

NMR Tutorial

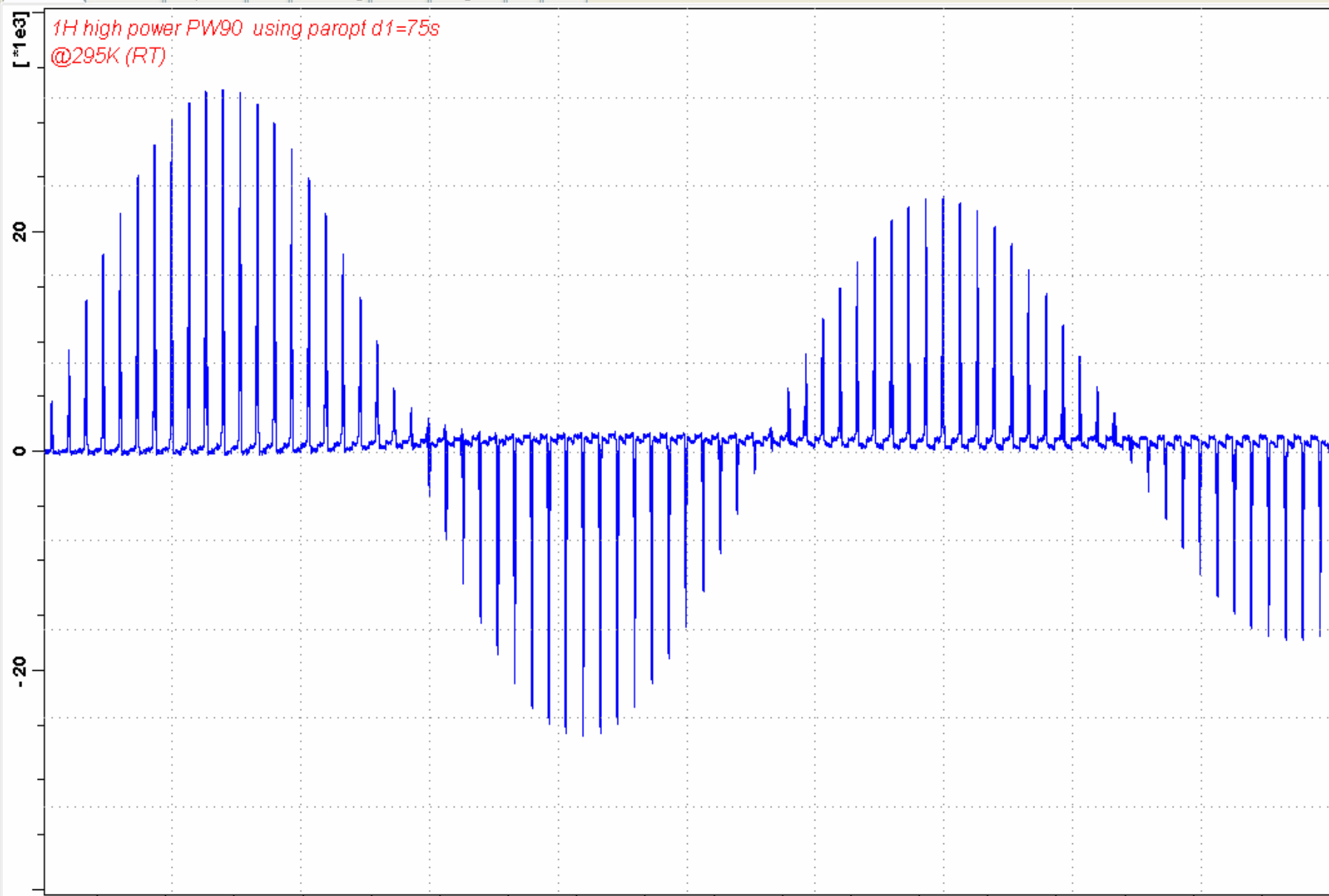
Mamata Joshi

NMR Facility, TIFR, Mumbai

1H pulse calibration

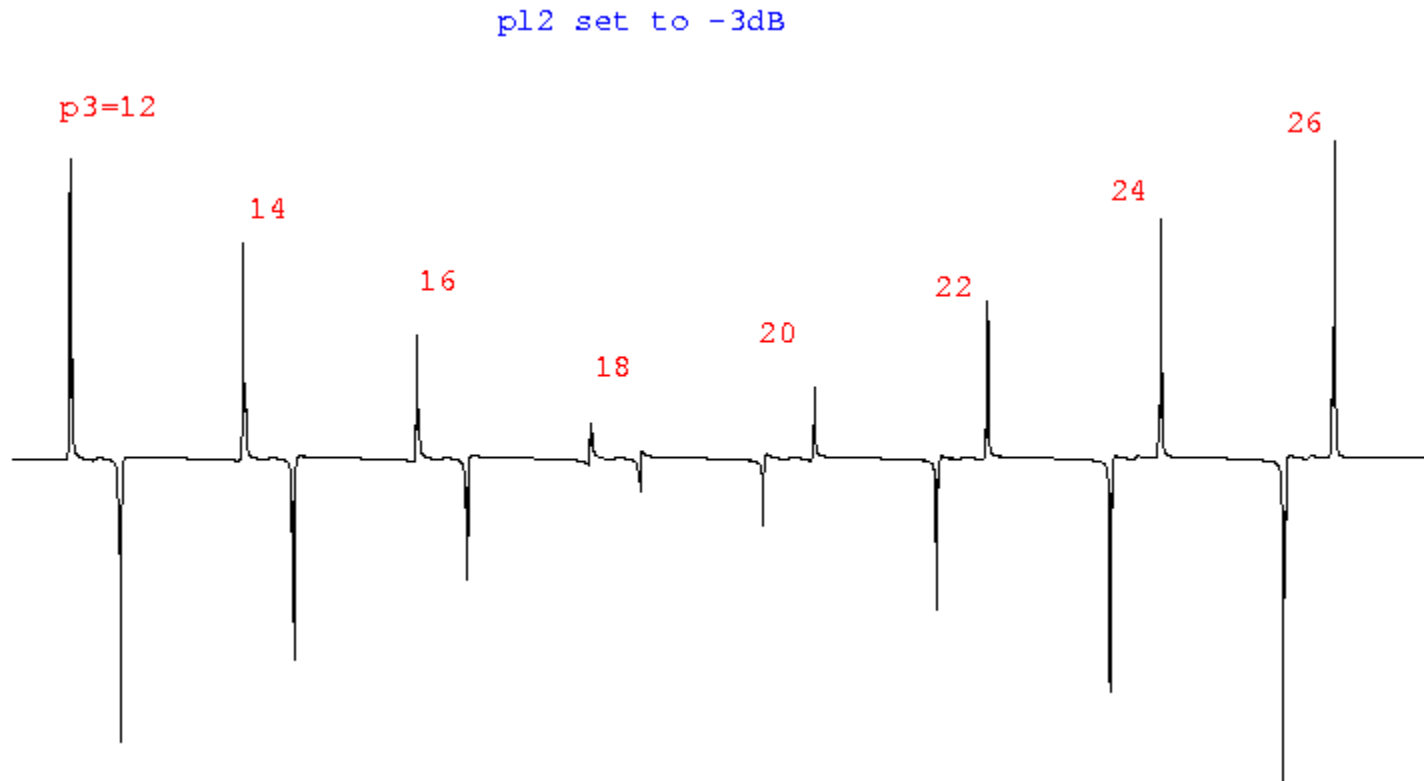
Spectrum ProcPars AcqPars Title PulsProg Peaks Integrals Fid Print

1H high power PW90 using paropt d1=75s
@295K (RT)



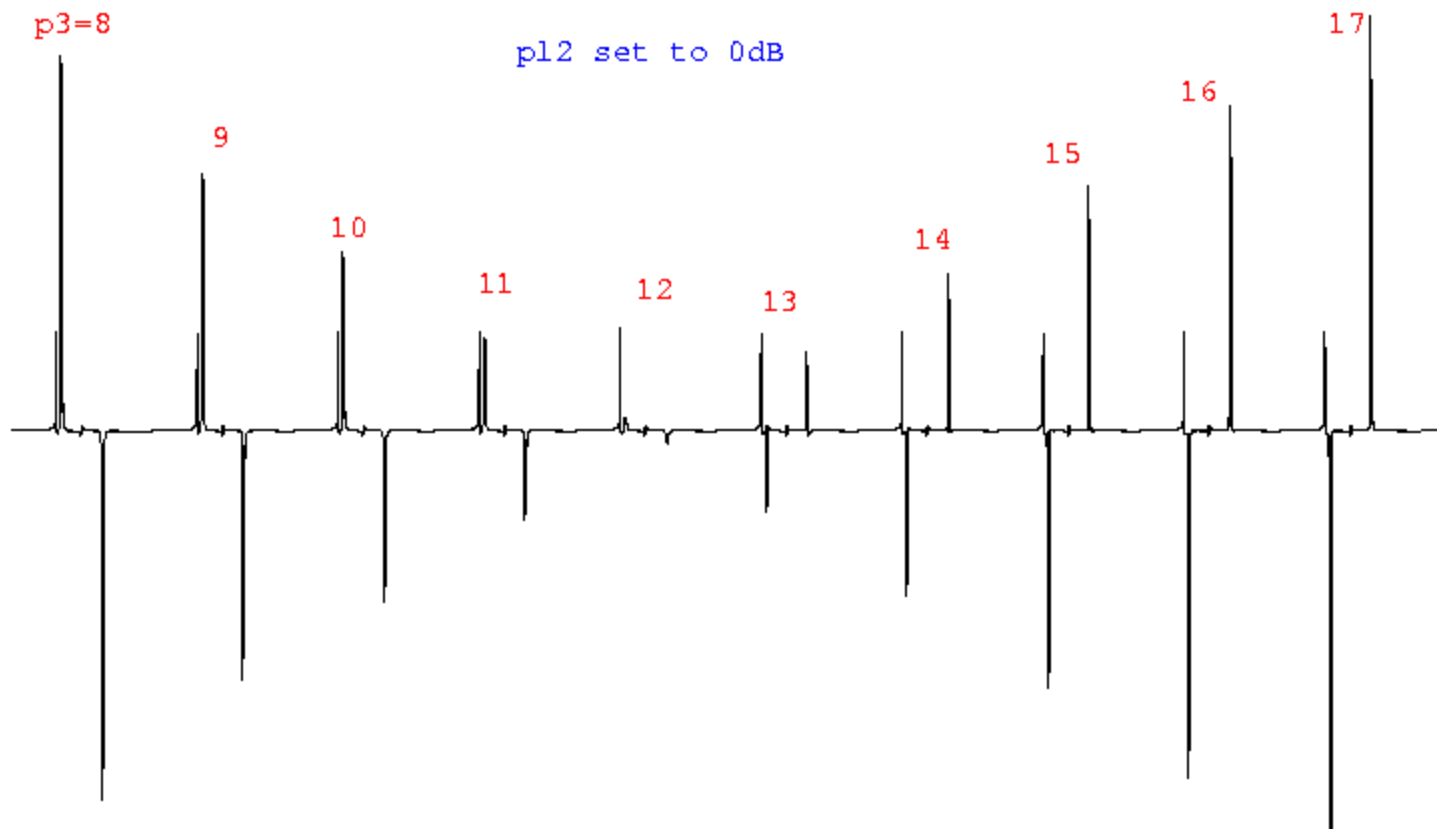
High-power ^{15}N decouple pulse calibration

