# 2D NMR FOR THE CHEMIST

*A Practical Description*
*And Experimental*
*Guide*

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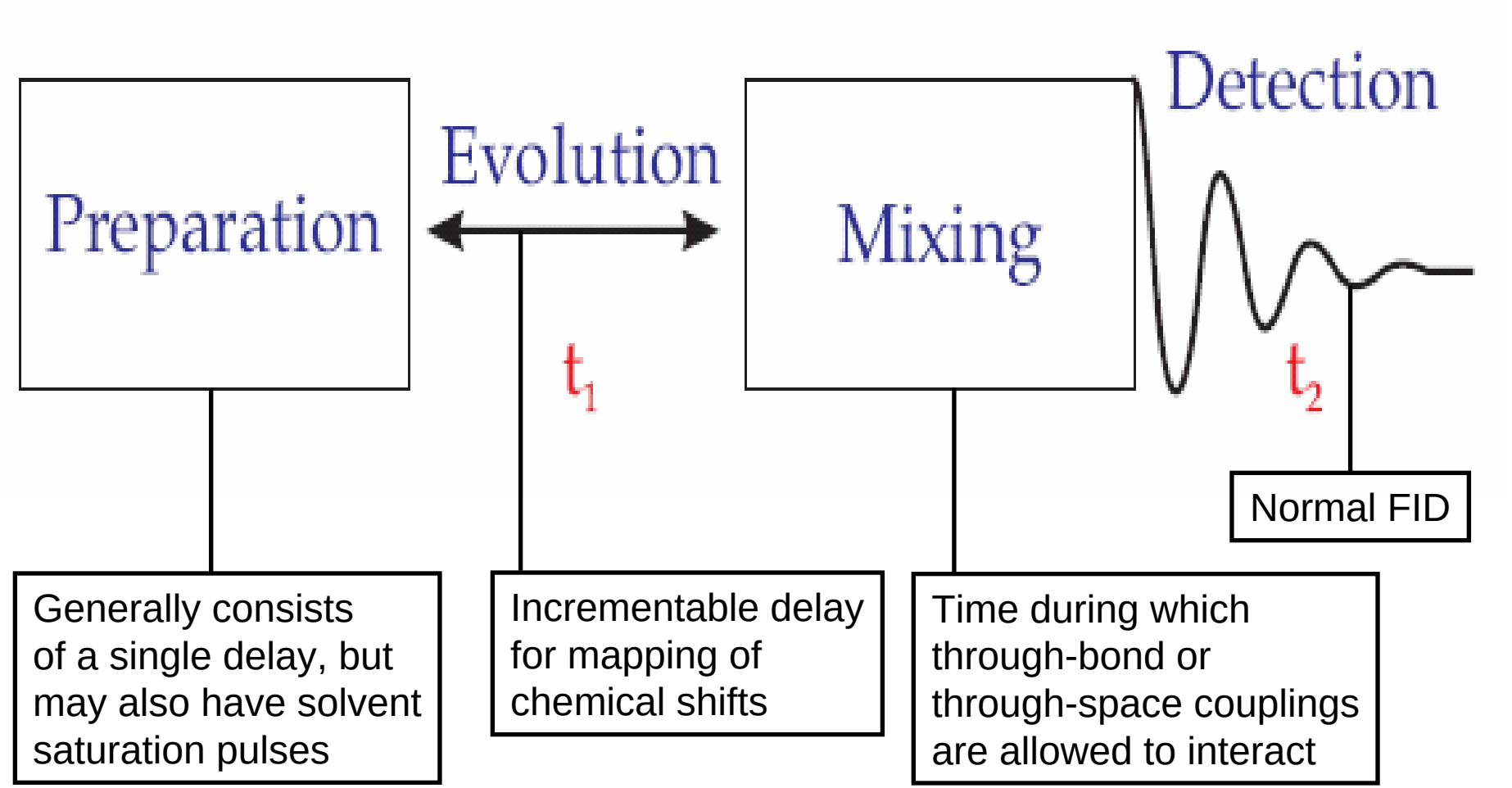
# Introduction to 2D NMR

- Varian software makes setting up, acquiring, and processing 2D NMR experiments easy
- Most 2D experiments are already set up, requiring only a minimum of user intervention for "routine" samples
- With a relatively small amount of experience, high quality data can be obtained
- Automated processing allows for minimal time spent on making 2D spectra ready for interpretation
- Most synthetic chemists in industry now run a series of 2D's on their compounds and decide later what they need

# Basics of *ANY* 2D NMR Experiment

General Schematic Description

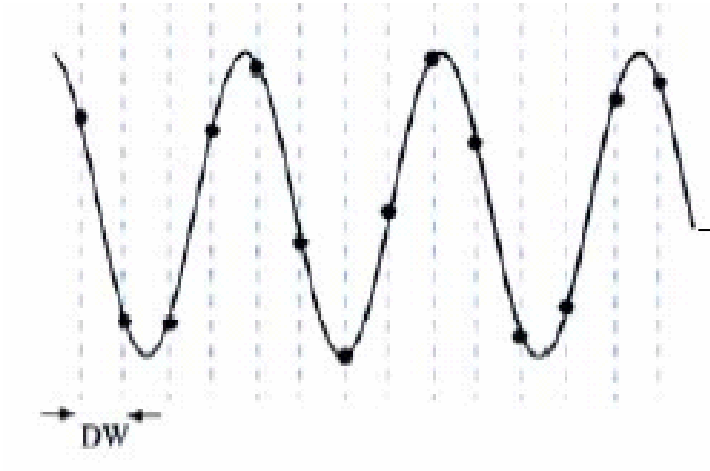


# Basics of 2D NMR

- All 2D experiments are a simple series of 1D experiments collected with different timing
- In general, 2D's can be divided into two types, homonuclear and heteronuclear
- Each type can provide either through-bond (COSY-type) or through space (NOESY-type) coupling information
- A 2D frequency correlation map is produced after a Fourier transform in both dimensions ($t_1$ and $t_2$).
- On modern spectrometers, only the proton 90 degree pulse width needs to be determined to run a full series of 2D experiments

# Foundations for 2D NMR

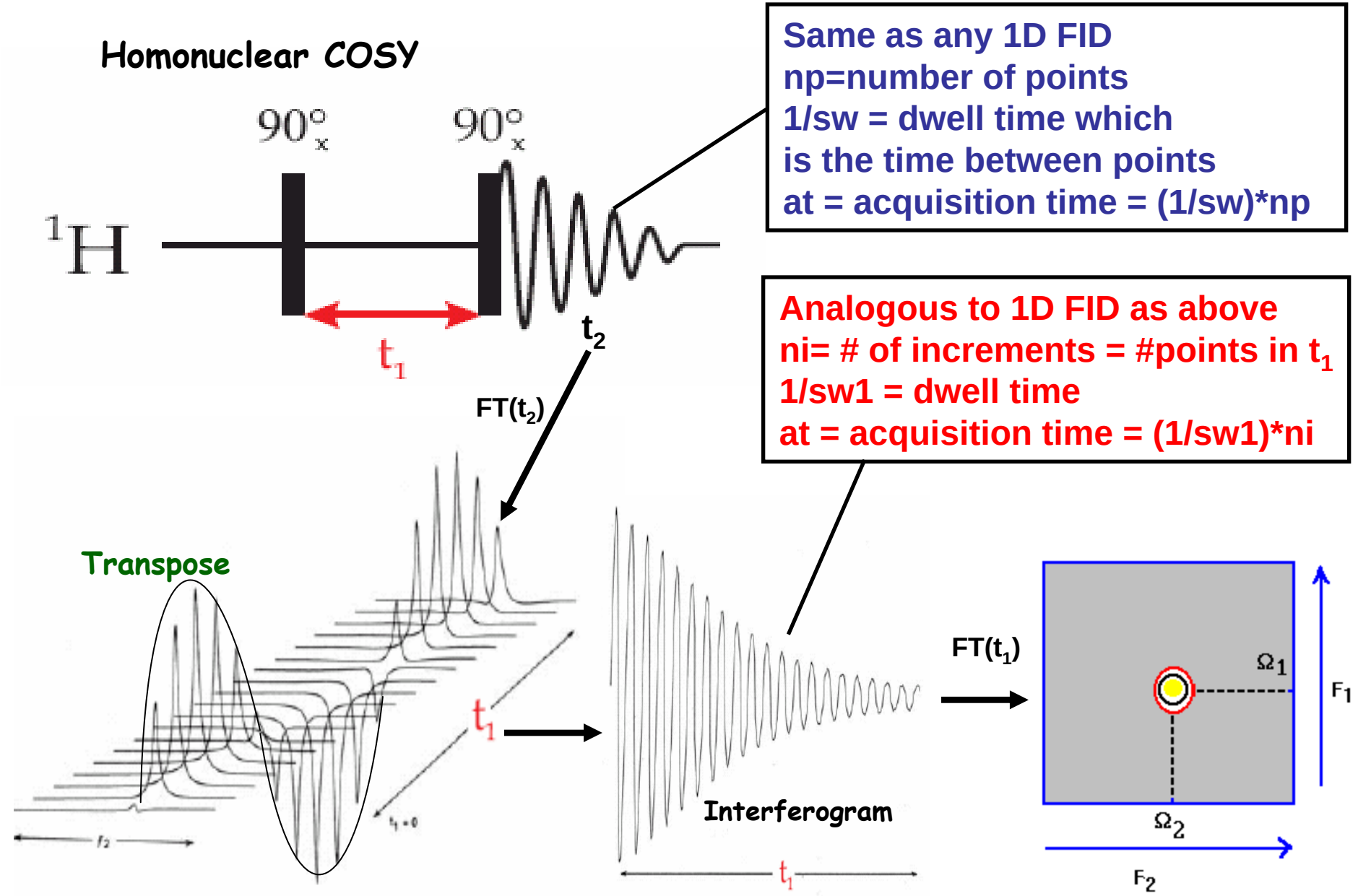
## Digital resolution and data sampling



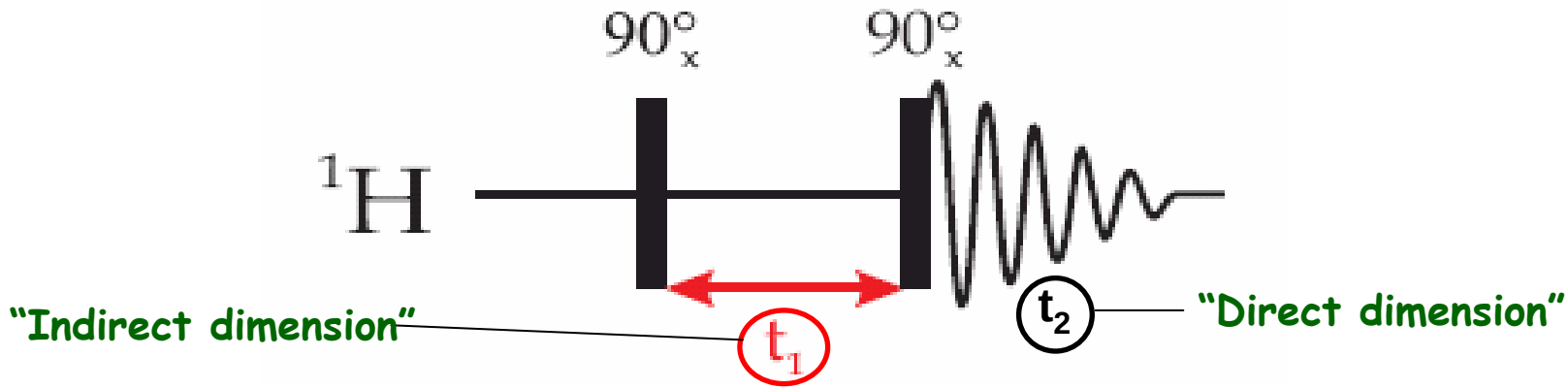

For any FID, 1D or 2D, the dwell time (dw) = 1/spectral width (sw). dw represents the maximum Frequency that can be digitized. This is called the Nyquist theorem.

