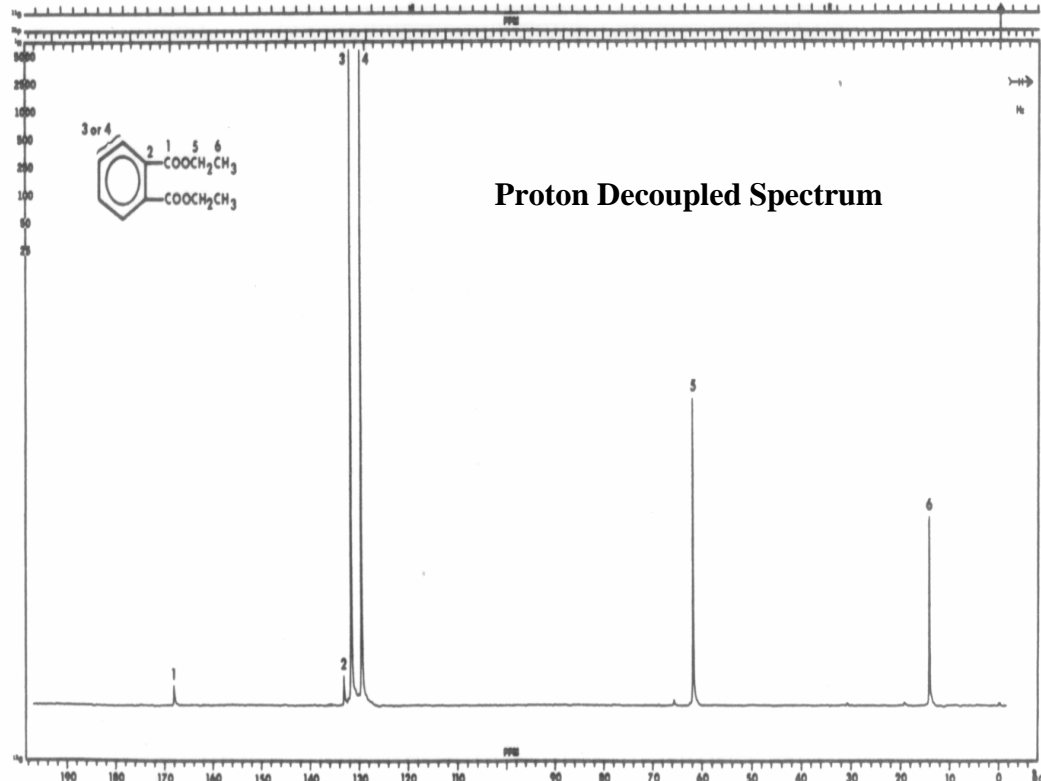
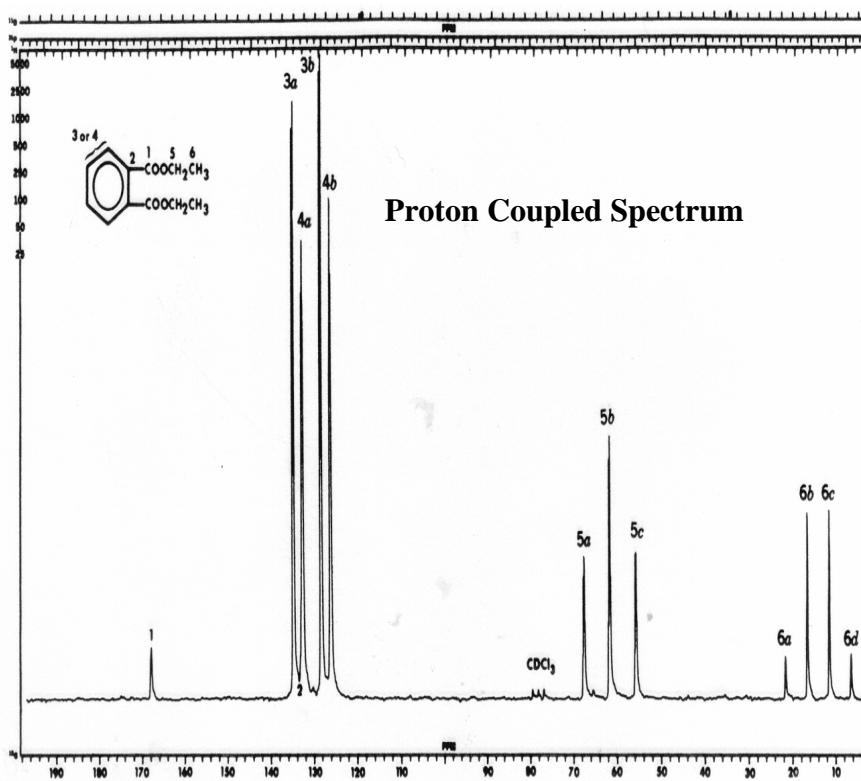


# NMR Concepts - Integration

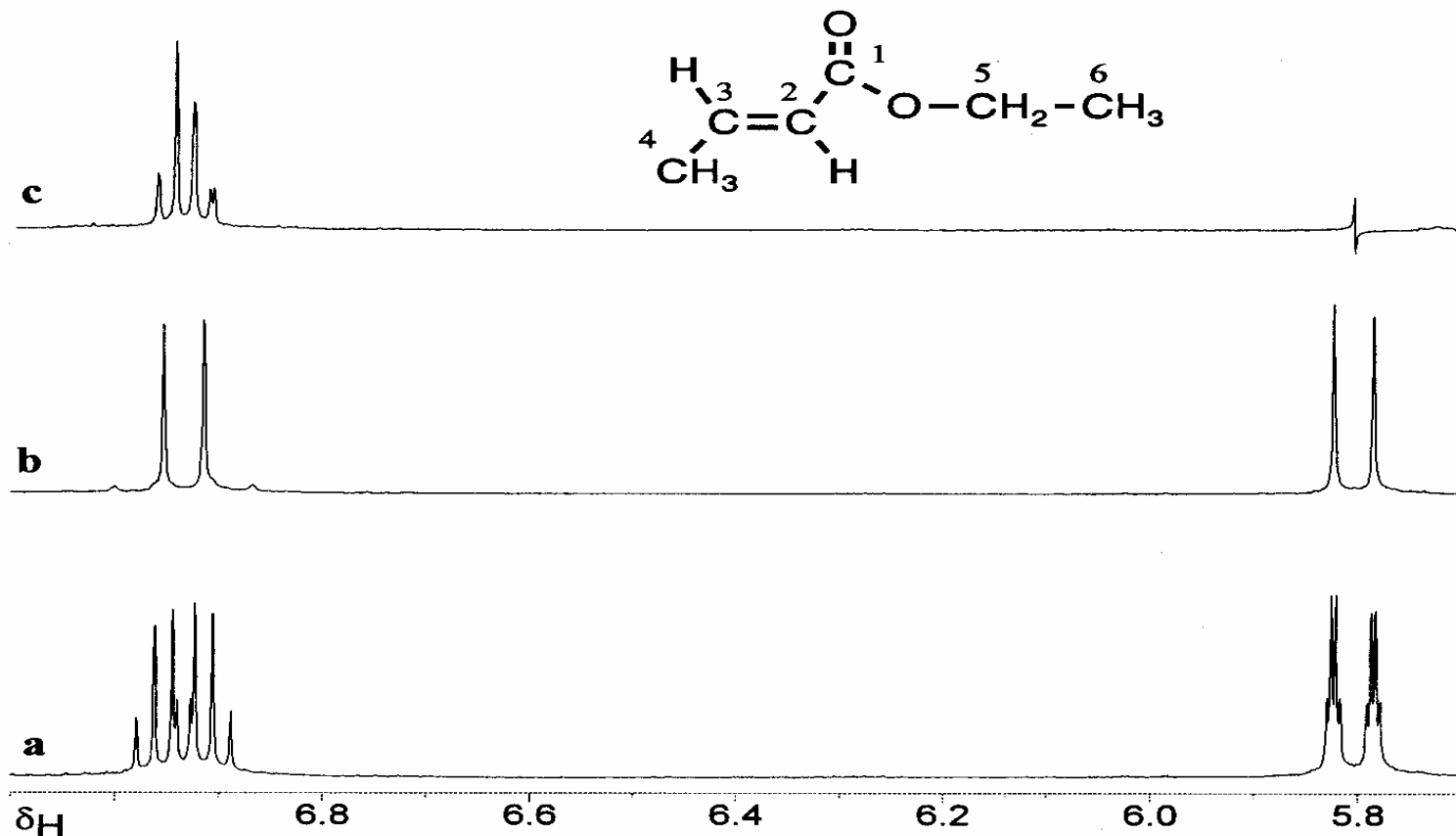


# $^{13}\text{C}$ NMR

- $^{13}\text{C}$  has  $I = 1/2$ ; its natural abundance is 1.1%;
- $^{13}\text{C}$  sensitivity is only 1/5700 that of  $^1\text{H}$ ;
- $^{13}\text{C}$  experiments require higher concentrations and more scans/time.
- S/N increases with square root of # of scans



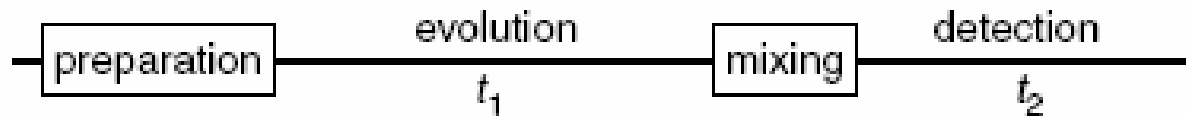
# THE HOMONUCLEAR DECOUPLING EXPERIMENT



- Expanded  $^1\text{H}$  spectrum for ethyl crotonate. (a) Control spectrum. (b) Spectrum with 4-Me group irradiated. (c) Spectrum with H-2 irradiated.

# NMR Concepts – Multiple Dimensions

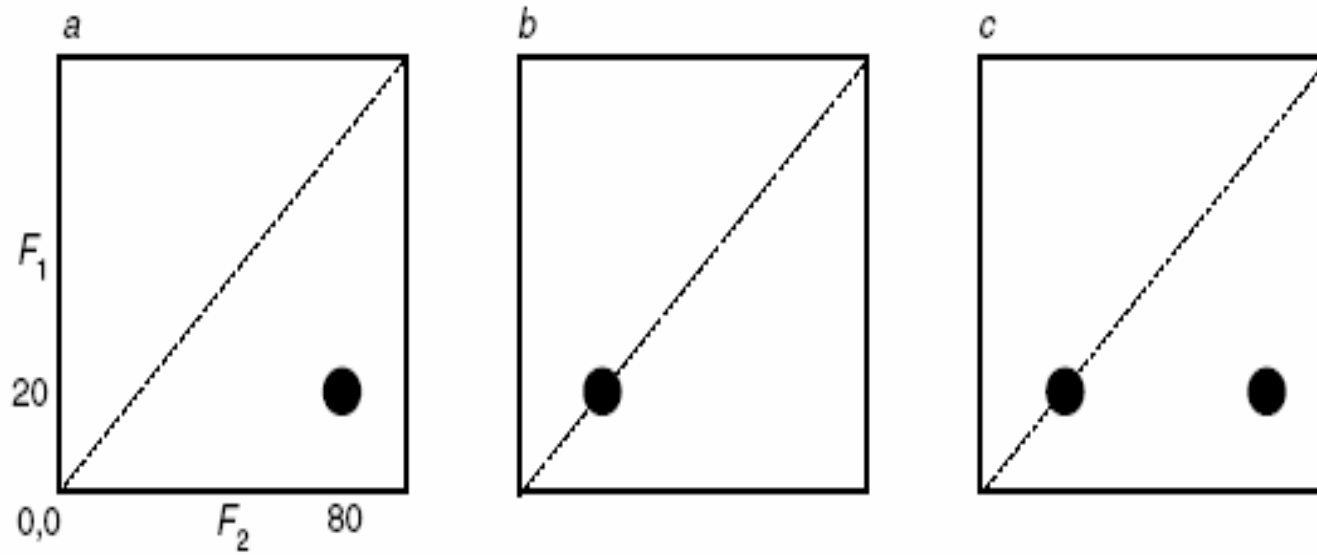
- 2-D NMR – Signal is recorded as a function of two time variables,  $t_1$  and  $t_2$ .



Pulse Sequence

- Rf pulses are generally applied during the preparation and mixing periods.

# NMR Concepts – Multiple Dimensions

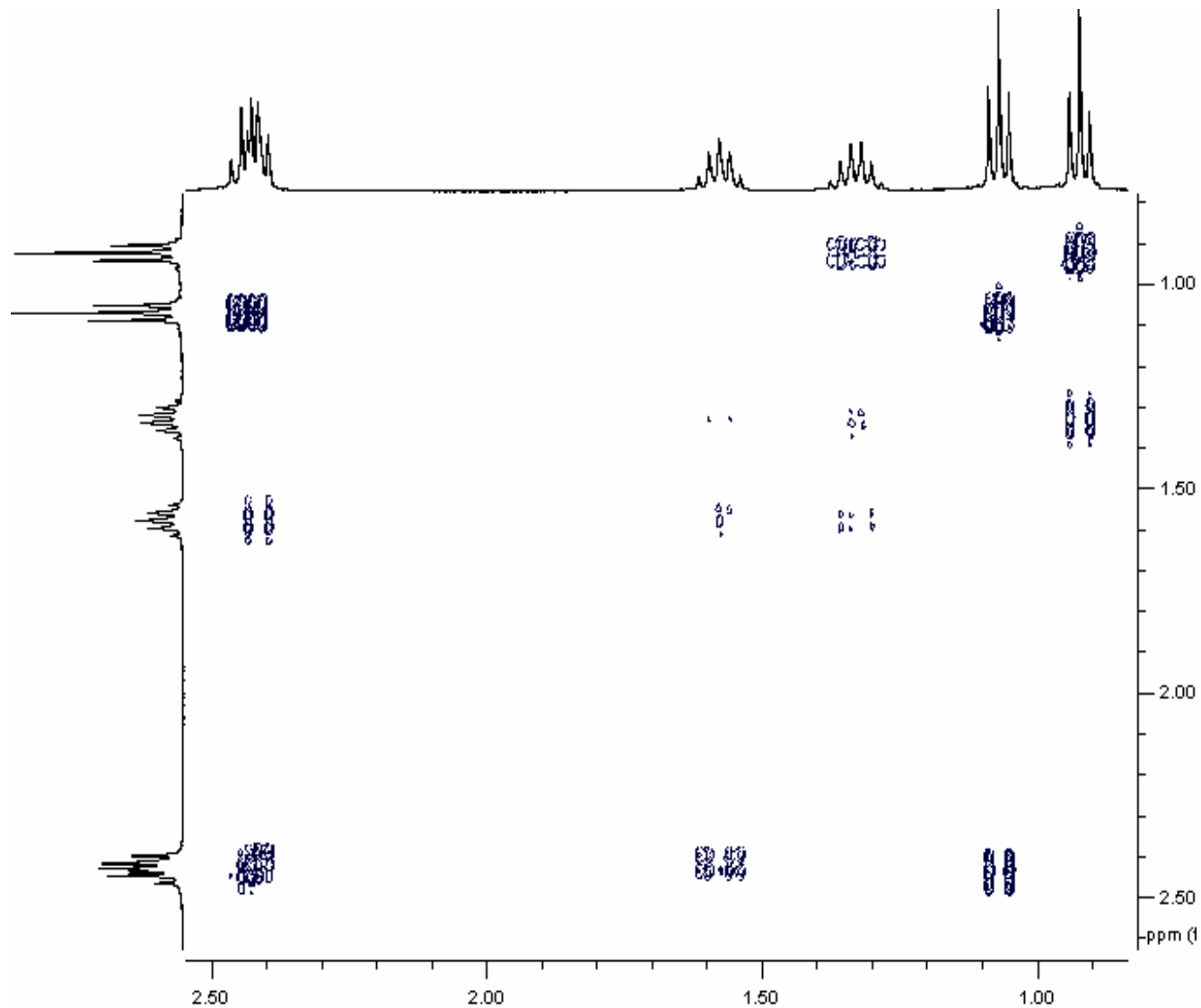


- Signal evolves at 20Hz during  $t_1$  and is transferred to a different signal evolving at 80Hz during  $t_2$ .
- Signal evolving at 20Hz during  $t_1$  was unaffected by mixing period and therefore continued evolving at 20Hz during  $t_2$ .
- Signal evolving at 20Hz during  $t_1$  imparted some of its magnetization onto a different signal evolving at 80Hz during the mixing period. Both signals are detected during  $t_2$ .

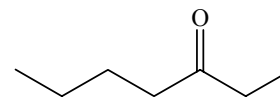
# NMR Concepts – Multiple Dimensions

- Routine 2-D NMR Experiments:
  - Correlation SpectroscopY (COSY) – Scalar Coupling
    - » Identifies all coupled spins systems.
  - Nuclear Overhauser Effect SpectroscopY (NOESY) – Dipolar Coupling
    - » Identifies neighboring spin systems ( $\leq 5 \text{ \AA}$ )
    - » Identifies chemical exchange.
  - Heteronuclear Multiple/Single Quantum Correlation (HMQC/HSQC) – Scalar Coupling
    - » Identifies coupling between heteronuclei (C-H, N-H).

