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**

- **Preparation**: Generally consists of a single delay, but may also have solvent saturation pulses.
- **Evolution**: Incrementable delay for mapping of chemical shifts.
- **Mixing**: Time during which through-bond or through-space couplings are allowed to interact.
- **Detection**: Normal FID

Diagram:
- Preparation
- Evolution ($t_1$)
- Mixing
- Detection ($t_2$)
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

• 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 $f1 = twice as long$).

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.
General Scheme for 2D NMR

Homonuclear COSY

Same as any 1D FID
np = number of points
1/sw = dwell time which is the time between points
at = acquisition time = (1/sw)*np

Analogous to 1D FID as above
ni = # of increments = #points in t₁
1/sw₁ = dwell time at = acquisition time = (1/sw₁)*ni

Interferogram

Transpose
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 (DQF-COSY)

(Most often used for assignment in small molecules)

Glycine - Simplest Spin System - AX

Phase-sensitive DQF-COSY

Measuring J-Couplings

Anti-phase, pure absorption line-shape
COSY Cross Peak Structure

Spin System - AMX

Serine

OD

Hα - C - Hβ

N - C - C

D - HX

Phase-sensitive COSY

COSY Cross Peak Structure
COSY Cross Peak Structure and Measuring J-Couplings

Spin System - AMX

Serine

OD
Ha-Ć-Hm
-Ν-Ć
D
Hx

active coupling

Ha-Ć-Hm

Ha-Ć-Hm

passive coupling

-Ć
Hx
-Ć
Hx

βH1

βH2

M

A
Measuring J-Couplings

Spin System - $A_3X$

Phase-sensitive COSY

Treat only one H of CH₃ as active pair

active coupling

passive coupling
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 $= 0$.

Geminal if not equivalent
Example of lysine spin system

Lysine

DQF-COSY

TOCSY

80msec mixing time

Hα  Hε  Hδ  Hβ  Hγ

Hαβγδε
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
Example of TOCSY Spectrum

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

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

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 for COSY, DQFCOSY, and TOCSY
These are suggestions only - Defaults should also work

**COSY**

F2 (Direct Dimension)

- \(sw\) = spectral width = 6000Hz (10 ppm) 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 (10 ppm) 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 sensitive FT 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.

**Artifact**

Removes intensities above a certain threshold if no symmetric partner exists on the other side of the diagonal. 2D matrix must be square (i.e. fn=fn1). Can be set in VNMRJ.

Use cautiously!! Only for homo 2D's
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.
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

<table>
<thead>
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<th>H</th>
<th>C</th>
<th>Assignment</th>
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<tr>
<td>6.6</td>
<td>113</td>
<td>8</td>
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<tr>
<td>6.5</td>
<td>120</td>
<td>7</td>
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<tr>
<td>5.7</td>
<td>133</td>
<td>3</td>
</tr>
<tr>
<td>5.3</td>
<td>128</td>
<td>5</td>
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<tr>
<td>4.8</td>
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<td>9</td>
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<td>56</td>
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<td>3.3</td>
<td>59</td>
<td>11</td>
</tr>
<tr>
<td>3.0 &amp; 2.3</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>2.6</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>2.6 &amp; 2.4</td>
<td>46</td>
<td>13</td>
</tr>
<tr>
<td>2.4</td>
<td>43</td>
<td>14</td>
</tr>
<tr>
<td>2.0 &amp; 1.8</td>
<td>36</td>
<td>17</td>
</tr>
</tbody>
</table>

Acquisition Parameters:
512 complex points in direct dimension
128 t1 increments
2 scans
2 sec. relaxation delay
Total acquisition time: ~ 10 min.

Processing:
sine squared window function in both dimensions(45°)
2x zero-fill in the indirect dimension
magnitude calculation (no phasing is required)
final data size 512 x 512
The sample is 3.3 mg codeine in ~ .65 ml CDCl₃ Total time = 20 minutes

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)

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

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
Analysis of HMBC Experiment

The sample is 3.3 mg codeine in ~0.65 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).
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 = 6000 Hz (10 ppm) 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 = 1–2 s (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 = 6000 Hz (10 ppm) 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 = 2 s (longer $d1$, less artifacts)
- $Mix$ = 70 ms (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 (\( \text{Sin}(t)/t \)) that will be convoluted into the lineshape after the FFT.
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.
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