A quick look at 2-D NMR techniques:

4. Nomenclature:
   - The first perturbation of the system (pulse) is called the *preparation* of the spin system.
   - The effects of this pulse are allowed to coalese; this is known as the *evolution time*, t_1 (NOT T_1 – the relaxation time)
   - During this time, a *mixing event*, in which information from one part of the spin system is relayed to other parts, occurs
   - Finally, an *acquisition period* (t_2) as with all 1-D experiments.

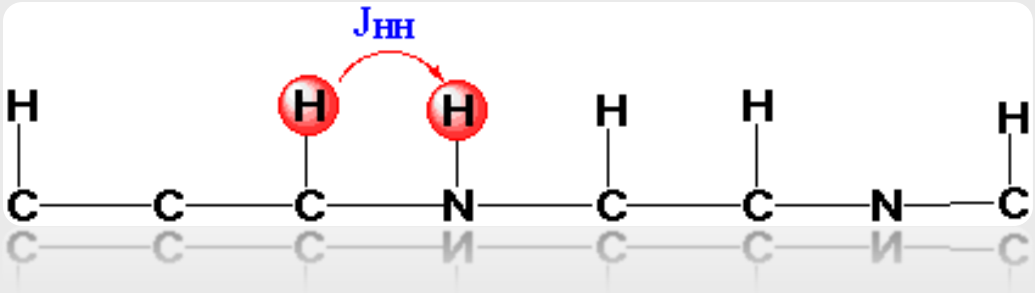
Graphically:

DESCRIPTION

The **2D COSY** experiment is the most simple and widely used 2D experiment.

It is an homonuclear chemical shift correlation experiment based on the transfer polarization by a mixing pulse between directly *J*-coupled spins.

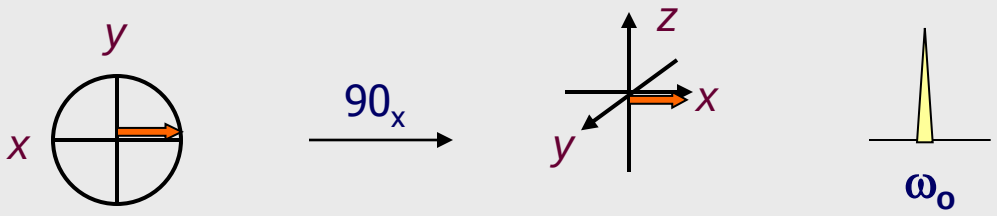
Thus, homonuclear through-bond interactions can be trace out by simple analysis of the 2D map providing a more general and more useful alternative to classical 1D homodecoupling experiments.



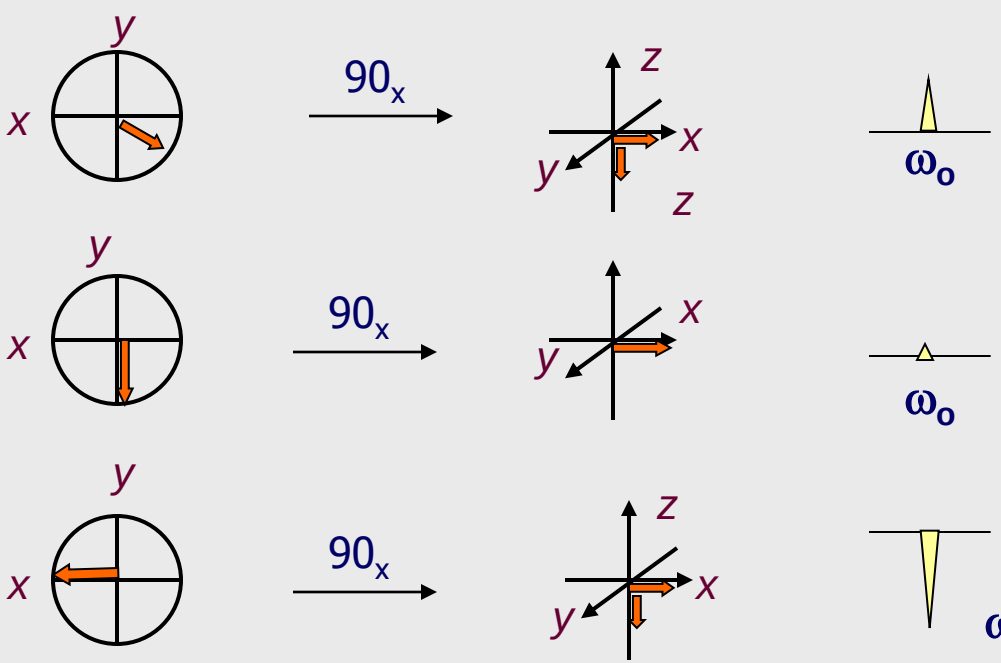
COSY:

1. H-H COrrelation SpectroscopY (COSY):
   d. Observe what occurs with several pulses in the x-plane with different t_1 times:

Pulse gives normal decay

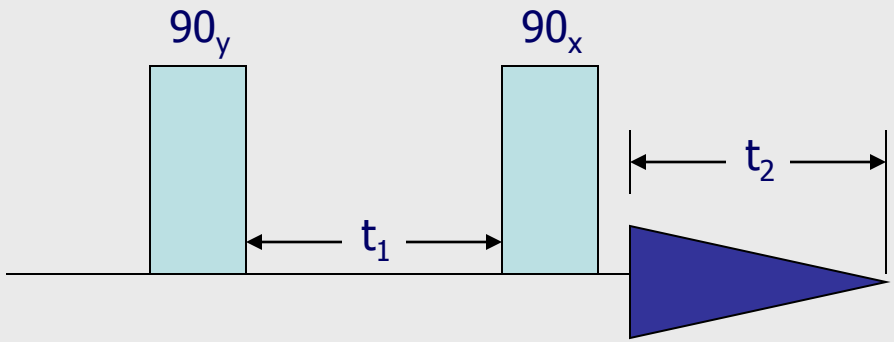


Next pulse (lower t_1) "catches" the relaxation out of phase – X-component decreased



COSY:

1. H-H COrrelation SpectroscopY (COSY):
   a. The pulse sequence for COSY is illustrated by the following:



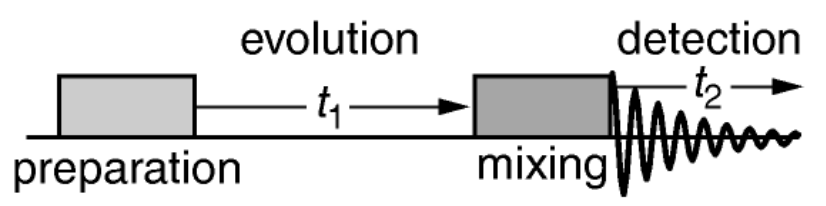
   b. A 90° pulse in the x-direction is what we used for 1-D ^{1}H NMR

   c. Here, after a variable "mixing" period, a 90° pulse in the y-direction is performed, followed by acquisition of a spectrum

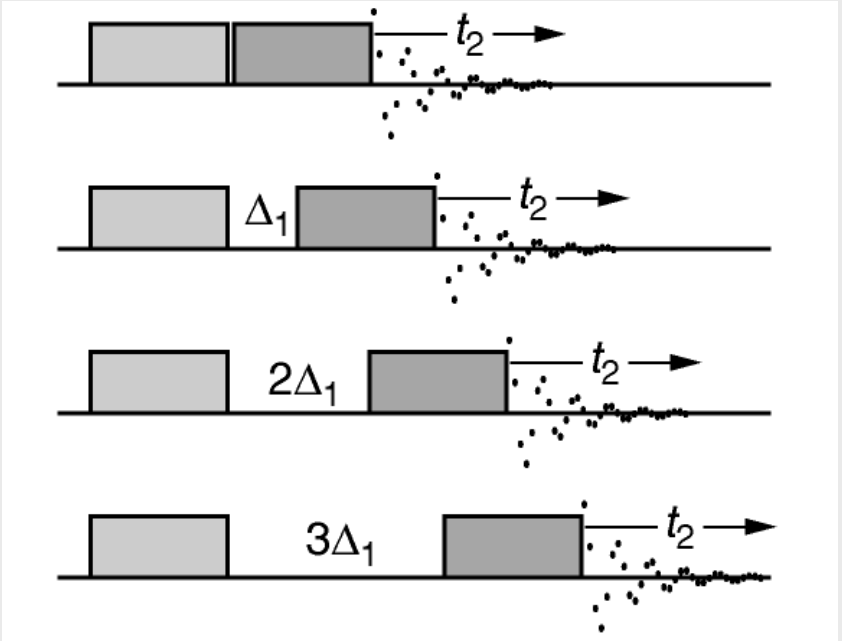
2D COrrelation SpectroscopY

The basis for this experiment;

- As with any 2-D technique we use a "pulse sequence"



- By varying the t_1 time, we allow the "prepared" protons to transfer their spin to their neighbors
- By exciting this population with another pulse and obtaining the FT we will observe the protons that are coupled
- For each individual t_1 we take an ^1H NMR, each will be transformed in the "normal" direction
- Across the array of t_1 we can take Fourier transforms and obtain the "cross" relationships

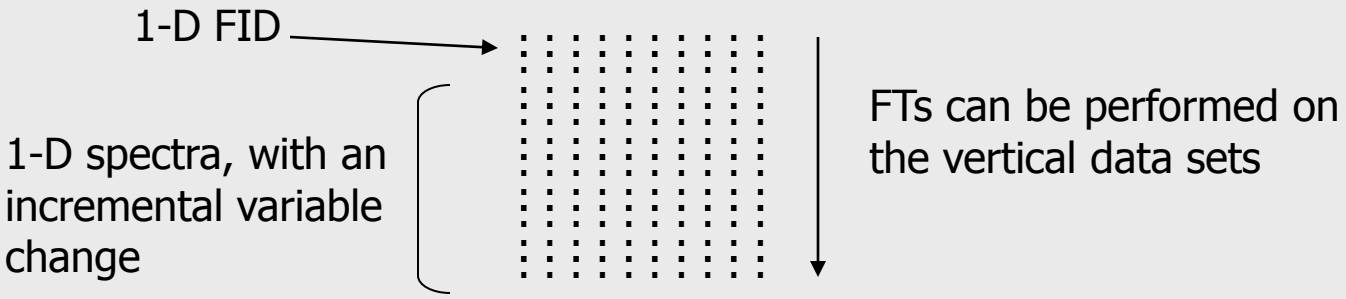


A quick look at 2-D NMR techniques:

1. In actuality, the techniques we have already covered ^{1}H, ^{13}C, and DEPT are 2-D (frequency vs. intensity) however, by tradition the intensity component is dropped when discussing dimensionality

2. In the following techniques, many FIDs (proto-NMR spectra) are taken one after another, with some acquisition variable or pulse sequenced varied by small increments