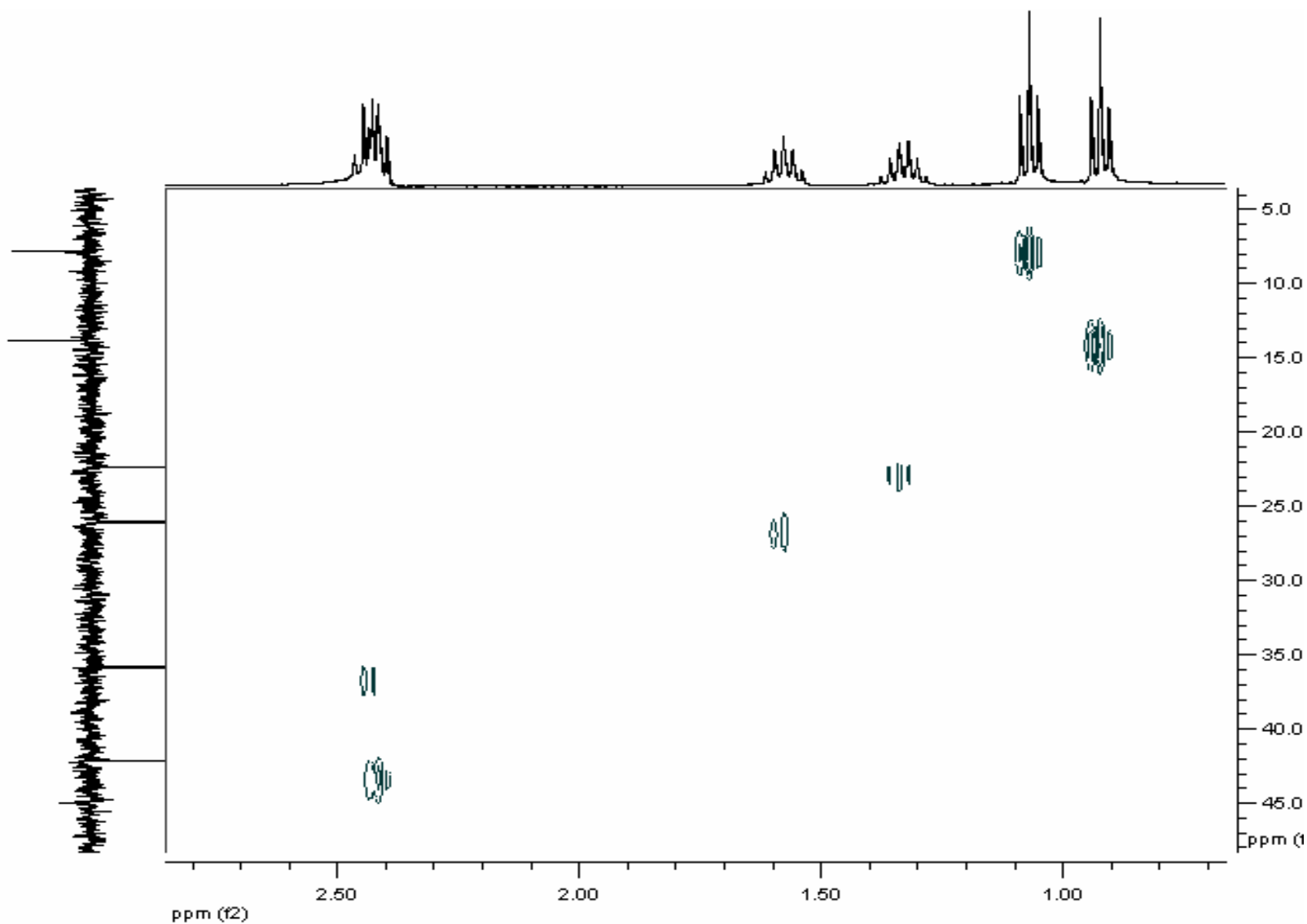
# NMR Concepts – COSY Experiment



COSY spectrum of 3-heptanone



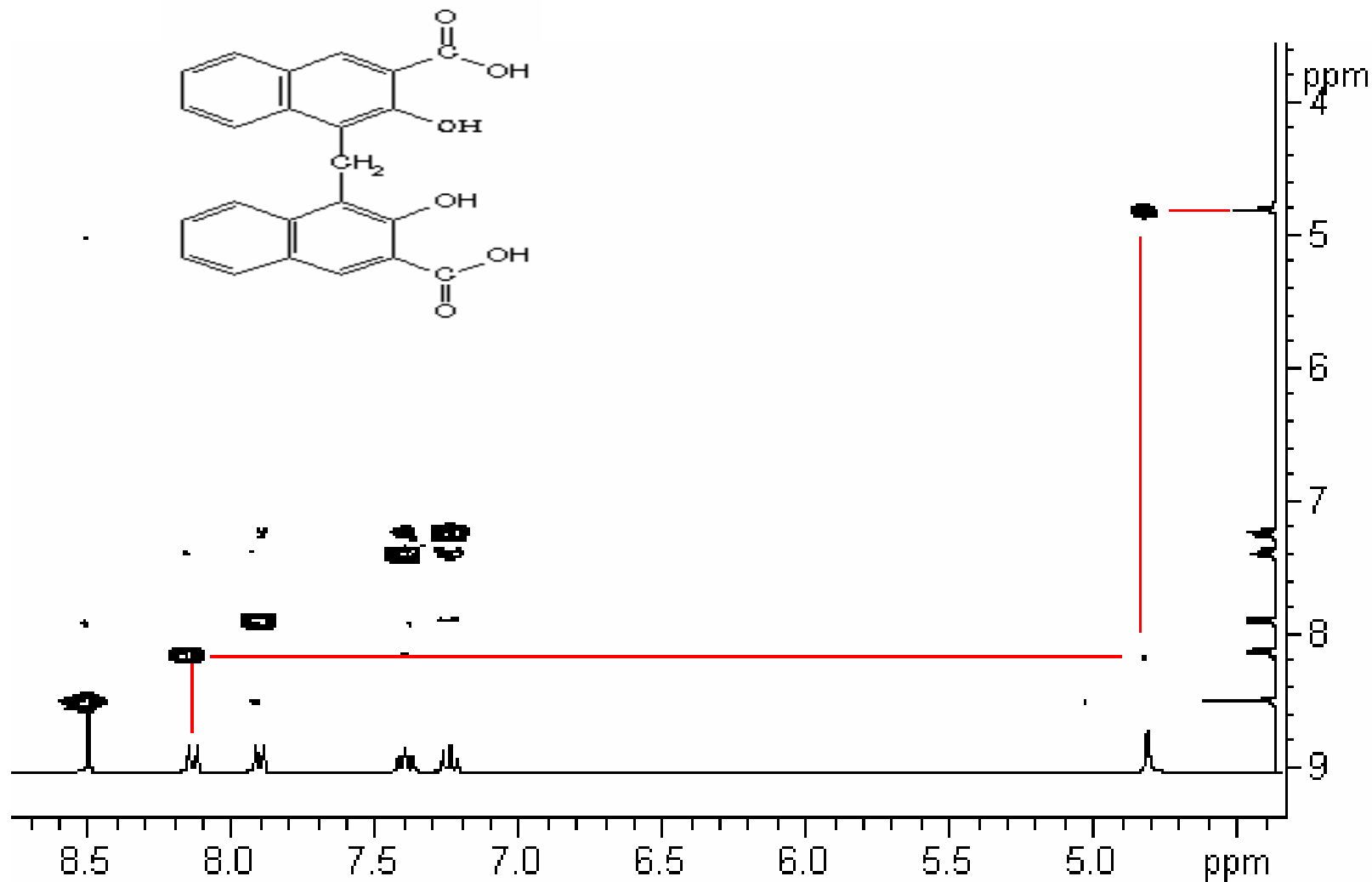
# NMR Concepts – HMQC Experiment



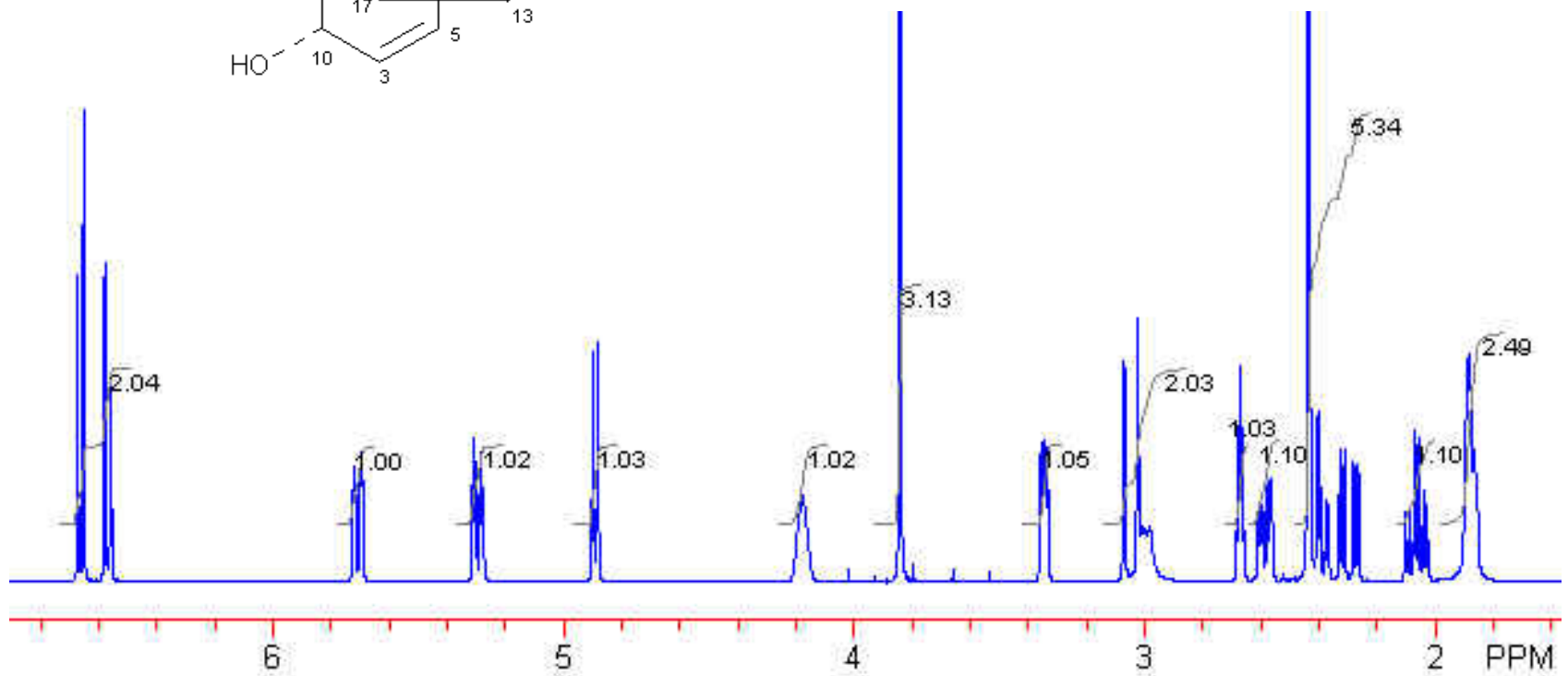
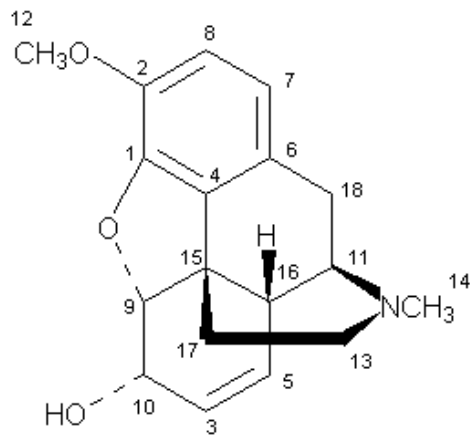
HMQC Spectrum of heptanone



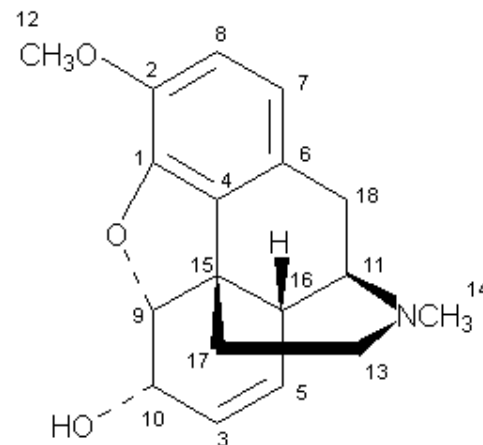
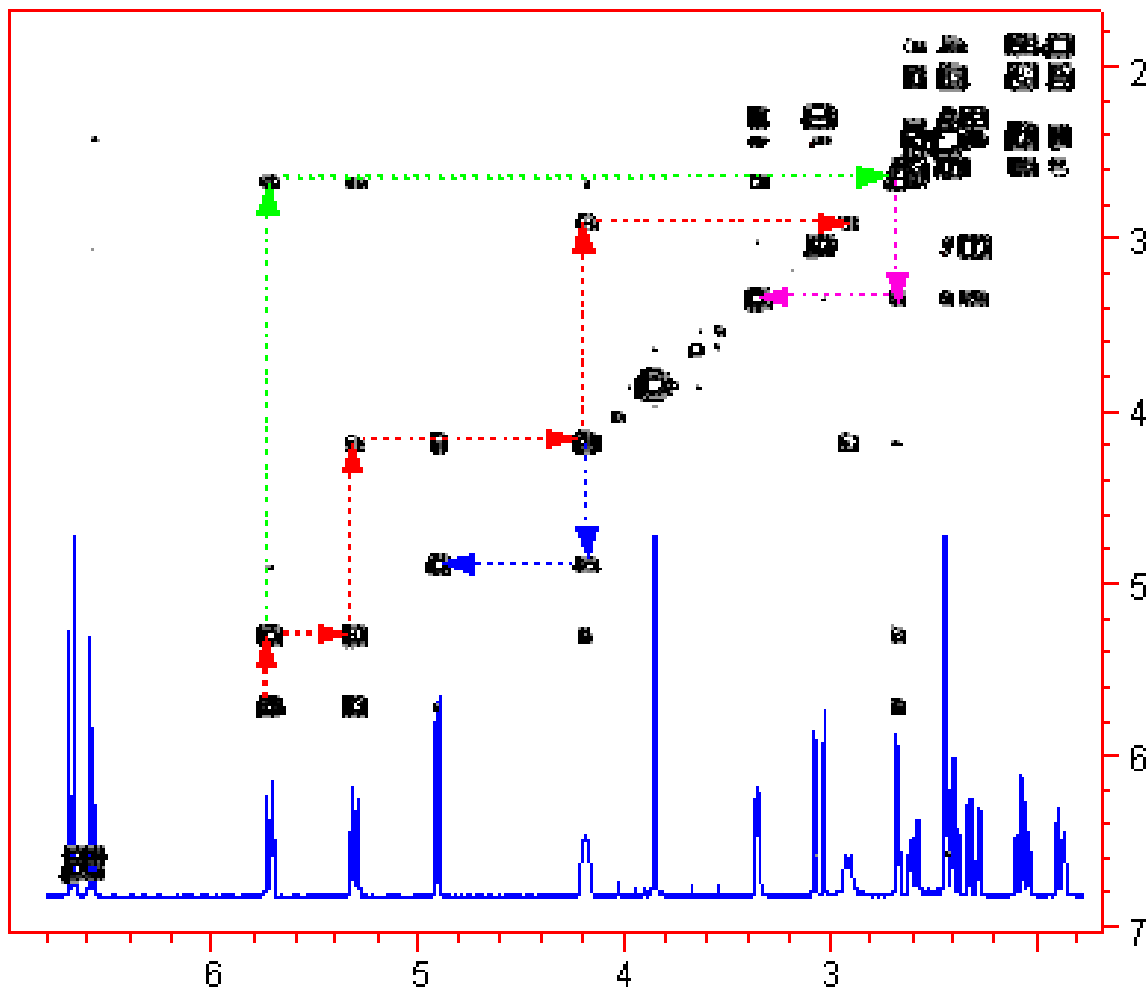
# NMR Concepts – NOESY Experiment



# Where to Begin?

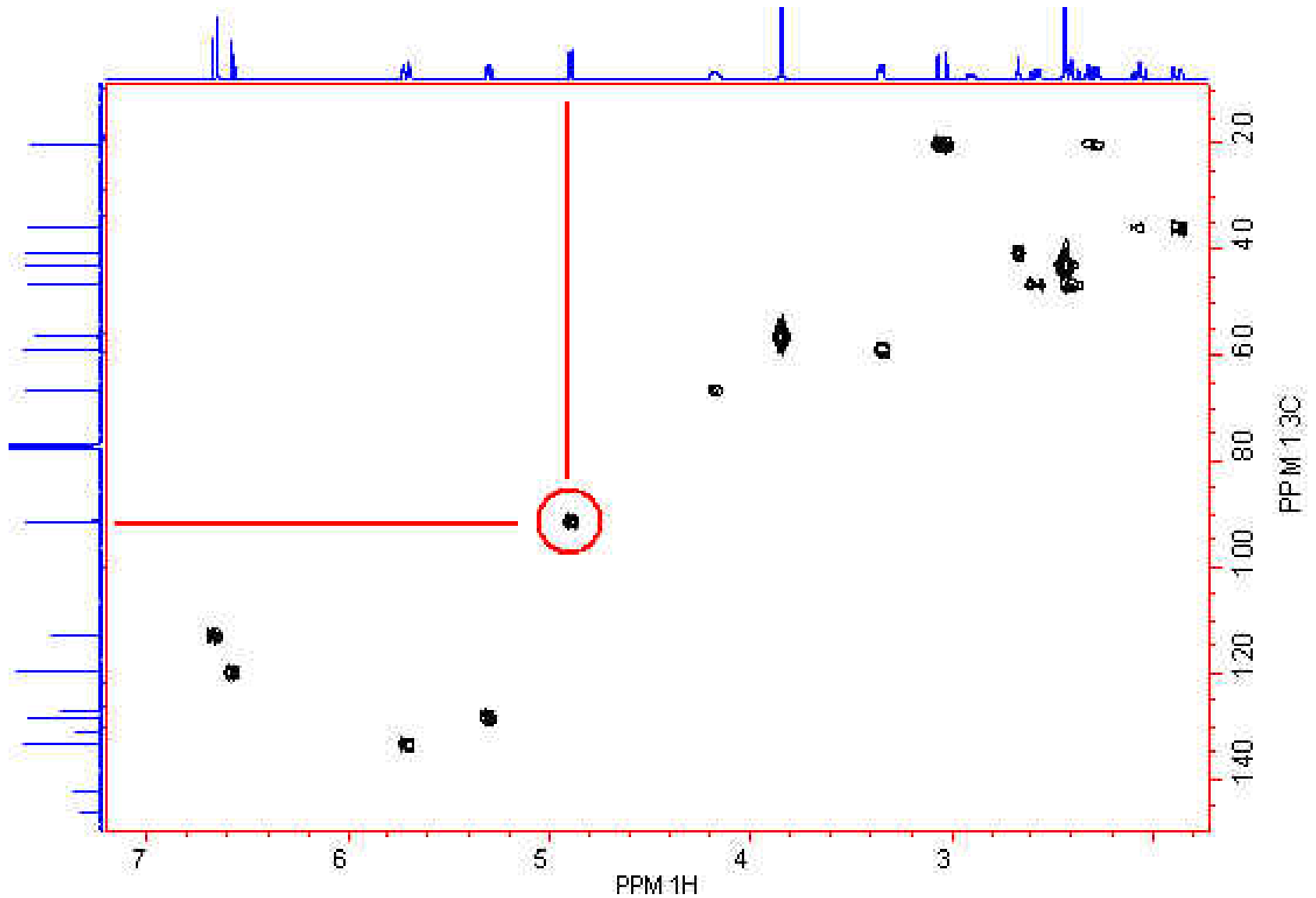


# COSY Spectrum of Codeine



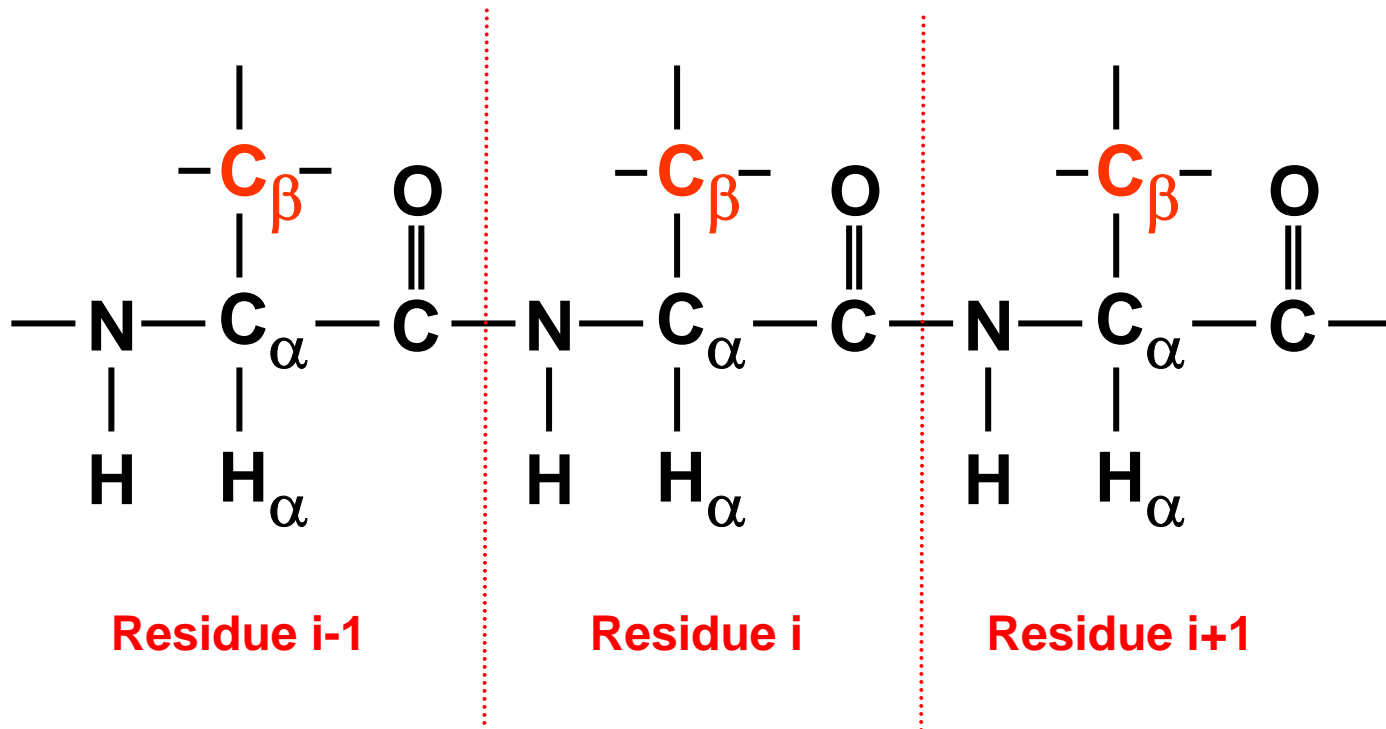
1H	13C	Assignment
6.6	113	8
6.5	120	7
5.7	133	5
5.3	128	3
4.8	91	9
4.2	66	10
3.8	56	12
3.3	59	11
3.0 & 2.3	20	18
2.6	40	16
2.6 & 2.4	46	13
2.4	43	14
2.0 & 1.8	36	17