- **All 2D experiments have a direct ($t_2$) and indirect ($t_1$) dimension, given by the Varian parameters at and d2.**
- **Digital resolution of a spectrum = # hertz/data point = sw/np for f2 and sw1/ni for f1 in any 2D experiment.**
- **As in a 1D experiment, the digital resolution in the indirect dimension of a 2D experiment must be great enough to resolve the correlations of interest.**
- ***Higher resolution in $t_2$ (direct dimension) costs little time, but higher resolution in the $t_1$ (indirect dimension) adds directly to the total time of the experiment (i. e. twice as many points in f1 = twice as long).***

# General Scheme for 2D NMR



# Homonuclear Proton-proton COSY




- Generates a 2d map which has cross peaks due to geminal and vicinal coupling ONLY

***Advantages***

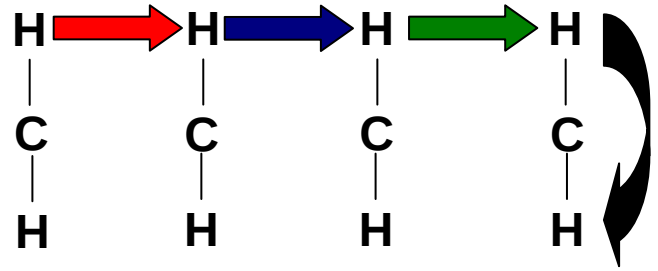
- Simplest type of 2D experiment
- Easiest to set up
- Forgiving of pulse width errors

***Disadvantages***

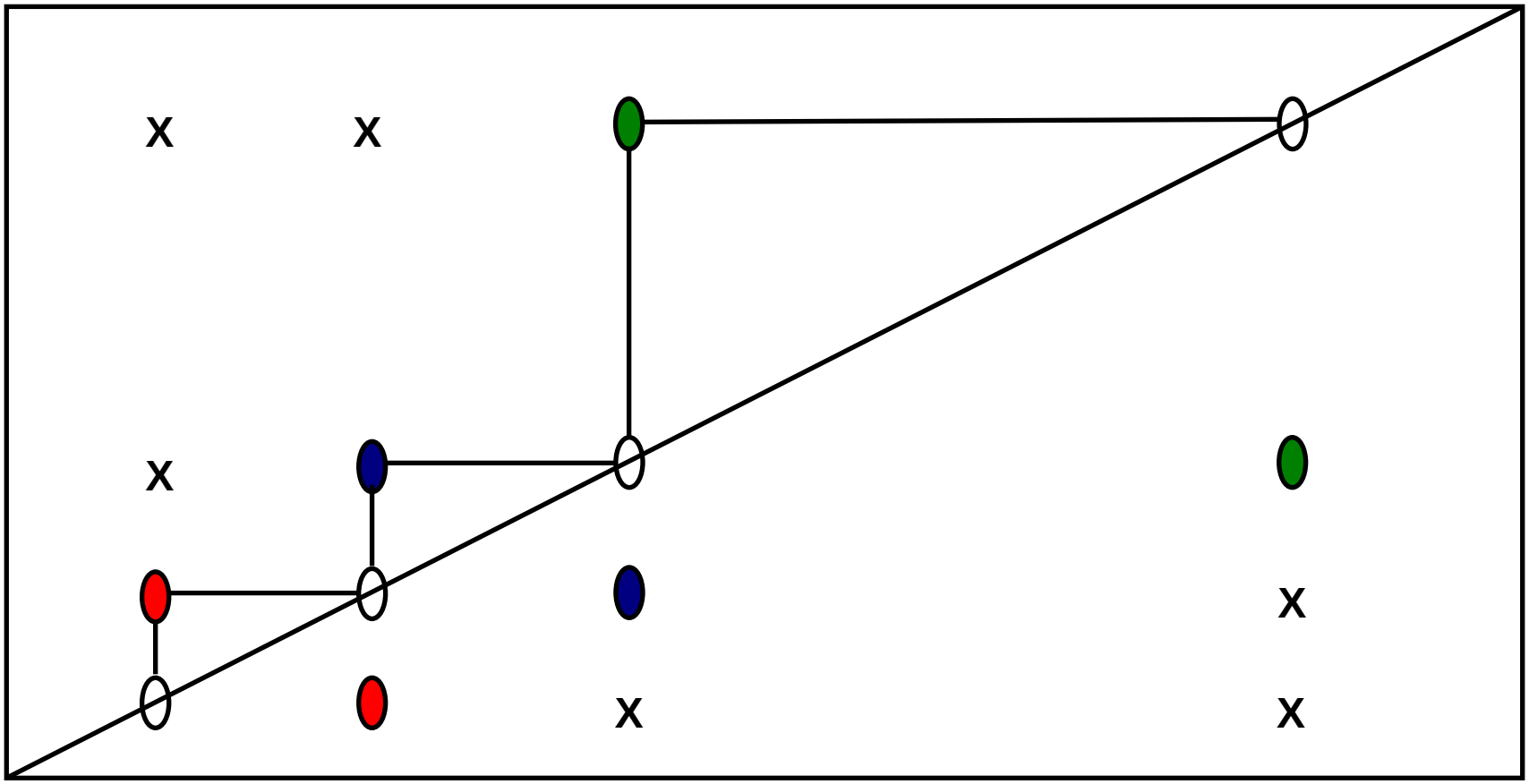
- Has inherently low resolution and relatively low sensitivity compared to other types of proton-proton 2D's
- Contains the least amount of information of proton-proton 2D experiments
- Should be used only for routine assignment of low molecular weight compounds that have little resonance overlap

# $^{1}H$-$^{1}H$ COSY and DQFCOSY Experiments

Geminal and vicinal Couplings only

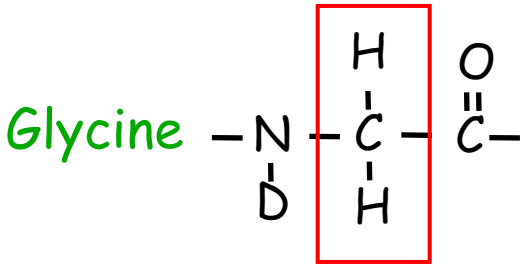



Geminal if not equivalent



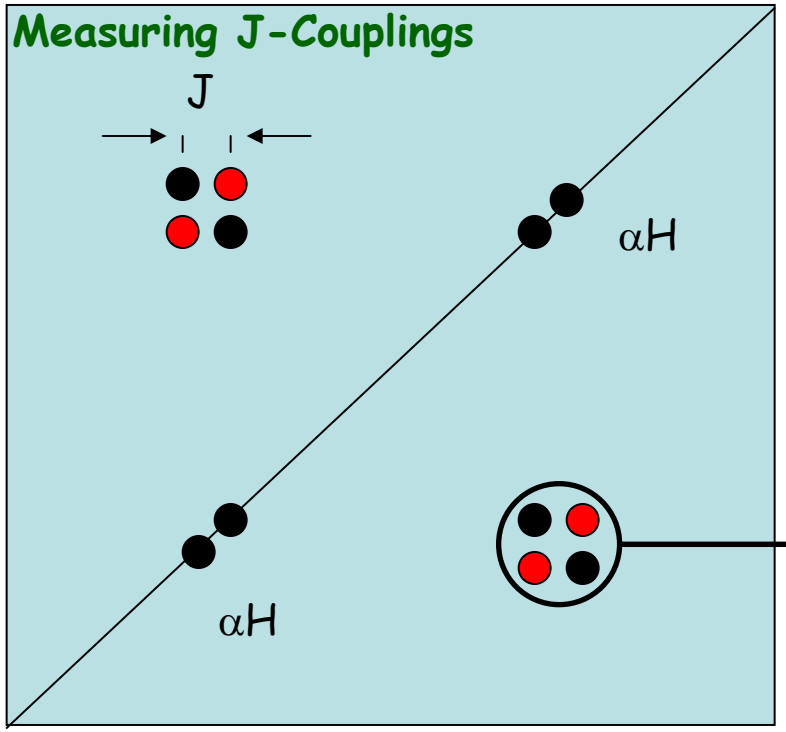
# Phase Sensitive COSY (DQFCOSY)

(Most often used for assignment in small molecules)




Simplest Spin System - AX

Phase-sensitive DQF-COSY

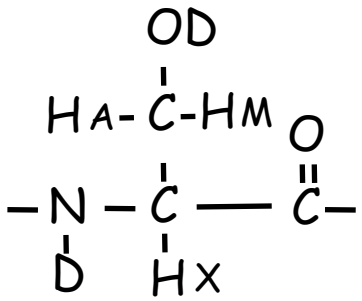



Anti-phase, pure absorption line-shape

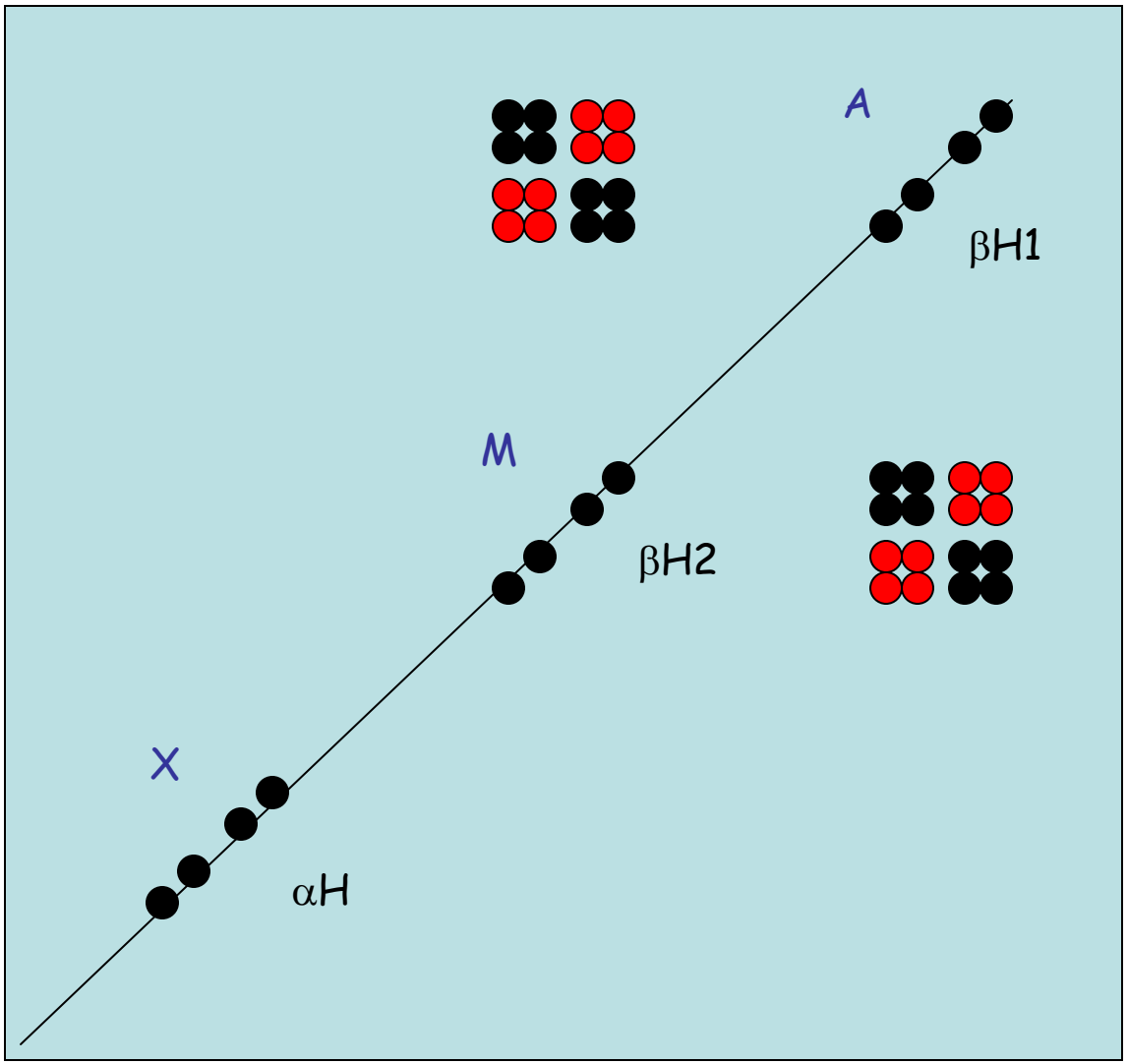
# COSY Cross Peak Structure

Spin System - AMX

Serine



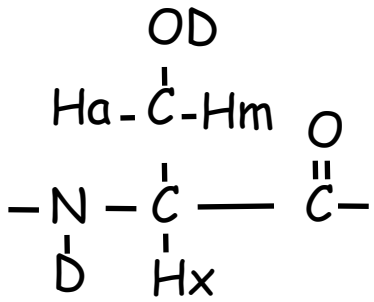

Phase-sensitive COSY



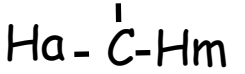
# COSY Cross Peak Structure and Measuring J-Couplings