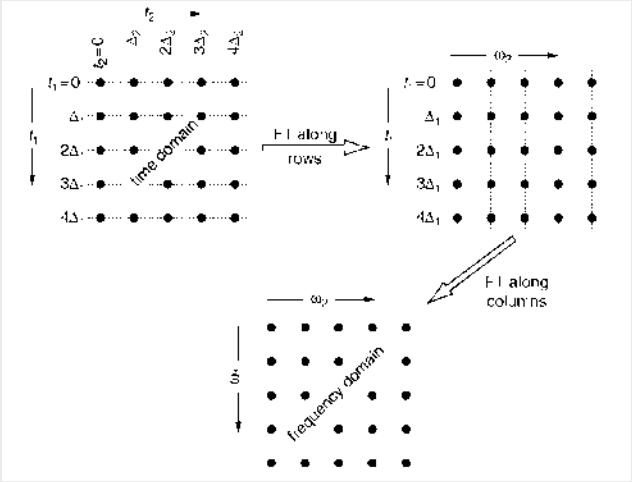
3. Since each FID is a collection of digitized data points in the first dimension (say 10 points to make a spectrum) if 10 spectra are accumulated with an incremental change in variable, an FT can be performed in the other dimension

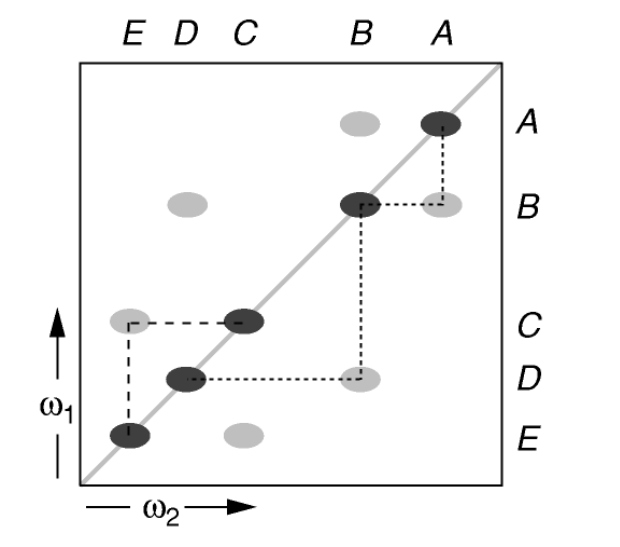


2D COrrelation SpectroscopY

The basis for this experiment;

- In the upper figure we see the array of data with two successive FTs
- In the lower diagram we see a cartoon of the result
- Diagonal peaks are created as full relaxation by the originally excited protons is not complete by t1, so they are reobserved following the second pulse
- Off diagonal peaks give the relationship between neighboring spin systems.
- We see that E-C are a coupled spin system which is isolated from the A-B-D coupled spin system (A is coupled to B and B is coupled to D)
- This molecule would be A-B-D-(group or quaternary carbon)-C-E





COSY:

1. H-H COrrelation SpectroscopY (COSY):
   d. The second π / 2 pulse acts only on the y axis component of the magnetization of the x-y plane.

   e. The x-axis component is not affected, but its amplitude will depend on the frequency of the line:

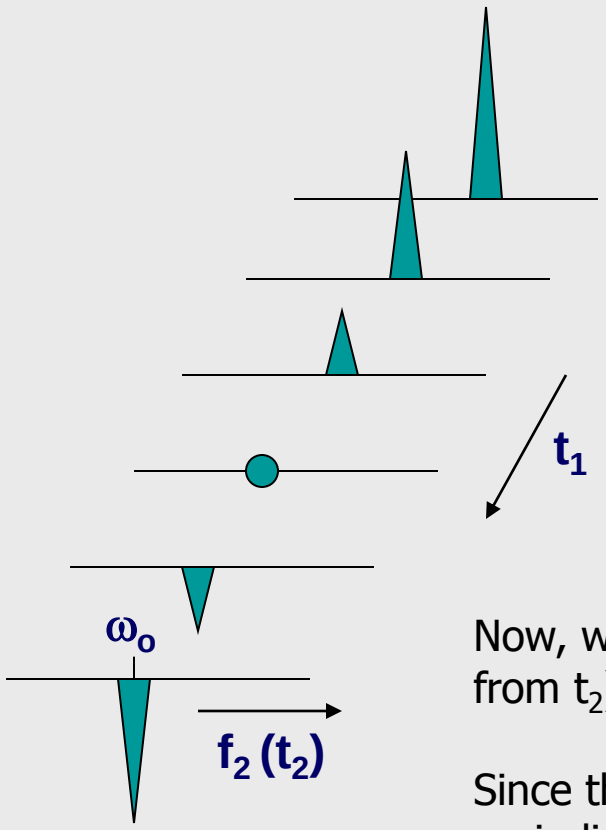
$$A(t_1) = A_o \cdot \cos(\omega_o \cdot t_1)$$