Sample: urea, $\text{H}_2\text{N-CO-NH}_2$ Pulse program: **decp90f3**

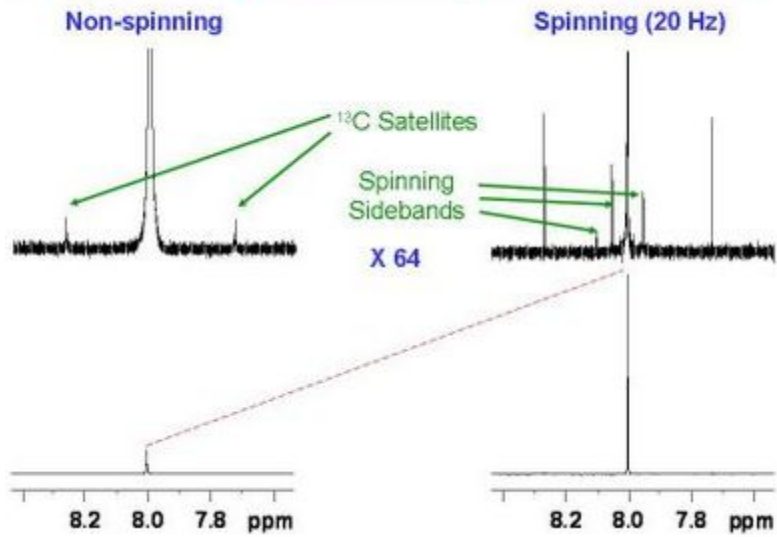


High-power ^{13}C decouple pulse calibration

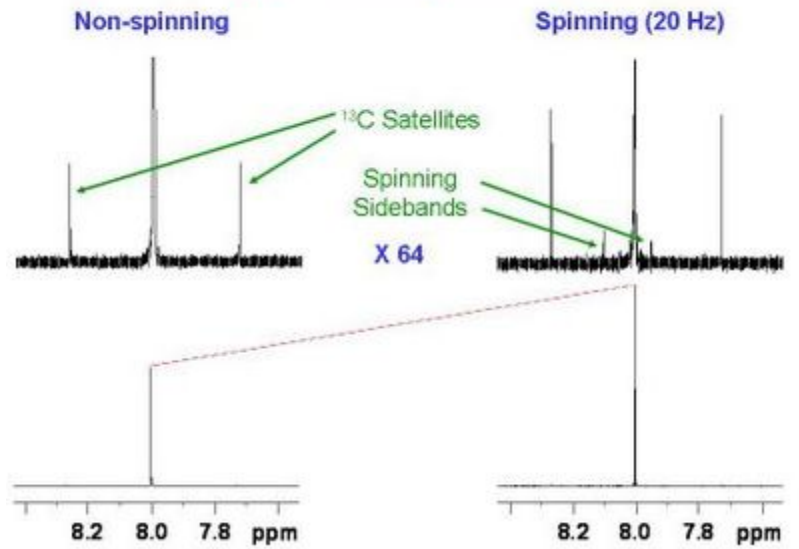
Sample: Methanol Pulse program: **dec90**



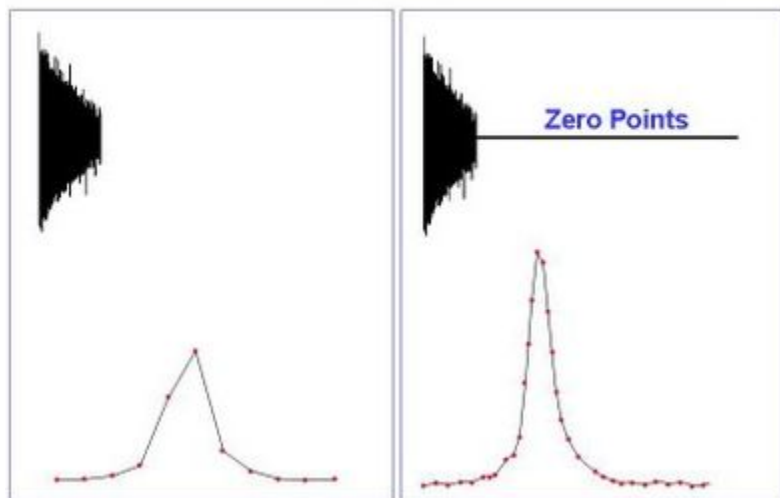
To Spin or not to Spin in an Inhomogeneous Magnetic Field?



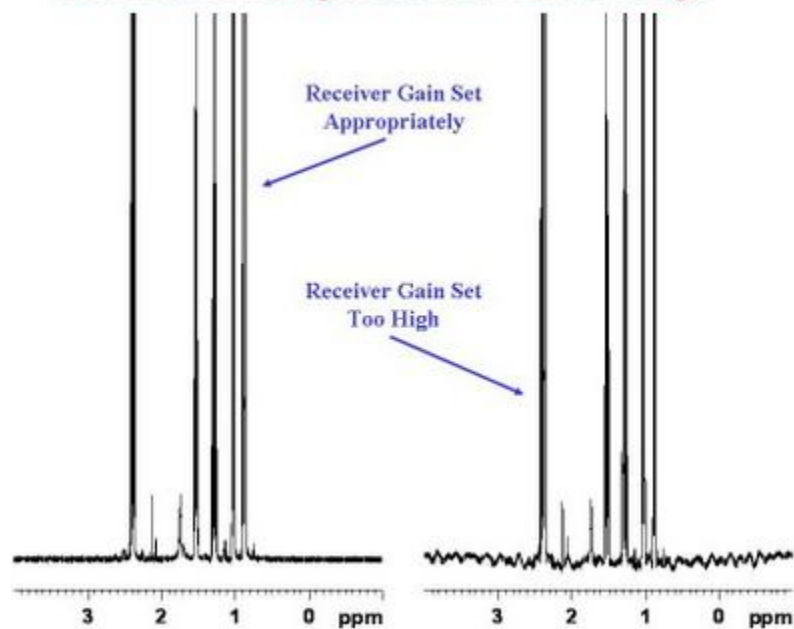
To Spin or not to Spin in a Homogeneous Magnetic Field?



