Two-Dimensional NMR: General Scheme

There are four sections in all 2D experiments:

- **Preparation**
- **Evolution**
- **Mix**
- **Detection**

For homonuclear COSY (the simplest example), this reduces to:

\[ \text{d1} \quad \text{p0} \quad \text{d0} \quad \text{p1} \quad \text{aq} \]
Two-Dimensional NMR: General Scheme

There are four sections in all 2D experiments:

preparation  evolution  mix  detection

\[ t_1 \]

\[ t_2 \]
$t_2$ to $F_2$ in 2D NMR

$t_1 = d0 + n/sw1$
$t_1$ to $F_1$ in 2D NMR
Fourier Transforms in 2D Spectroscopy (topspin)
Resolution in 1D Spectroscopy

Resolution in 1d NMR is usually determined by the acquisition time (but limited by shim quality and relaxation time):

$$1d \text{ (true) resolution} \sim \frac{1}{aq}$$

Resolution depends on an ability to differentiate two very similar time-varying signals.
Resolution in 1D NMR is usually determined by the acquisition time (but limited by shim quality and relaxation time):

\[ 1d \text{ (true) resolution} \sim \frac{1}{aq} \]

The Fourier transform provides a simpler representation of the data for humans to “see” the difference.
Resolution in 1D Spectroscopy

Resolution in 1d NMR is usually determined by the acquisition time (but limited by shim quality and relaxation time):

1d (true) resolution $\sim 1/aq$

The fundamental limitation of natural linewidth remains.
Resolution in 2D Correlation Spectroscopy

1d (true) resolution $\sim 1/aq$

2D NMR works identically, but limitations on data set sizes (td and td1) make the digital resolution a key factor. It may appear that:

$$\text{dig. res. F2} \sim \frac{\text{sw}}{\text{td}/2} = \frac{1}{aq} = \frac{\text{dresF2}}{}$$

but the 1st full zerofill does assist. Zerofilling is typically not done in $t_2(F2)$, however, so the equation above is (with no zerofill) correct.
Resolution in 2D Correlation Spectroscopy

A minimum of one zerofill is always performed in $t_1(F1)$, however:

$$\text{dig. res. } F1 \sim \frac{sw1}{2 \times td1/2} = \frac{1}{2 \times at1} \equiv dresF1 = \frac{sw1}{td1}$$

$$= 2 \cdot \frac{SW1}{td1} = \frac{SW1}{td1}$$

with one zerofill
A minimum of one zerofill is always performed in $t_1(F1)$, however:

\[ \text{dig. res. F1} \sim \frac{sw1}{2 \times td1/2} = \frac{1}{2 \times aq1} \]

Typical values for 1H cosy:

- $sw = sw1 = 8$ ppm
- $td1 \sim 256@300$ MHz; $td1 \sim 512@500$ MHz
- $dresF1 \sim 9$ Hz/pt

Resolution in 2D Correlation Spectroscopy

---

- $4800$ Hz
  - $400$ pts
  - $12$ Hz/pt
- $16000$ Hz
  - $400$ pts
  - $40$ Hz/pt

- $4800$ Hz
  - $2048$ pts
  - $2$ Hz/pt
Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy

Although we are using J-couplings in a correlation (cosy) 2D experiment, we do not need to resolve the coupling.
Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy

Although we are using J-couplings in a correlation (cosy) 2D experiment, we do not need to *resolve* the coupling.

The experiment instead must *evolve* the coupling during $t_1$, and thus produce a crosspeak: $I_y$ during $t_1(F1)$, and $S$ in $t_2(F2)$.

The J-coupling evolution creates an antiphase spin state $I_xS_z$:

$$I_y(t_1) = I_y \cos(\pi J t_1) + I_x S_z \sin(\pi J t_1)$$
Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy

5.7ppm proton in $t_1$
5.7ppm proton in $t_2$ or 3.0ppm proton in $t_2$

start with I proton, evolve to S
Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy

start with S proton, evolve to I

3.0 ppm proton in \( t_1 \)
3.0 ppm proton in \( t_2 \) or 5.7 ppm proton in \( t_2 \)
Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy

The J-coupling evolution creates an antiphase spin state $I_xS_z$:

$$I_y(t_1) = I_y \cos(\pi J t_1) + I_x S_z \sin(\pi J t_1)$$

The trigonometric terms are multipliers to the intensity of diagonal peaks and crosspeaks in the 2d spectrum. The closer $t_1$ gets to $1/2J$, the larger the crosspeaks will become.
The J-coupling evolution creates an antiphase spin state $I_x S_z$:

$$I_y(t_1) = I_y \cos(\pi J t_1) + I_x S_z \sin(\pi J t_1)$$

It is not necessary to achieve full antiphase creation with $t_1=1/2J$, but only that the $t_1$ evolution be sufficiently long to produce observable crosspeak intensity (i.e., crosspeaks unambiguously bigger than the noise).

$$\Rightarrow \sin(\pi J t_1) \ll 1 \quad \text{is OK.}$$
The J-coupling evolution creates an antiphase spin state $I_xS_z$:

$$I_y(t_1) = I_y \cos(\pi J t_1) + I_x S_z \sin(\pi J t_1)$$

It is not necessary to achieve full antiphase creation with $t_1 = 1/2J$, but only that the $t_1$ evolution be sufficiently long to produce observable crosspeak intensity (i.e., crosspeaks unambiguously bigger than the noise).

$$\Rightarrow \sin(\pi J t_1) \ll 1 \; \text{is OK.}$$

**But how much < 1 is OK?** Crosspeaks will always be observed (on any properly functioning, high-field spectrometer) when:

$$\sin(\pi J t_1) \geq 0.25$$

in proton-proton COSYs, and within $t_1 = aq1/2$ because of the sinebell apodization.
From the considerations stated on the previous page, we can arrive at an important empirically verified rule-of-thumb for 1H COSY. Start with:

\[
\sin(\pi J_{t_1}) \geq 0.25
\]

make some substitutions:

\[
\sin(\pi J_{t_1}) = \sin(\pi J \times at_1/2) = \sin(\pi J \times td_1/4sw_1)
\]

and now solve for \( J \):

\[
J \geq \frac{4 \times sw_1}{\pi \times td_1} \times \arcsin(0.25) \sim \frac{sw_1}{3 \times td_1}
\]

Thus crosspeaks will be observed when:

\[
J_{\text{observed}} \geq \frac{sw_1}{3 \times td_1} = \frac{1}{6 \times aq_1}
\]

Better quality data (reduced artifacts, superior sequence, more ns) will allow less-intense crosspeaks to be observed (e.g., those \( \geq 10\% \)), increasing the factor of 6 to perhaps 15). Smaller \( J \)'s can then be observed for the same \( td_1 \) and \( sw_1 \).
Summary of Evolution for 2D NMR Experiments

For 1H COSY:

Typical: $J_{\text{obs}} \geq 3 \text{ Hz} \approx \text{sw1}/(6 \times \text{td1})$

Increasing td1 decreases $J_{\text{obs}}$.

Increasing the evolution time $t_1$ decreases $J_{\text{obs}}$ (long-range COSY).

For heteronuclear 1-bond cosy, HSQC:

Fix delays to $1/2J_{\text{CH}}$, so only chem shift (digital) resolution is an issue.

td1 is set as a compromise between experiment time and signal-to-noise.

For heteronuclear n-bond cosy, gHMBC:

Delays become long, so experiment is modified from HSQC.

Still fixed delays at approx $1/2^nJ_{\text{CH}}$, so td1 is a compromise between exp. time and s/n.
S/N, *ns* and *td1*: Time of 2D Experiments

1D: \[ \frac{S}{N} \propto \sqrt{ns} \]

2D: \[ \frac{S}{N} \propto \sqrt{(td1 \cdot ns)} \]

Can increase *td1* or *ns* to improve S/N.

Time 1D: \[ \approx (aq + d1) \cdot ns \]

Time 2D: \[ \approx (aq + d1 + \frac{td1}{2 	imes sw1}) \cdot td1 \cdot ns \]

The S/N improves for all peaks during the complete 2D experiment.

*T_2* (linewidth) can restrict from this important use of instrument time. For large MW (small *T_2*), increasing *ns* is usually best. For small MW, increasing *td1* is most often best, as this improves both S/N and resolution.