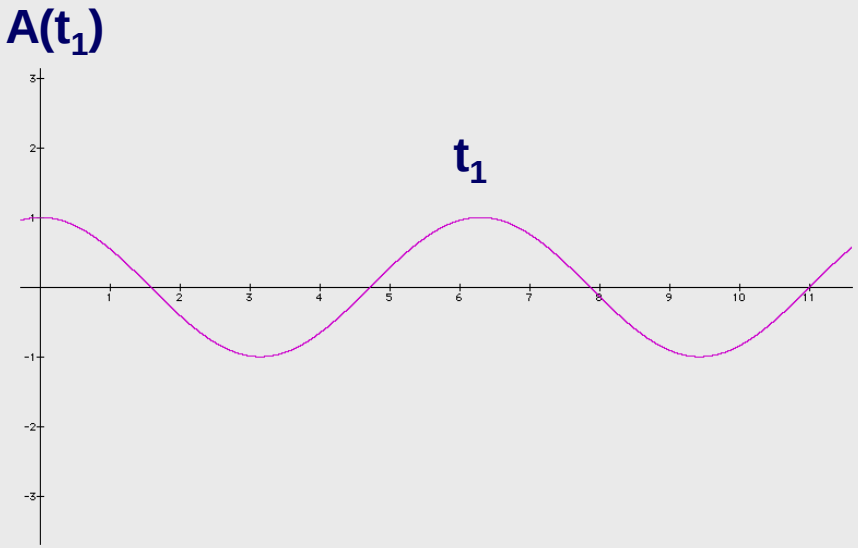
   – COSY (black box) looks for where the relationship between two protons that are coupled to one another will demonstrate a coherence in the 2nd dimension by this equation

   – If there is a coherence, a FID in the 2nd dimension will afford a peak – remember the FT of a periodic function gives a line

COSY:

1. H-H COrrelation SpectroscopY (COSY):
   f. Looking at the plot of stacked spectra:





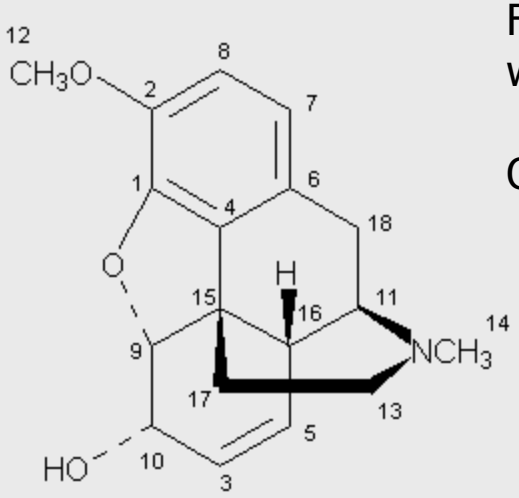
Now, we have frequency data in one axis (f_2, which came from t_2), and time domain data in the other (t_1).

Since the variation of the amplitude in the t_1 domain is also periodic, we can build a pseudo FID if we look at the points for each of the frequencies or lines in f_2

COSY:

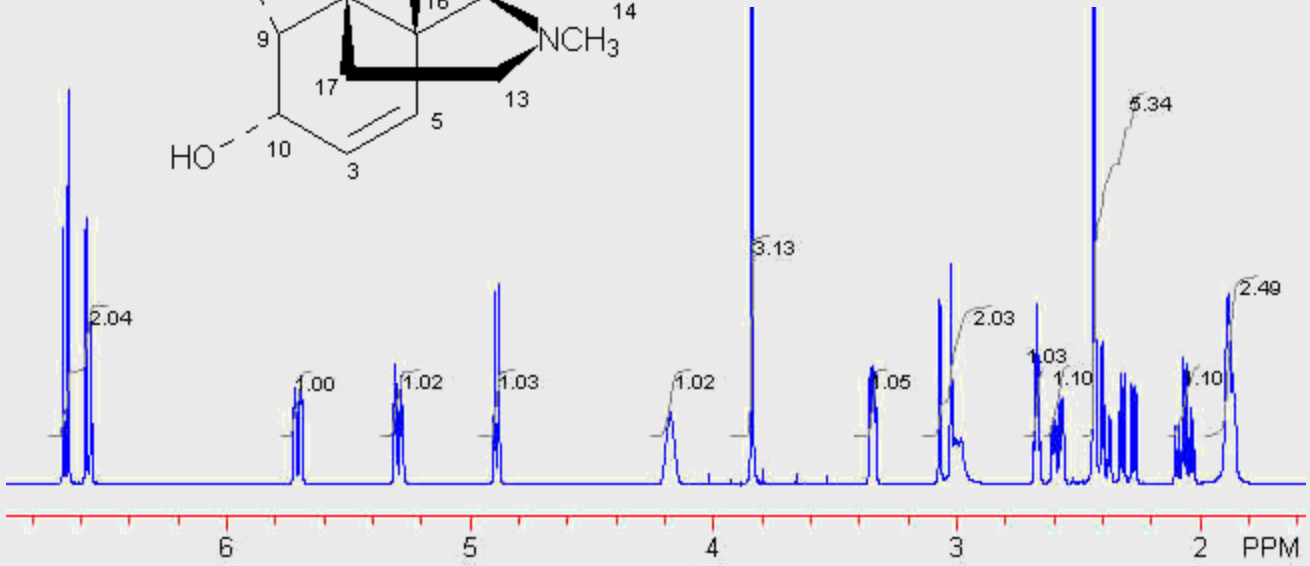
2. Performing an experiment on a real molecule

codeine



For complex molecules, ^{1}H-COSY shows you which protons are coupled

Observe the complexity of the 1-D ^{1}H-NMR:



COSY:

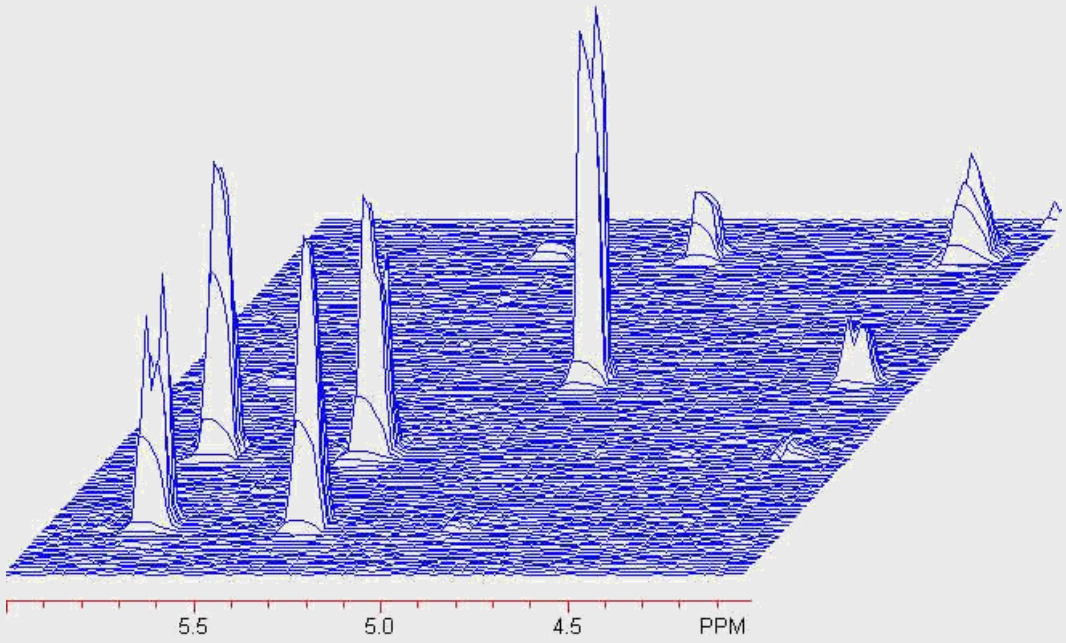
2. Performing an experiment on a real molecule

   a. The parameters used for this example:
      512 points in the "normal" dimension" (loss of some resolution)
      128 t_1 increments – 2 scans each
      1 sec T_1 delay
      Total acquisition time (500 MHz) 5 min
      Sample size 3.3 mg in .65 mL $CDCl_3$

   b. You can see there is some cost of resolution/sensitivity with regards to the normal 1-D method (2 scans, short delay, low resolution) to keep the acquisition time short, and the data file small (less a problem in 2005 – big problem in 1990)

COSY:

2. Performing an experiment on a real molecule
   c. Here is the "real" COSY spectrum:

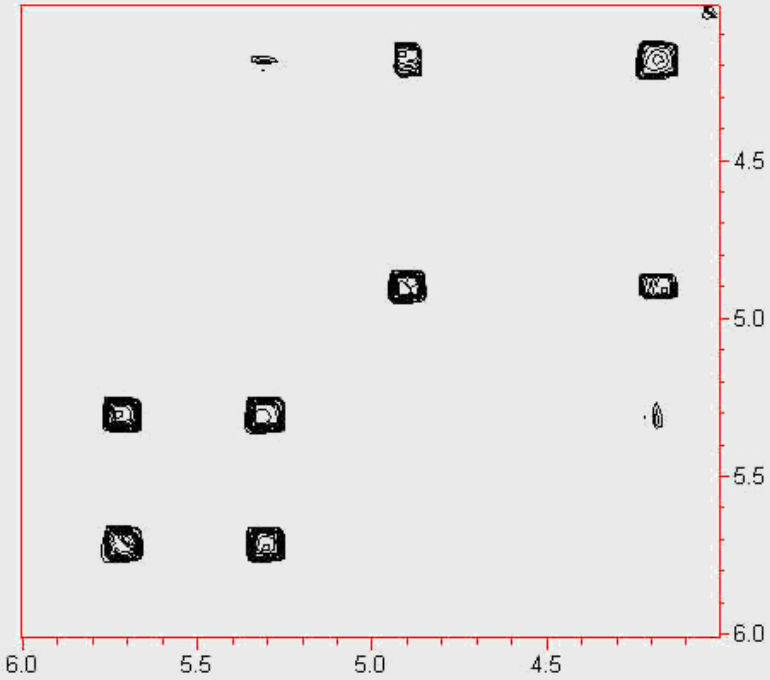


   d. Observe how this is cumbersome to use in the 3rd dimension

   e. The spectrum is converted to a "contour plot" similar to a flat map of a mountainous region....

COSY:

2. Performing an experiment on a real molecule
   e. Here is the same region in 2-D as a contour map:

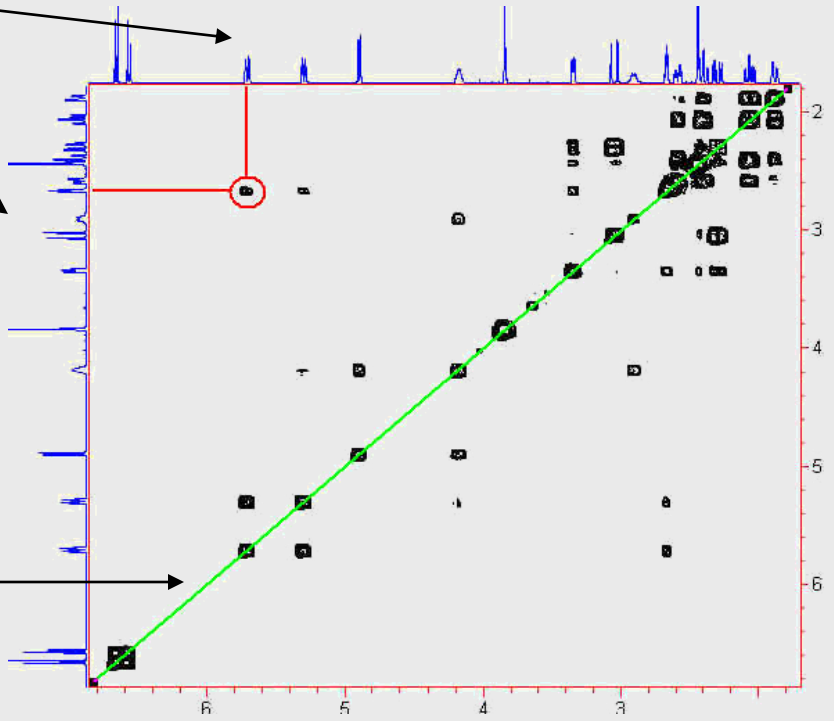


   f. Now let's see what all the mountains mean....

COSY:

2. Performing an experiment on a real molecule
   g. Looking at the entire spectrum as it would be analyzed:

On each of the two axis, there is a plot of the "normal" 1-D ^{1}H NMR spectrum



The contour plot is symmetrical about the diagonal (green), these contour peaks are meaningless for our analysis

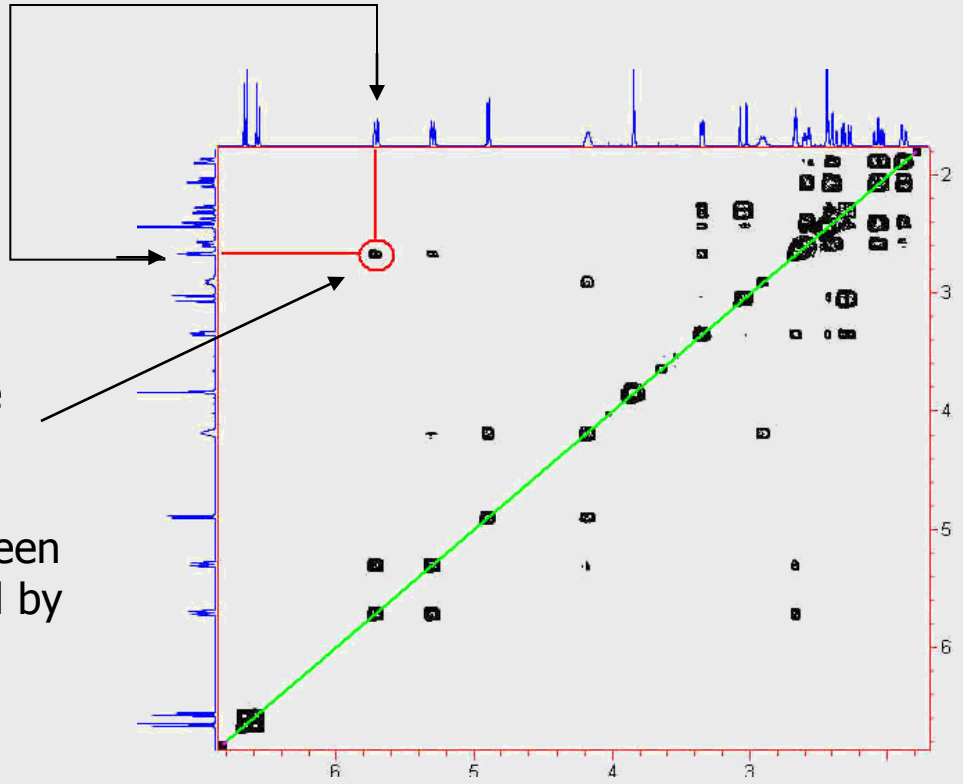
COSY:

2. Performing an experiment on a real molecule
   g. Looking at the entire spectrum as it would be analyzed:

These two protons are coupled

no n+1 rule, no tree-diagrams

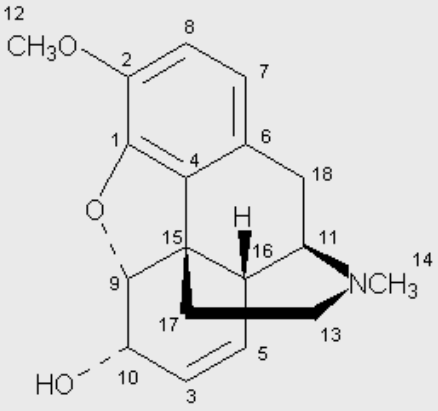
The off-diagonal peaks are the power of the COSY spectrum – here there is a coupling relationship between the two protons connected by the red line