# HMQC of Codeine



Break

# Proteins



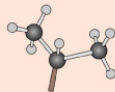
# 20 Amino Acids



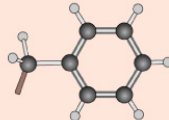
## Hydrophobic



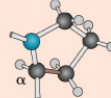
Alanine  
Ala  
A



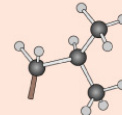
Valine  
Val  
V



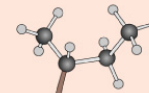
Phenylalanine  
Phe  
F



Proline  
Pro  
P



Leucine  
Leu  
L

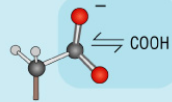


Isoleucine  
Ile  
I

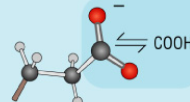
## Hydrophilic



Arginine  
Arg  
R



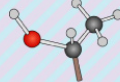
Aspartic acid  
Asp  
D



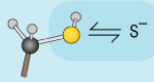
Glutamic acid  
Glu  
E



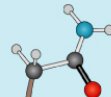
Serine  
Ser  
S



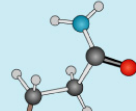
Threonine  
Thr  
T



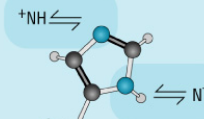
Cysteine  
Cys  
C



Asparagine  
Asn  
N

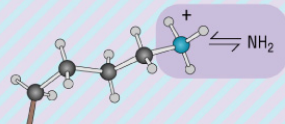


Glutamine  
Gln  
Q

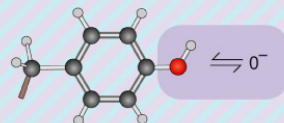


Histidine  
His  
H

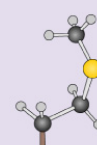
## Amphipathic



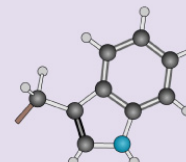
Lysine  
Lys  
K



Tyrosine  
Tyr  
Y



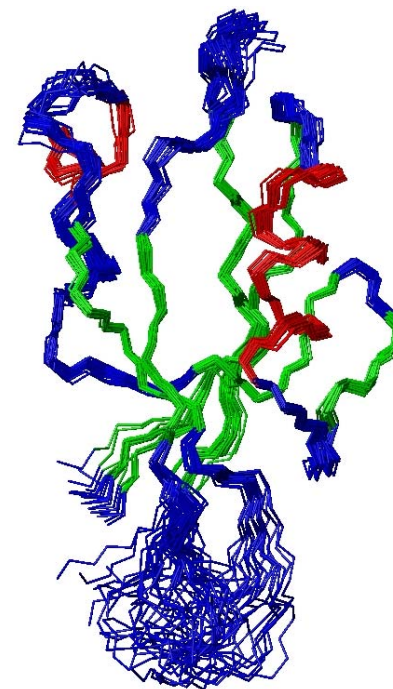
Methionine  
Met  
M



Tryptophan  
Trp  
W

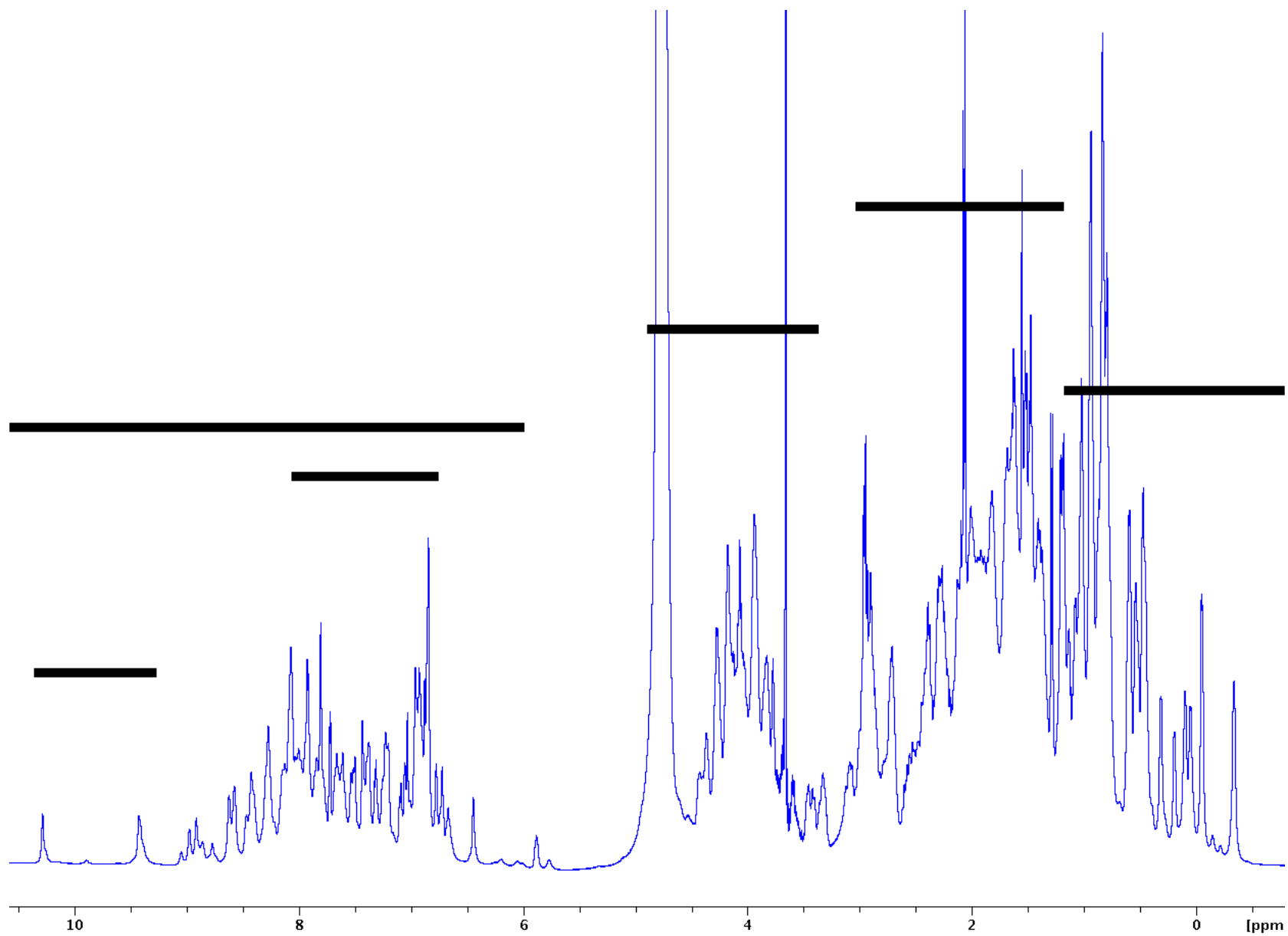
# Sequence .....> Structure?

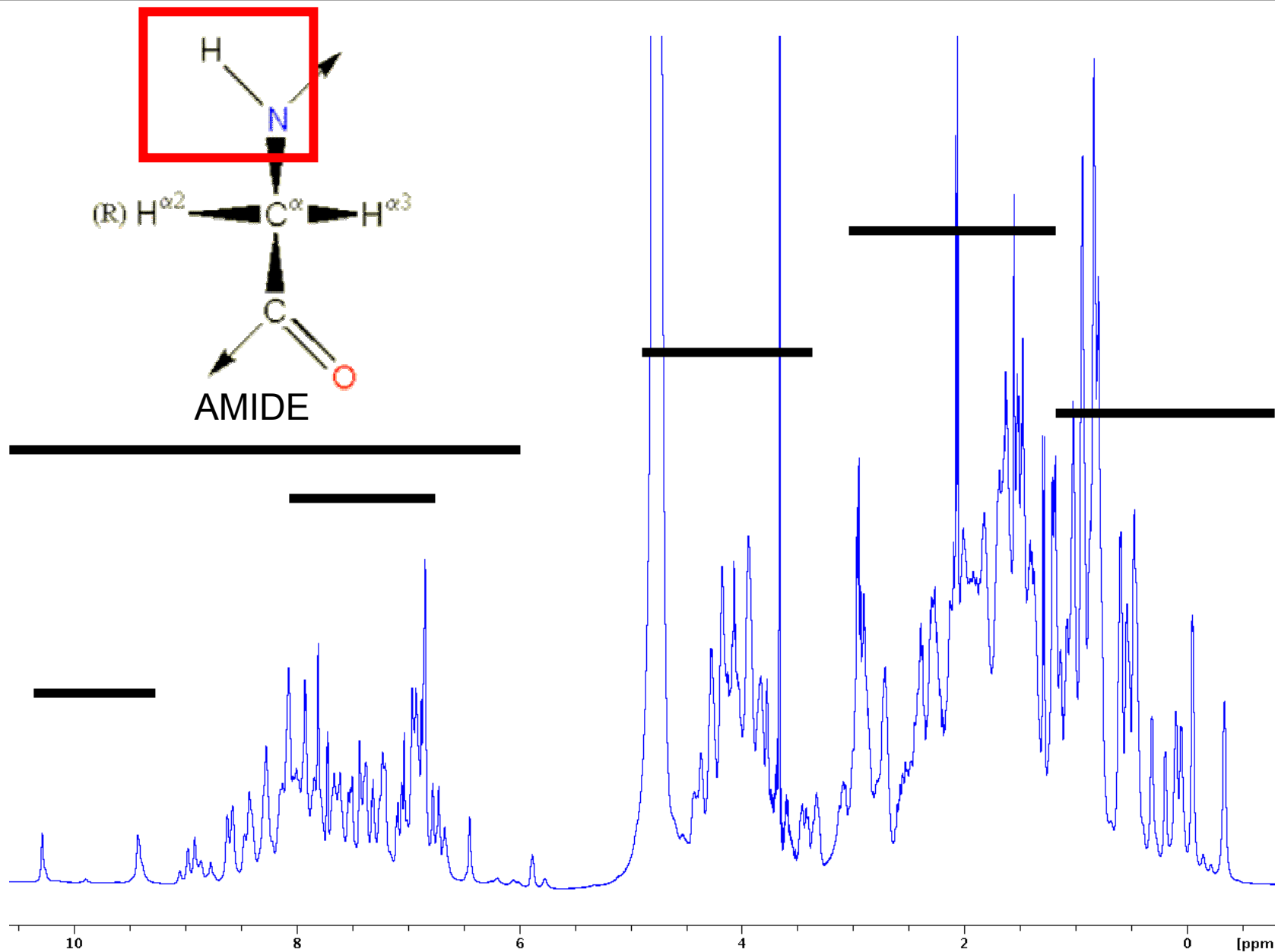
```
GHMELFPVELEKDEDGL  
GSIIGMGVGADAGLEKL  
GIFVKTVTEGGAAQRDG  
RIQVNDQIVEVDGISLVG  
VTQNFAATVLRNTKGNV  
RFVIGREKP
```