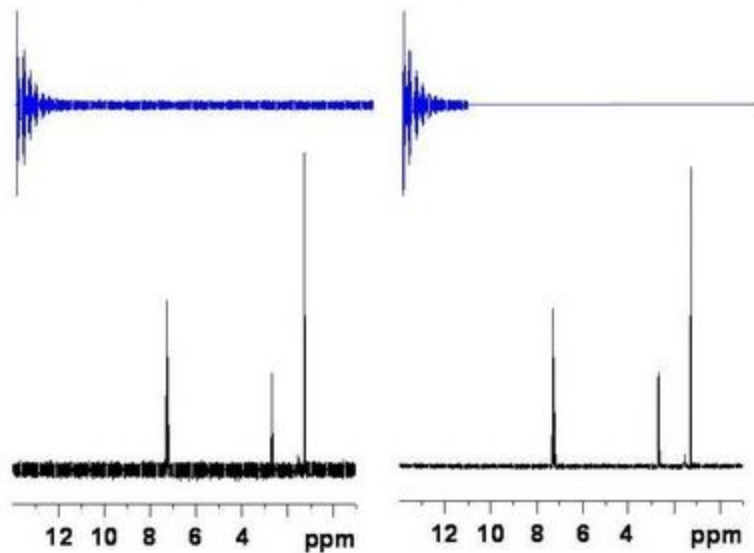
Zero Filling



The Result of Setting the Receiver Gain Too High

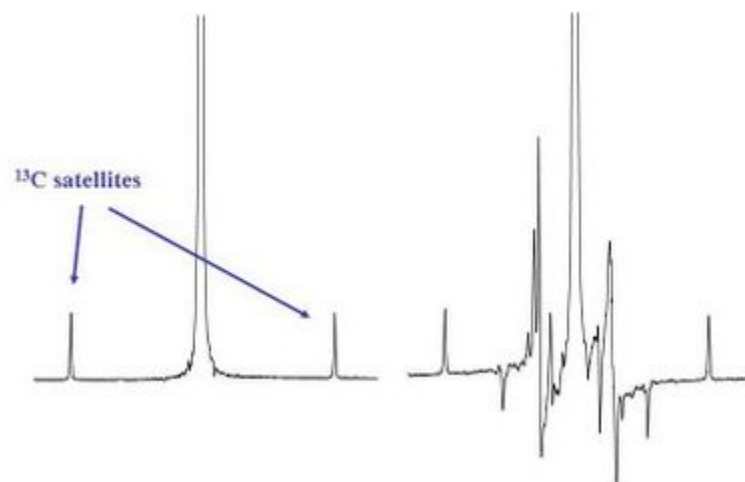


Throwing Away Noise to Improve Your Data



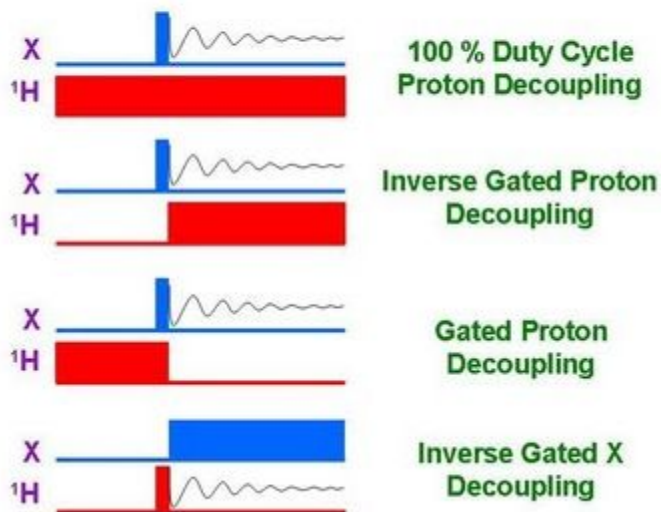
^1H NMR Chloroform

With Vibration Isolation Without Vibration Isolation

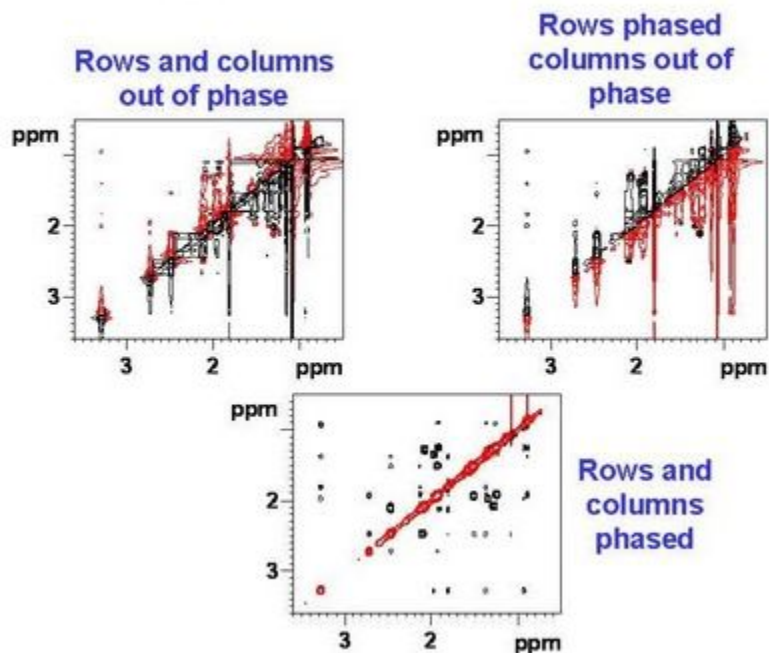


Modes of Broadband Heteronuclear Decoupling

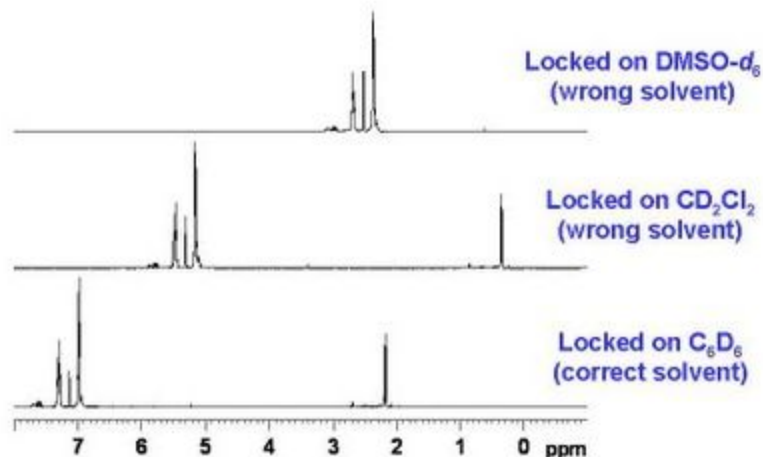
(X = ^{13}C , ^{31}P , ^{15}N ,)



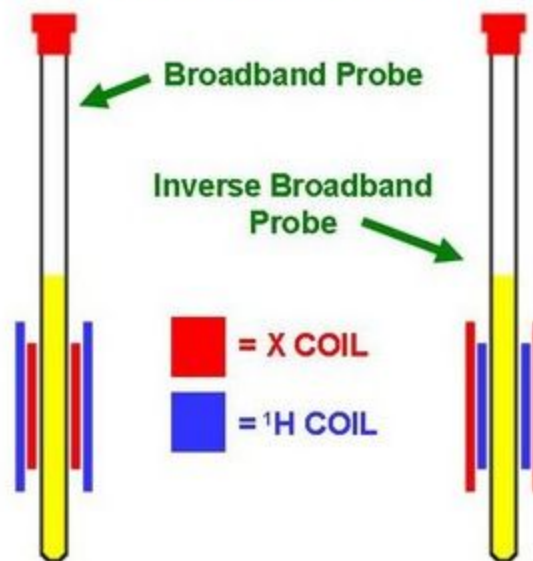
Phasing NOESY Data in TOPSPIN



The Consequence of Selecting the Wrong Solvent When Establishing the Deuterium Lock

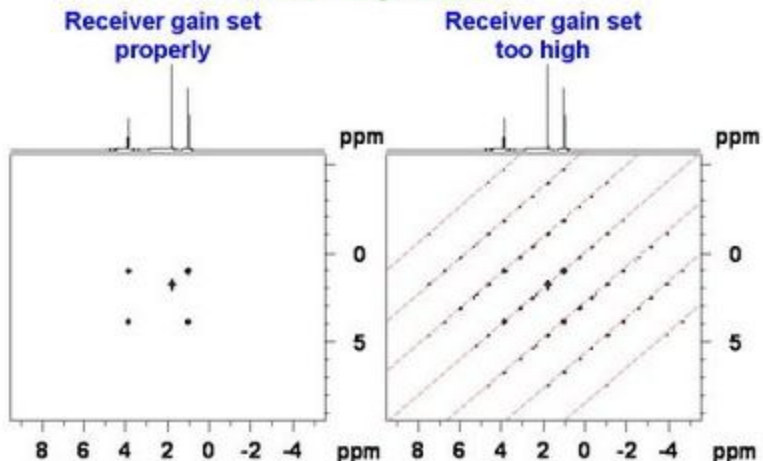


Broadband Probes vs. Inverse Broadband Probes



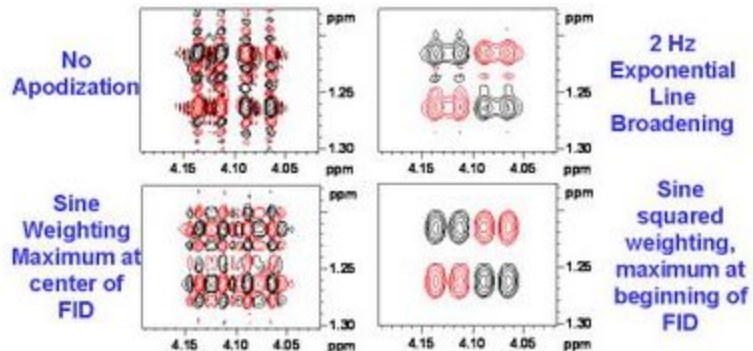
The Effect of Setting the Receiver Gain Too High in Homonuclear 2D NMR Experiments

¹H COSY Ethyl Acetate

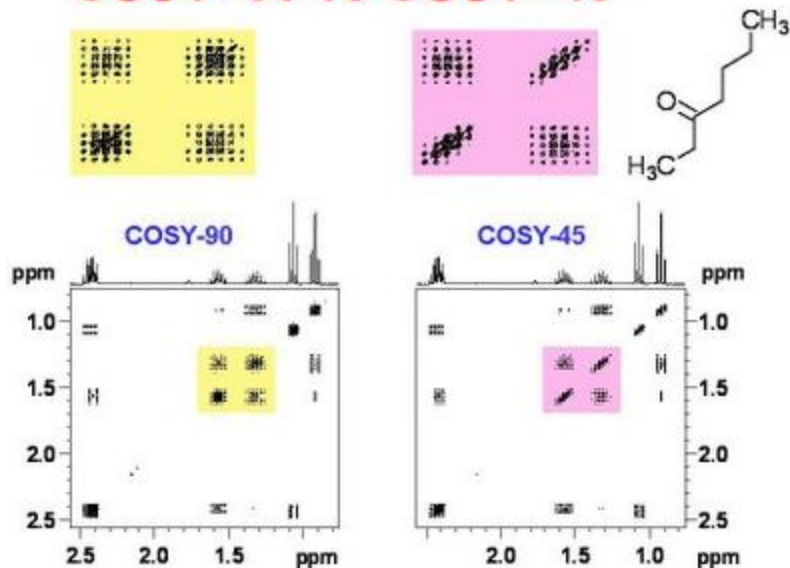


The importance of Choosing an Appropriate Apodization Function for 2D Data

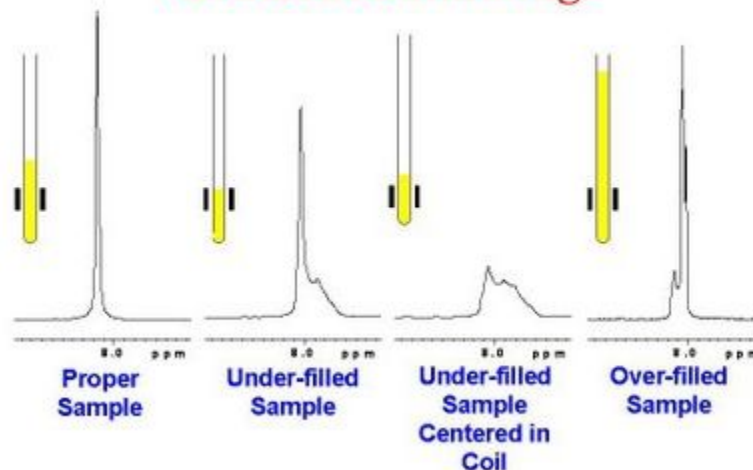
Cross Peak in the Phase Sensitive COSY Spectrum of Ethyl Acetate