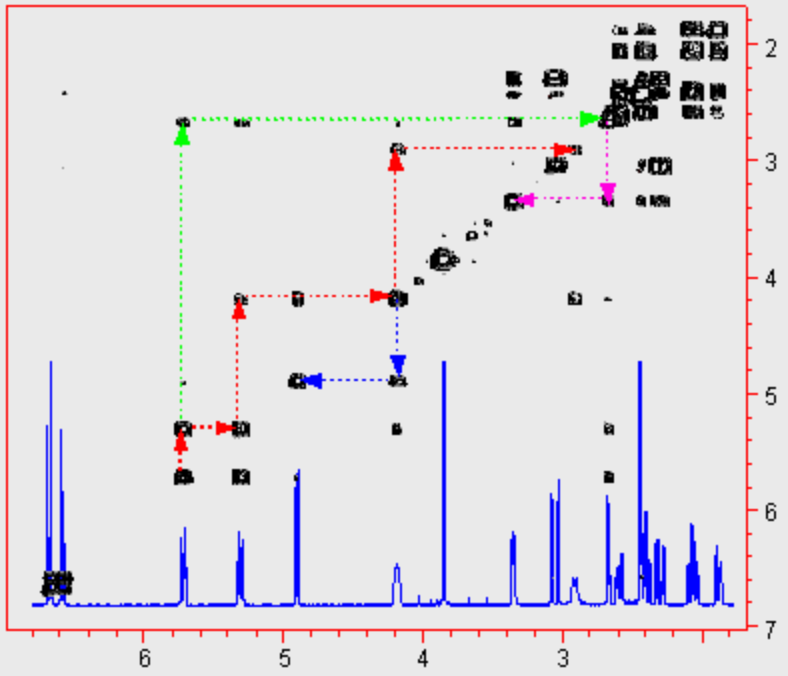


COSY:

2. Performing an experiment on a real molecule
   g. Looking at the entire spectrum as it would be analyzed:

A complete analysis would involve connecting each of the off-diagonal peaks to complete each coupling chain