Spin System - AMX

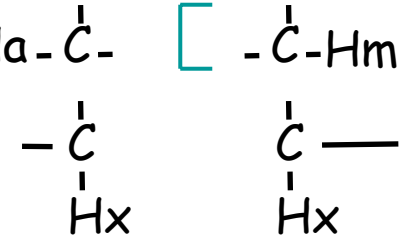
Serine

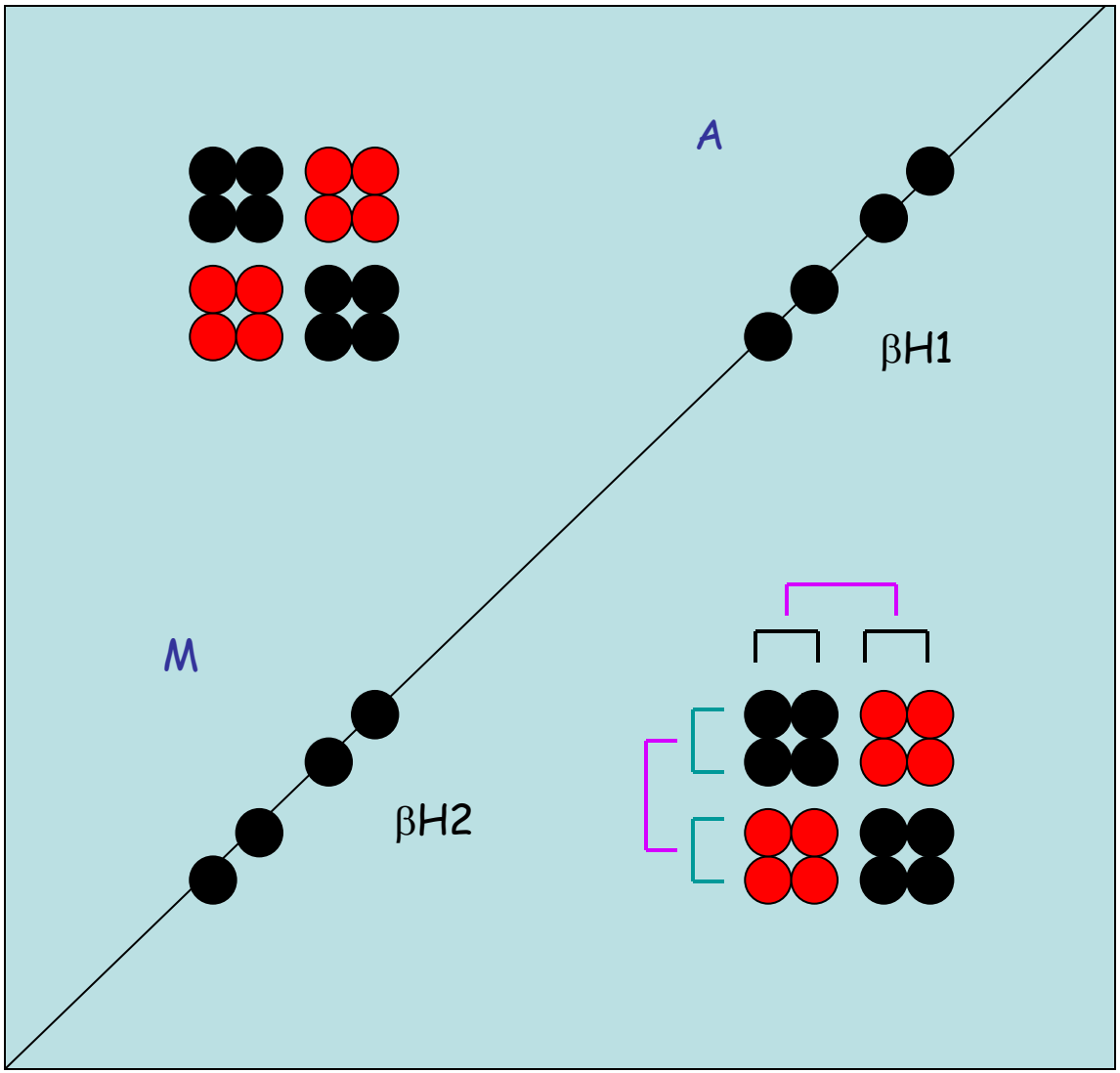



active coupling



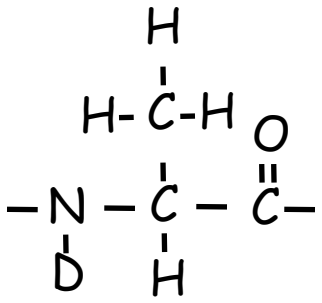

passive coupling



# COSY Cross Peak Structure and Measuring J-Couplings

Alanine

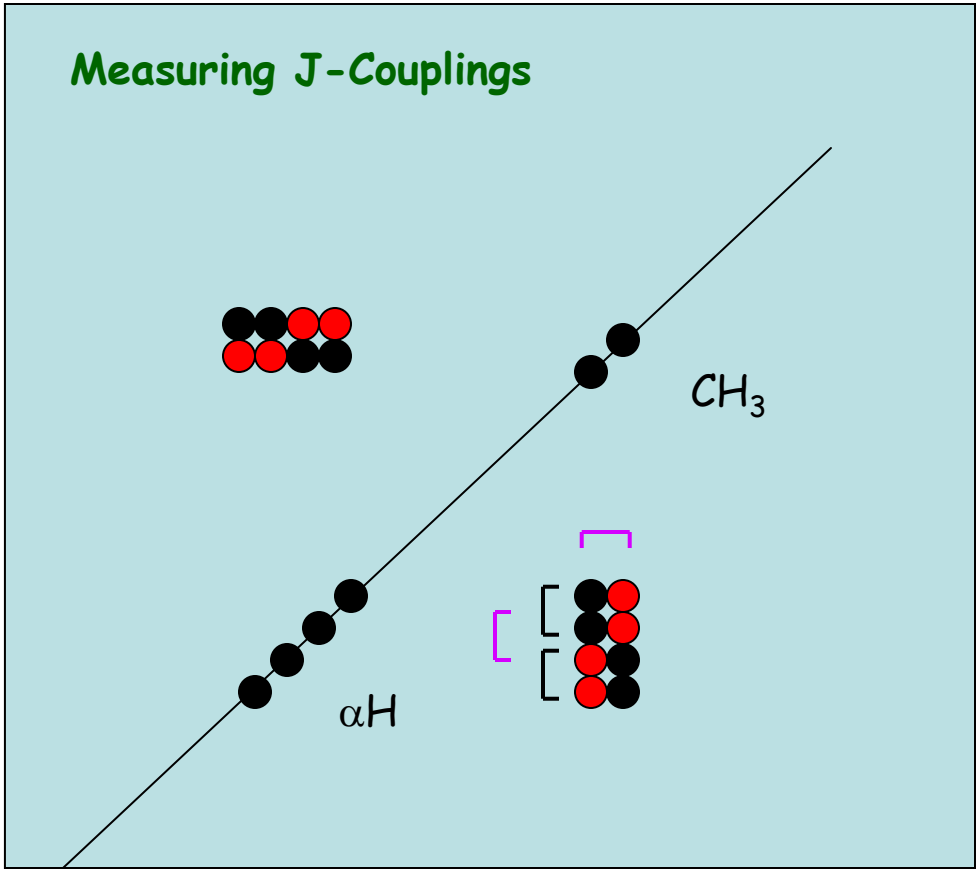


Spin System - $A_3X$

active coupling

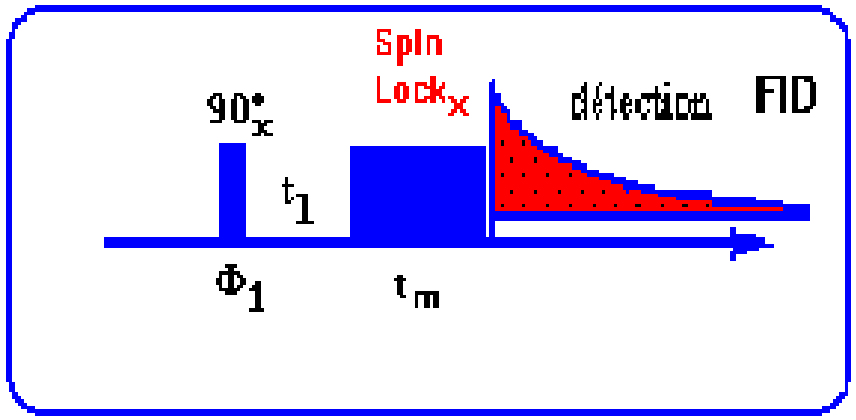
passive coupling

Treat only one H of $CH_3$ as active pair




Phase-sensitive COSY

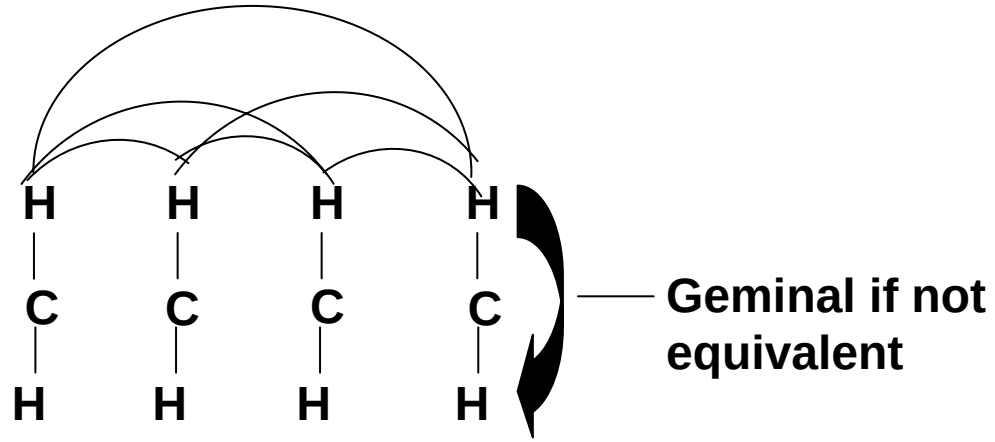
# Total Correlation Spectroscopy - TOCSY

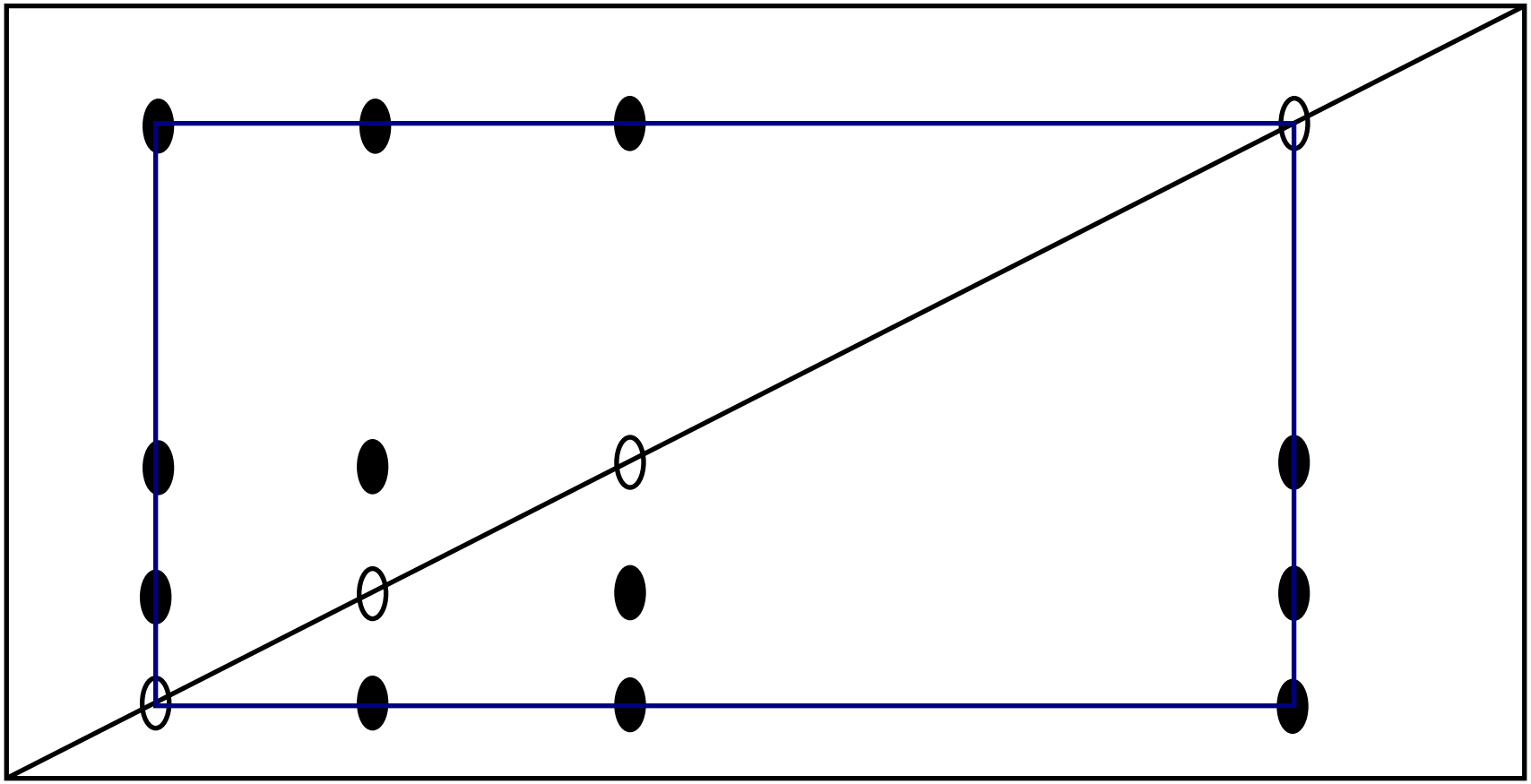



- Powerful variant of the COSY experiment
- Transfers magnetization throughout a spin system, provided that no coupling = 0
- Length of the mixing time determines how far the magnetization is transferred (i.e. how many bonds)
- Longer mixing = greater transfer, but < signal
- Typical mixing times are 30-200msec
- Magnitude of mixing time related to 1/2J for smallest coupling