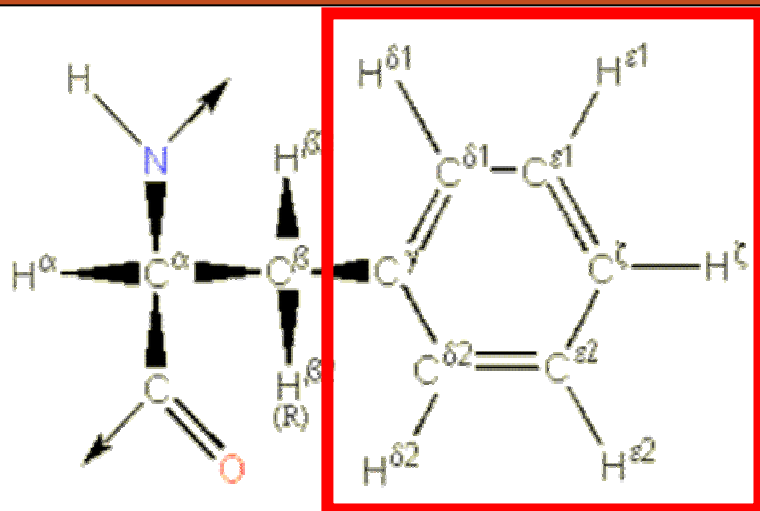


1. Chemical shift assignment
2. Distance measurements (NOE)
3. Structure calculation
4. Structure refinement



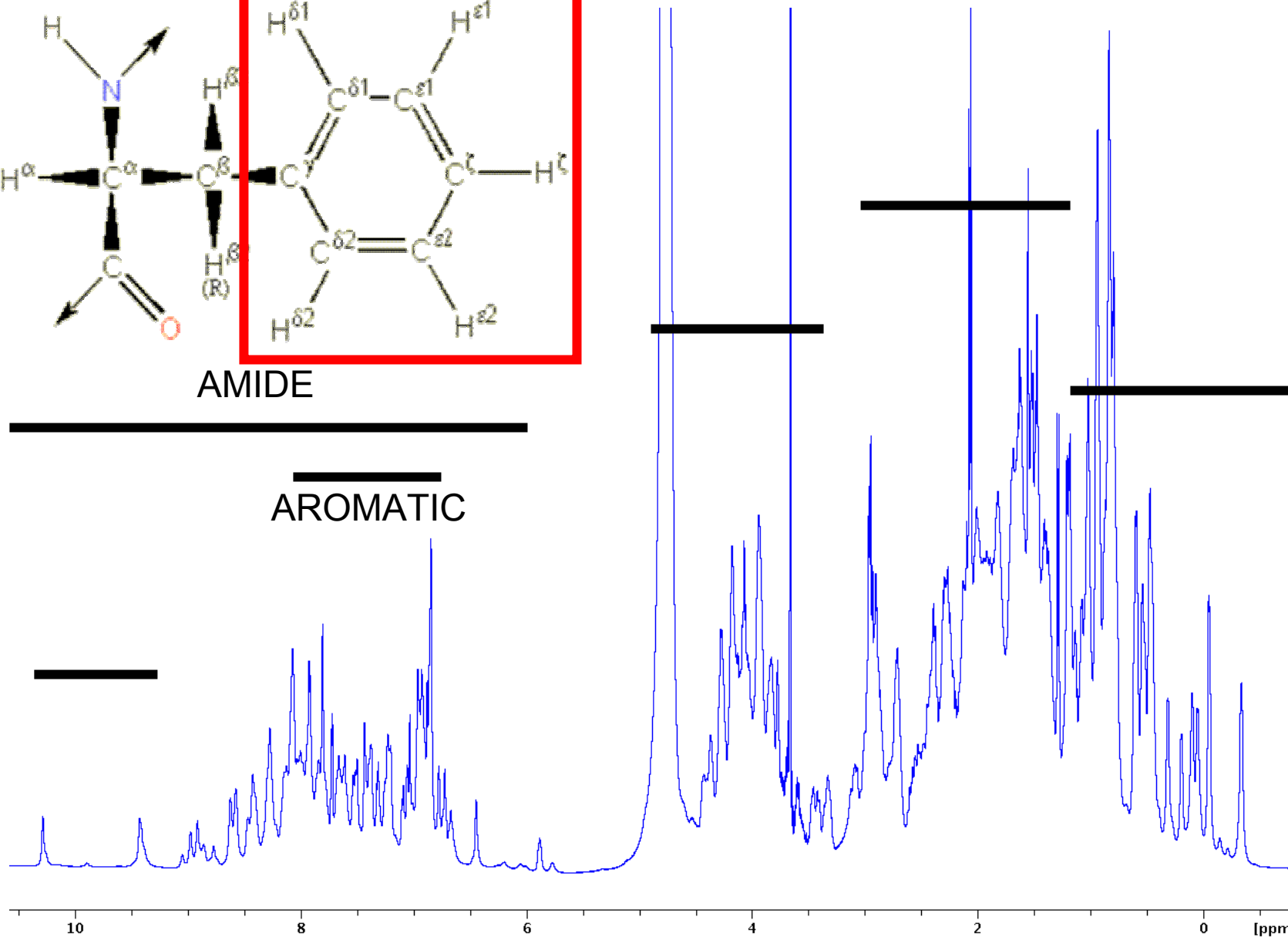




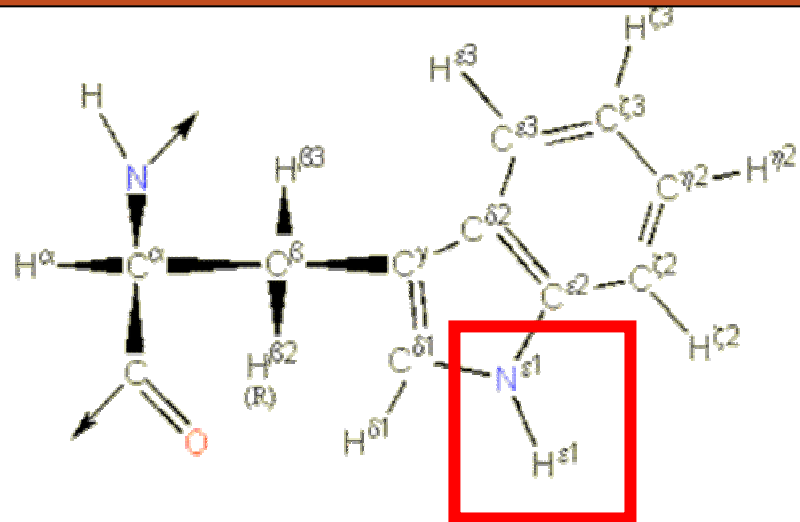


AMIDE

AROMATIC



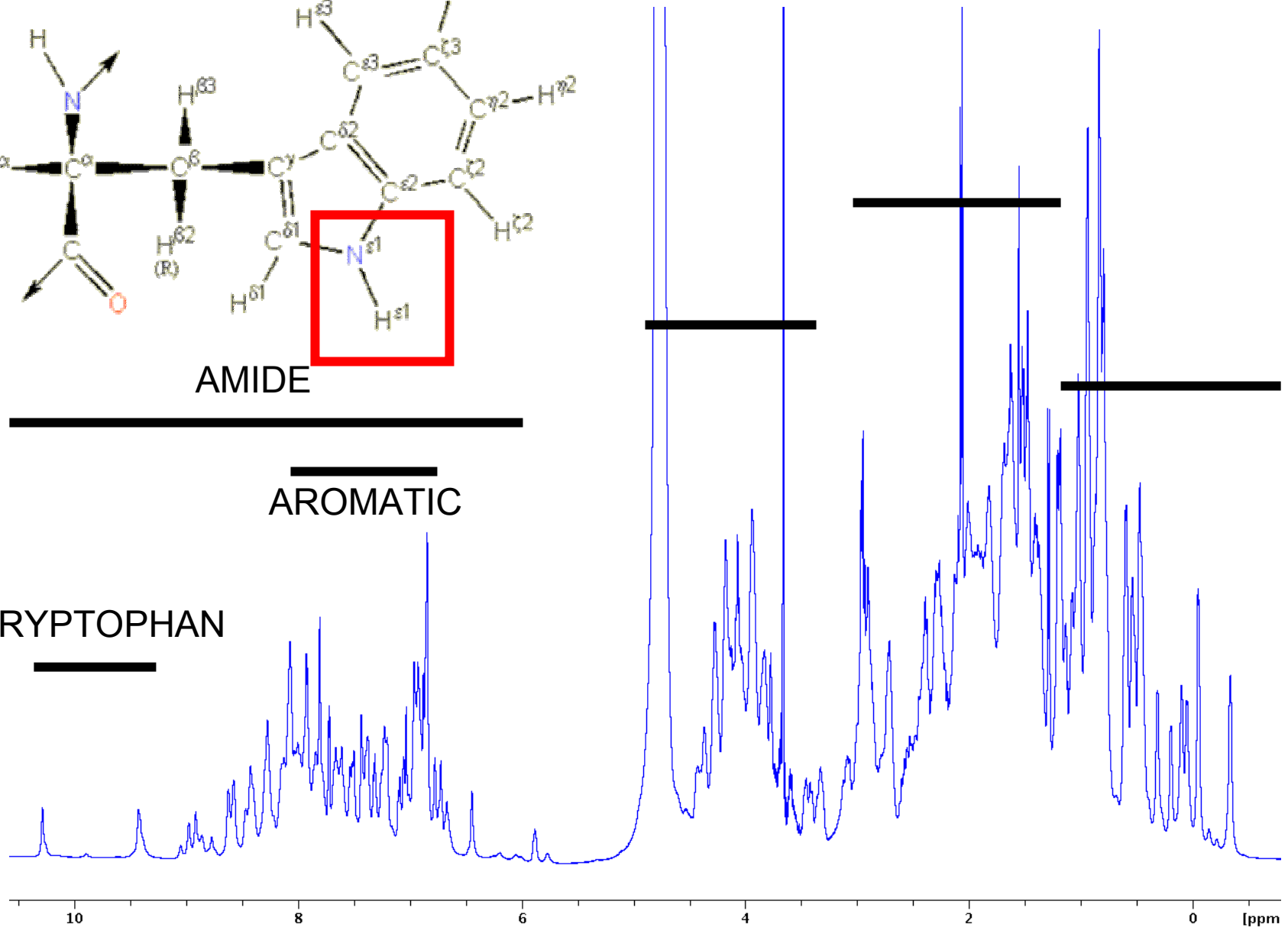
# NMR – W. Peti

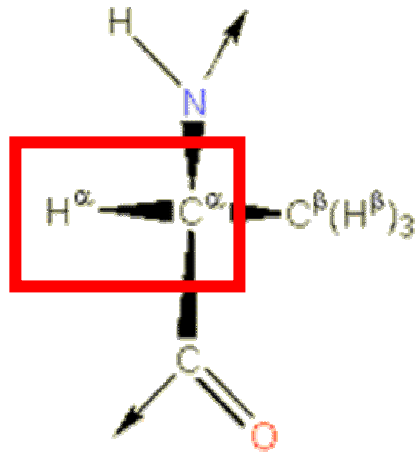


AMIDE

AROMATIC

TRYPTOPHAN



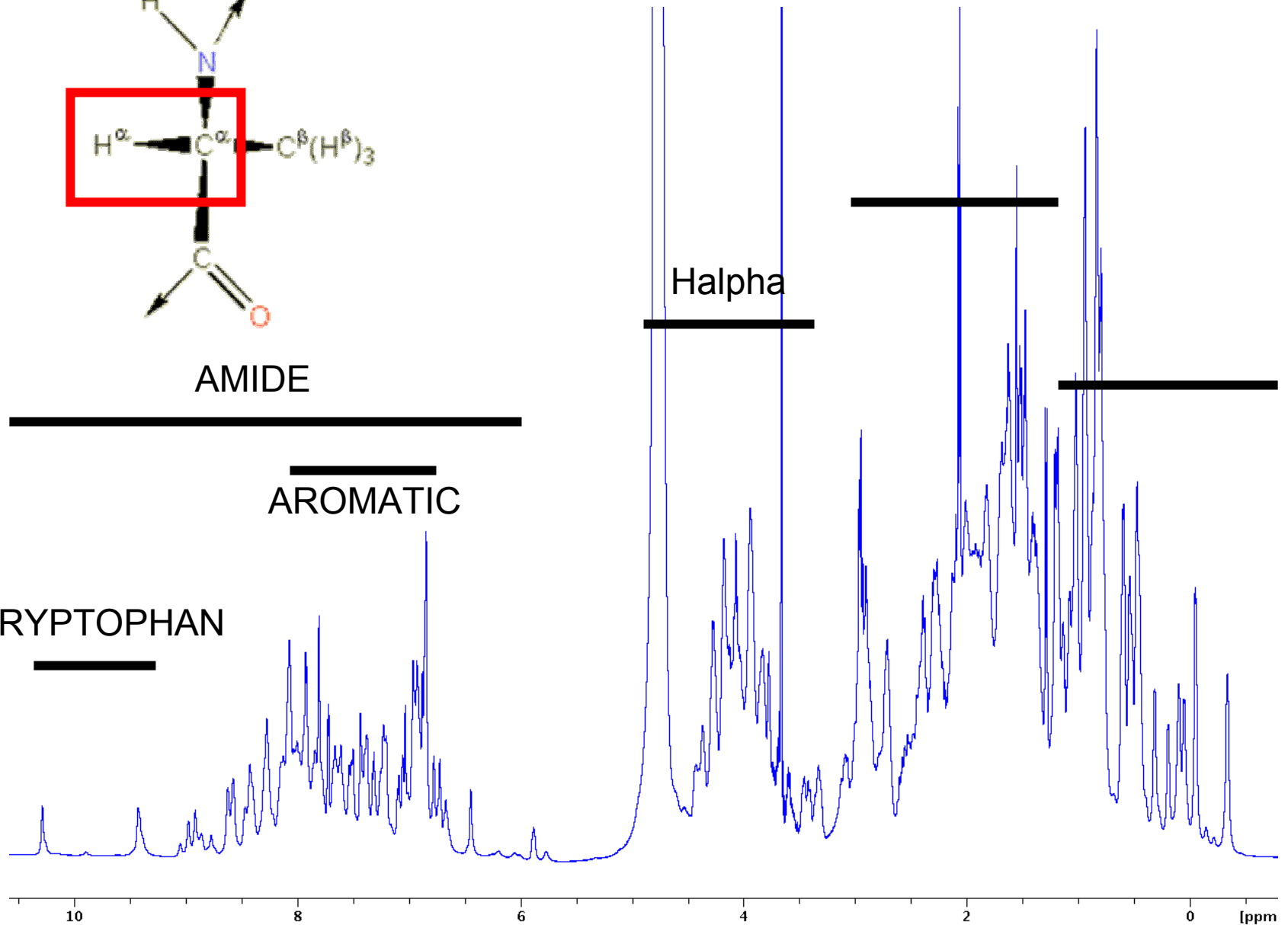


AMIDE

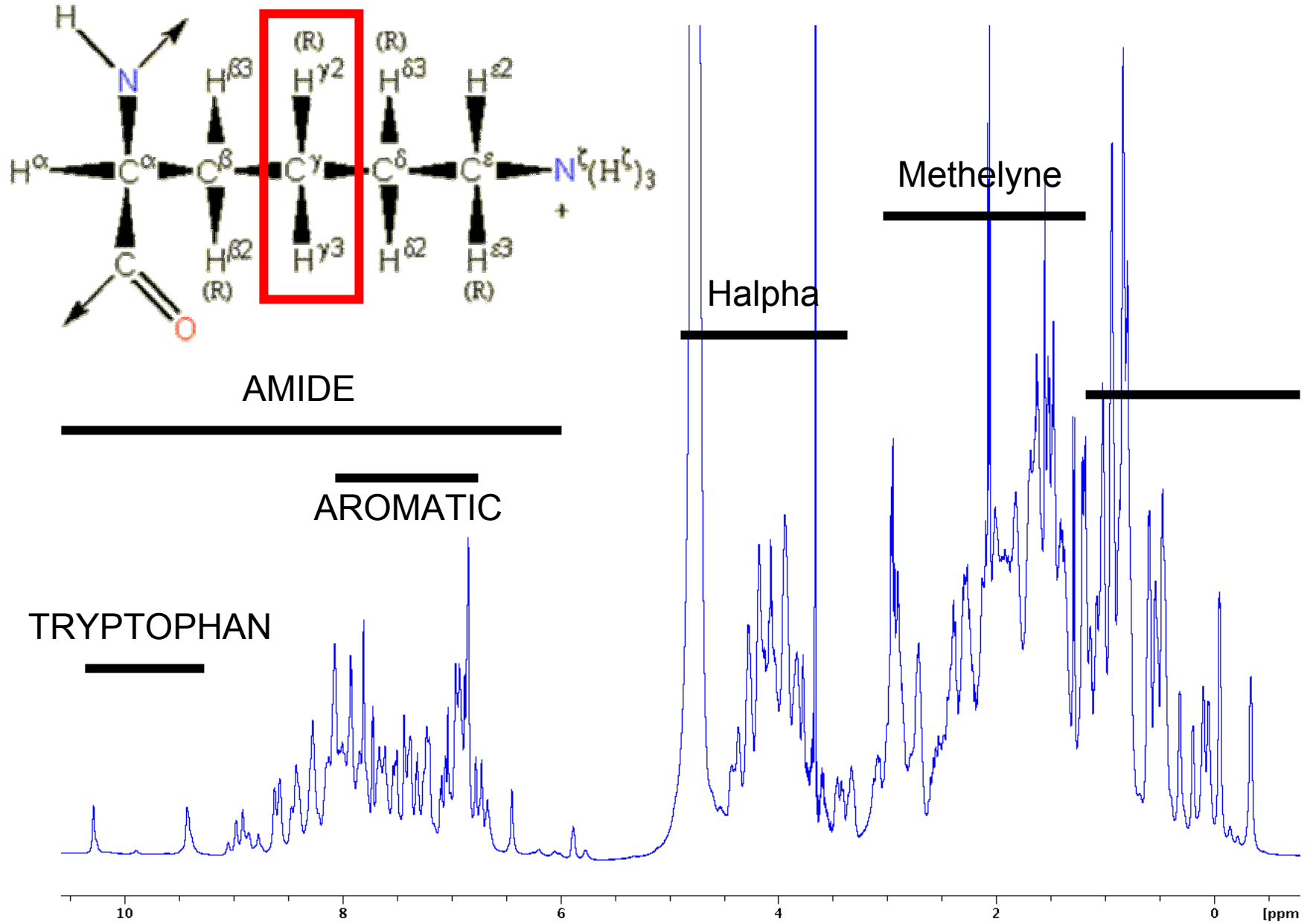
AROMATIC

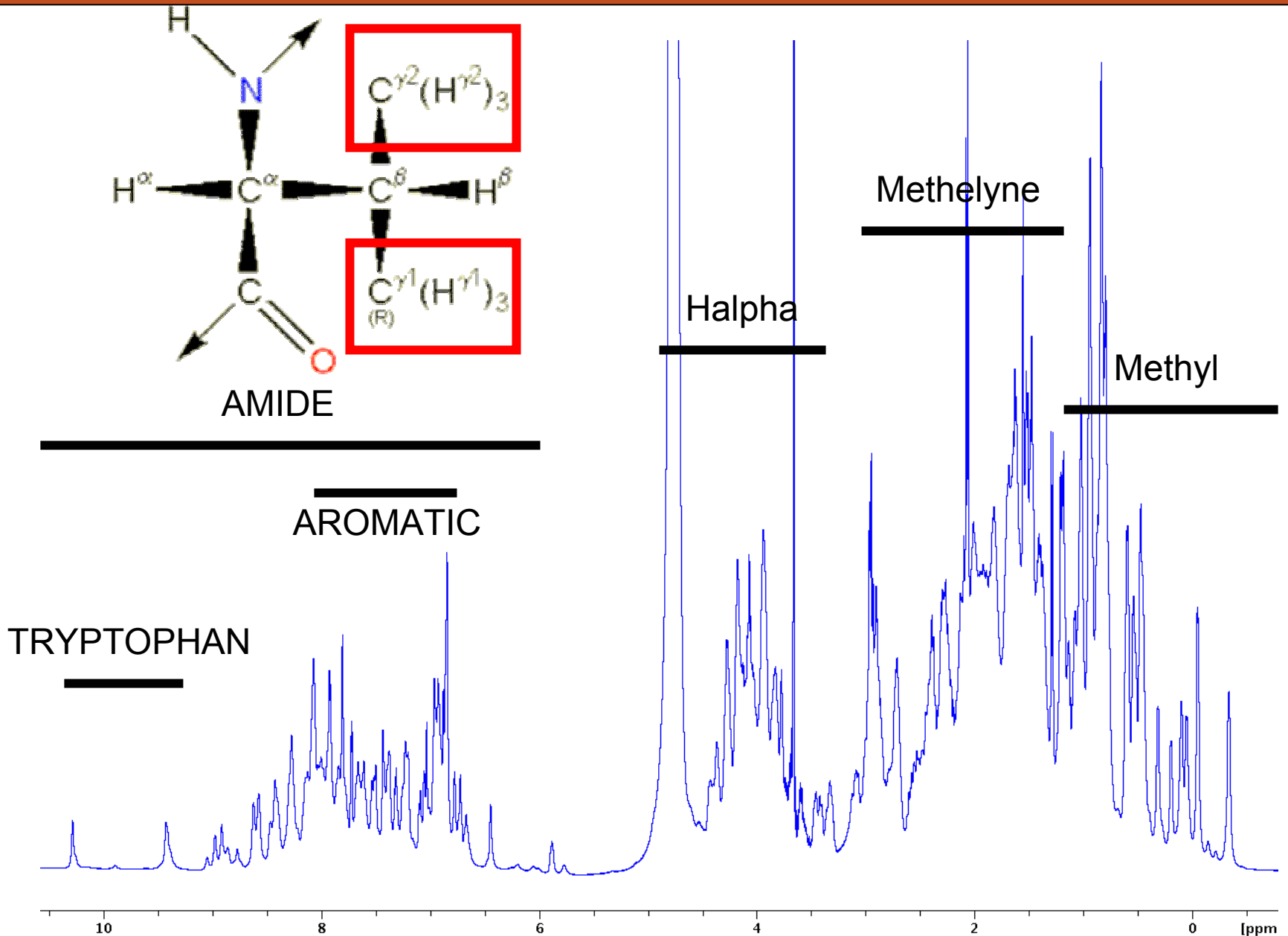
TRYPTOPHAN

$H^{\alpha}$

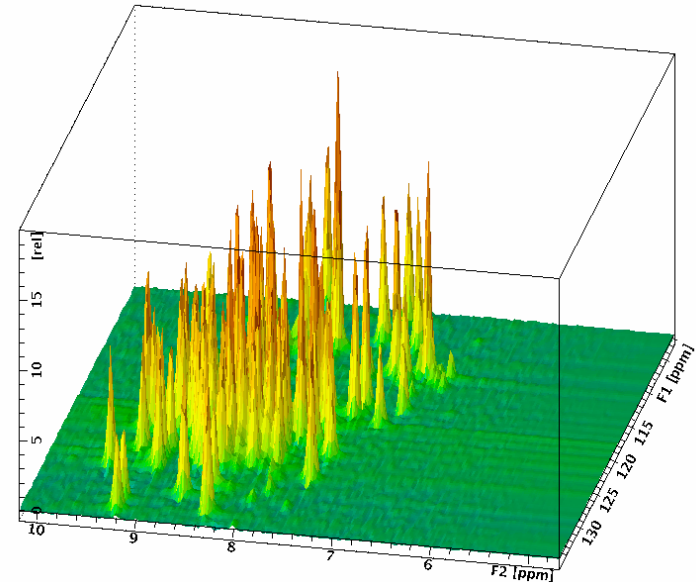
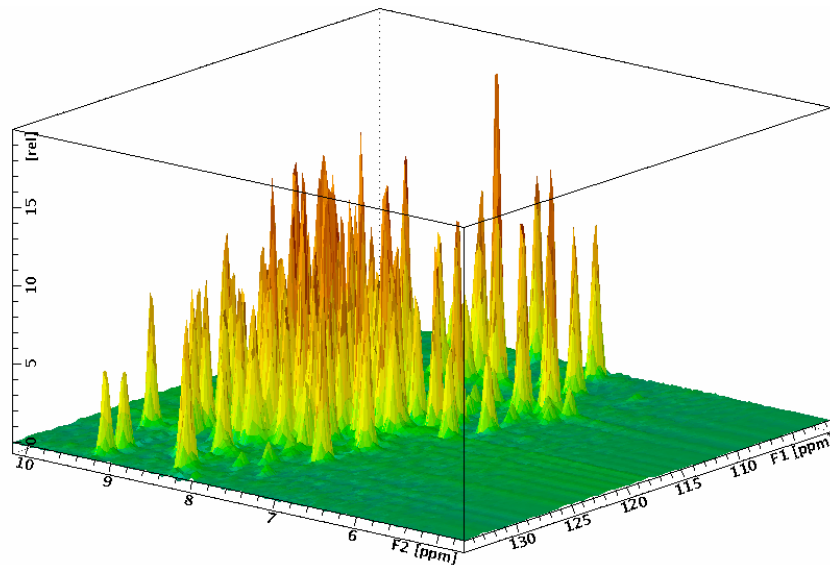
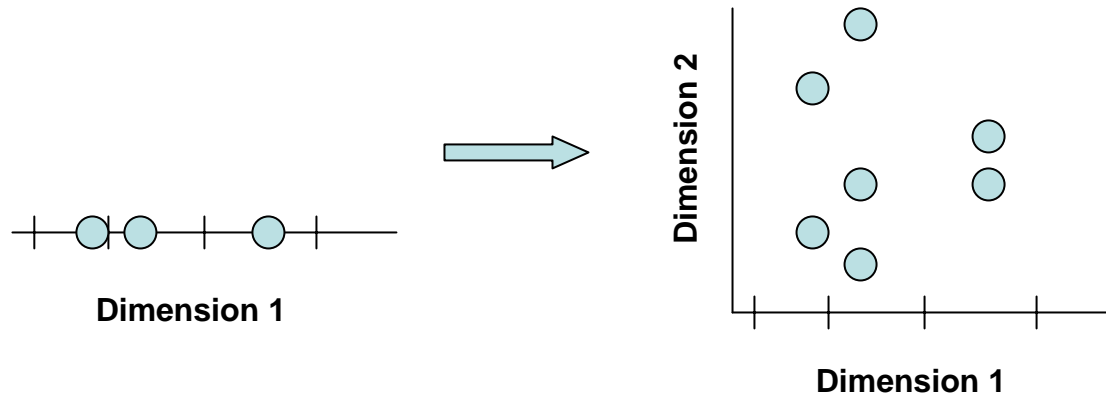