COSY-90 vs COSY-45

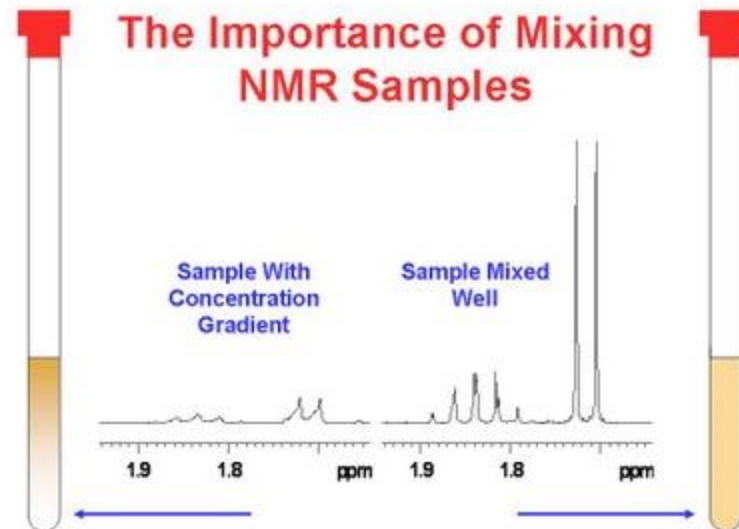
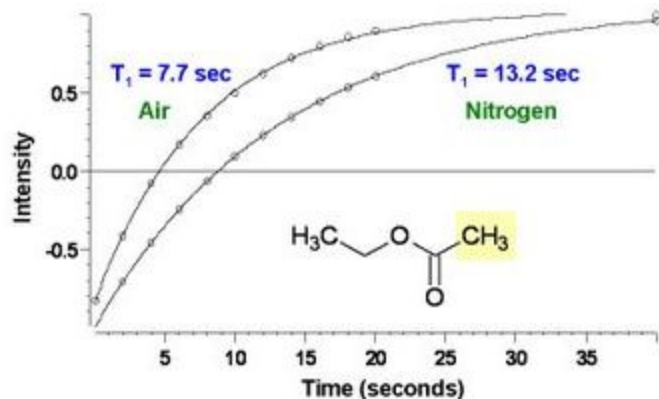


The Effect of Sample Volume on Gradient Shimming

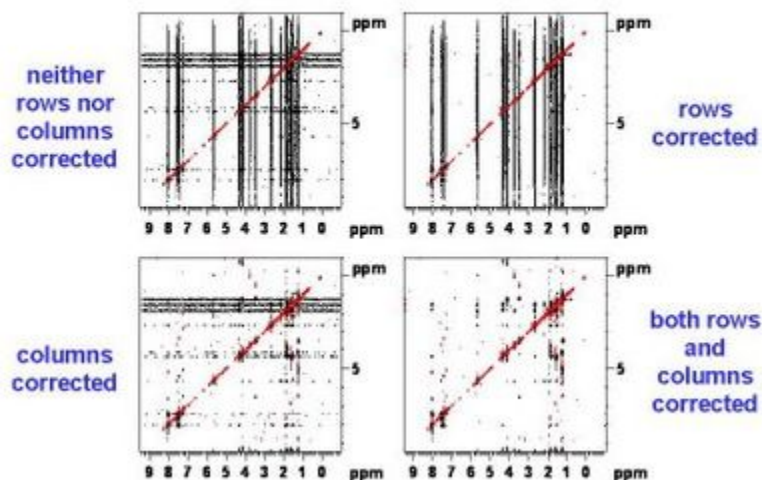


The Effect of Oxygen on T_1 Relaxation Times

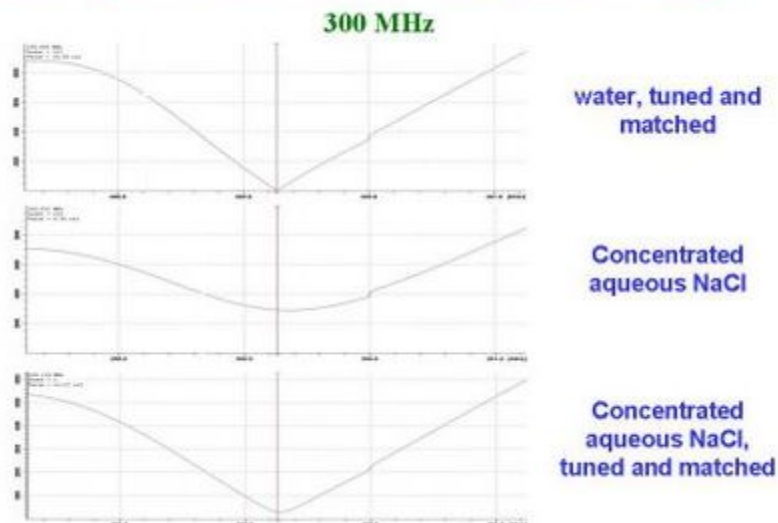
^1H T_1 Measurement for the Acetate Methyl Protons of Ethyl Acetate in Acetone- d_6 - Samples in Air and Nitrogen



Baseline Correction in 2D NMR Spectra



Tuning Problems for Samples of High Ionic Strength



Probe cleaning

- Over time, the "stuff" from your hands and residue on the outside of your NMR tubes builds up on the inside of the inserts inside the coil of the NMR probe.
- This "stuff" contains protons and results in a background signal in all subsequent NMR spectra.
- Periodically NMR probes must be cleaned to remove this offensive residue. This can usually be accomplished by gently inserting and removing a cotton swab soaked in alcohol inside the coil insert.

¹H NMR Spectra of an Empty 5 mm Tube in an NMR Probe Before and After the Probe Was Cleaned



1) **Dispersion** is a term used to express the notion of how well resonances in an NMR spectrum are separated from one another (in Hz) - it is a qualitative term expressing the ease with which signals can be distinguished. Two signals occur at 2.1 and 2.3 ppm in the proton spectrum in a spectrometer operating at 200 MHz for ^1H .

- i) What is the frequency difference between the resonances in Hz?
- ii) What is their frequency difference (in Hz) in a spectrometer operating at 600 MHz for ^1H observation?
- iii) For a given type of nucleus, what is the general relationship between "dispersion" and the magnetic field strength of the NMR spectrometer?

i)
$$\text{Chemical shift} = \frac{\text{Frequency of signal} - \text{Frequency of reference (Hz)}}{\text{spectrometer frequency}}$$

At 200 MHz 1ppm=200 Hz, 0.1ppm=20Hz Difference in Hz=.2 ppm =40Hz

ii) At 600 MHz .1 ppm = 60Hz, .2 ppm = 120 MHz

- 2) If you measure an NMR spectrum for an alcohol like ethanol, and then add a few drops of deuterium oxide, D_2O , to the solution, allow it to settle and then re-measure the spectrum, the -OH peak disappears! Why?

4) In a magnetic field of strength 2.349 T, the resonance frequency of ^{15}N nuclei is 10.13 MHz. What is the resonance frequency of ^{15}N in a magnet of 11.745T ?

$$\omega = \gamma B_0$$

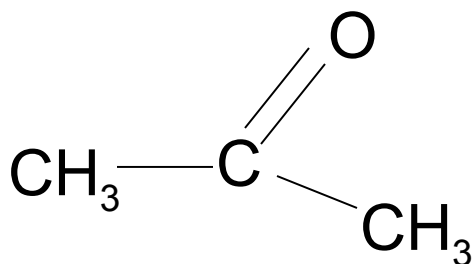
$$11.745/2.349 = 5$$

$$\omega \text{ at } 11.745 = 10.13 \times 5 = 50.65 \text{ MHz}$$

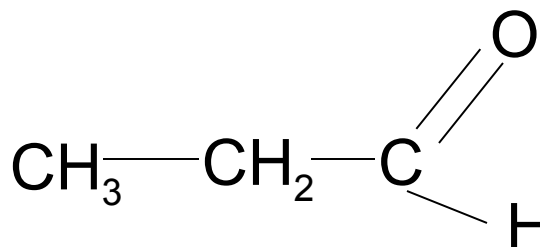
5) The intensity of the ^1H NMR signal is determined in part by the population difference between the α and β states, does the sensitivity of the experiment increase, decrease or stay the same when :

- The strength of the magnet is increased.
- The temperature of the sample is increased.
- ^2H was observed instead of ^1H .
- A sample of 10 mm diameter is used instead of 5 mm.
- Concentration of sample is increased

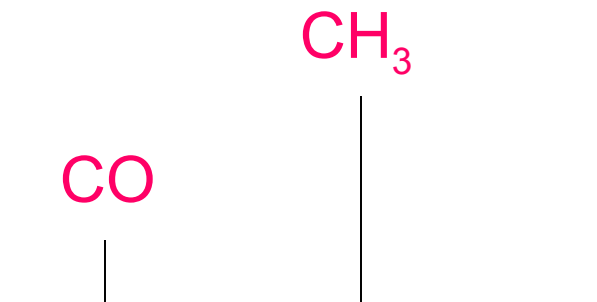
6) How could you tell from just a quick look at a C-13 NMR spectrum (and without worrying about chemical shifts) whether you had propanone or propanal (assuming those were the only options)?