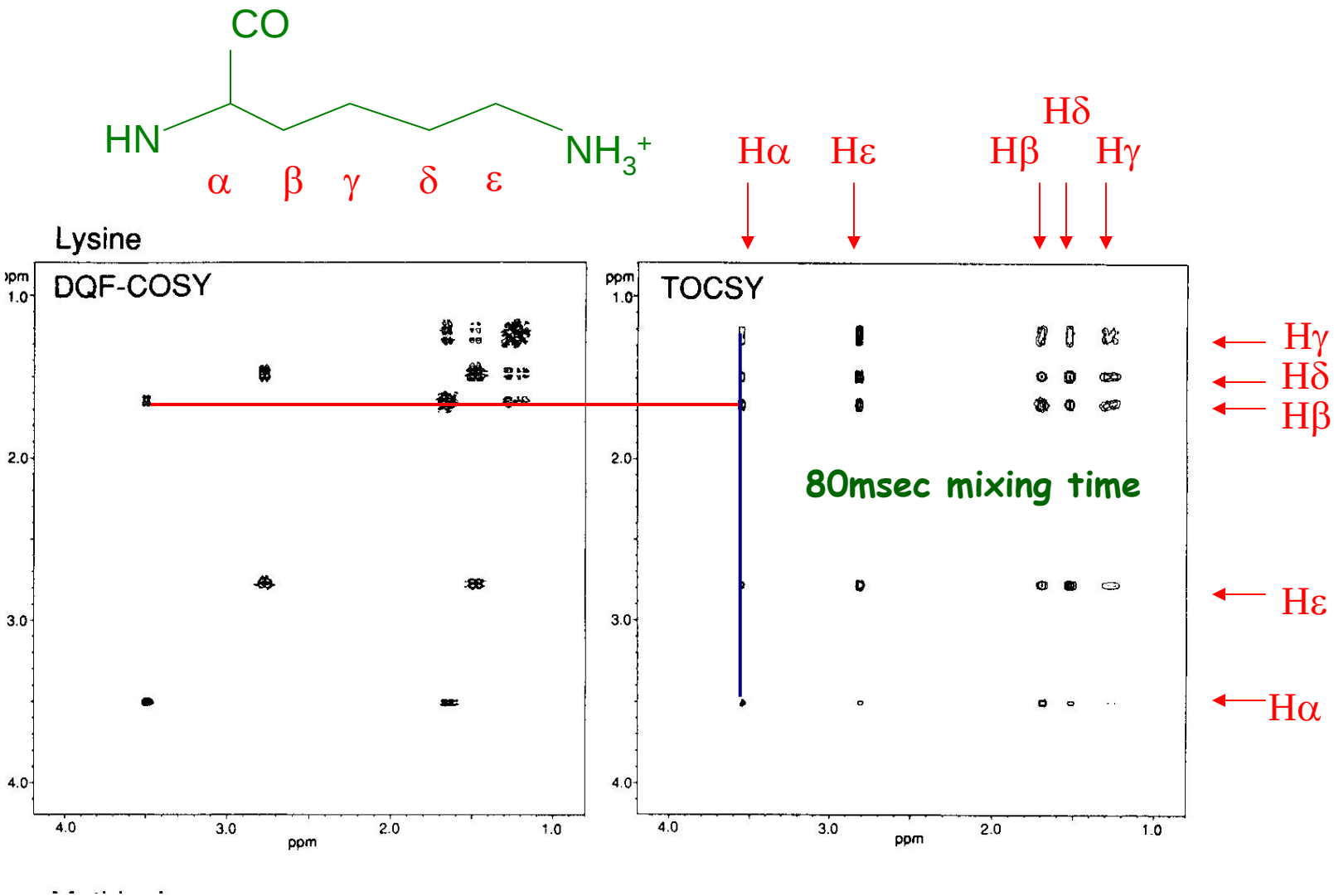
# TOCSY Experiment

In general, the TOCSY mixing time determines the number of bonds over which signal can be Transferred, assuming that none of the coupling Constants = 0



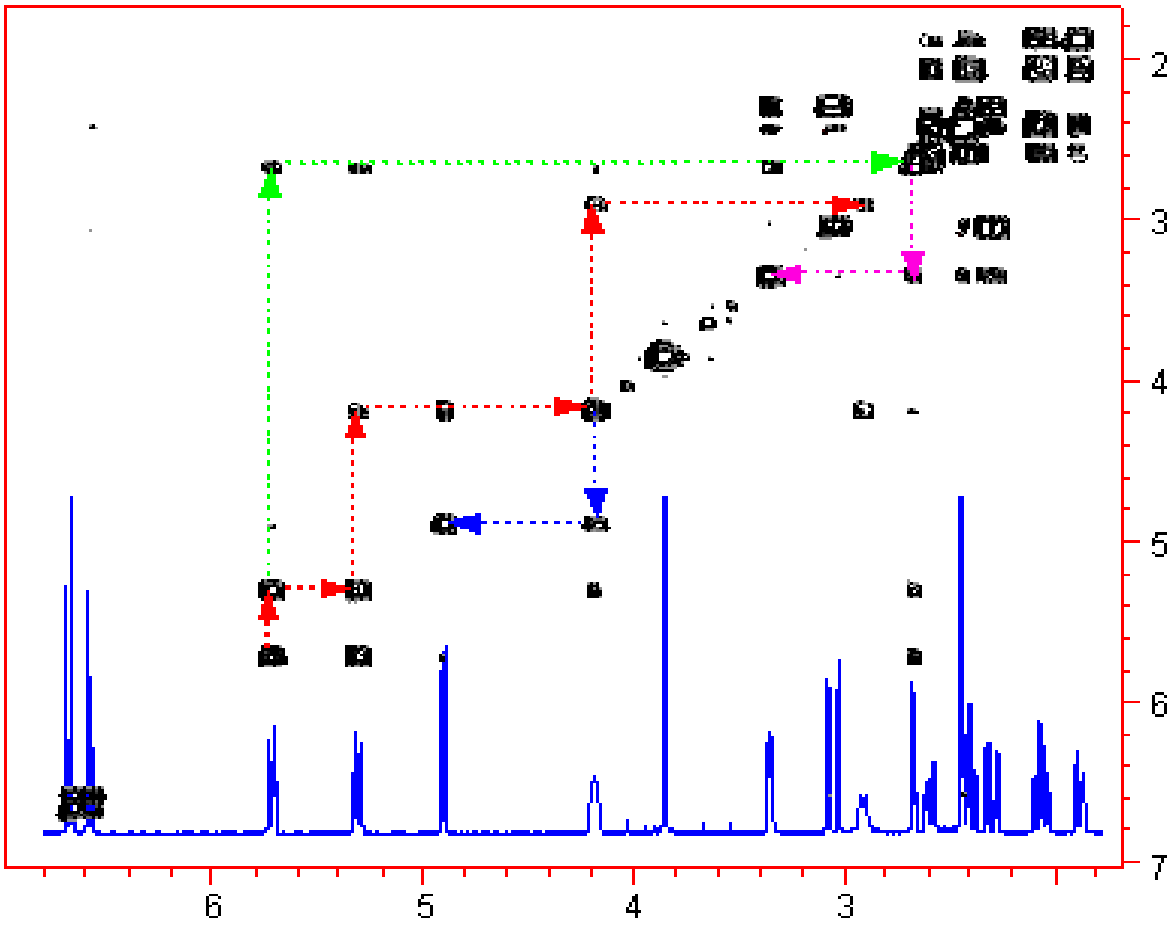
# Example of lysine spin system

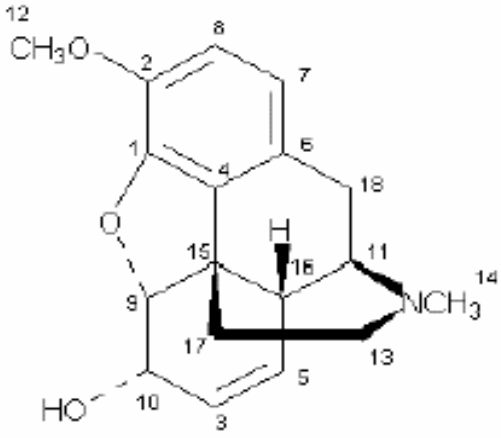


# Example of COSY Spectrum

***The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 5 minutes!!***

512 complex points in direct dimension
128 t1 increments
2 scans
1 sec relaxation delay
Total acquisition time: 5 min




**H-3 —> H-5 —> H-10 —> OH**

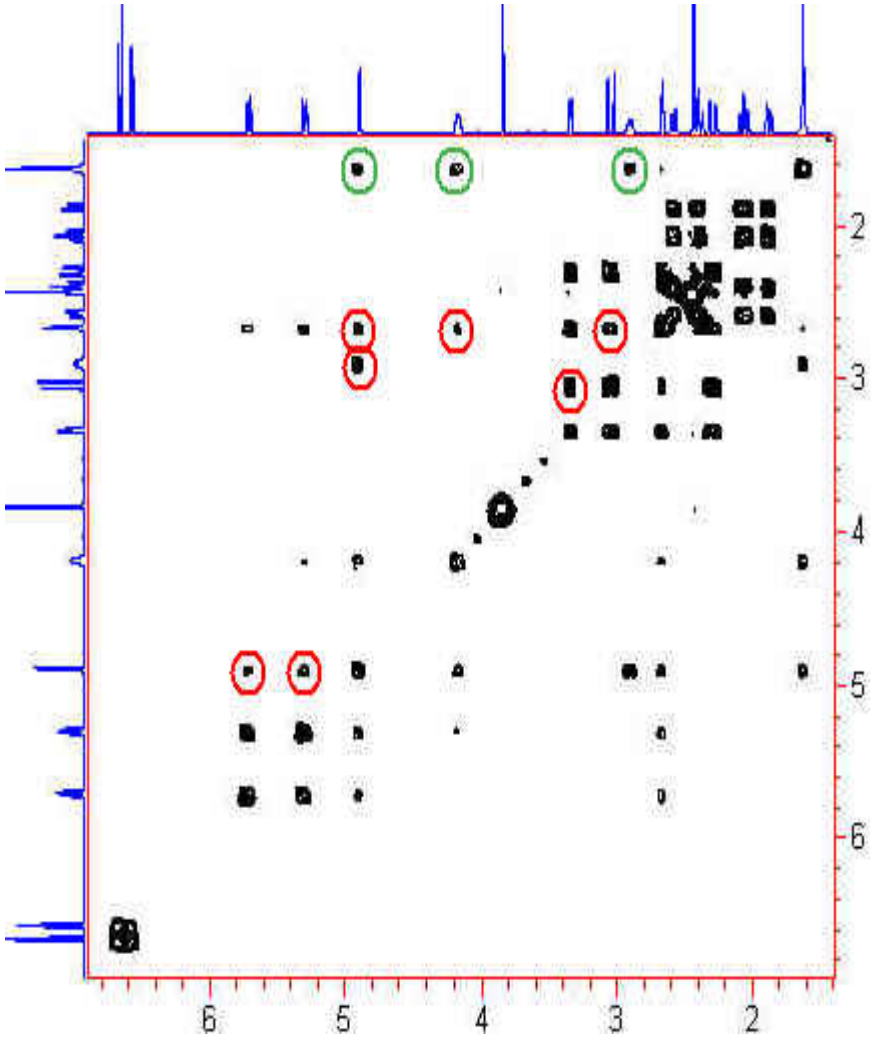
**H-10 -> H-9**

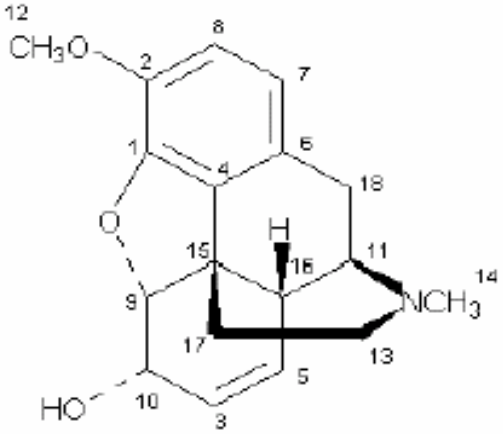
**H-3 —> H-16**

**H-16 —> H-11**

# Example of TOCSY Spectrum

***The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 20 minutes***




8 --> 7
3 --> 5, 9, 10, 16
5 --> 9, 10, 11, 16
9 --> 10, 16, OH, H2O
10 --> 16, OH, H2O
11 --> 16, 18, 18'
18 --> 16, 18'
16 --> 18'
13 --> 13', 17, 17'
13' --> 17, 17'
17 --> 17'

**Acquisition parameters:**

512 complex points in the direct dimension
128 t1 increments
mixing time 70 ms
4 scans
2 sec relaxation delay
Total time: ~20 min.