# NMR – W. Peti



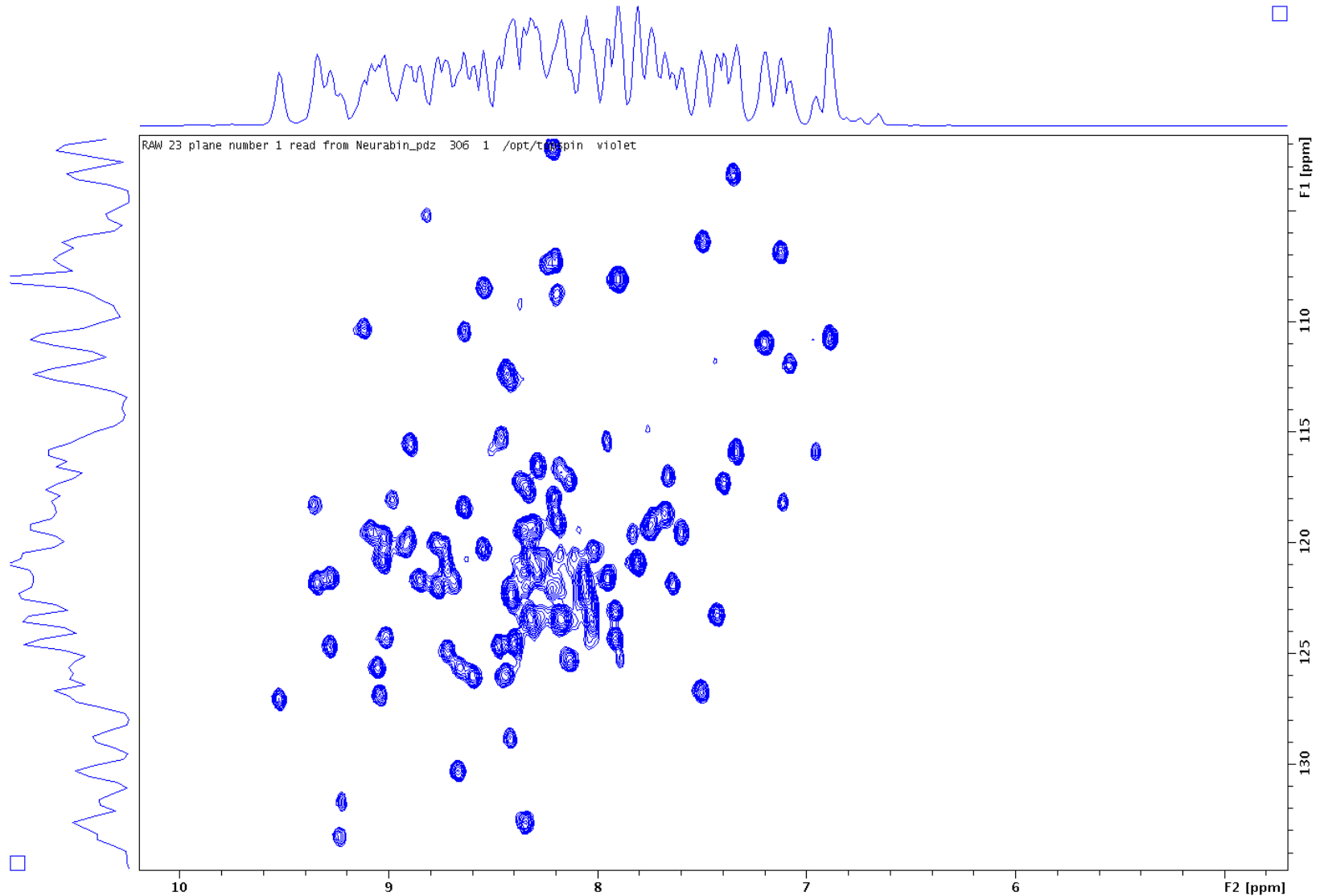


# 2D NMR solves overlap

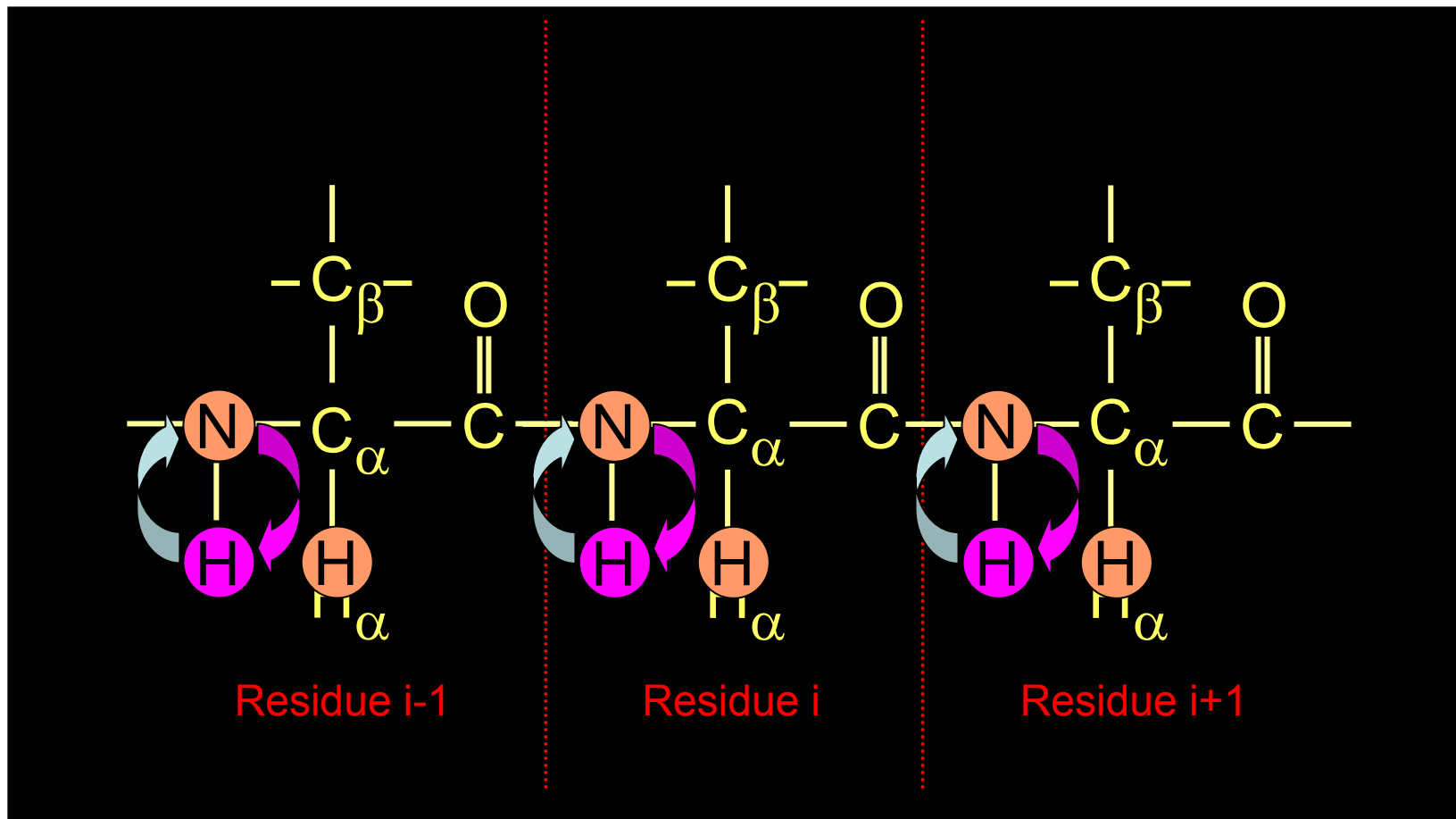




# 2D Protein Spectrum

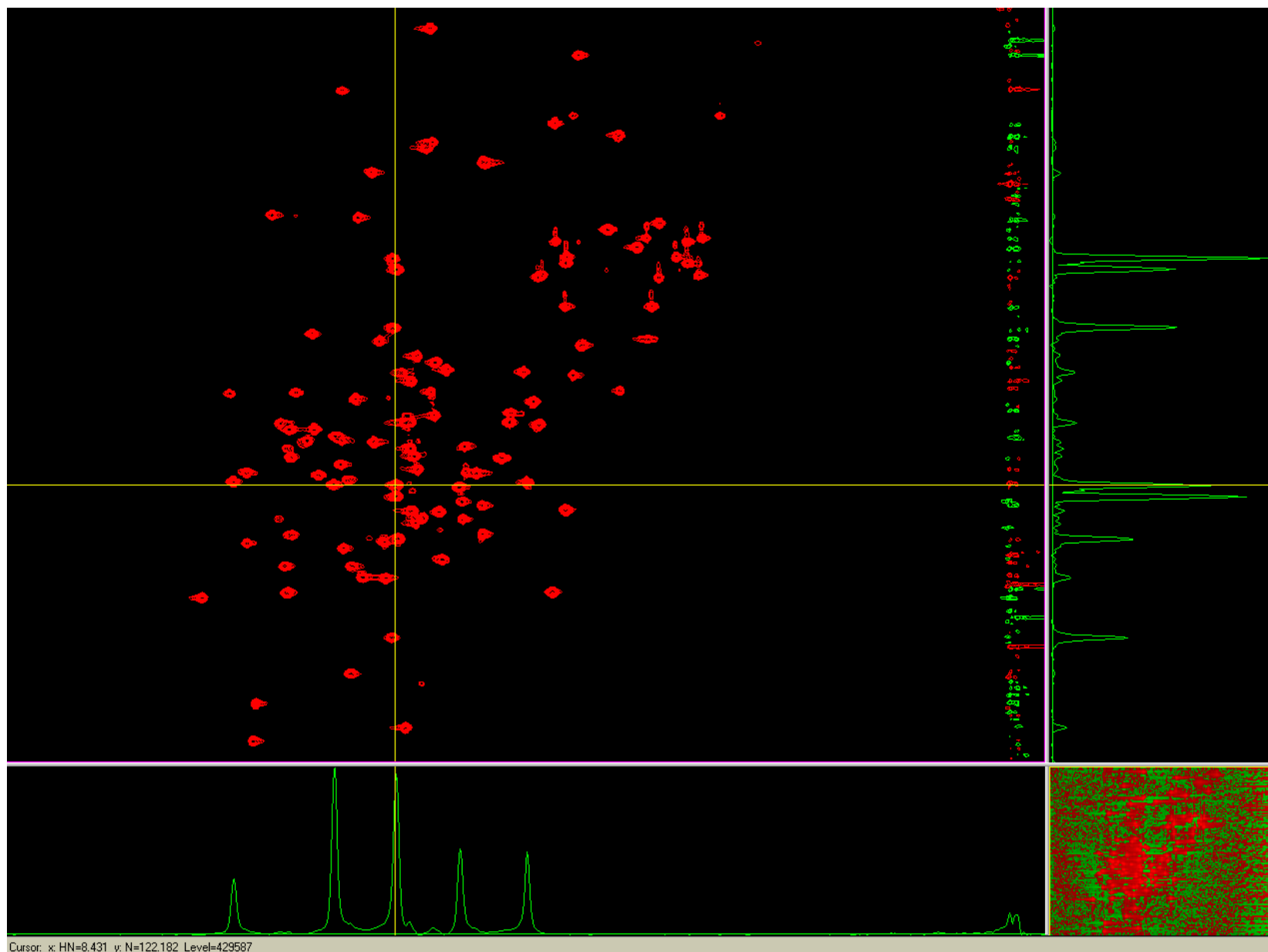


# 2D [ $^1\text{H}$ , $^{15}\text{N}$ ] HSQC



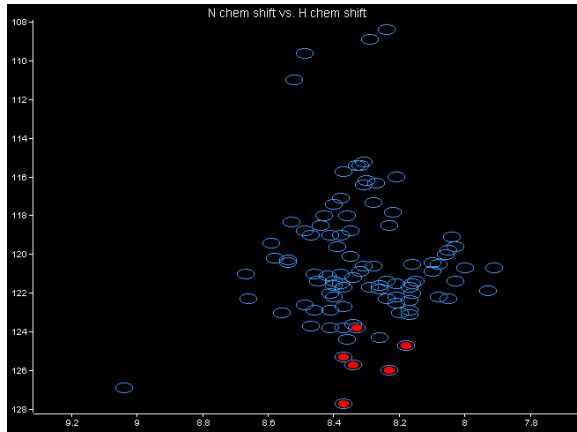
See one peak at intersection of H and N chemical shifts for each amino acid residue (except proline).

Also see side chain NH<sub>2</sub> groups

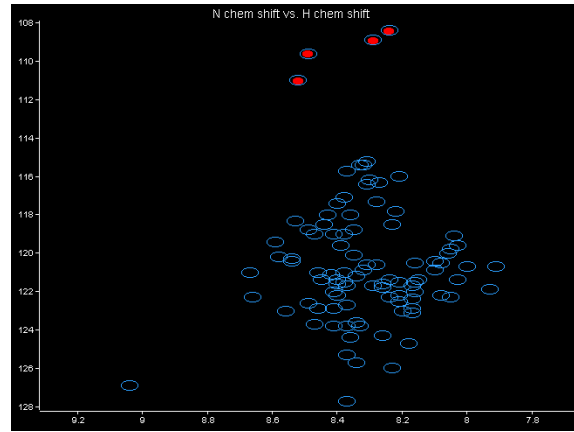


# 2D [ $^1\text{H}$ , $^{15}\text{N}$ ] HSQC

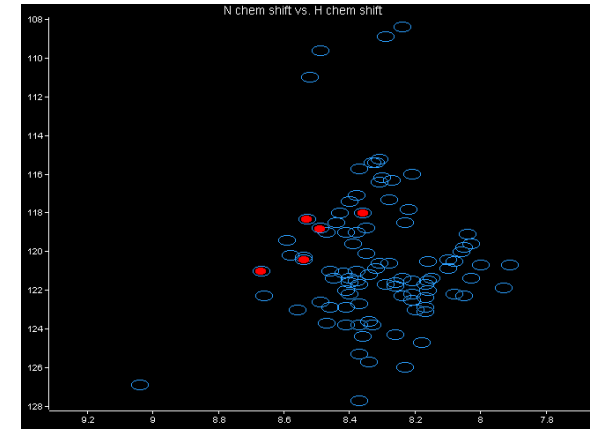
**Alanines**



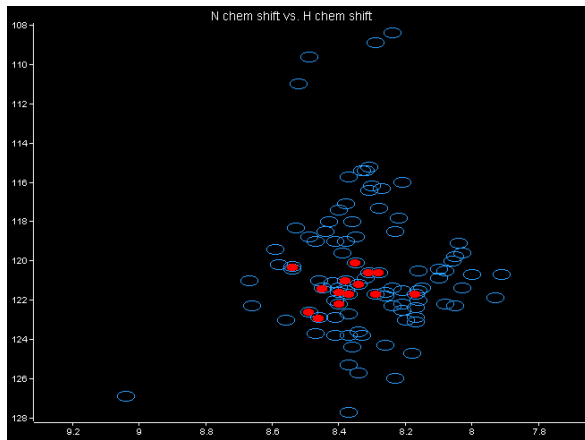
**Glycines**



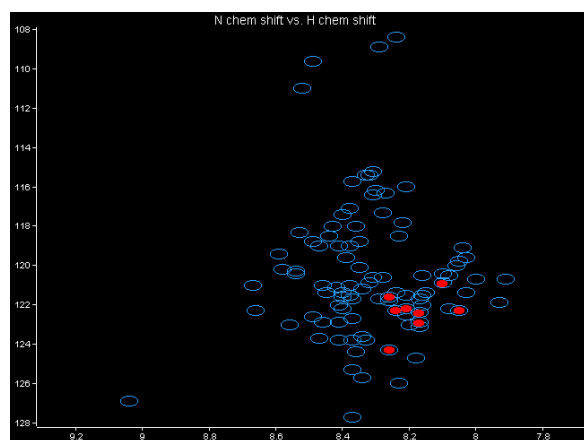
**Histidines**



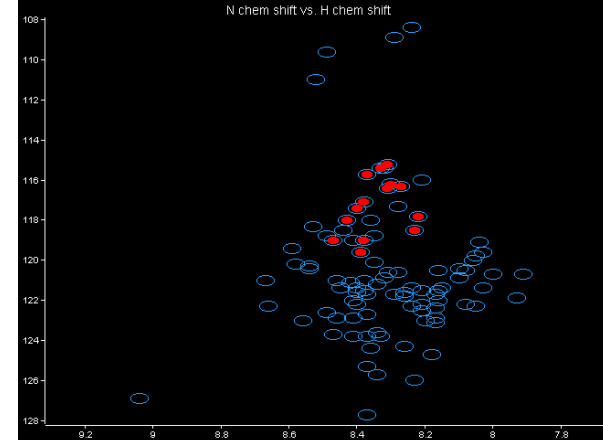
**Glutamic Acids**



**Leucines**



**Serines**



# From 2D to 3D – Improving Resolution

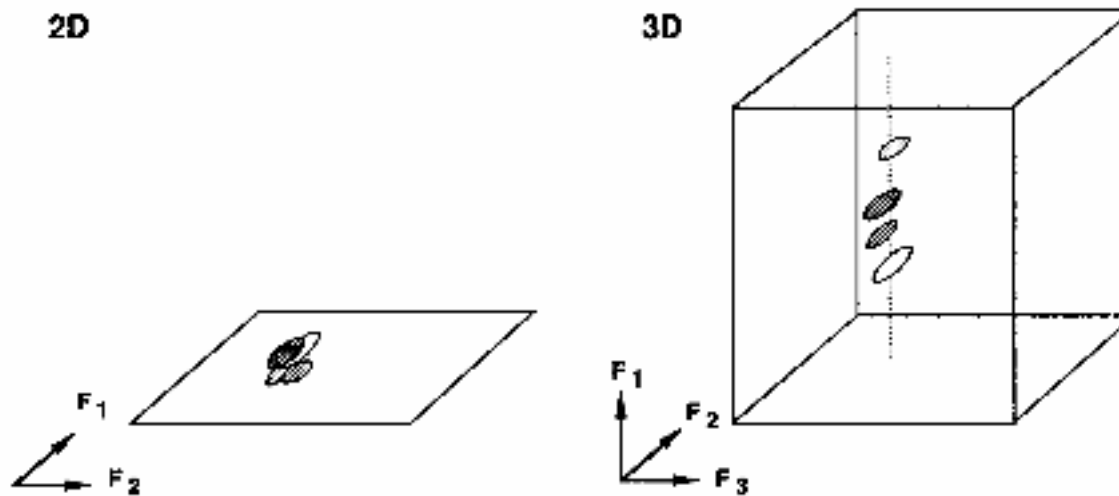
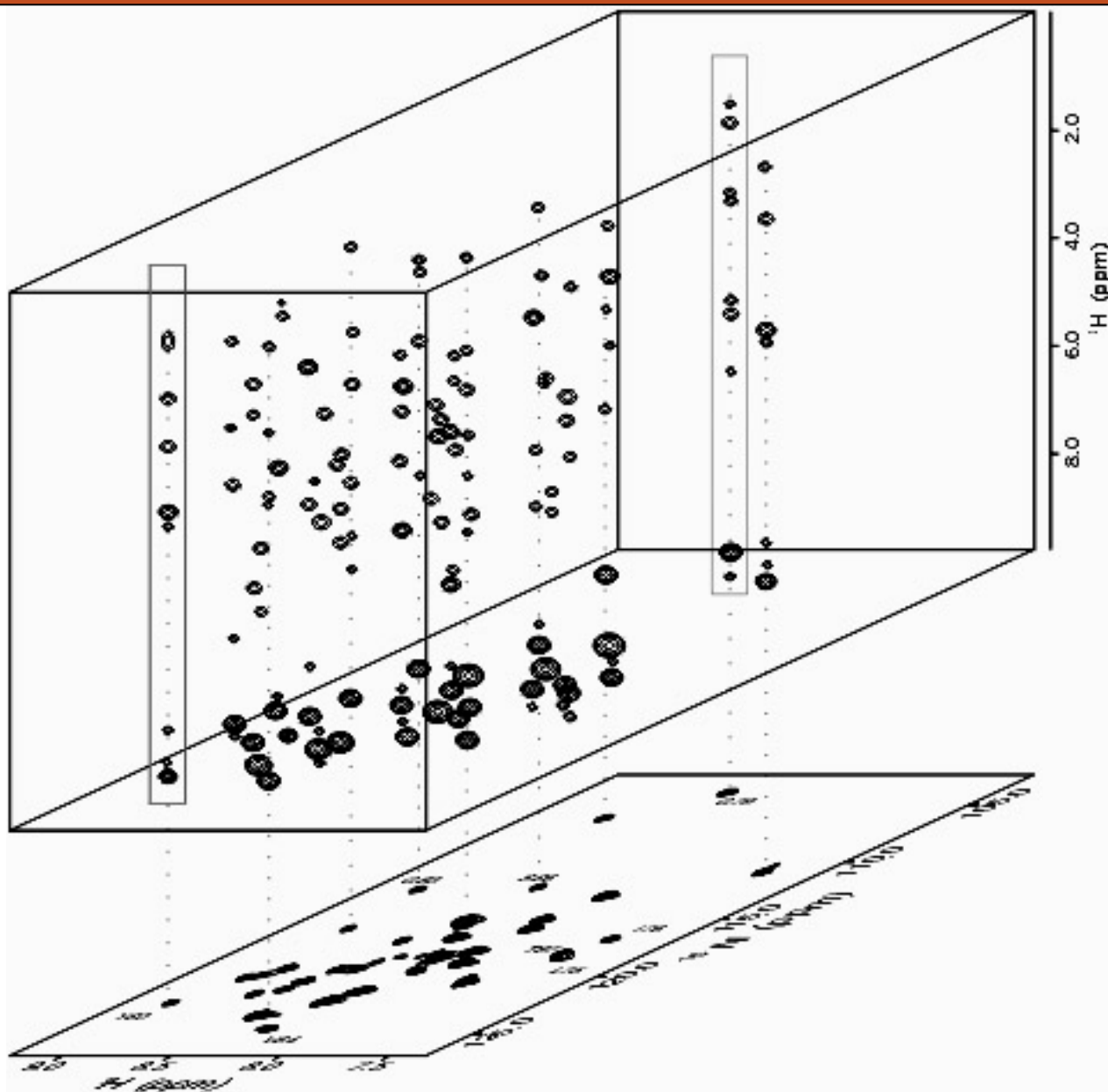