Propanone



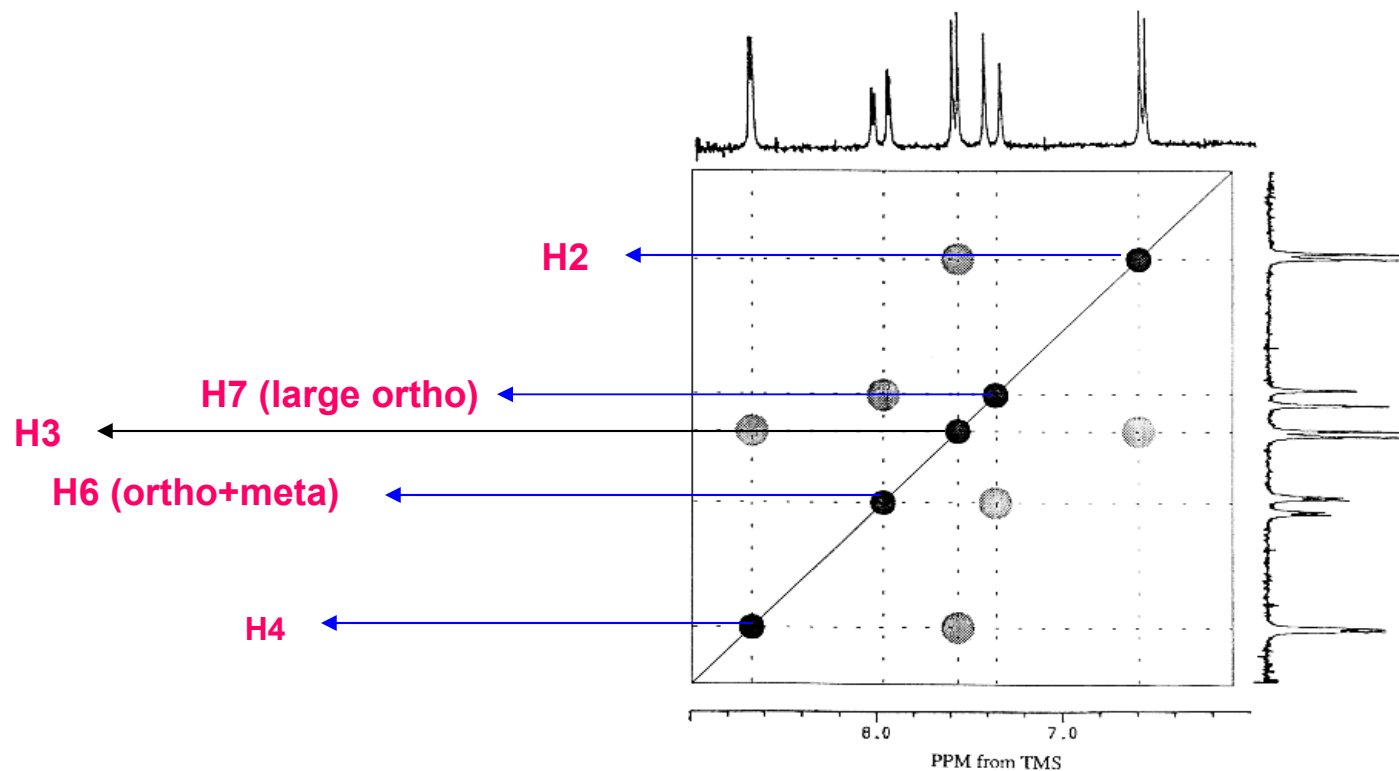
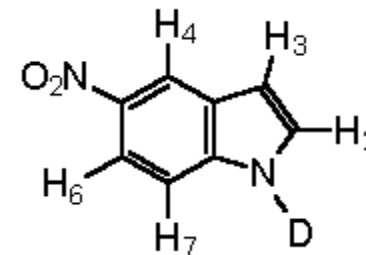
Propanal



7) The ^1H NMR spectrum of 5-nitroindole (D_2O exchanged) shows 5 resonances (d 8.7, d 8.0, d 7.5, d 7.4 and d 6.6 ppm). Given below is a schematic representation of the NOESY spectrum of 5-nitroindole, where the shaded circles represent cross peaks in the two-dimensional spectrum.

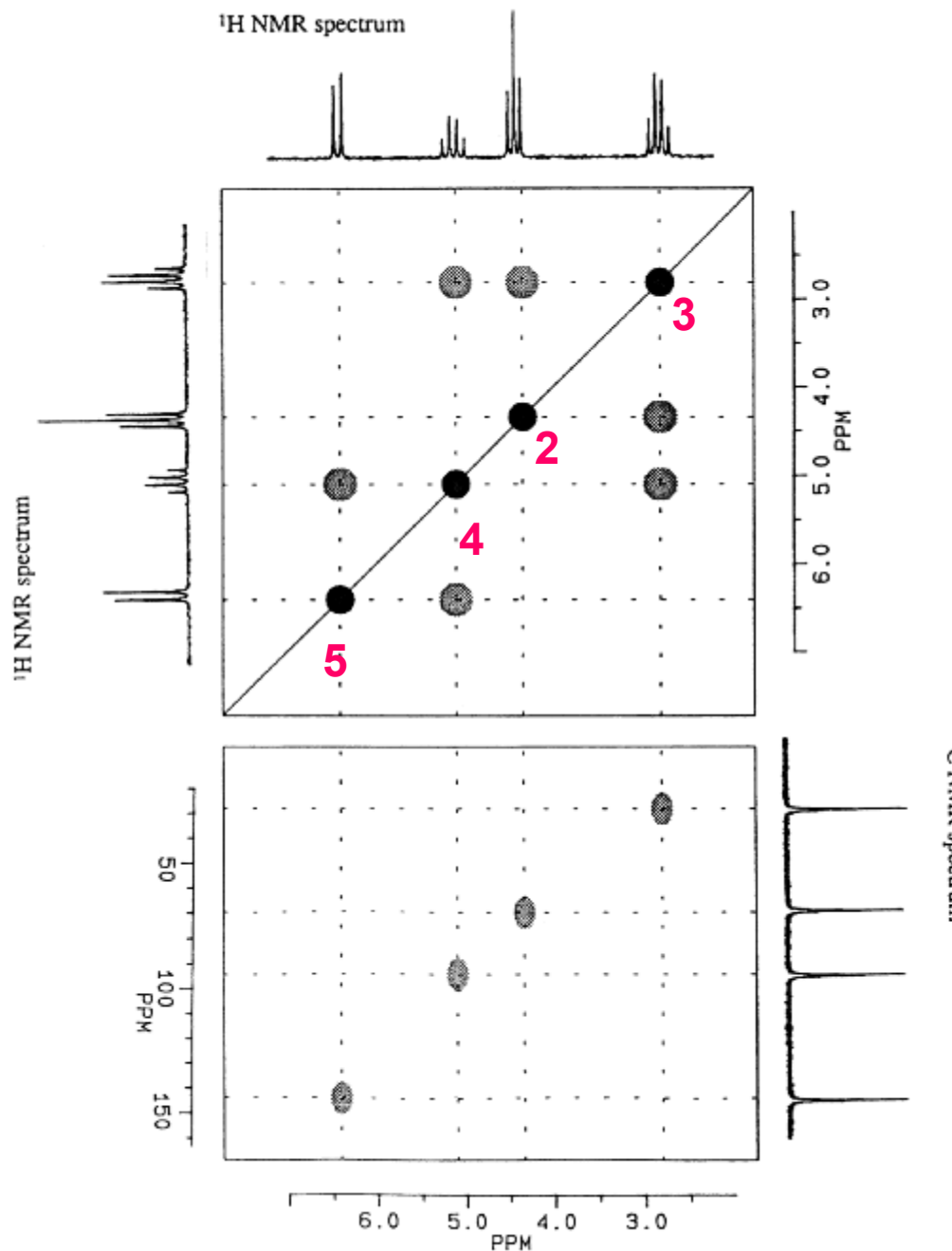
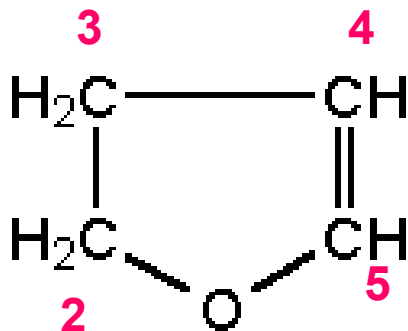
(i) Explain how the NOESY spectrum can be used to assign the signals in the ^1H spectrum.

(ii) Assign the resonances for H2, H3, H4, H6 and H7



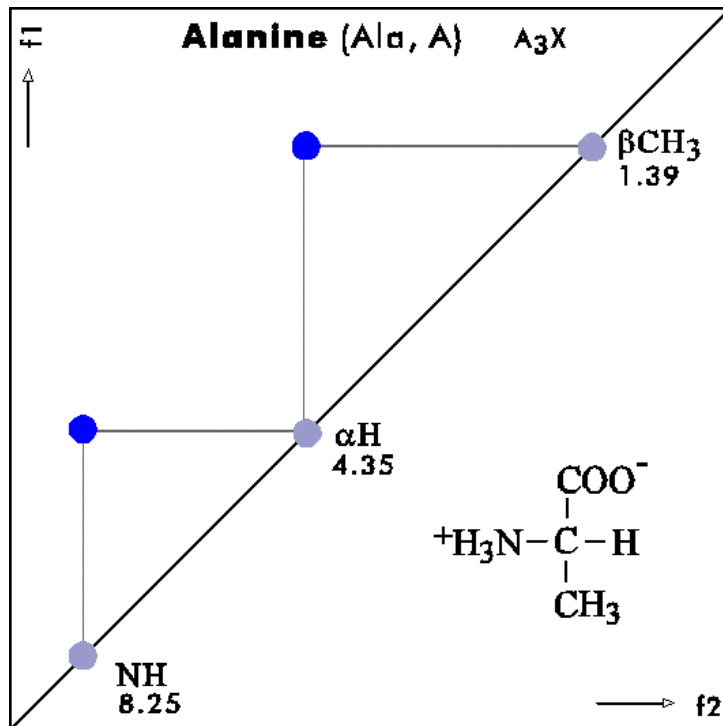
8) The ^1H NMR spectrum of 2,3-dihydrofuran (F) shows resonances at δ 2.6, δ 4.2, δ 4.9, δ 6.2 PPM. Given below is a schematic representation of the COSY spectrum of (F). The ^{13}C spectrum of (F) contains cross peaks at δ 2.6, δ 4.2, δ 4.9, δ 6.2 PPM

Explain how you could use the COSY and the C-H correlation spectrum to assign the ^1H and ^{13}C spectra (i.e. establish which ^{13}C and ^1H spectrum belongs to which specific site in the molecule.)