**Processing parameters:**

sine squared window function (0 degree phase shift)
in f1 and f2 2x zero-fill in the indirect dimension
magnitude calculation (no phasing needed)
final size 512 x 512

# Acquisition Parameters for COSY, DQFCOSY, and TOCSY

## These are suggestions only – Defaults should also work

### COSY

F2 (Direct Dimension)

**sw** =spectral width=6000Hz (10ppm) more or less depending on chemical shifts

**np**=2048(only costs disk space)

**pw**=pw90=90 degree pulse

**nt**=minimum of 4, multiples of 4 for greater S:N

**d1**=relaxation delay =1-2s (longer d1, less artifacts)

F1 (Indirect Dimension)

**sw1** =sw because f1 is also proton

**ni**=# points in f1=128-1024 depending on desired resolution

### DQFCOSY and TOCSY

F2 (Direct Dimension)

**sw** =spectral width=6000Hz (10ppm) more or less depending on chemical shifts

**np**=4096(only costs disk space)

**pw**=pw90=90 degree pulse

**nt**=minimum of 4, multiples of 4 for greater S:N

**d1**=relaxation delay =2s (longer d1, less artifacts)

**Mix**=70ms (30-150) for TOCSY

F1 (Indirect Dimension)

**sw1** =sw because f1 is also proton

**ni**=# points in f1=128-1024 depending on desired resolution

# Processing Parameters for COSY, DQFCOSY, and TOCSY

## These are suggestions only – Defaults should also work

### COSY

#### F2 (Direct Dimension)

fn – zero-filling parameter. Set=np or up to 4*np for > resolution

pmode='partial' – no phasing

sb=-at (sine bell)

dmg='av' $(R^2+Im^2)^{1/2}$ – forces all signals to be positive

wft2d –command to process data. Performs a 2D FT

wft1d-performs an FT in $t_2$ only

#### F1 (Indirect Dimension)

fn1=ni or up to 4*ni as above

proc1='lp' (linear prediction) – better resolution

sb1=-(1/sw1*ni)/2 =-at for $t_1$ dimension

### DQFCOSY and TOCSY

#### F2 (Direct Dimension)

fn – Set=np-4*np for > resolution

pmode='full' – phase sensitive

sb and sb1=-at (squared sine bell with 90 degree shift)

dmg='ph' – data can be phased

wft2da –phase sensitive 2D FT

wft1da-phase sensitiveFT in $t_2$

#### F1 (Indirect Dimension)

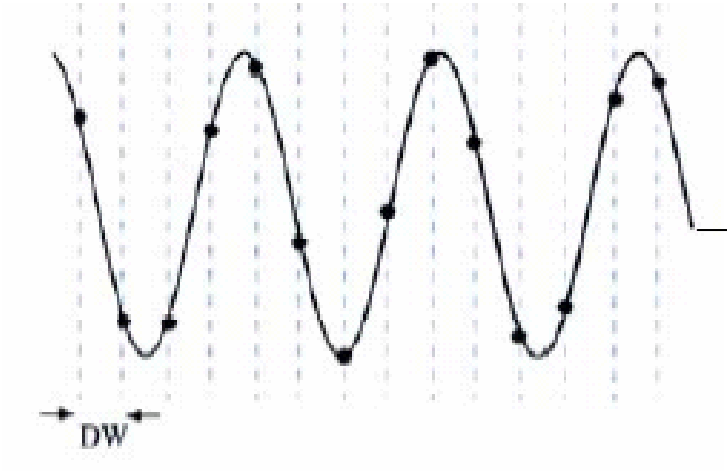
fn1=ni or up to 4*ni as above

proc1='lp' (linear prediction) – better resolution

sb1 and sbs1=-1/sw1*ni =-at for $t_1$ dim.

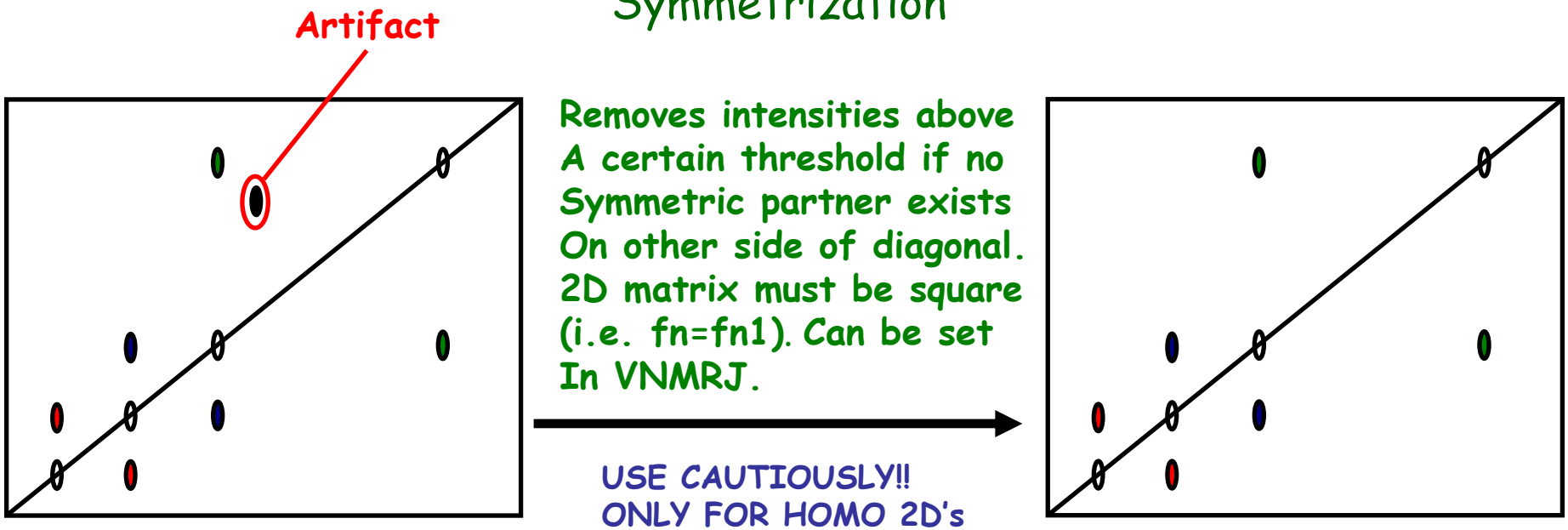
# Processing Techniques for 2D NMR Experiments

## Linear Prediction (lp)



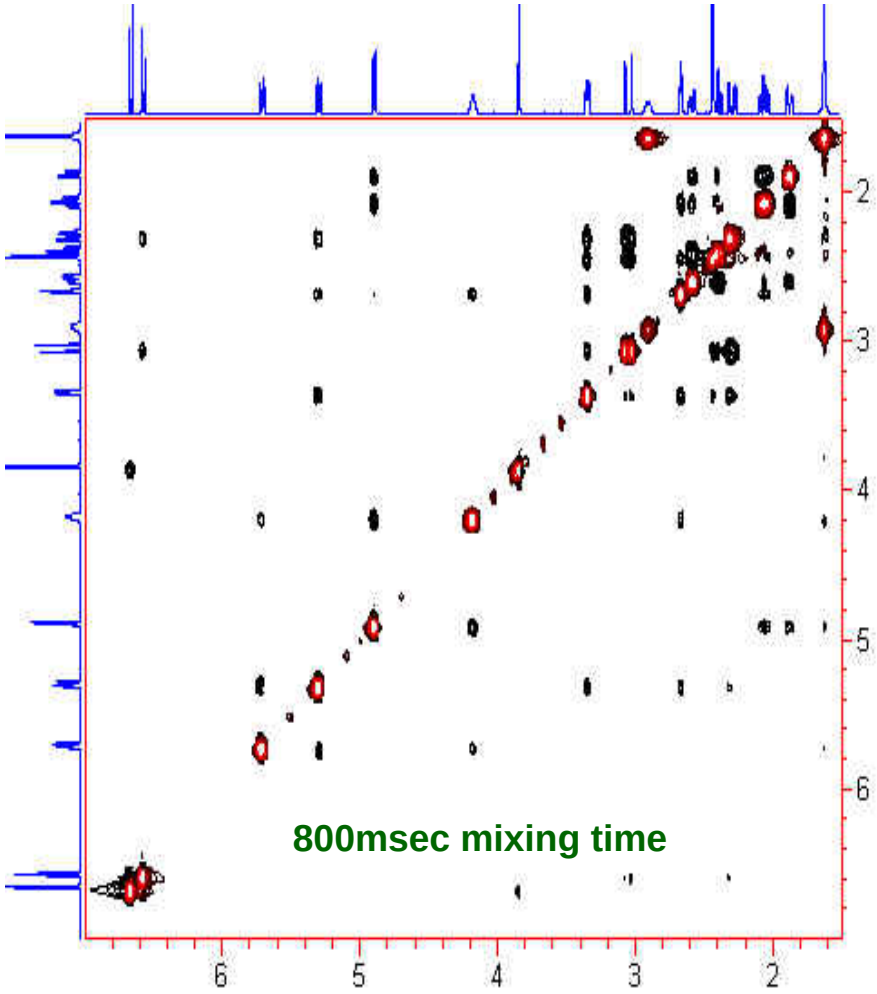

Attempts to "extend" the FID by mathematically predicting points at either the beginning (backward lp) or at the end (forward lp). Can greatly improve resolution. Is part of the "process" macro in VNMRJ.

## Symmetrization



# 2D NOESY – Through Space Coupling

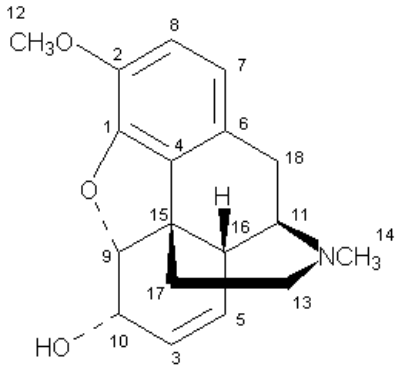
*The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 5 hours*




The interesting information is contained in the "cross-peaks", which appear at the coordinates of 2 protons which have an NOE correlation.

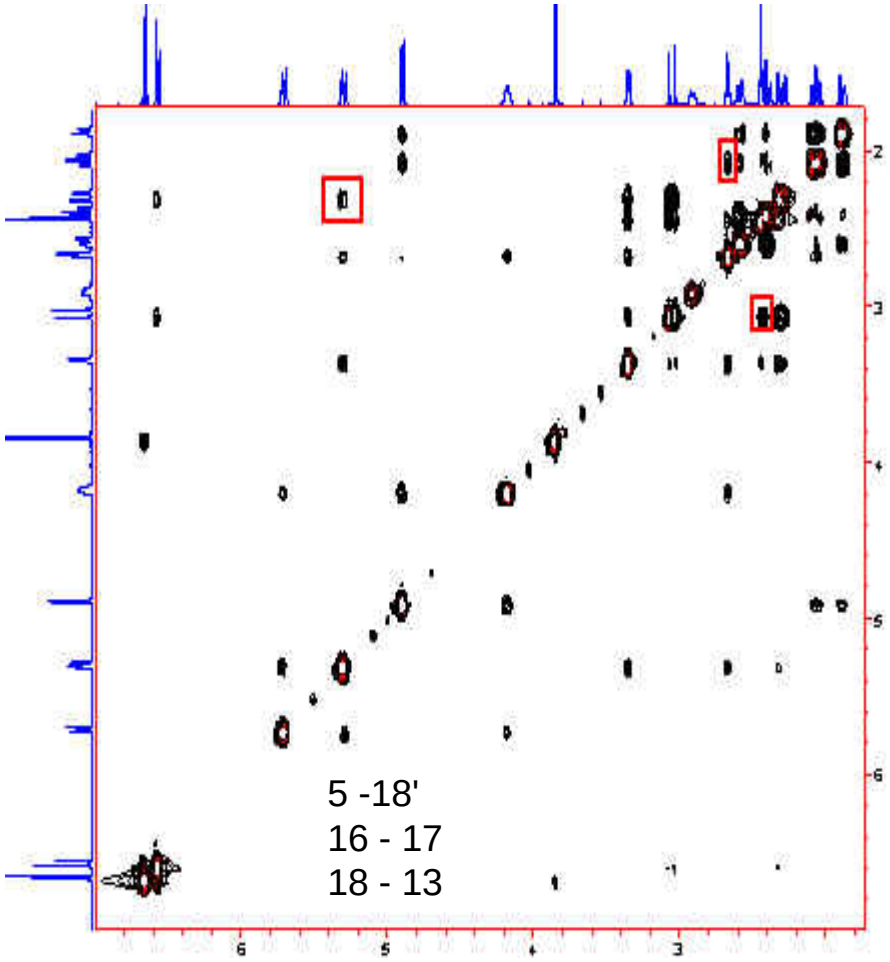
For small molecules, the NOE is positive. Exchange peaks have the opposite sign from NOE peaks, making them easy to identify. The water peak at 1.5 ppm exchanges with the OH at 2.9 ppm, shown here in red.