Figure 1.2.4



# Dynamic NMR

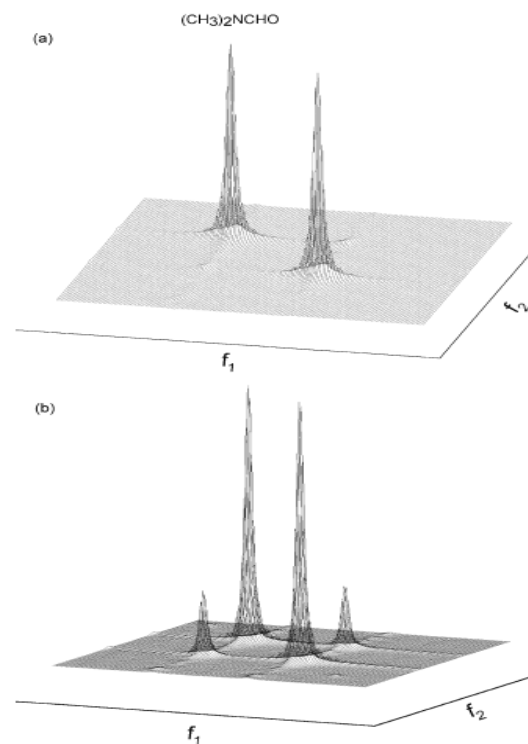
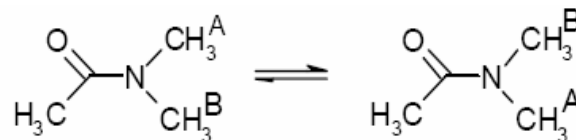
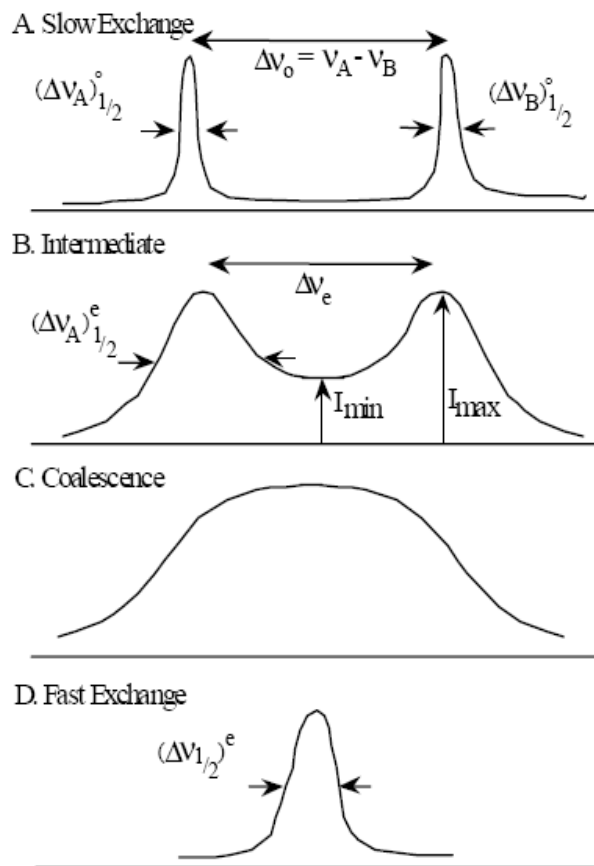
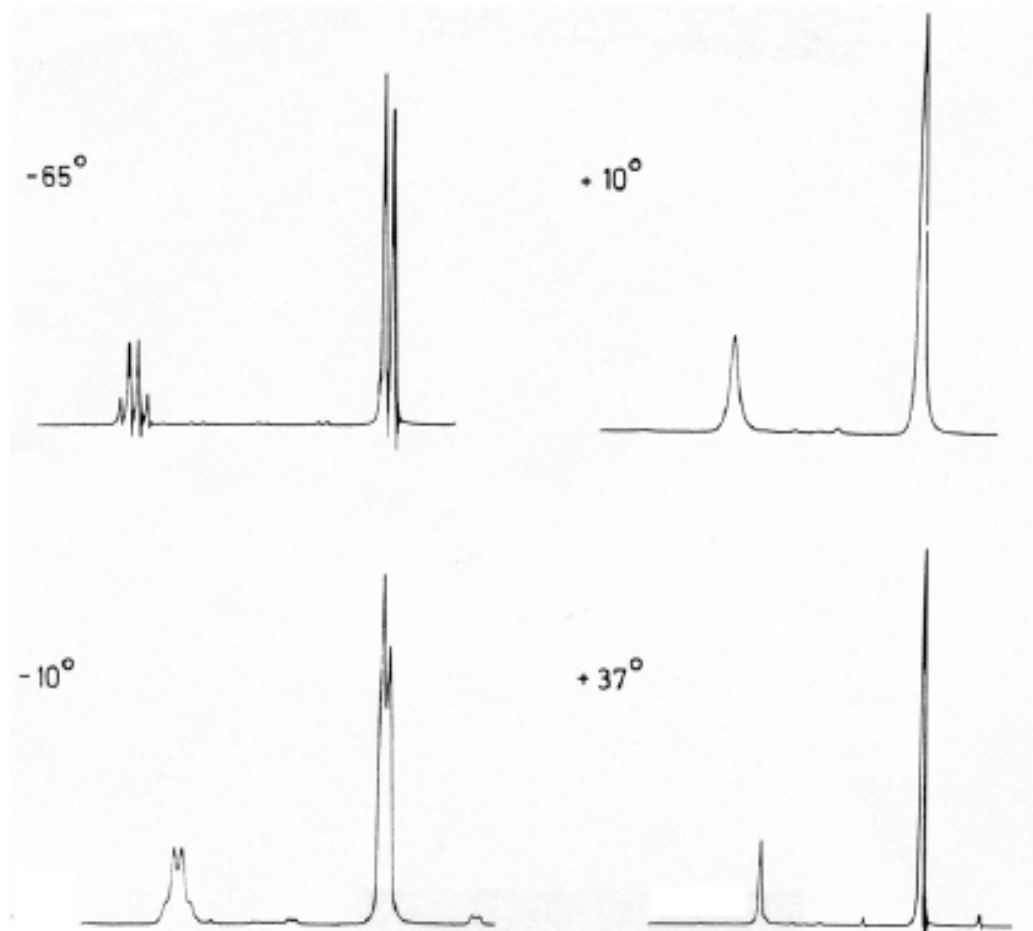


Figure 1. Effect of exchange of chemically equivalent nuclei on NMR line shapes.

$$k = \frac{t_m}{2 \log\left(\frac{1+r}{1-r}\right)}$$

# Dynamic NMR

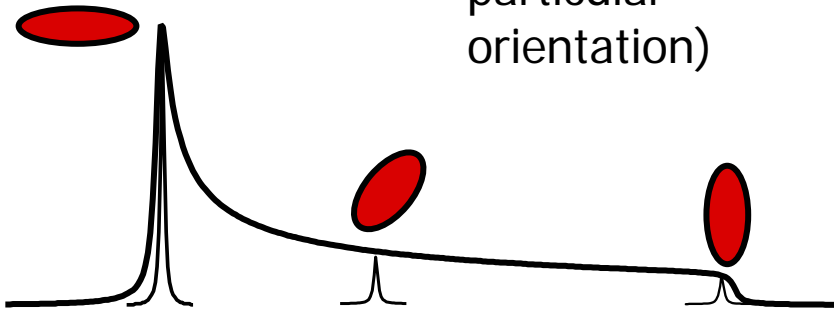
methanol:





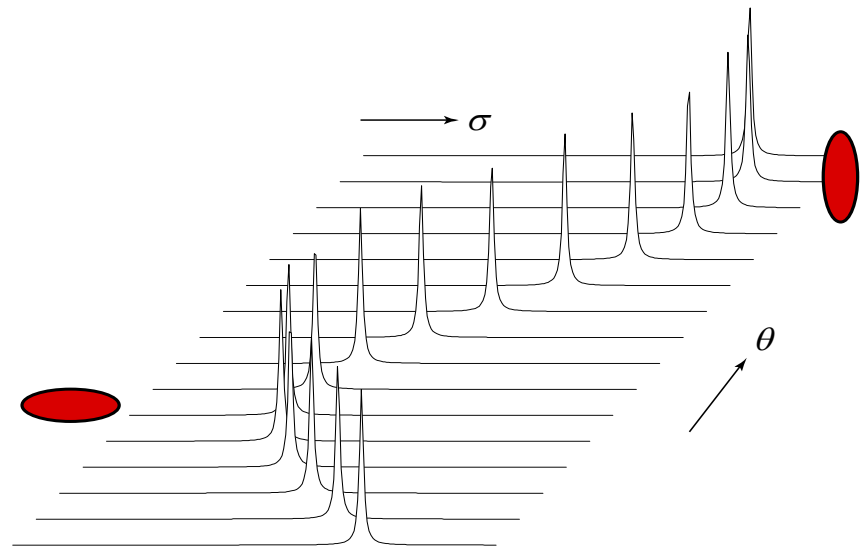
# Solid State NMR

(Shape reflects probability of particular orientation)

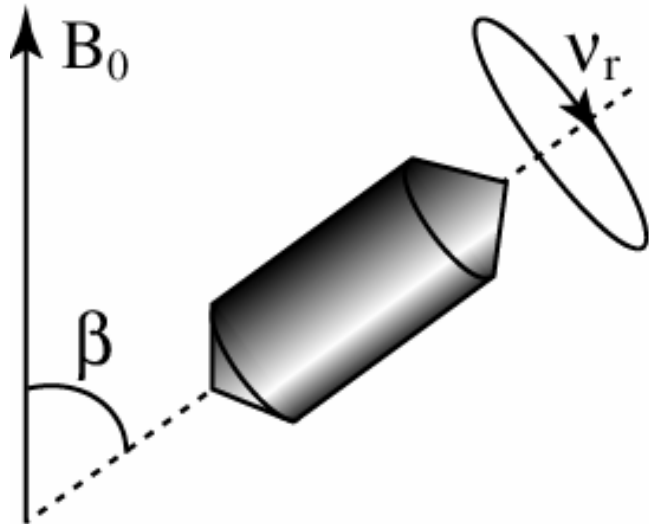


Chemical Shift Depends on Orientation of Molecule with Respect to External Field.

Typical Solid State NMR Powder Spectrum Appearance



# Magic Angle Spinning



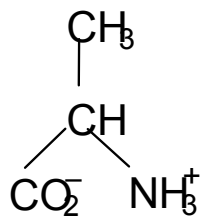
For “fast” spinning, anisotropic interactions are scaled by

$$(3 \cos^2 b - 1) / 2$$

which is zero for  $\beta = 54.7^\circ$  (magic angle)

“Spinning sidebands” appear at slower speeds

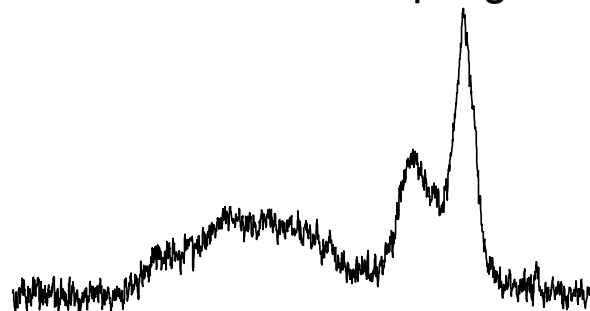
# Solid State NMR – Effect of Magic Angle Spinning and $^1\text{H}$ Decoupling



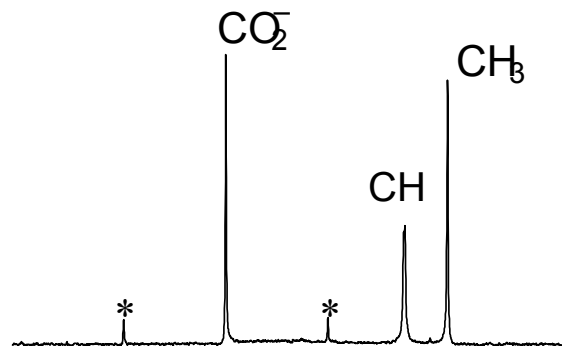
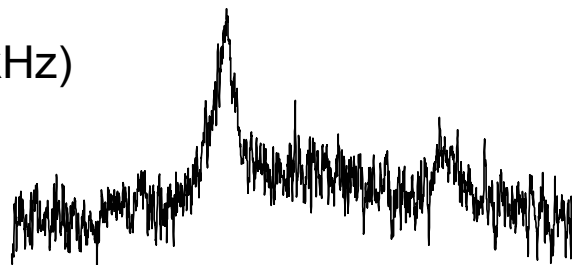
without decoupling

with  $^1\text{H}$  decoupling

static



spinning (5 kHz)



# Inorganic NMR

## P-31 Spectrum of $\text{PF}_2\text{H}(\text{NH}_2)_2$ labeled with $^{15}\text{N}$

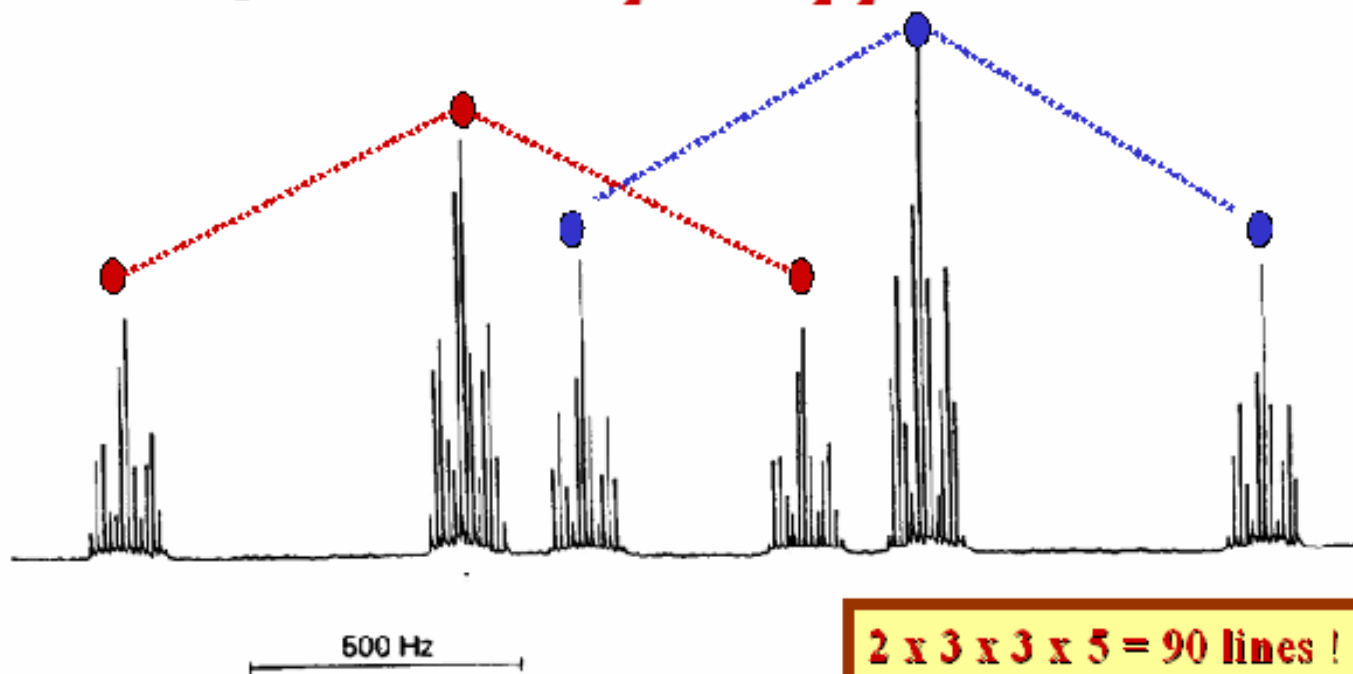


Fig 2.9  $^{31}\text{P}$  NMR spectrum of  $\text{PF}_2\text{H}(\text{NH}_2)_2$ . It is a doublet ( $J_{\text{PH}}$ ) of triplets ( $J_{\text{PF}}$ ) of triplets ( $J_{\text{PN}}$ ) of quintets ( $J_{\text{PH}}$ ) – 90 lines in all.