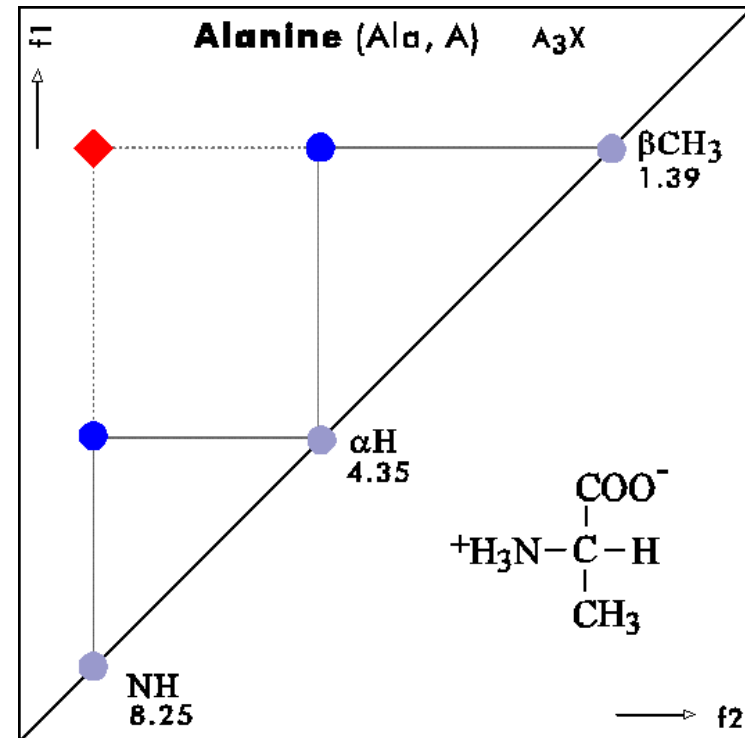


SPIN SYSTEM IDENTIFICATION IN PEPTIDES

COSY

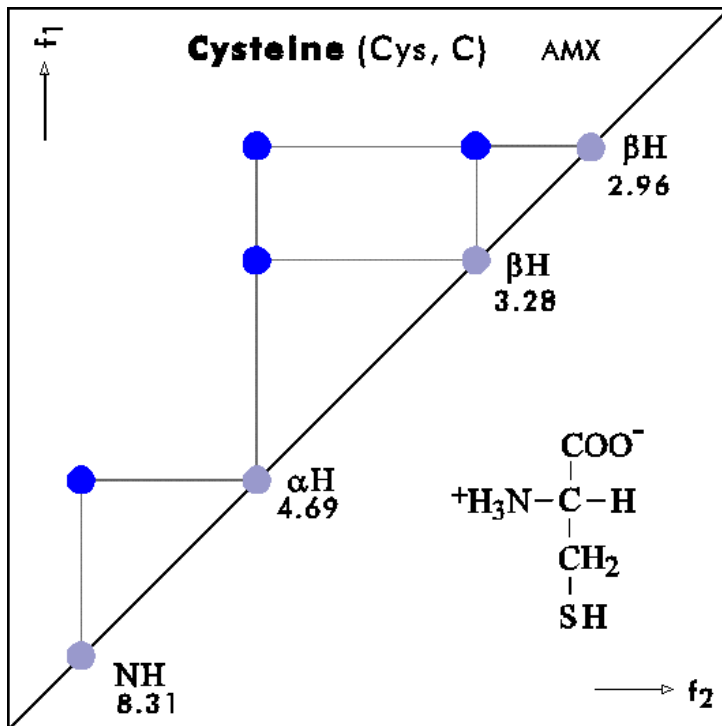


TOCSY

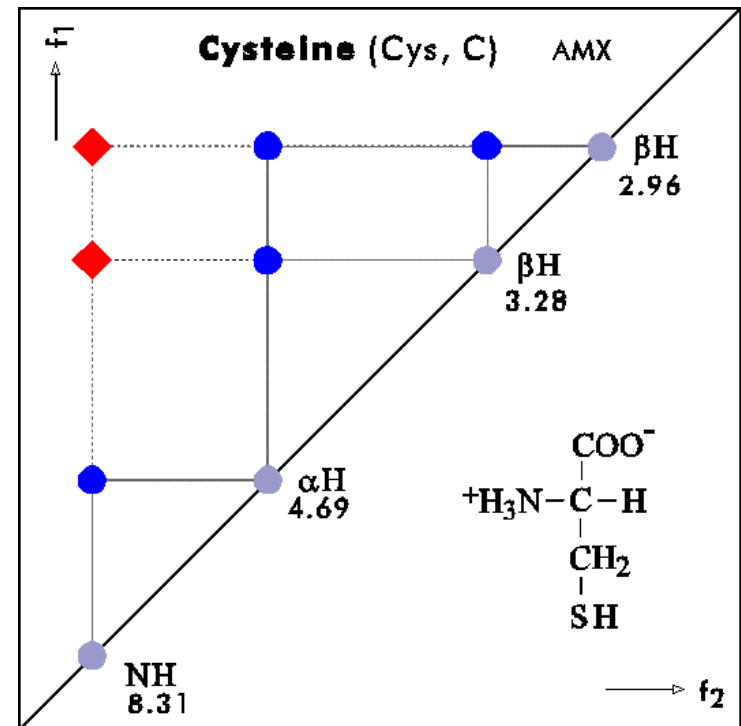


SPIN SYSTEM IDENTIFICATION IN PEPTIDES

COSY

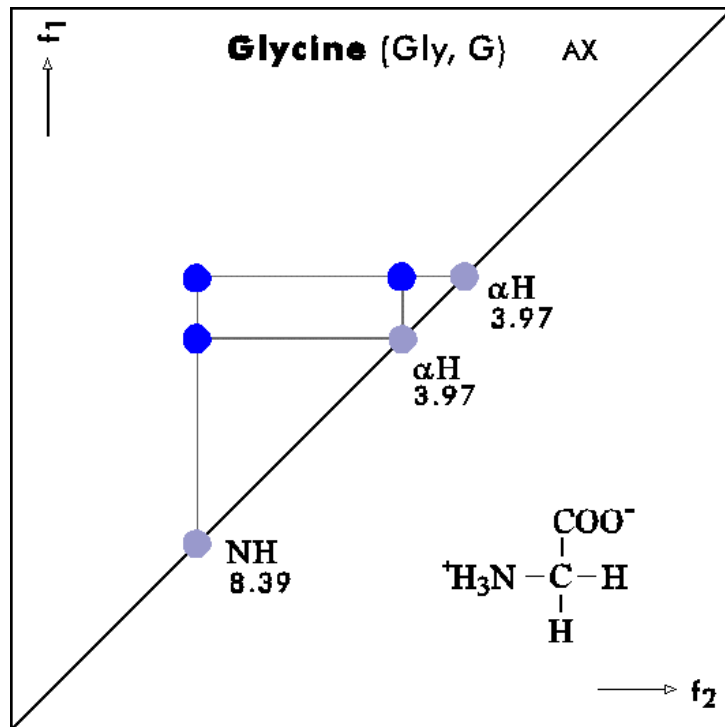


TOCSY

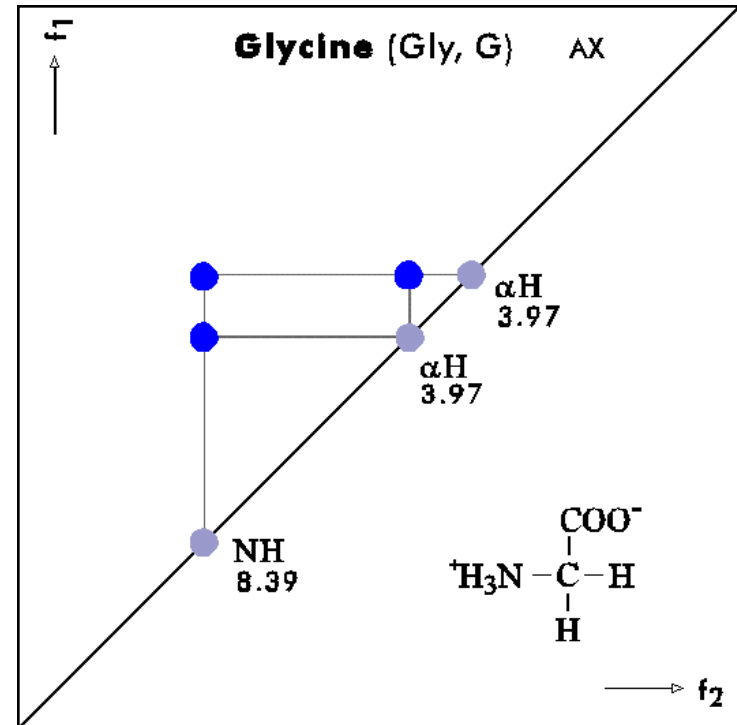


SPIN SYSTEM IDENTIFICATION IN PEPTIDES

COSY

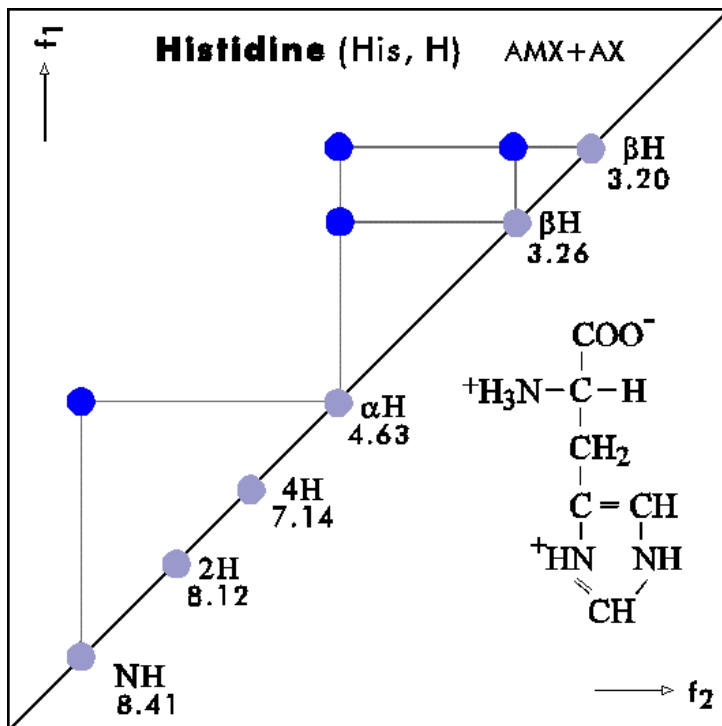


TOCSY

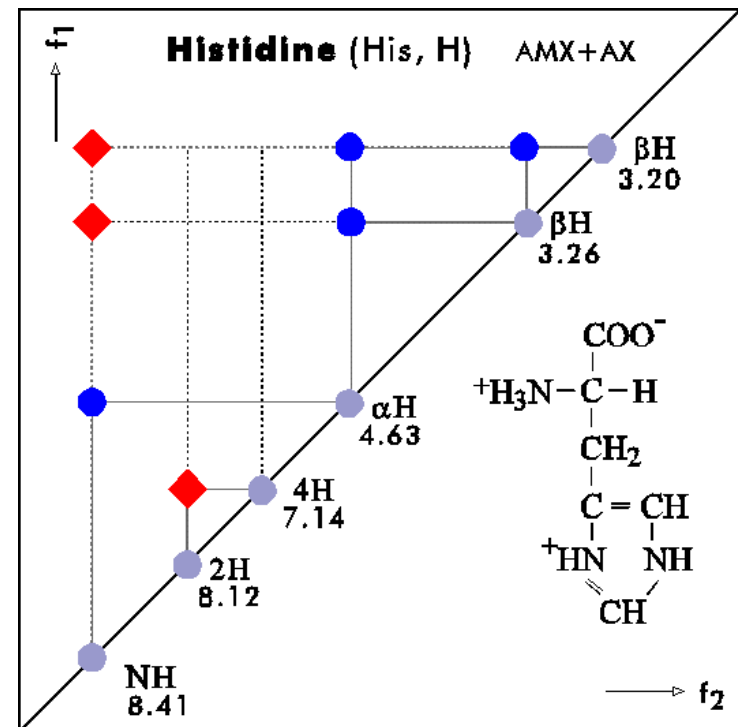


SPIN SYSTEM IDENTIFICATION IN PEPTIDES

COSY

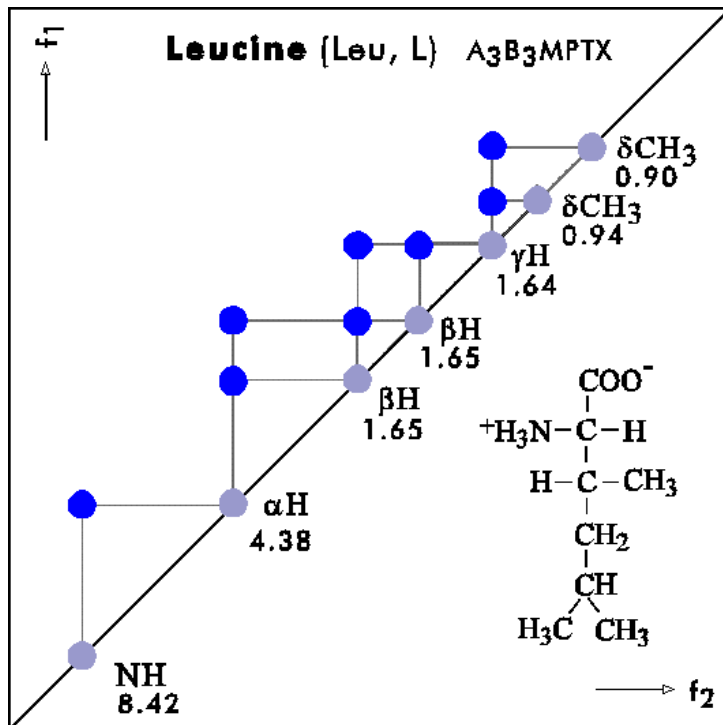


TOCSY

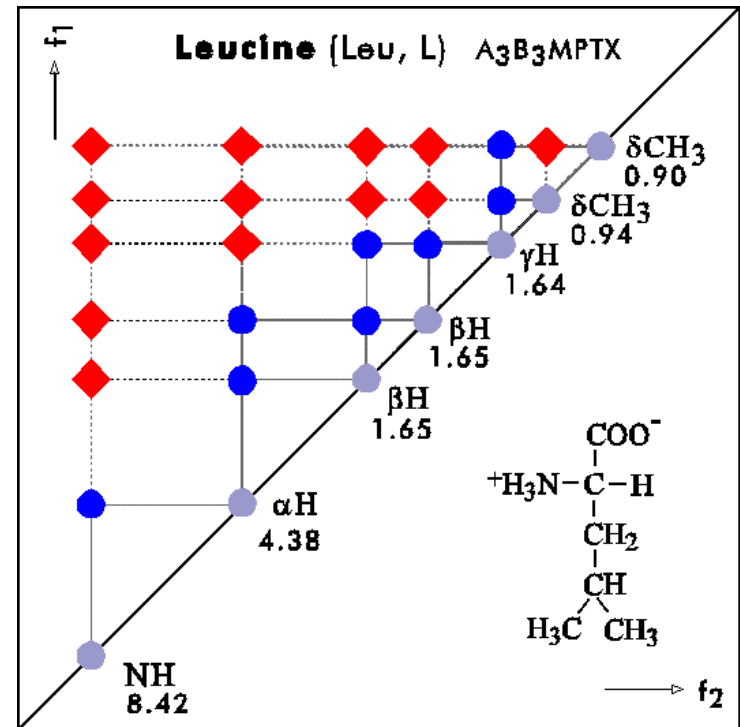


SPIN SYSTEM IDENTIFICATION IN PEPTIDES

COSY

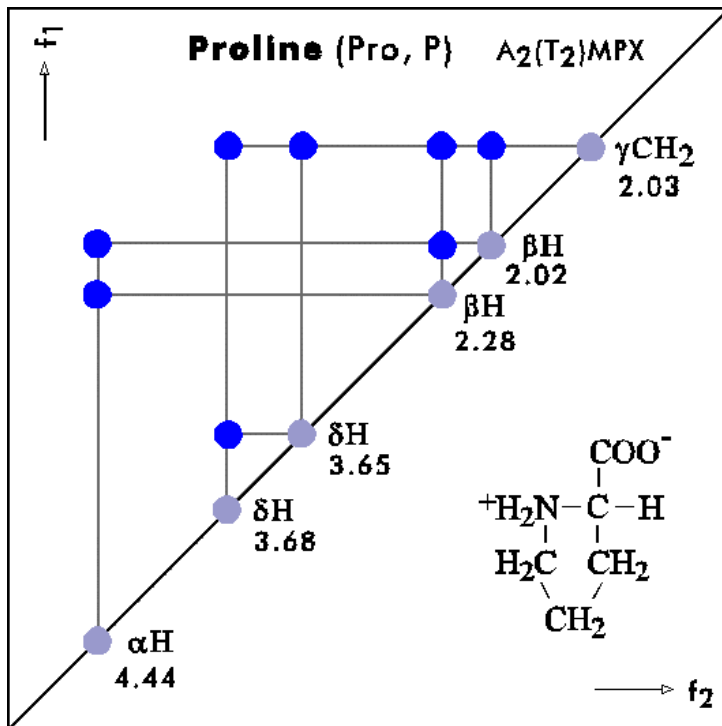


TOCSY

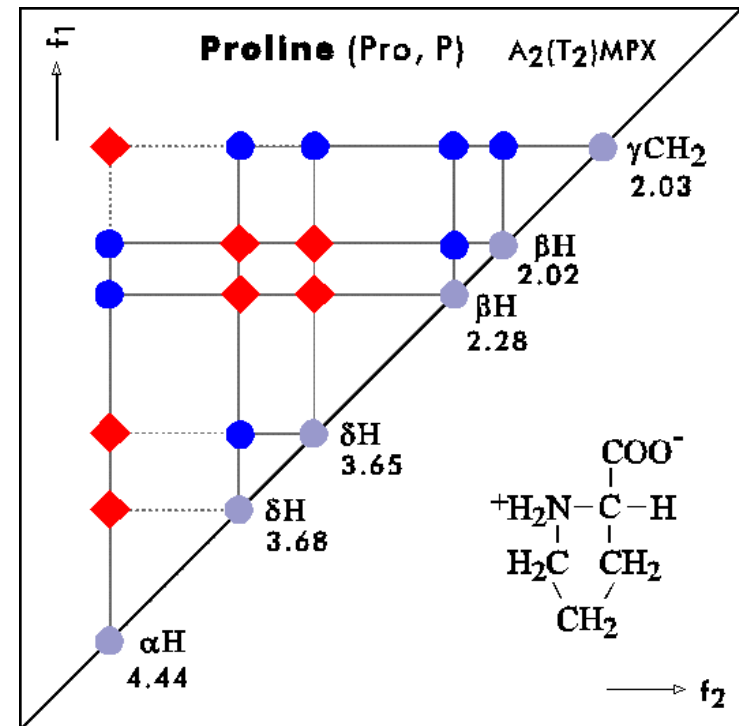


SPIN SYSTEM IDENTIFICATION IN PEPTIDES

COSY

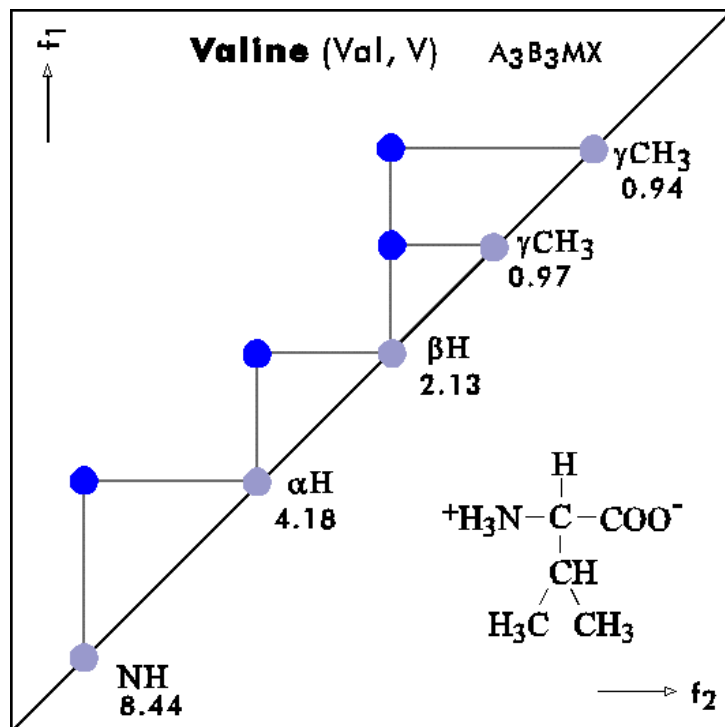


TOCSY

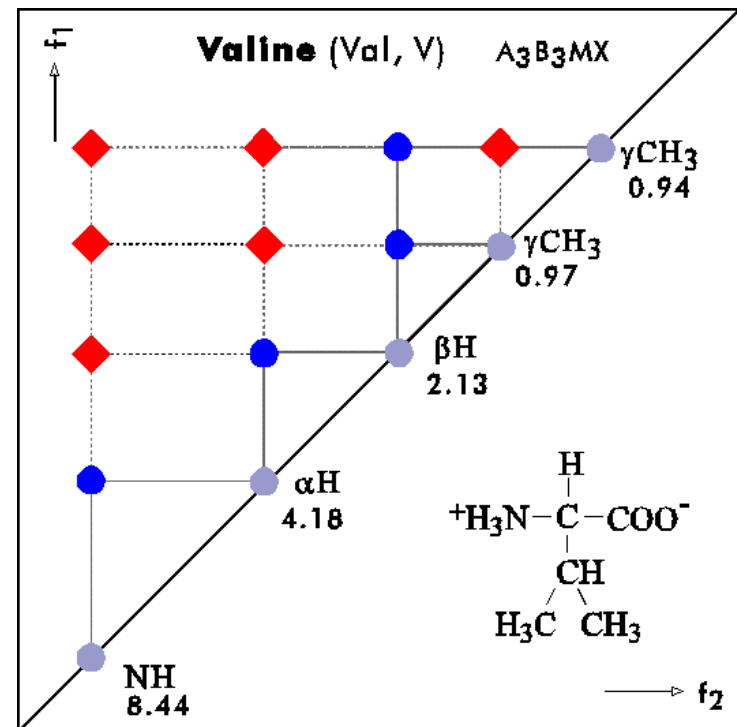


SPIN SYSTEM IDENTIFICATION IN PEPTIDES

COSY



TOCSY



Pulsed Field Gradients and Solvent Suppression.

Reading:

Pulsed Field Gradients: Cavanagh et. al, pp. 220-224

Selective Pulses and Solvent Suppression: Derome, pp. 172-178, Cavanagh et. al, pp. 144-160

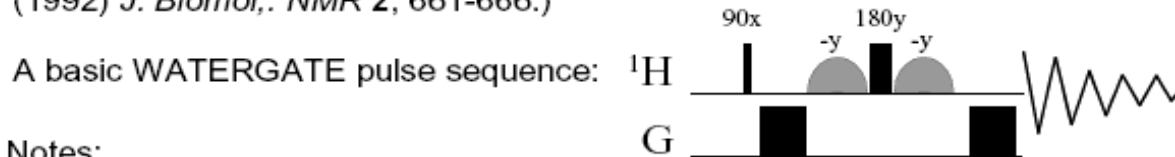
By adding another coil of wire in the probe, and running a direct current through it, one can create a magnetic field gradient across the sample, ruining the high magnetic field homogeneity that we usually struggle so hard to achieve. Surprisingly, this turns out to be a very useful thing to do.