The spectrum is phased with the large diagonal peaks inverted (shown in red here), so the NOE cross-peaks are positive

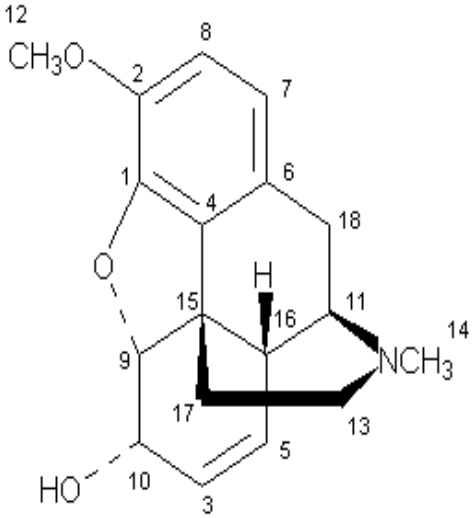


# 2D NOESY – Through Space Coupling

***The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 5 hours***




**In addition to confirming assignments, the NOESY spectrum allows stereospecific assignments of methylene Hs. The 3 cross-peaks indicated in red on the plot below distinguish between the 3 CH2 pairs:**

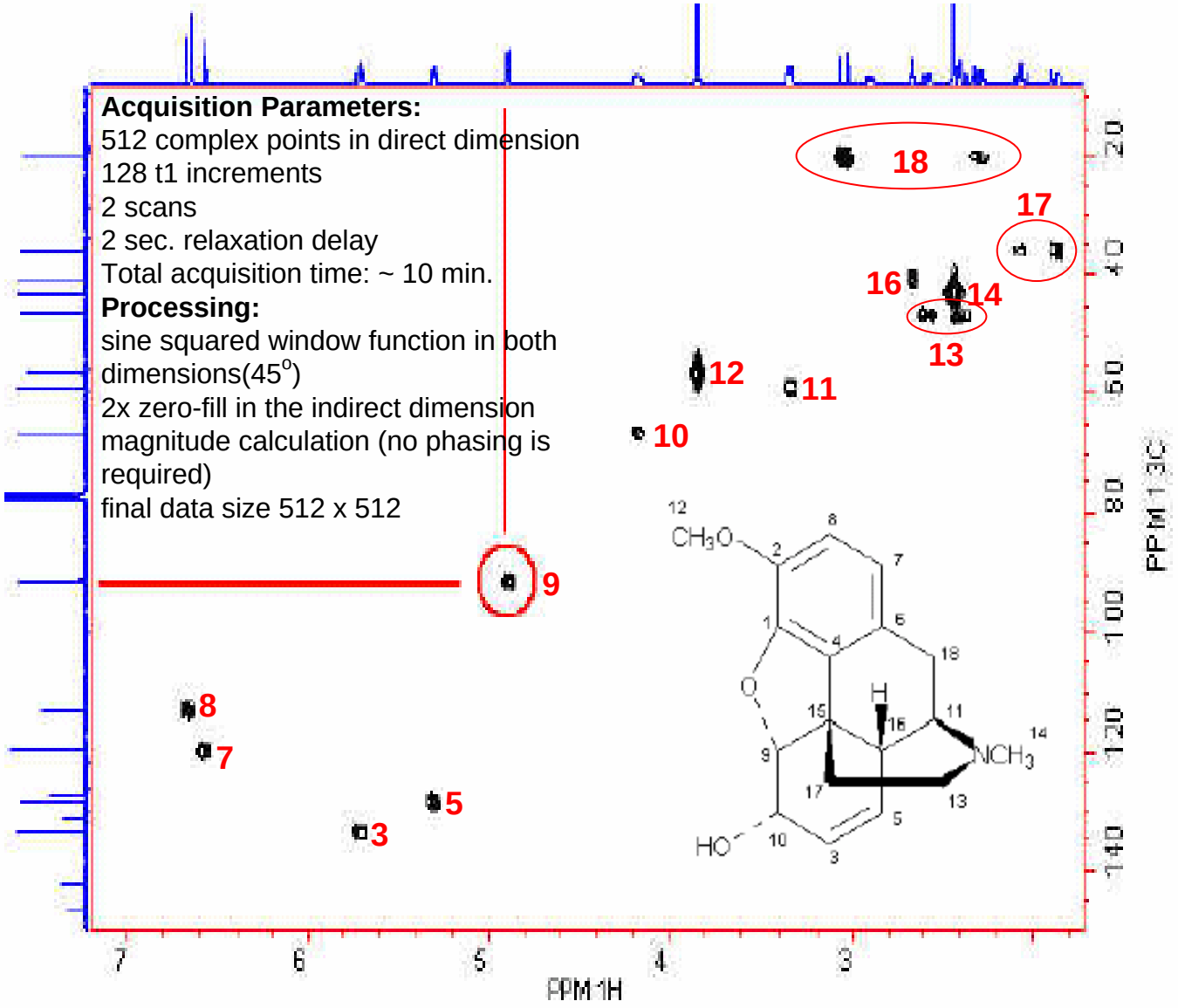



Acquisition parameters:
512 points in t2. 256 in t1
mixing time: 0.8 sec.
phase sensitive 16 scans
2 sec relaxation delay
Total time: 5 hrs.
Processing parameters:
cosine squared window function (sine function with 90 degree phase shift) in both dimensions
phased so all peaks in first slice are inverted
2x zero-fill in the indirect dimension
final size 512 x 512

# Heteronuclear Proton-Carbon HMQC

***The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 10 minutes***

| $^{1}H$ | $^{13}C$ | Assignment |
|---|---|---|
| 6.6 | 113 | 8 |
| 6.5 | 120 | 7 |
| 5.7 | 133 | 3 |
| 5.3 | 128 | 5 |
| 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 |



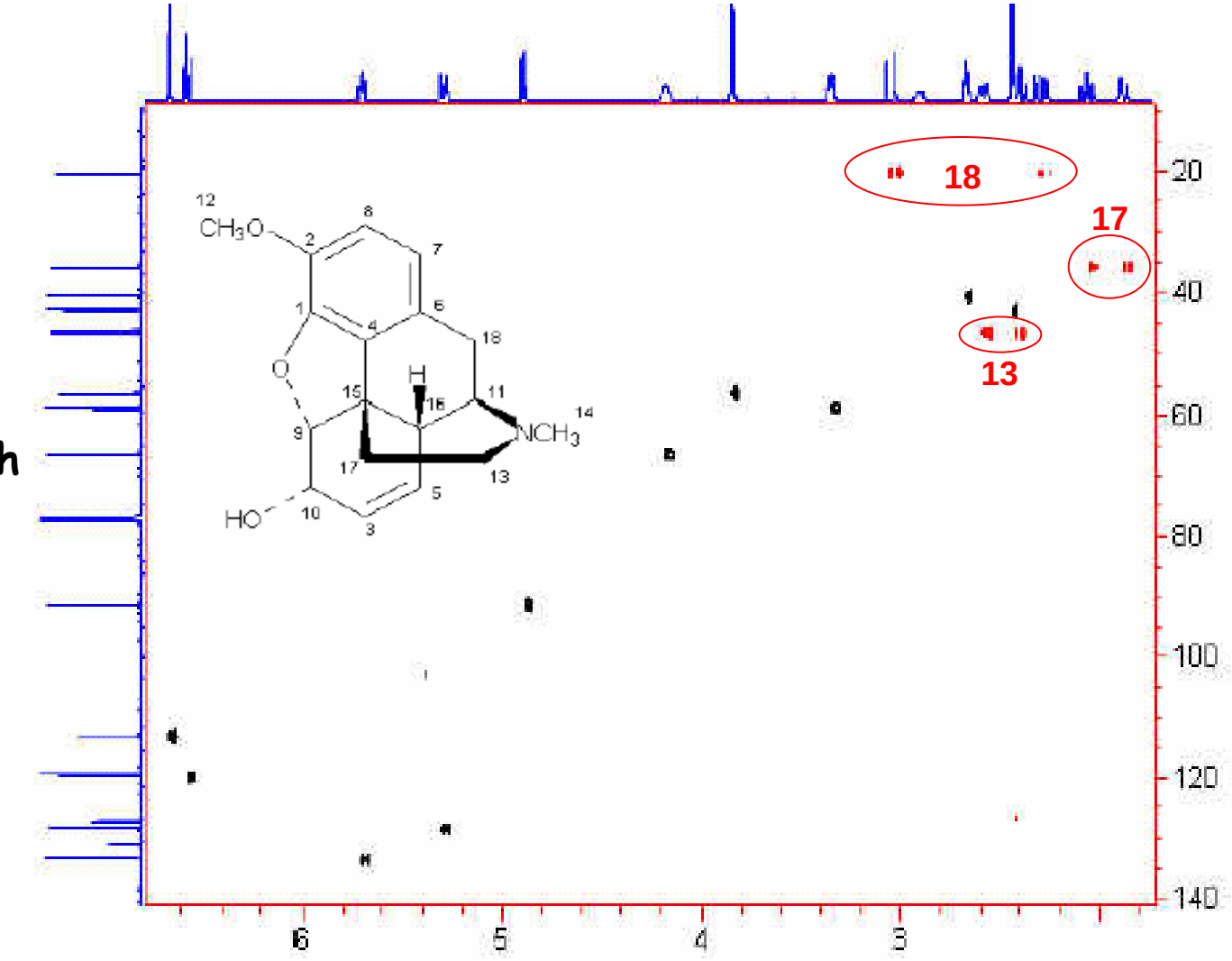
# Heteronuclear Proton-Carbon HMQC-DEPT

*The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 20 minutes*

● — $CH_2$

● — $CH$, $CH_3$

Can also be run with usual types of dept editing. In general, the sensitivity is about ½ that of unedited HMQC



# General Parameters for 2D HMQC or HSQC Spectra

*The sample is 3.3 mg codeine in ~ .65 ml CDCl3*

**Acquisition Parameters:**

512 complex points in direct dimension
128 t1 increments
2 scans (4 scans for HMQC-DEPT)
2 sec. relaxation delay
Total acquisition time: ~ 10 min.