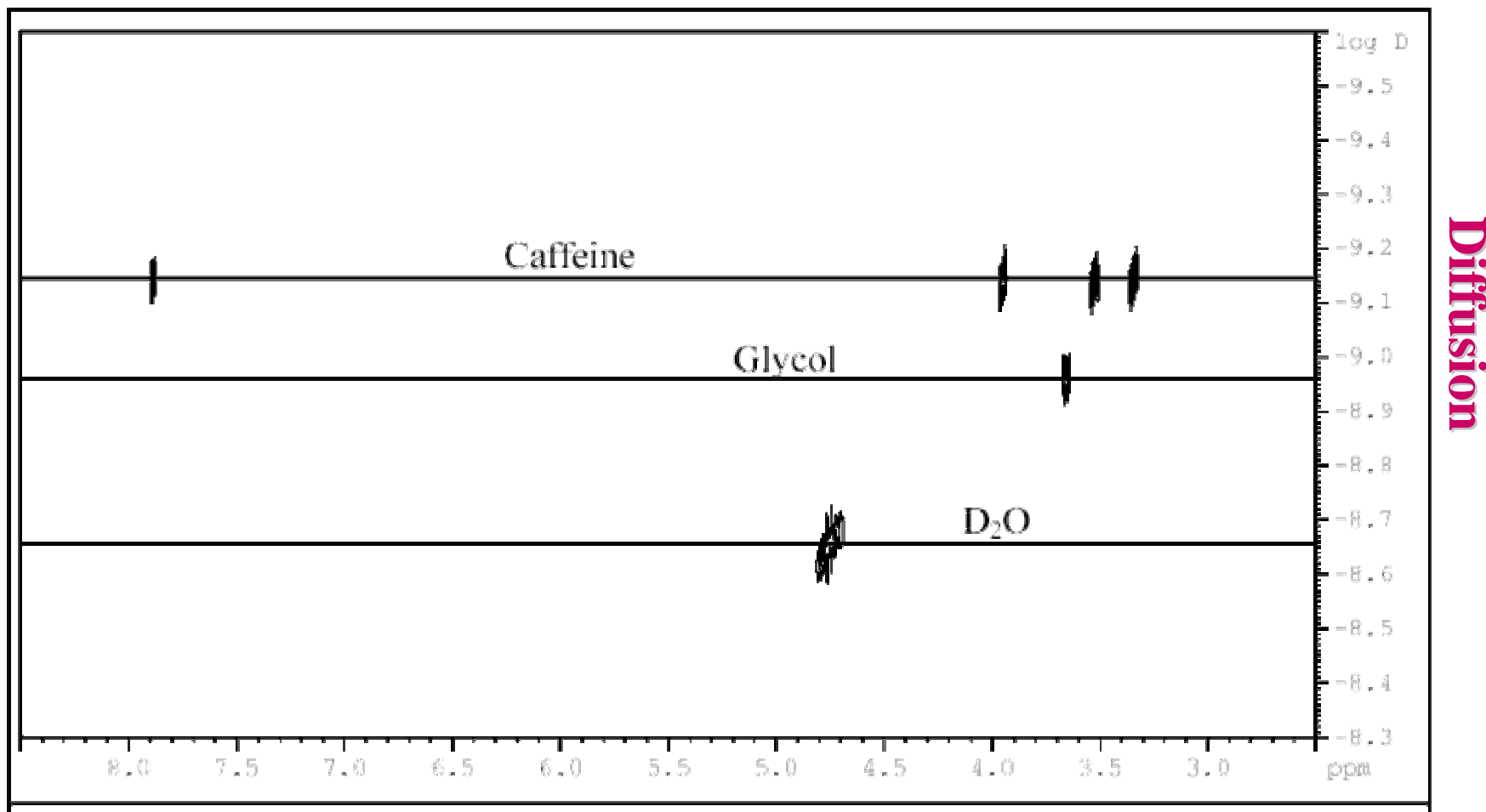
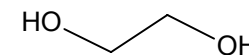
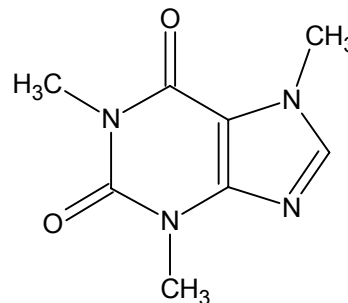
**coupling with H (largest coupling : Doublet)**

**then we see triplet with large coupling with fluorine**

With further **Coupling to 2 N produce triplets, further coupled to 4 protons => quintets**

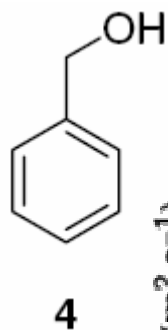
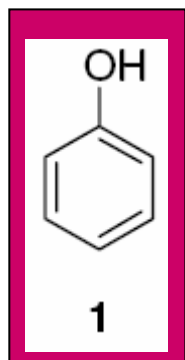
# DOSY Diffusion-Ordered Spectroscopy

Mixture of **Caffeine**, **Glycol** and **D<sub>2</sub>O**

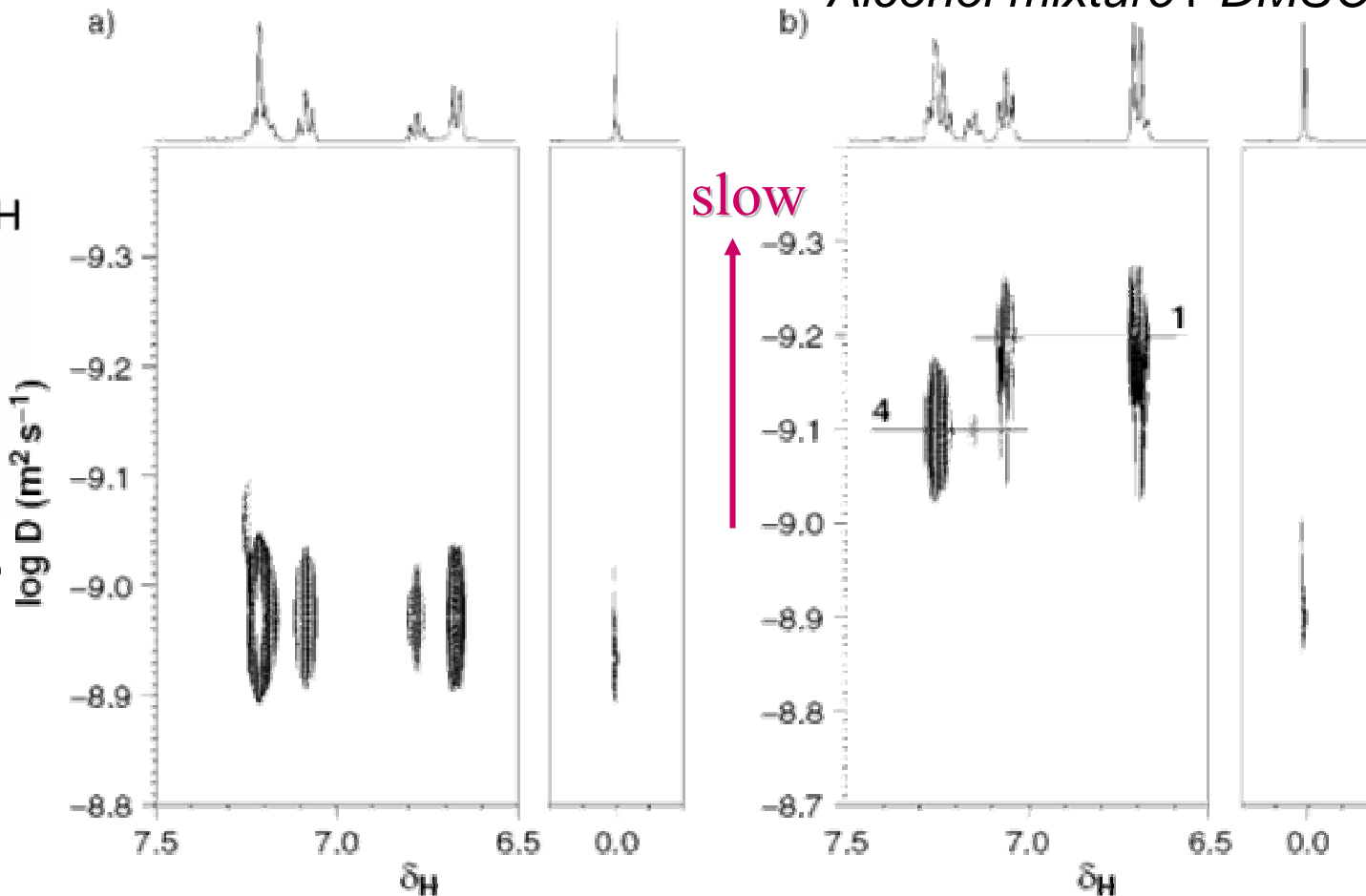


# Relative H-bond acidity probed by diffusion

*Alcohol mixture + DMSO*



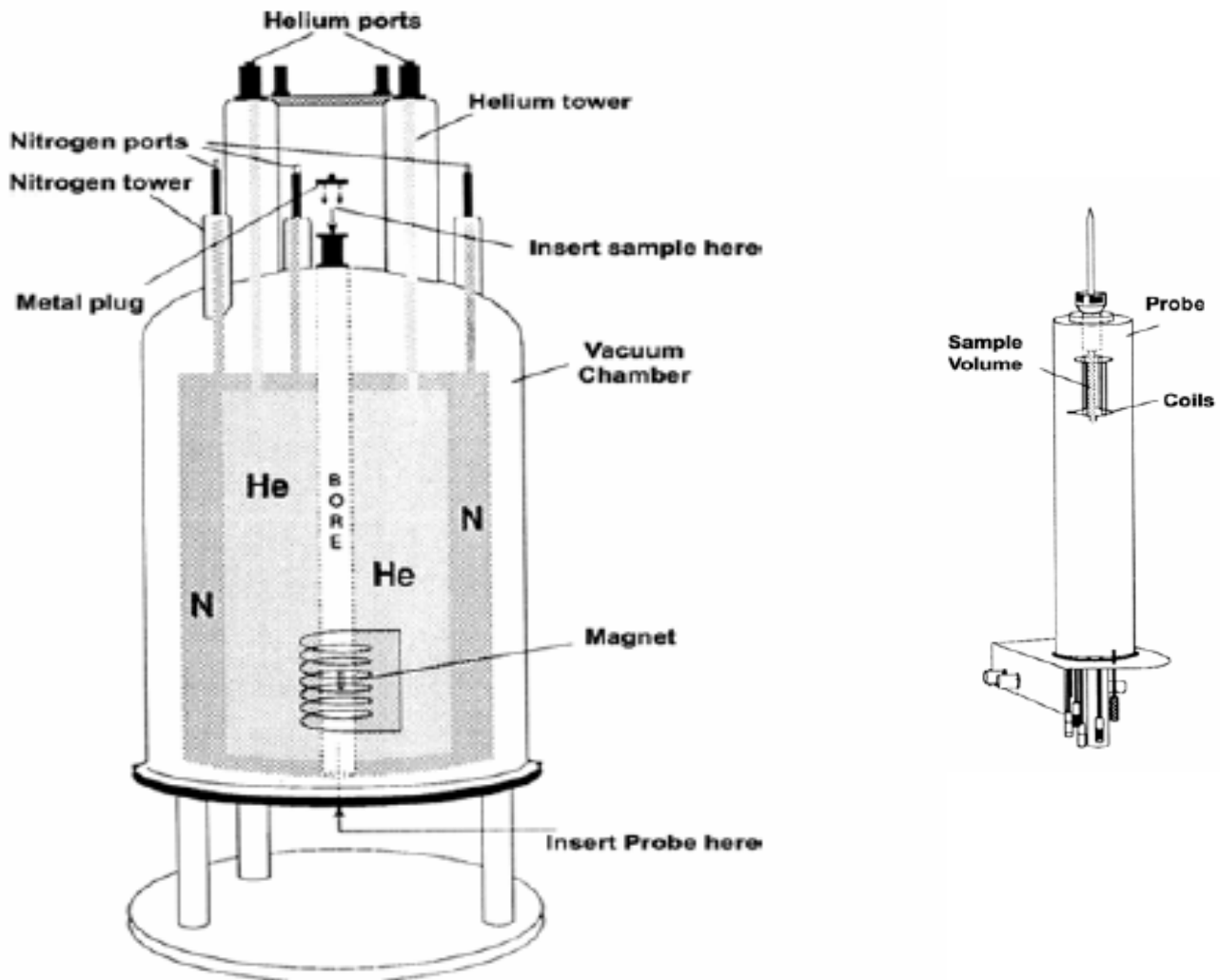
*Alcohol mixture*



- initial mixture, the two alcohols have identical diffusion coefficients
- DOSY plot shows immediately that the two compounds experience **different interactions with the H-bond acceptor DMSO** since they no longer have the same diffusion coefficient. **Compound 1 becomes slower than 4** as a result of a **stronger association with DMSO**,

# The NMR Spectrometer

Figure 4.4. Superconducting Magnet

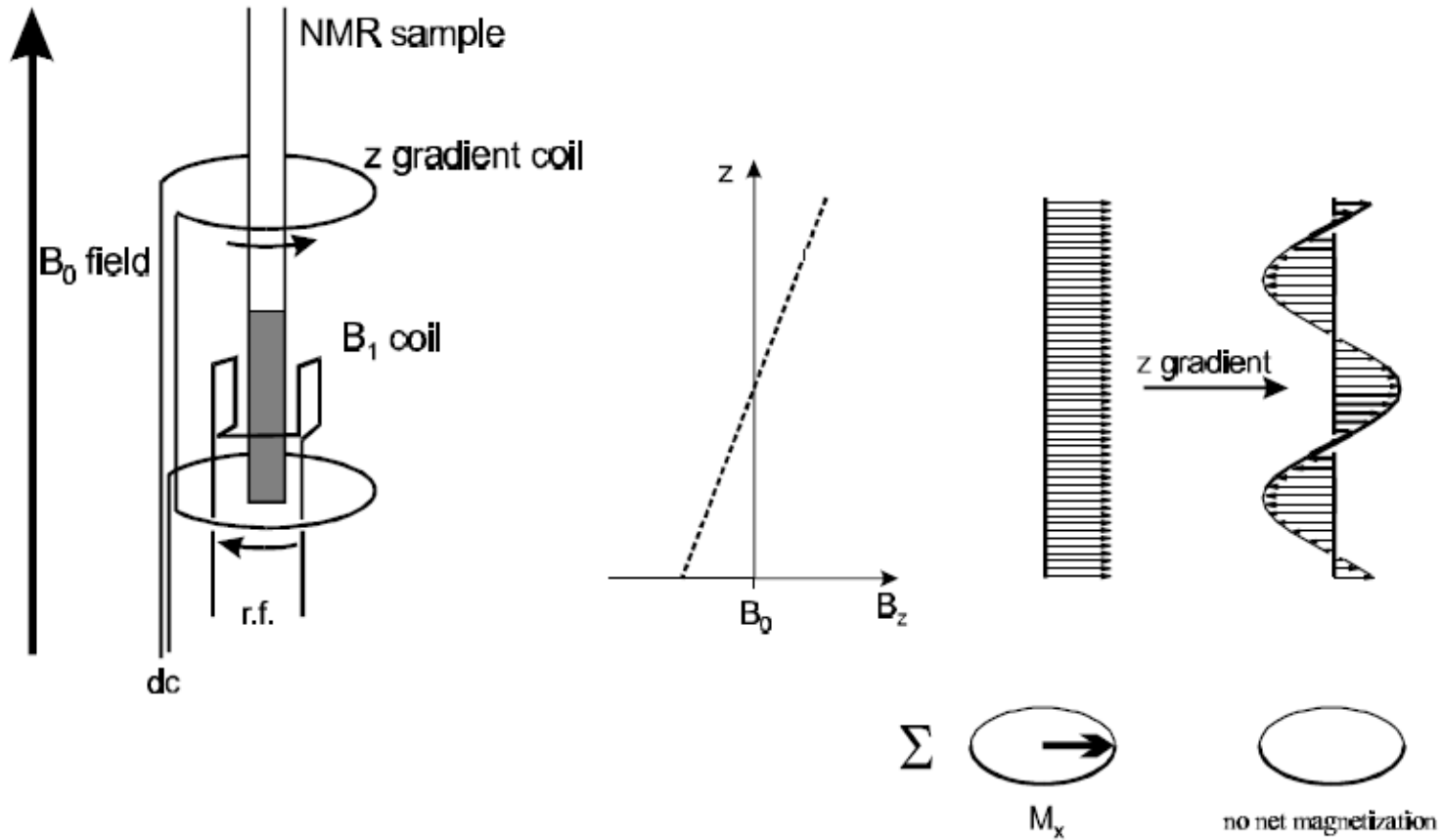


# Common NMR Probes

- BBI – Broad Band Inverse Detection  
1H on inner coil – most sensitive for 1H, HSQC, HMBC type experiments.  
This probe can be found on the 300MHz in GC410.
- BBO– Broad Band Observe  
Broad Band on inner coil – most sensitive for direct observe heteronuclear).  
This type of probe is found on the 400MHz in GC410 and the 300MHz in MM311.
- TXI - Triple Resonance  
Required for some 3-D experiments including protein/nucleic acid studies.  
Requires extra amplifier/transmitters setup in console.
- Microcoil –  
Low volume probe proven beneficial for mass limited samples and high throughput screening in automation settings.
- Cryoprobes  
3-4 X increase in sensitivity over room temperature probes but significantly more expensive to obtain and maintain.
- CPMAS – Cross Polarization Magic Angle Spinning  
Recommended for solid state NMR.



# Gradient Spectroscopy

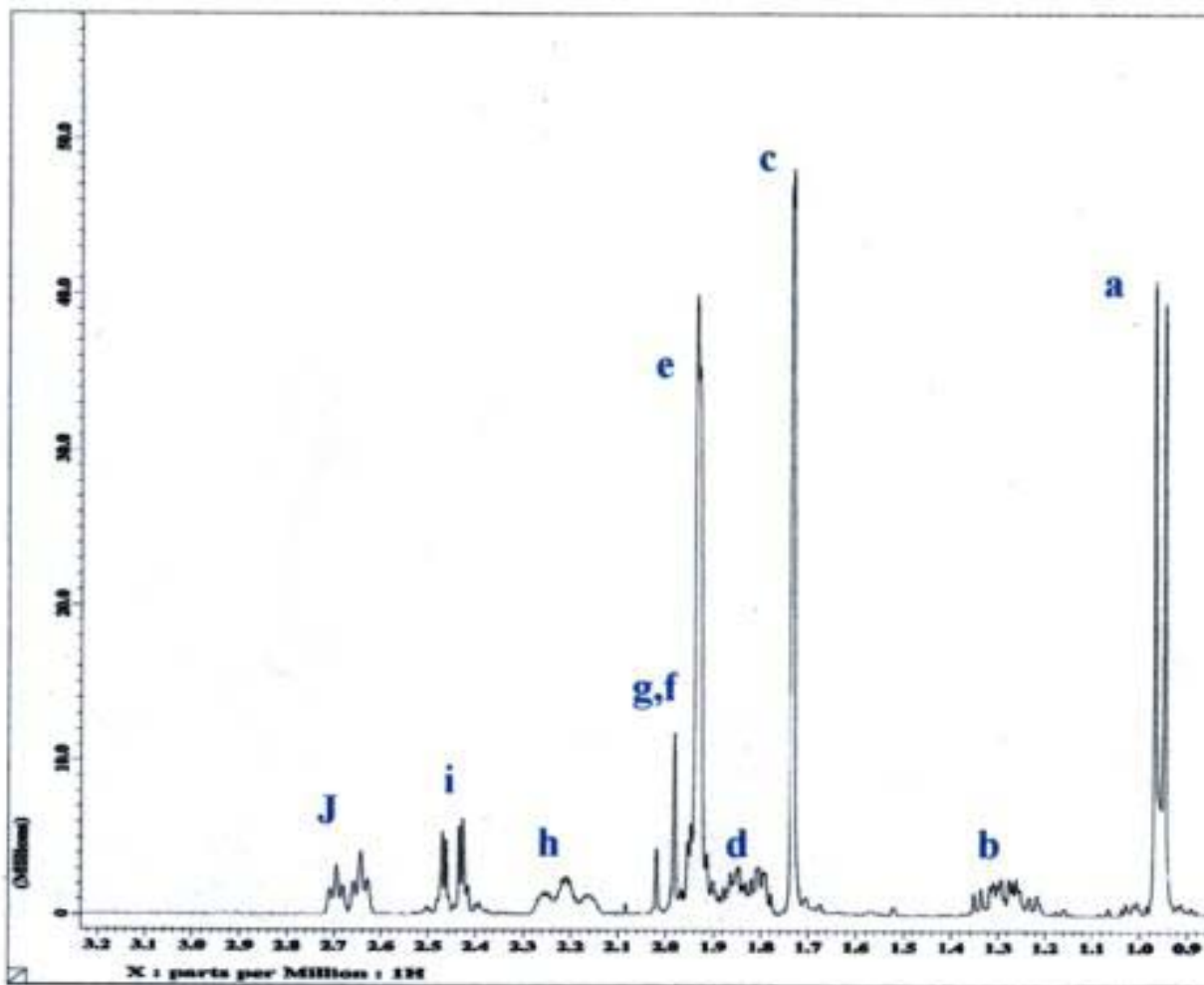


# Gradient Spectroscopy

- Significantly reduces experiment time by removing the requirement of multiple scans for phase cycling in 2D experiments .
- Selectively removes unwanted signals by coherence selection or through purge gradients yielding excellent solvent suppression, reduced artifacts, and cleaner spectra
- Improves dynamic range as the receiver gain can be optimized on the desired magnetization.
- Allows for diffusion measurements.
- Easy to use.