We'll examine linear magnetic field gradients along the z-axis, parallel to **BO**. x- and y-axis gradients are also available on many newer spectrometers, but not heavily used.

- The strength and sign (positive to negative, or negative to positive) of the gradient can be varied.
- The magnetic field at any position along the z-axis in the sample, $B(z) = B_0 + zG_z$, where G_z is the gradient strength (usually in Gauss/cm), z is the position along the z-axis in the sample, and the gradient strength is zero at the center of the sample.
- Typical gradient strengths are 1-100 Gauss/cm. Remembering that 1 Tesla = 10⁴ Gauss, the gradient strength is $\leq 0.1\%$ that of static magnetic field, B_0 .
- The gradient is gated on and off (hence "pulsed" field gradient). Typical lengths for gradient pulses are 100 μ s to 5 ms

Solvent (water) Suppression.

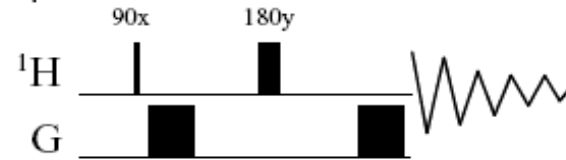
One of the best and most common current method of solvent suppression is called "WATERGATE" (**W**ATER suppression through **Gr**ADient **T**ailored **E**xcitation, Piotto et al, (1992) *J. Biomol., NMR* 2, 661-666.)



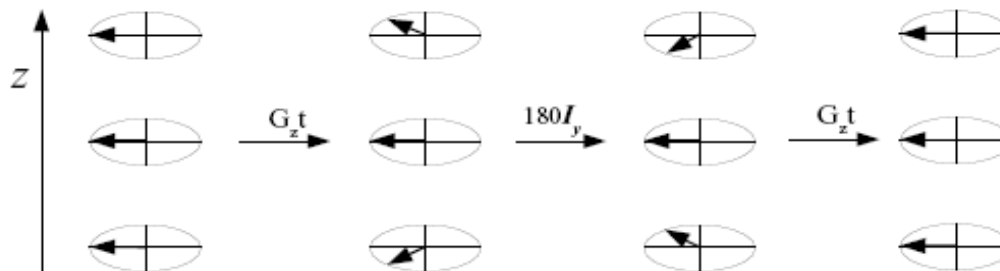
Notes:

- The rectangular proton pulses are "hard" non-selective 90_x and 180_y pulses.