**Processing Parameters:**

sine squared window function in both dimensions with 45 degree phase shift
2x zero-fill in the indirect dimension
magnitude calculation (no phasing is required) final data size 512 x 512

# Heteronuclear Multiple Bond Correlation (HMBC)

**Acquisition Parameters:**
512 complex points in direct dimension
128 t1 increments
8 scans
2 sec. relaxation delay
Total acquisition time: 35 min
**Processing:**
sine squared window function in both dimensions
with 0 degree phase shift in t2 and 90 degree
phase shift in t1. 2x zero-fill in the indirect dimension
magnitude calculation (no phasing is required)
final data size 512 x 512

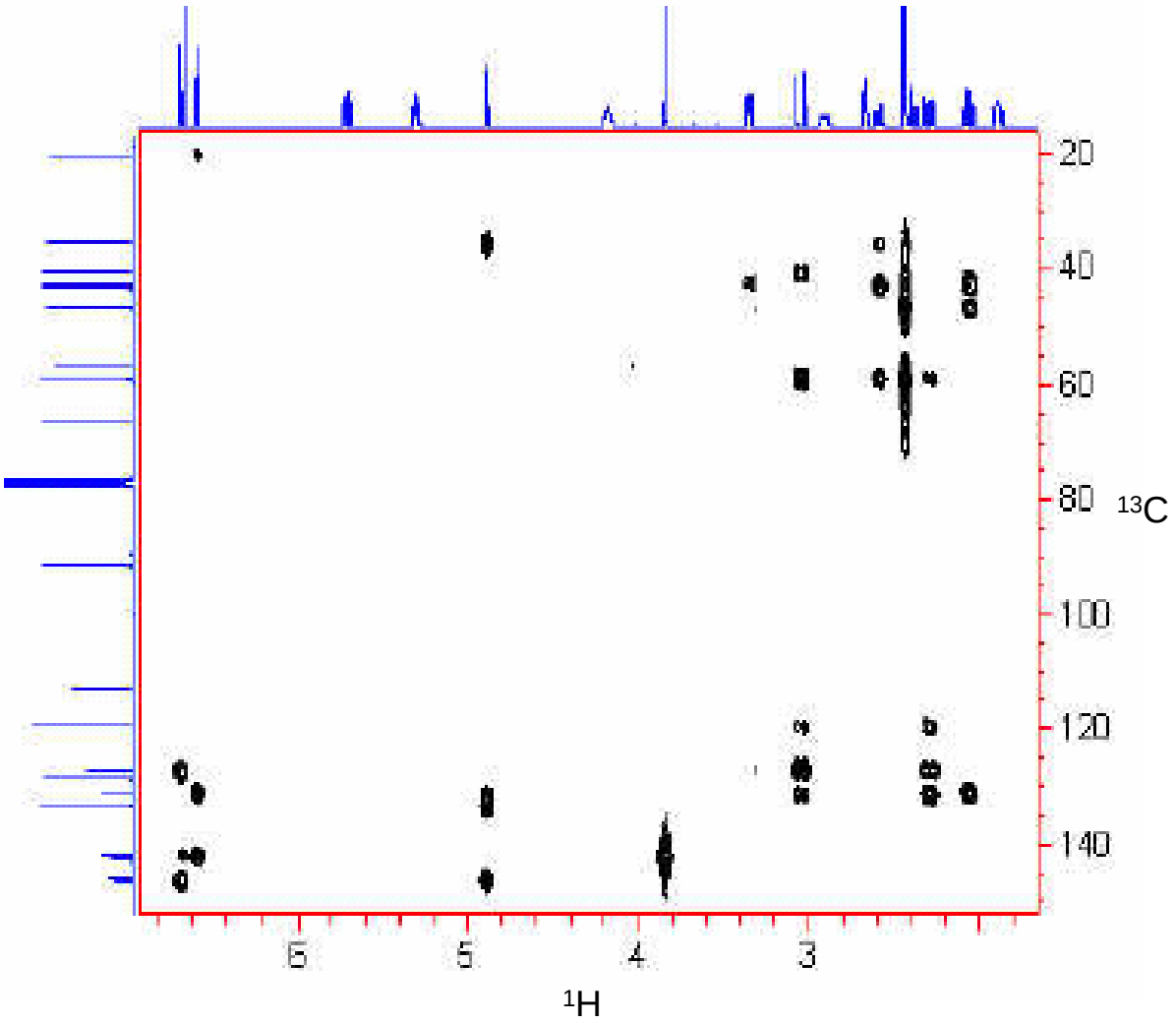
**Shows crosspeaks for protons and carbons separated by 2 and 3 bonds. The one bond correlations are suppressed.**

**"Tuning" may be done to emphasize 2 or 3 bond crosspeaks**

**The intensity of the crosspeaks depends on the magnitude of the long range proton-carbon coupling constants (5-20Hz)**

**Several variations are possible**

***The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 40 minutes***



# Analysis of HMBC Experiment

*The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 40 minutes*

Red lines show correlations from aromatic proton H-8 to aromatic carbons C-1 and C-6 (3-bond couplings) and a weak correlation to C-2, (2-bond coupling)

Green lines show correlations from proton H-9 to carbons C-1, C-3 and C-4 (all are 3-bond couplings).

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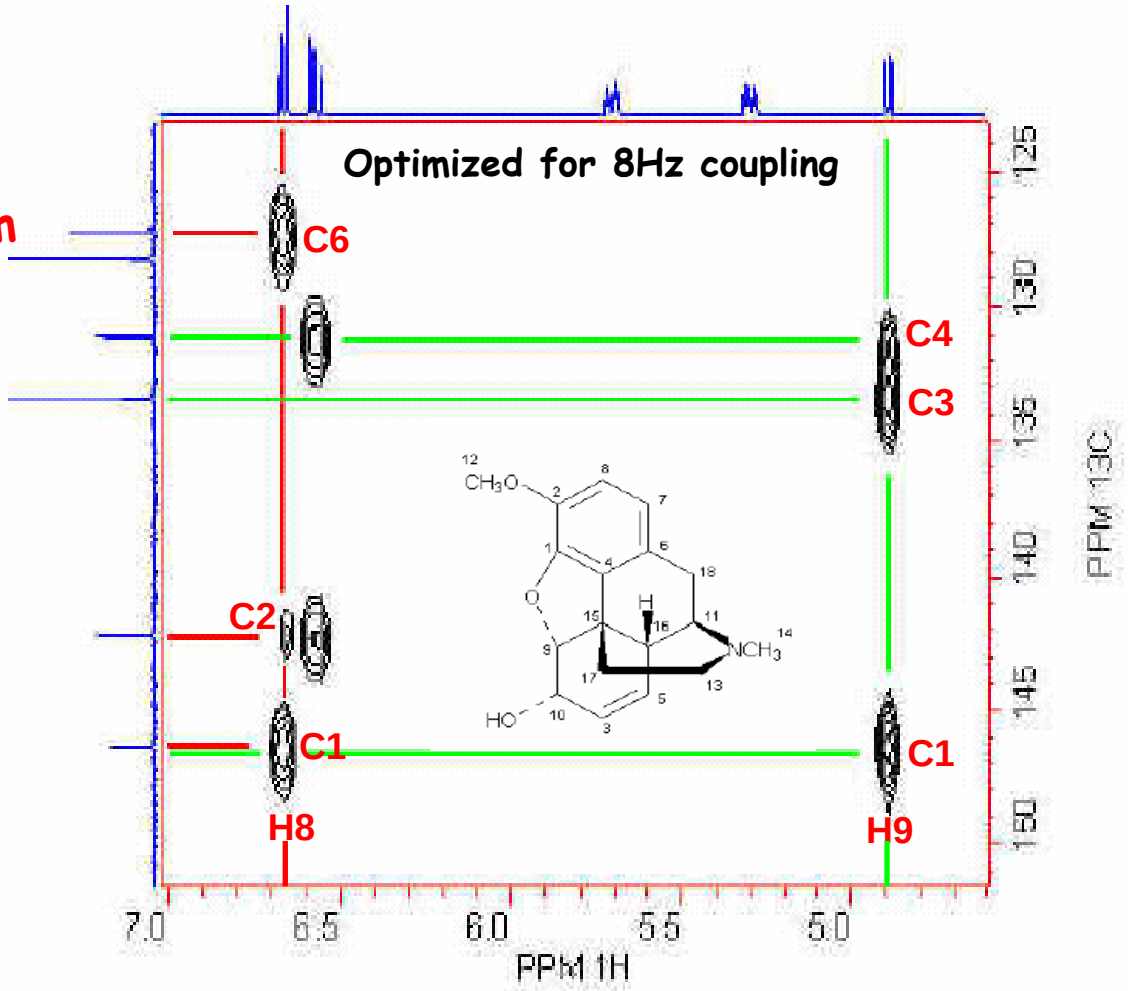
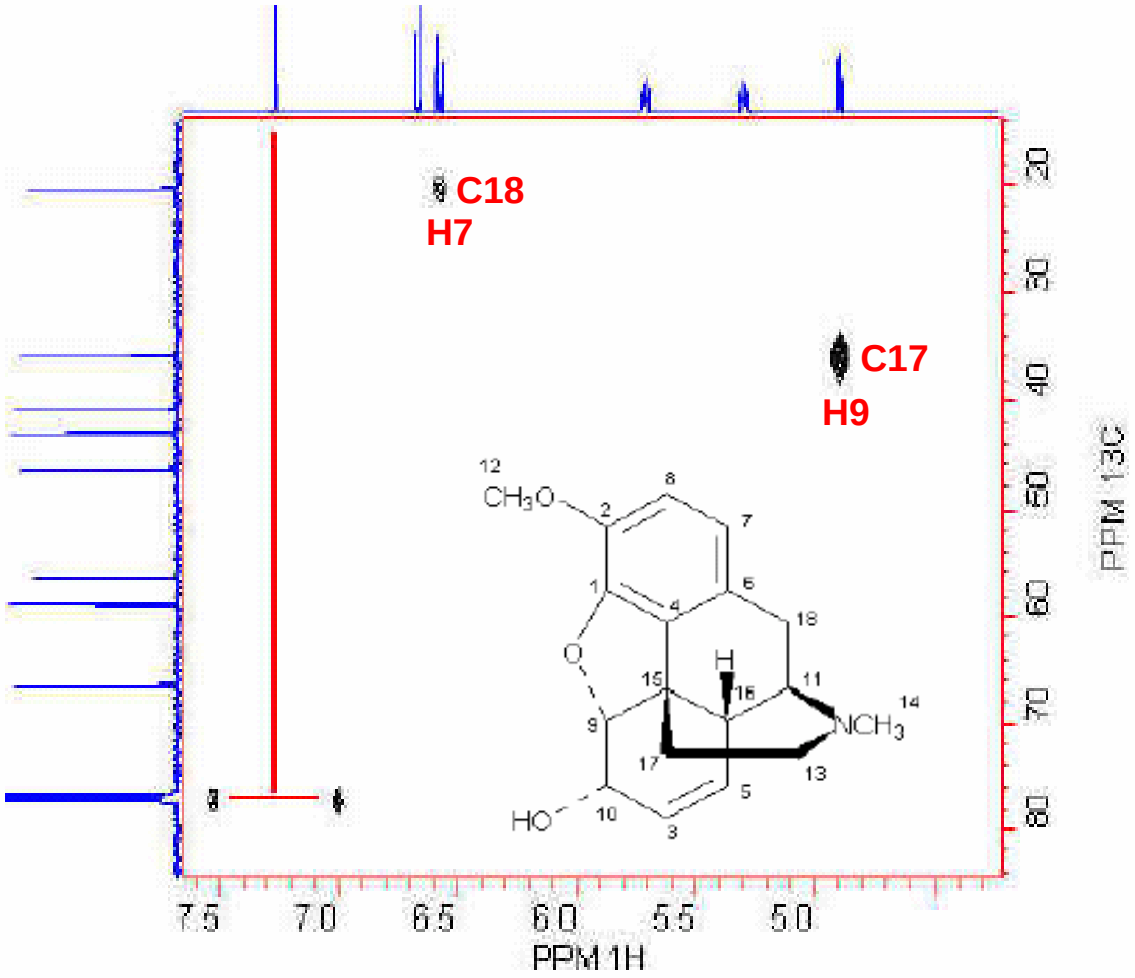


# Analysis of HMBC Experiment

*The sample is 3.3 mg codeine in ~ .65 ml CDCl3 Total time = 40 minutes*

## Artifacts

The peaks indicated by red lines are due to 1-bond coupling in CHCl3 solvent. Note that the pair of peaks don't line up with any H peaks, but are symmetrically located about the CHCl3 peak, with a separation equal to the 1-bond C-H coupling constant.



# Acquisition Parameters for Heteronuclear Experiments

## These are suggestions only – Defaults should also work

### Gradient HMQC or HSQC

F2 (Direct Dimension)

sw =spectral width=6000Hz (10ppm) more or less depending on chemical shifts

np=NEVER EXCEED 2048!!!! (because of carbon decoupling)

pw=pw90=90 degree pulse

nt=minimum of 2, multiples of 2 for greater S:N

d1=relaxation delay =1-2s (longer d1, less artifacts)

F1 (Indirect Dimension)

sw1 =range of protonated carbons

ni=# points in f1=128-1024 depending on desired resolution

### HMBC

F2 (Direct Dimension)

sw =spectral width=6000Hz (10ppm) more or less depending on chemical shifts

np=4096 or 8192

pw=pw90=90 degree pulse

nt=minimum of 4, multiples of 4 for greater S:N

d1=relaxation delay =2s (longer d1, less artifacts)

Mix=70ms (30-150) for TOCSY

F1 (Indirect Dimension)

sw1 =full carbon range

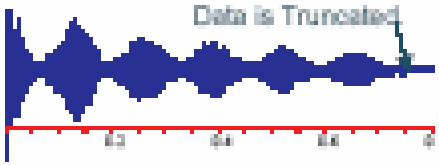
ni=# points in f1=128-1024 depending on desired resolution

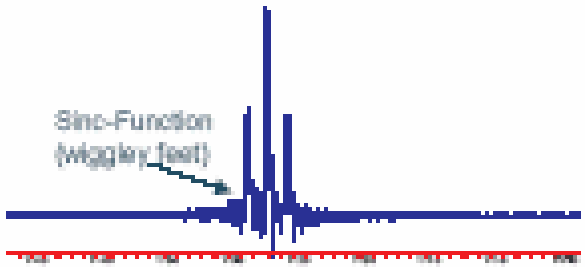
# Processing Concepts for 2D NMR

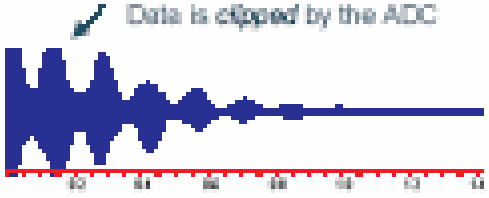
## Truncation Artifacts....