# Structure Determination Exercise-1

## $^1\text{H}$ NMR Spectrum of Unknown



Integration Values

A = 3H

B = 1H

C = 3H

D = 1H

E = 3H

F = 1H

G = 1H

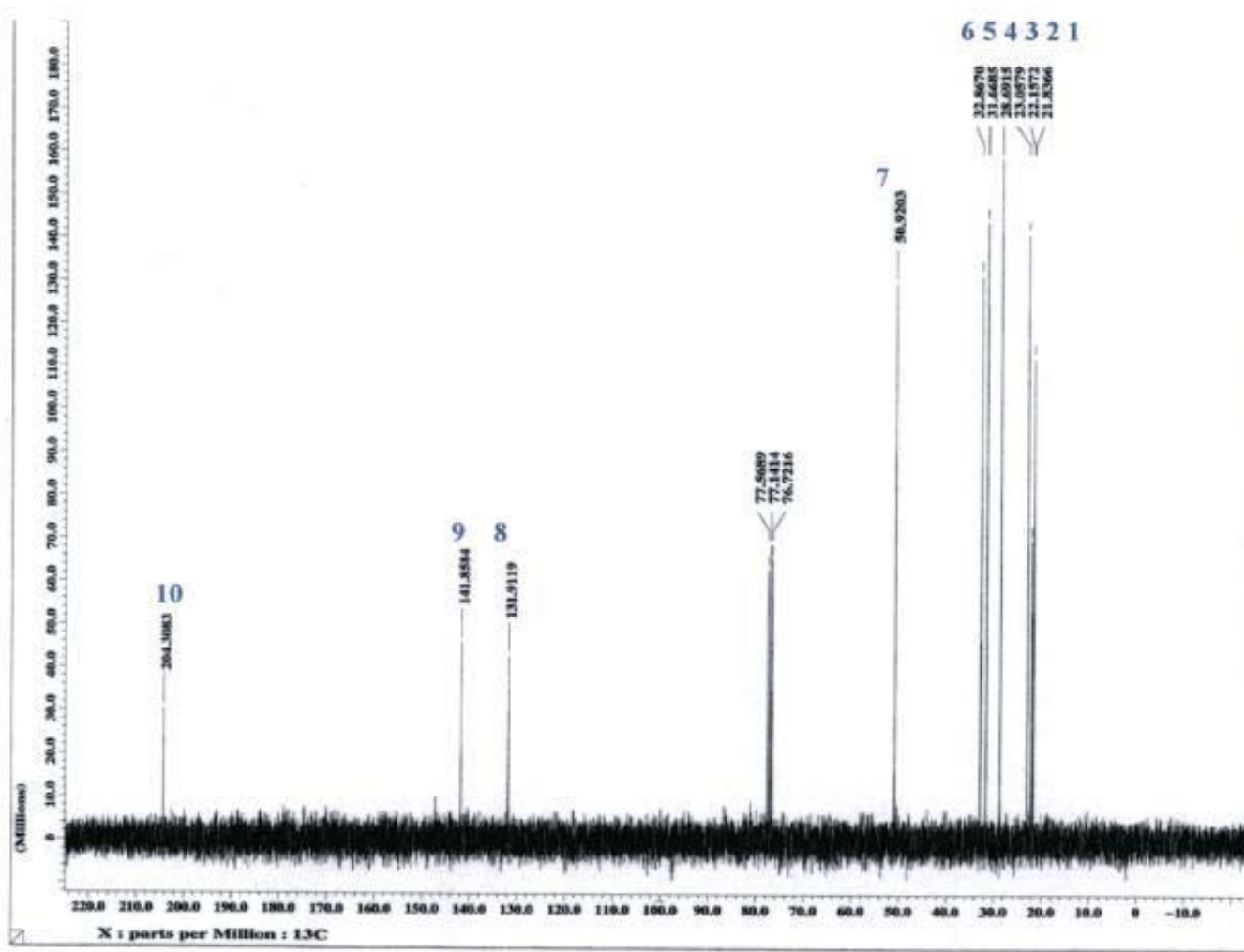
H = 1H

I = 1H

J = 1H

# Structure Determination Exercise-1

## $^{13}\text{C}$ Spectrum

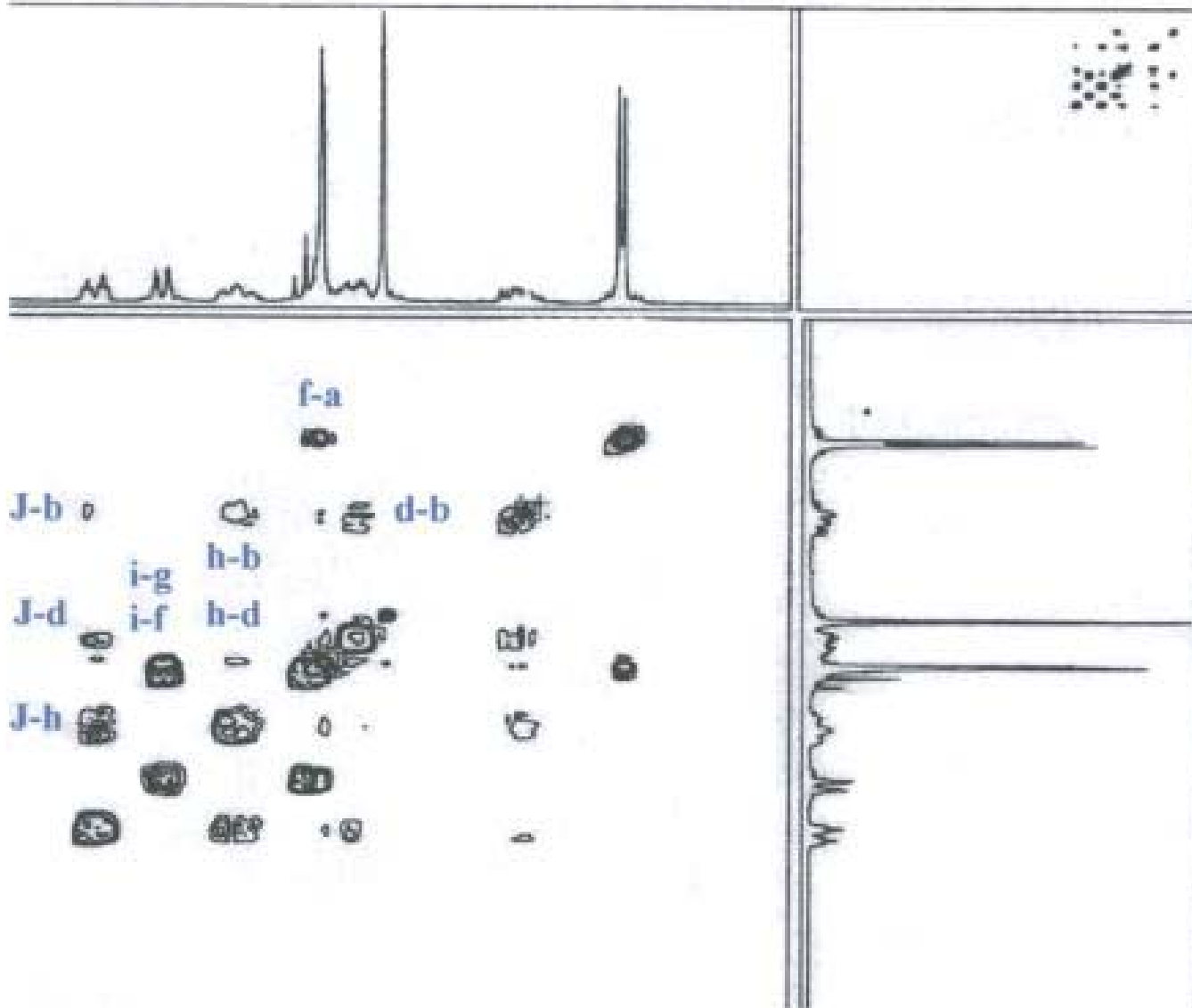


# Exercise-1

- 10 Carbons/16 Protons  $\rightarrow$  index of H deficiency = IHD =  $0.5 * [2c+2-h-x+n] = 3$
- From  $^1\text{H}$  NMR
  - 3 methyl groups; one split into a doublet (typical alkane chemical shift), two deshielded methyls (typical of attachment to double bond).
- From  $^{13}\text{C}$  NMR
  - 1 carbon at  $\sim 204\text{ppm}$  typical chemical shift of carbonyl carbons
  - 2 carbons at  $\sim 141$  &  $131\text{ppm}$  typical chemical shift of  $\text{sp}^2$  carbons (with no evidence of olefinic  $^1\text{H}$  attached from the proton spectrum).
- From the  $^1\text{H}$  and  $^{13}\text{C}$  alone, we suspect 1 alkene double bond, 1 carbonyl double bond, and 1 ring.
- Run COSY and HMQC.

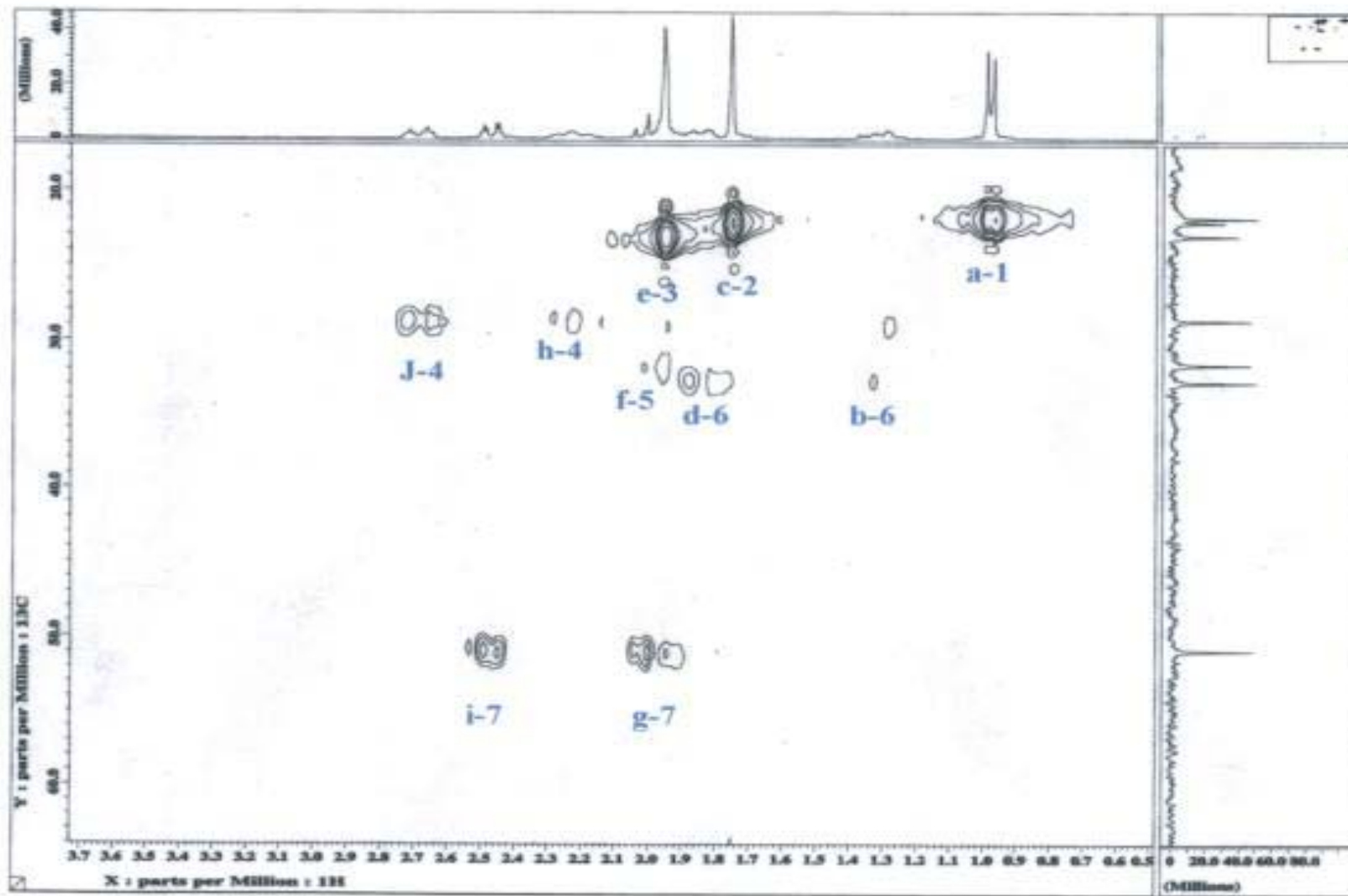
# Structure Determination Exercise

## COSY Spectrum



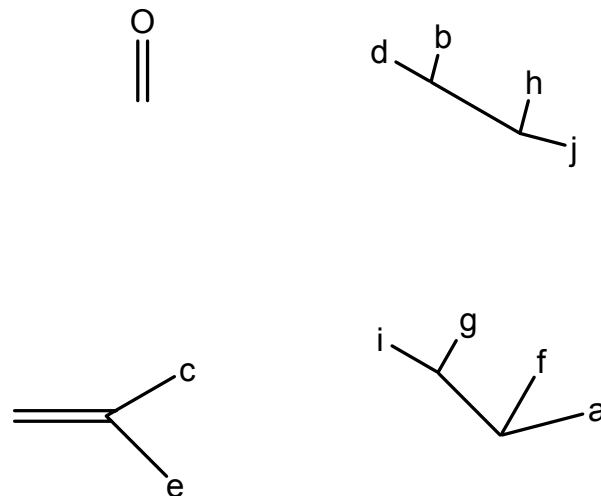
# Structure Determination Exercise

## HMQC Spectrum



# Structure Determination Exercise

- From HMQC –
  - Assign methylene protons –
    - j & h = carbon 4
    - d & b = carbon 6
    - i & g = carbon 7
  - Assign remaining protons
    - a = carbon 1
    - c = carbon 2
    - e = carbon 3
    - f = carbon 5
- From COSY –
  - Map out scalar couplings
    - j/h coupled only to d/b
    - i/g coupled only to f
    - f coupled to a (weak coupling to b)
    - c and e show no coupling



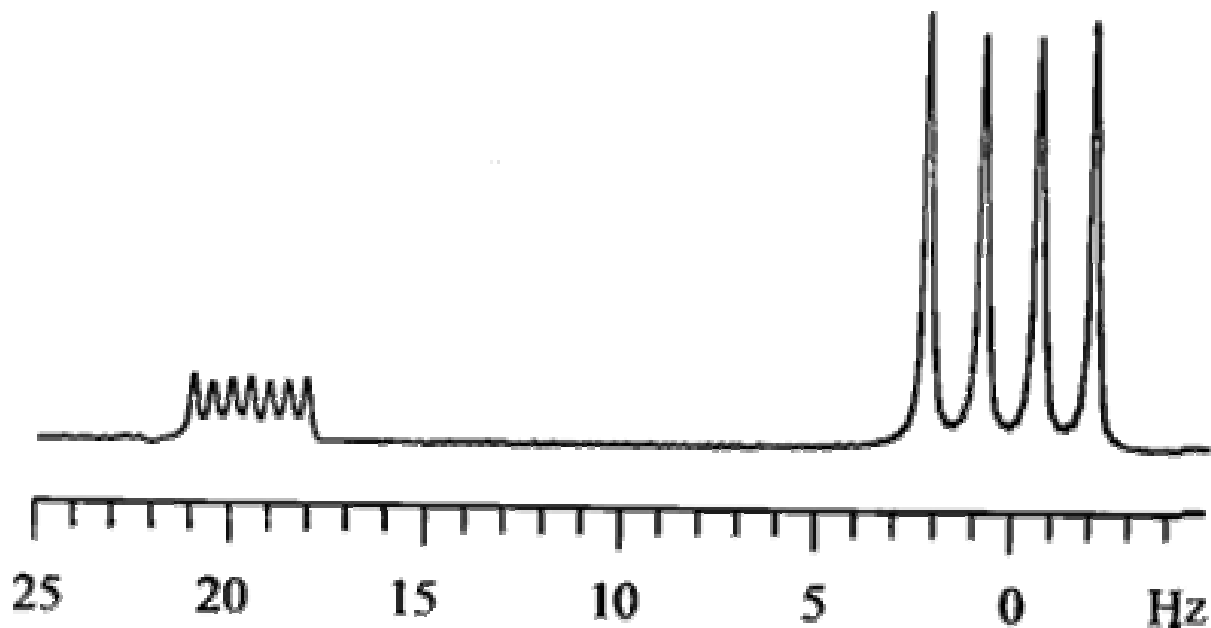


# Exercise-2 Inorganic NMR Problem

- $^{19}\text{F}$  NMR of  $\text{NH}_4\text{BF}_4$

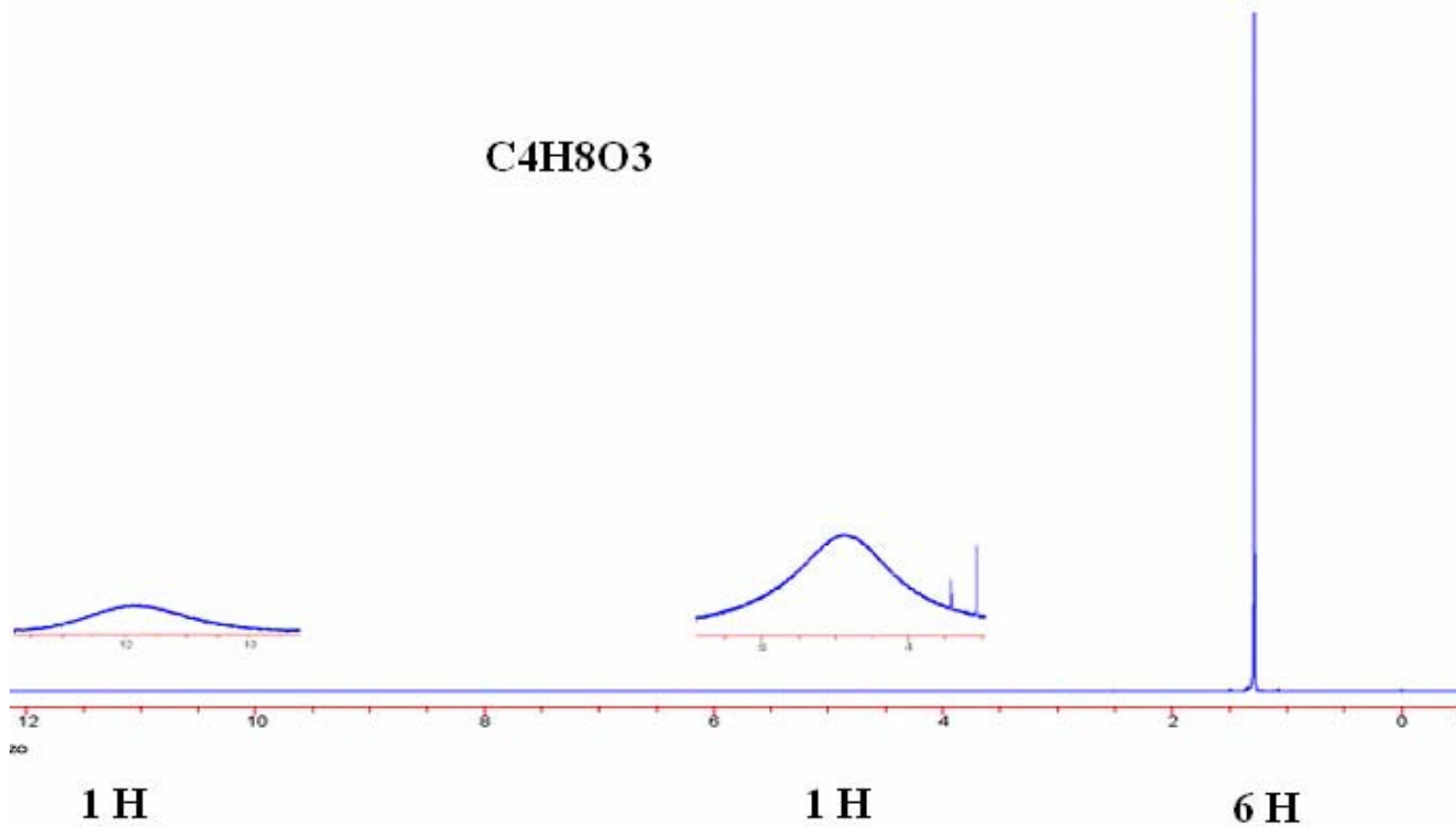
$^{10}\text{B}$ :  $Q = 8.5 \times 10^{-2}$     $a = 19.6\%$     $I = 3$

$^{11}\text{B}$ :  $Q = 4.1 \times 10^{-2}$     $a = 80.4\%$     $I = 3/2$



Explain the observed splitting pattern.

# Exercise -3



# Exercise - 4