- The shaped proton pulses are applied at the solvent (water) frequency, and don't affect other protons.
- The gradient pulses both have the same strength, sign, and duration.
- What happens is that most protons see a gradient- 180 -gradient, and are refocussed, while the solvent experiences a gradient- 0 -gradient, and is dephased.

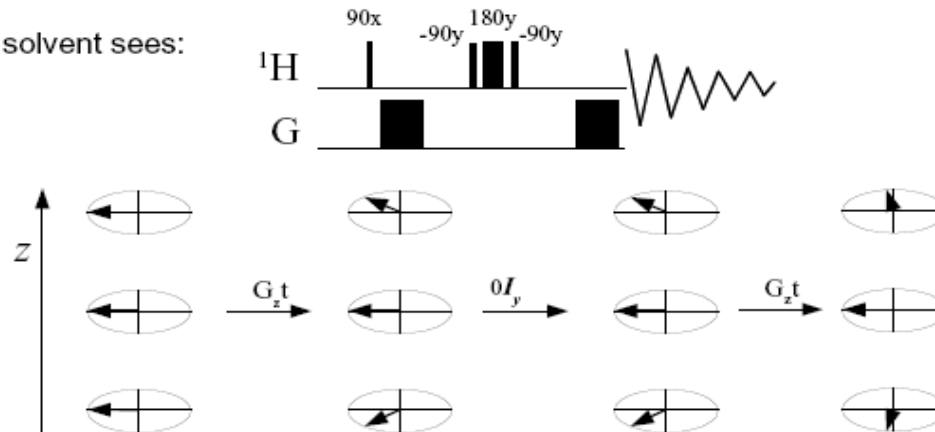
Looking separately at sample and solvent, the sample (solute) sees:



Which, as we've seen is a "gradient echo":



... but the solvent sees:



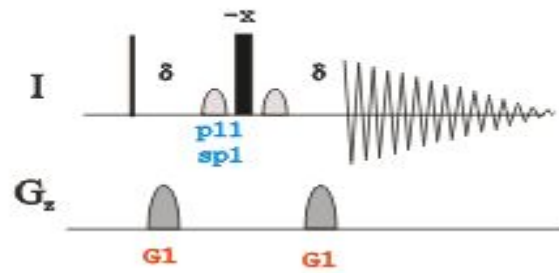
... and is completely dephased.

Advantages of WATERGATE:

- Fast solvent suppression (solvent is suppressed in milliseconds, (compare to ~ 1 sec for "presat")
- It works very well, usually much better than presaturation.
- This pulse sequence forms an element or module. It can be appended to almost any 1D, 2D, or 3D experiment to give solvent suppression.

There are also other solvent suppression methods. If time remains at the end of the lecture we'll go through the "Jump and Return", which works by not exciting the solvent at all. It is particularly useful for observing protons that are in rapid exchange with water (dephasing the water over milliseconds would dephase their magnetization as well, if they exchanged on that time scale).

zggpwg



zgesgp