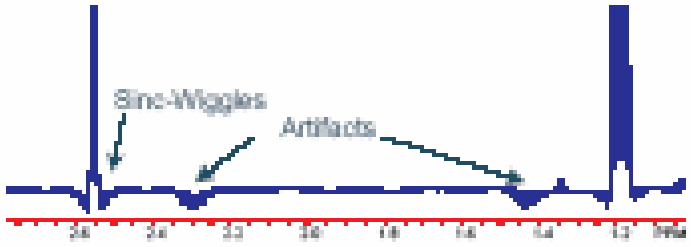
- Too short of Acquisition Time will truncate the end of the FID.
- If the amplitude is too high, the early part of the FID will be truncated by the digitizer.

- Any truncation will result in a *sinc* function (Sin(t)/t) that will be convoluted into the lineshape after the FFT



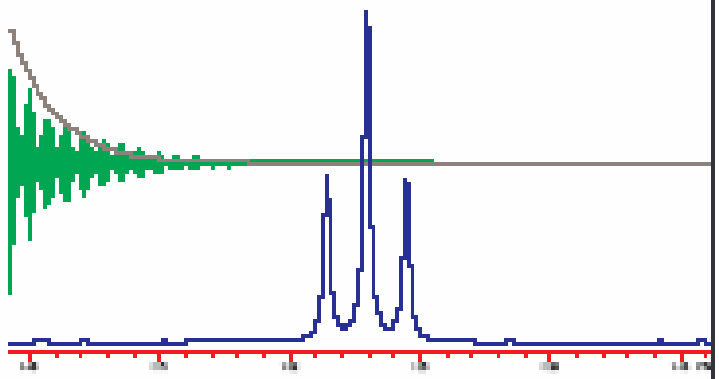



# Processing Concepts for 2D NMR

## Manipulation of FID's

- To prevent truncation artifacts, when the data is truncated at the end of the FID, we multiply the FID by a function, which decays smoothly to Zero.
- There are many commonly used functions, including: exponential, gaussian, sine-bell, shifted-sine-bell, trapezoidal, ..etc.
- The weighting function can determine the lineshape.
- The term *apodization* comes from the concept of "removing the feet" from the peaks.

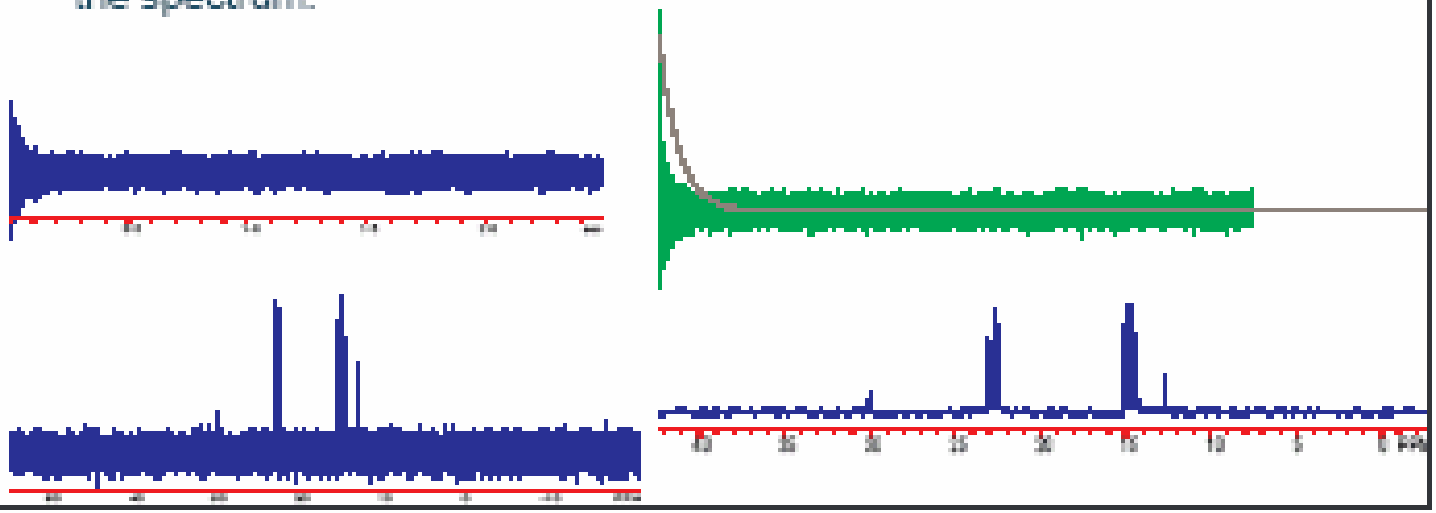


# Processing Concepts for 2D NMR

## Manipulation of FID's

Sensitivity Enhancement using the weighting function...

- The Signal-to-Noise in the Frequency spectrum results from the relative cross-section of the FID that is Signal vs. Noise.
- If the Signal decays quickly, but a long acquisition time is used, the end of the FID contains nothing but noise! This noise is *convoluted* into the entire frequency spectrum, and can seriously degrade the S:N ratio in the spectrum.

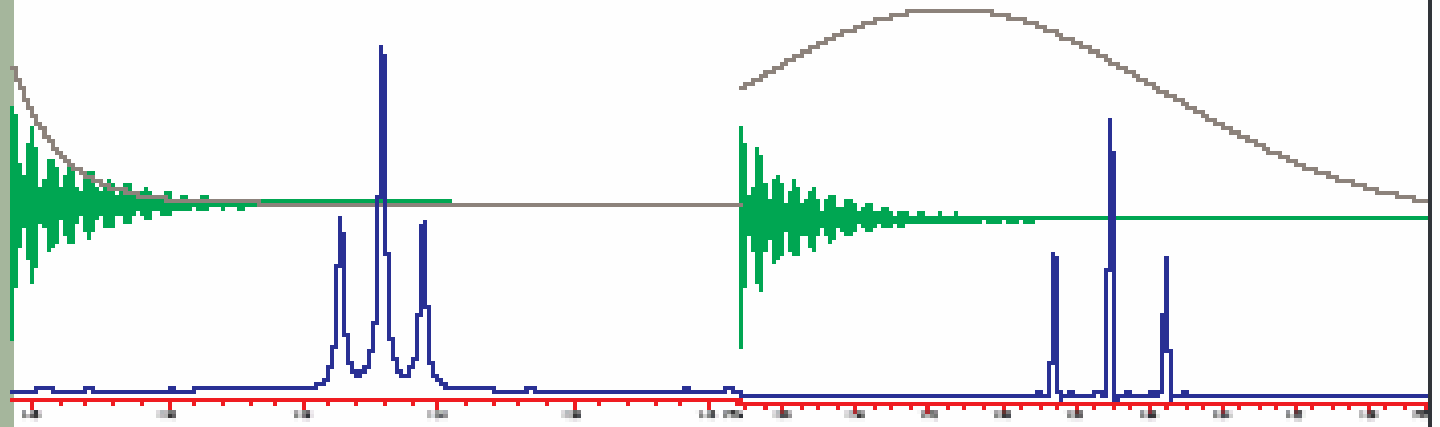


# Processing Concepts for 2D NMR

## Manipulation of FID's

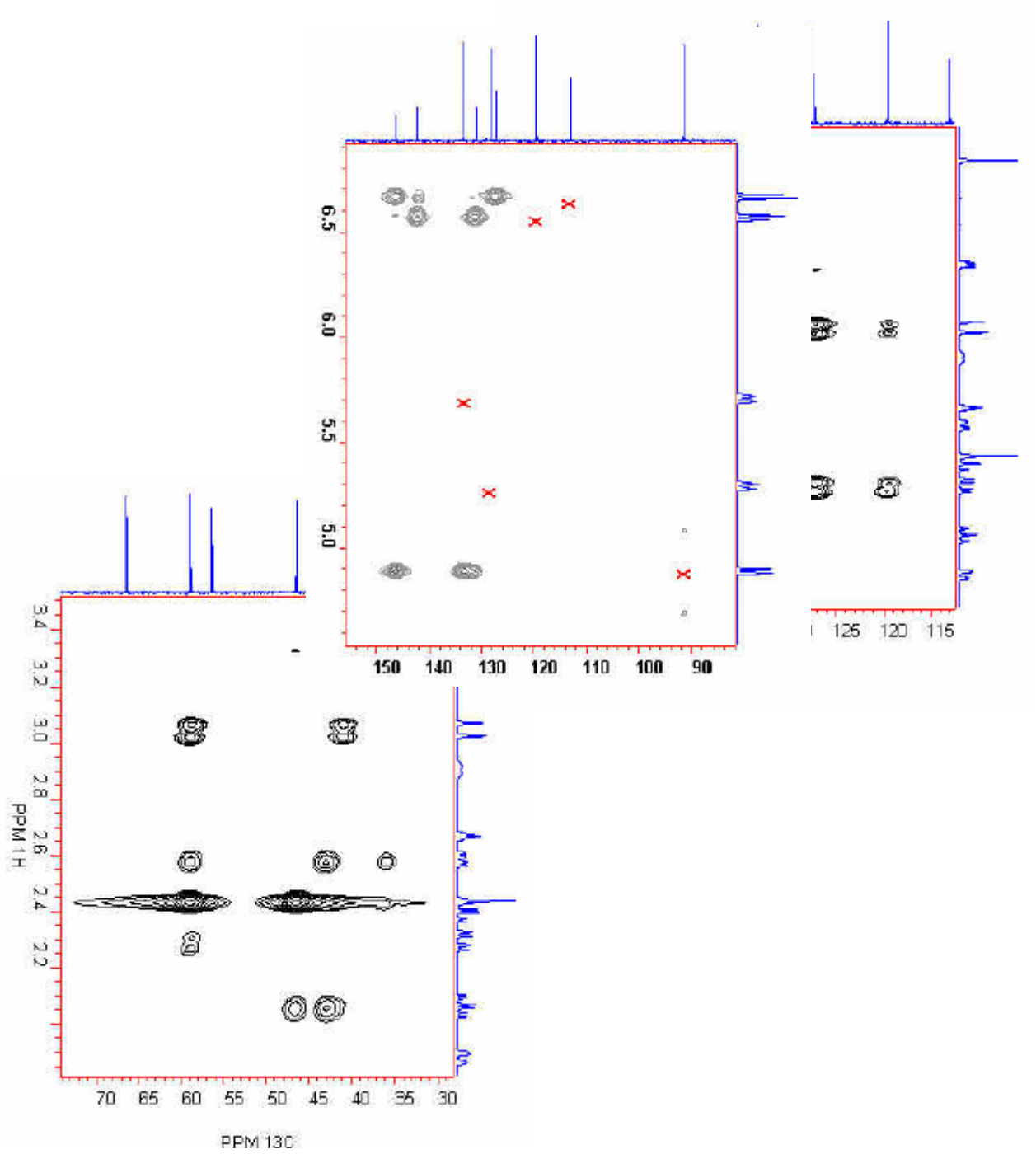
Now, let's sacrifice S:N to improve Resolution!!

- A FID decays according to $T_2^*$; however, we can use weighting functions to effectively "un-do" the natural decay!
- Imagine multiplying the decaying signal, with a reverse-exponential function that cancels-out the natural decay.



# Conclusions

- We have covered a series of 2D experiments that are useful for routine assignment of simple small molecules
- A large amount of information can be obtained in a short period of time with judicious choice of parameters
- The trade-offs are always between sensitivity, time, and resolution
- There are MANY variations of these experiments which are tailored for a particular application, but the basic concepts are the same
- For routine samples, the automated acquisition and processing routines usually work well


6.5
6.0
5.5
5.0
150 140 130 120 110 100 90
125 120 115
3.4
3.2
3.0
2.8
2.6
2.4
2.2
PPM 1H
70 65 60 55 50 45 40 35 30
PPM 13C