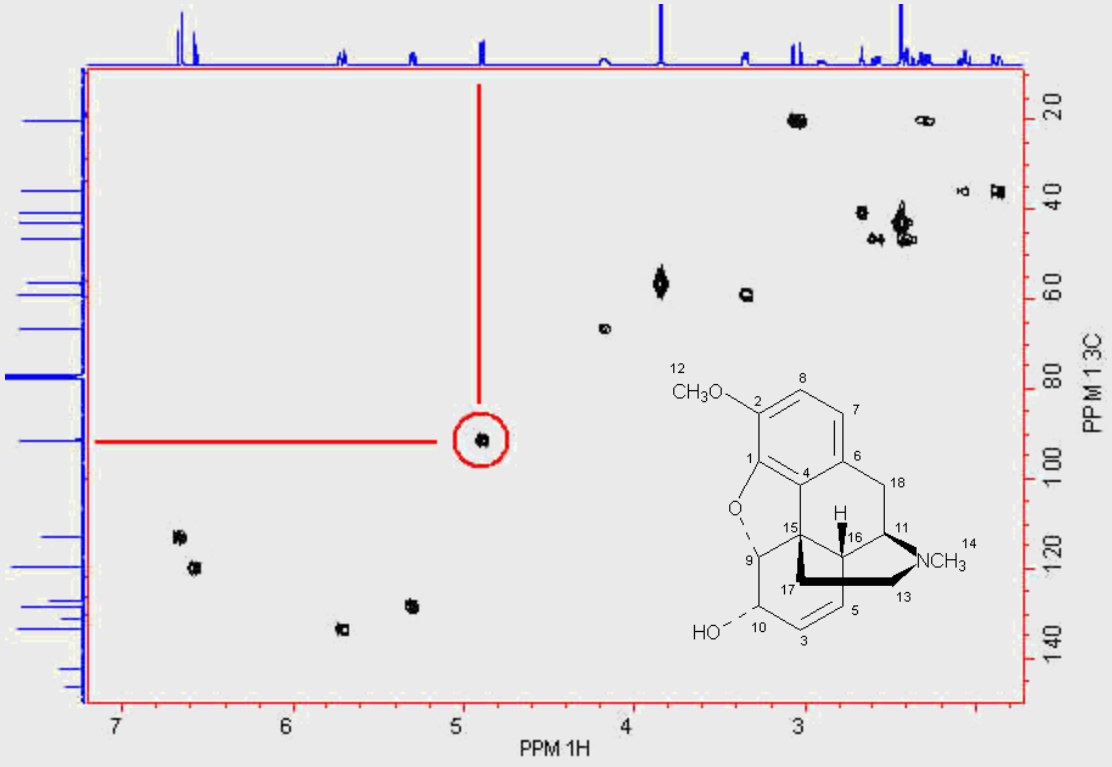




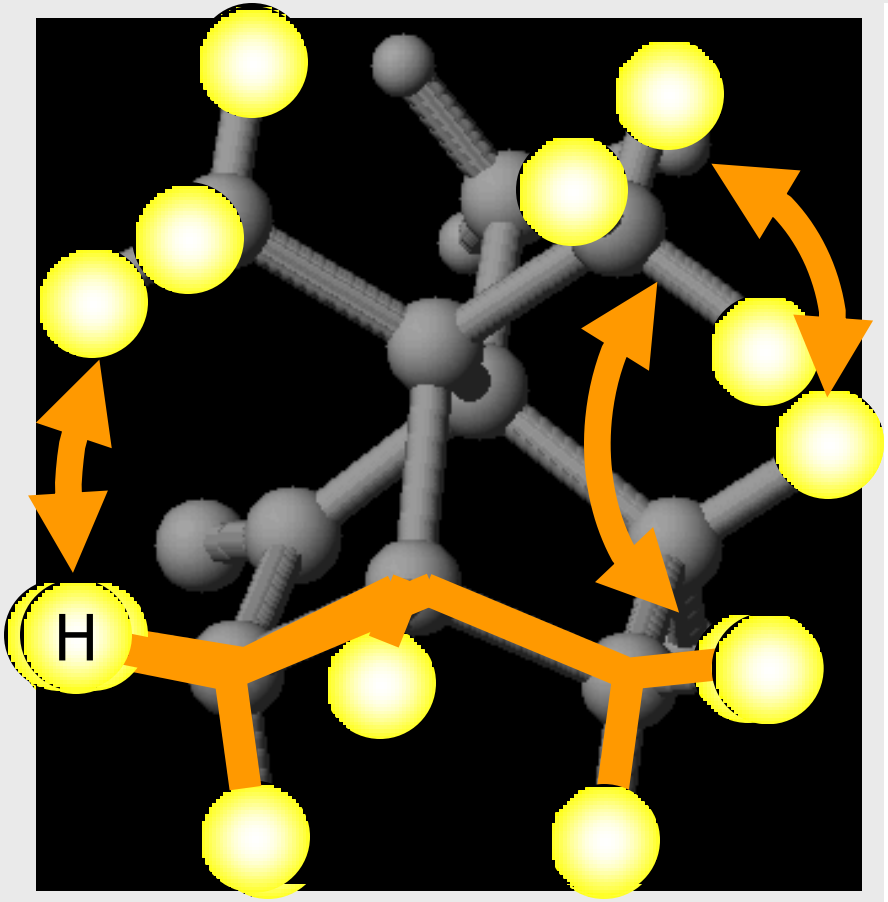
Codeine has many such "chains"
Green $H_3 - H_{16}$ (allylic)
Blue $H_{10} - H_9$
Red $H_5 - H_3 - H_{10} - OH$
Violet $H_{16} - H_{11}$

HETCOR:

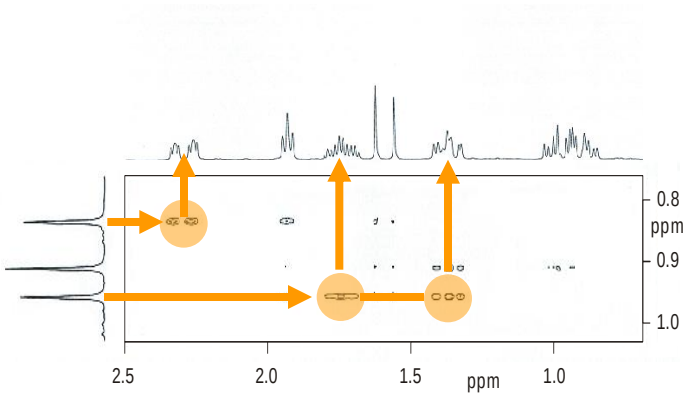
1. Also called ^{1}H-^{13}C COSY – HETeronuclear CORrelation spectroscopy
   a. The only difference on the spectral end, is that one axis is a ^{13}C spectrum
   b. From this data, you can identify which protons are bound to which carbons; again for simple structures this method is unnecessary, but for complex compounds, it is essential



Structure determination by NMR



Structure



NOESY

R. Meusinger, A. M. Chippendale, S. A. Fairhurst,
in "Ullmann's Encyclopedia of Industrial Chemistry", 6th ed., Wiley-VCH, 2001

Other Methods:

1. This lecture is by no means thorough; it is meant to give a "taste" of the power and specialization of advanced methods

2. Here is a compilation of other things that are routinely done; anyone with a knowledge of NMR theory can always devise a new experiment to see something unique! First the 1-D techniques:

Experiment	Nuclei	Types of information, applications
J-modulated spin-echo (attached proton test, APT)	^{13}C	CH and CH_3 carbon nuclei give positive signals, 4° and CH_2 carbons give negative signals. An aid to assignment.
INEPT	$^{1}H - ^{13}C$	The INEPT pulse sequence is used as a component of many 2D experiments e.g. HSQC
DEPT	^{13}C	Tells how many hydrogen atoms are directly bonded to a carbon nucleus: CH, CH_2, CH_3. Disadvantage: no signals from 4° carbon atoms.
Selective TOCSY	^{1}H	Allows identification of all the protons belonging to a common coupled spin system.
1D-INADEQUATE	^{13}C	Exact ^{13}C-^{13}C coupling constants without the need to synthesise ^{13}C enriched compounds.

Other Methods:

3. 2-D Methods and Applications

Experiment	Nuclei	Types of information, applications
Heteronuclear *J*-resolved ^{13}C NMR spectroscopy	^{13}C	^{13}C-^{1}H coupling constants, number of directly bonded protons (as in DEPT).
Homonuclear *J*-resolved ^{1}H NMR spectroscopy HOMO2DJ	^{1}H	Useful in determining chemical shift values in complicated spectra, identifying peaks of multiplets.
^{1}H-^{1}H COSY	^{1}H	Assigning signals in complicated spectra.
Long-range COSY	^{1}H	Assigning signals of protons separated by four or more bonds where the couplings are small.
^{1}H-^{13}C COSY (HMQC, Heteronuclear Multiple Quantum Coherence)	^{1}H ^{13}C	Assigning signals in proton and carbon spectra, starting from known signals.
HMBC (Heteronuclear Multiple Bond Correlation)	^{1}H ^{13}C	Assigning ^{1}H and ^{13}C signals on the basis of long range couplings.
2D-TOCSY	^{1}H	Allows identification of all the protons belongng to a common coupled spin system.
NOESY	^{1}H	Gives evidence for spatial proximity of nuclei using nuclear Overhauser effect.
EXSY	^{1}H	Qualitative evidence of exchange processes.
2D-INADEQUATE	^{13}C	Assigning signals by detecting couplings between adjacent ^{13}C nuclei.