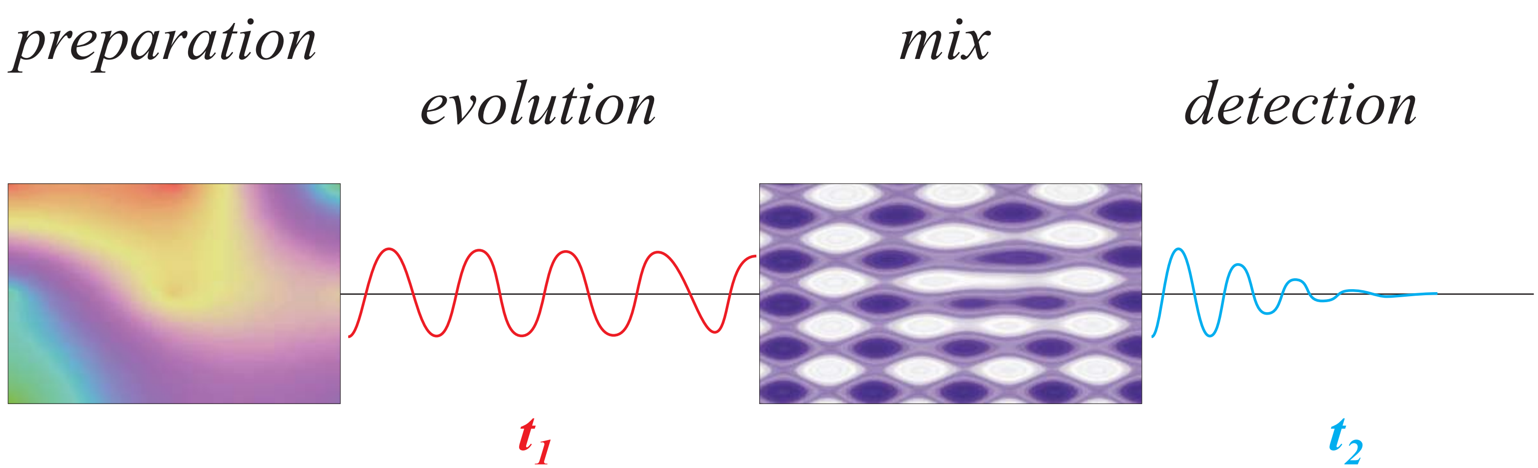
Two-Dimensional NMR: General Scheme

There are four sections in all 2D experiments:



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

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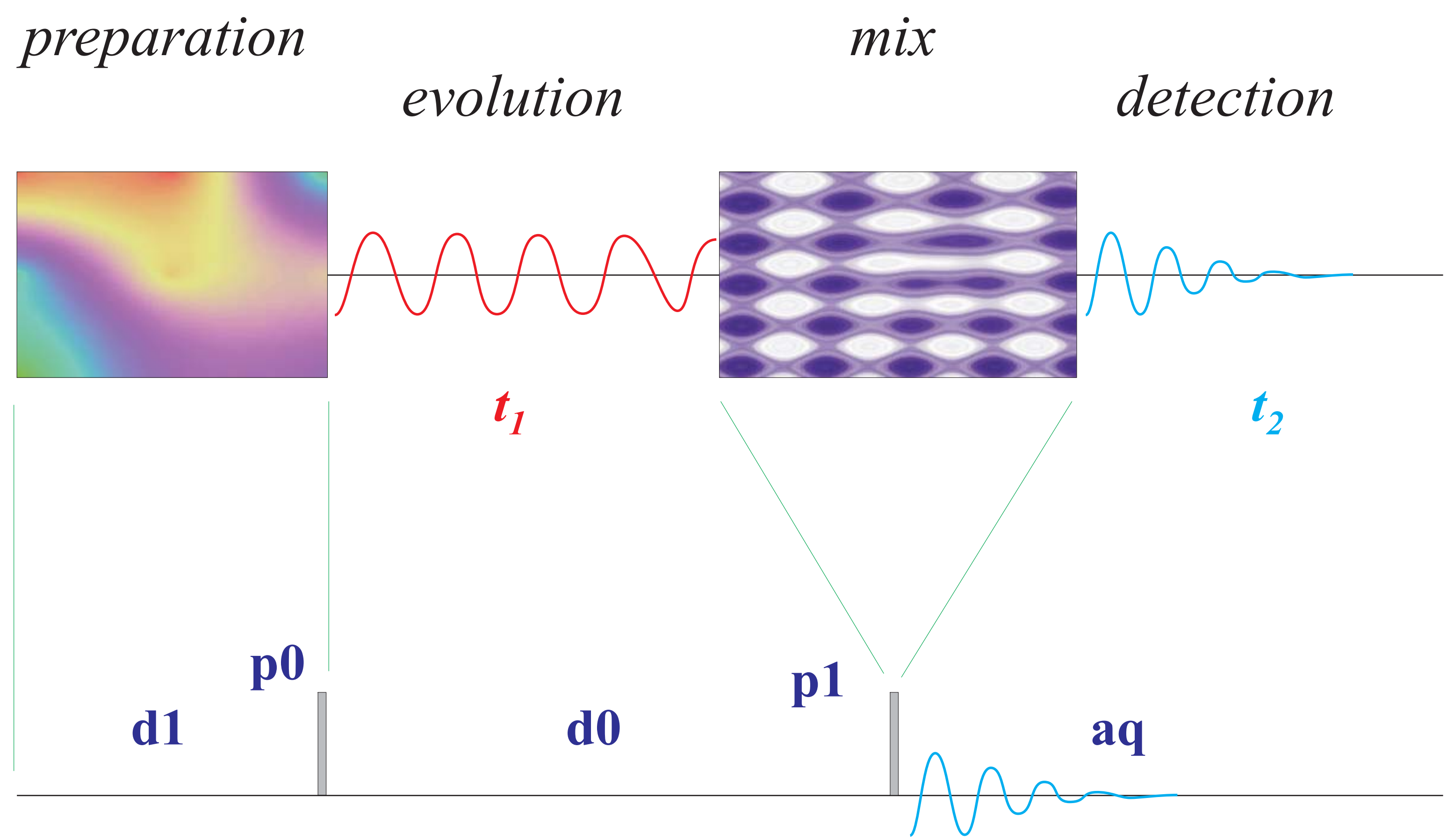
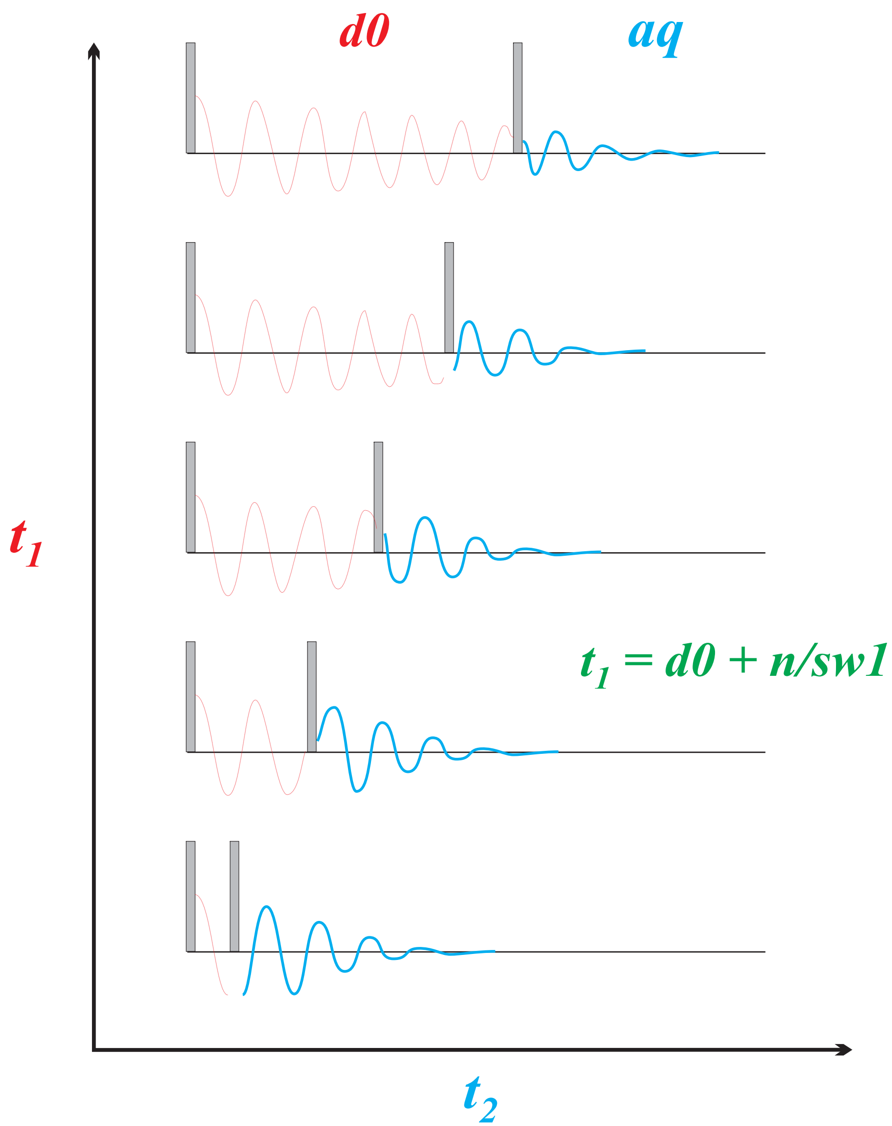
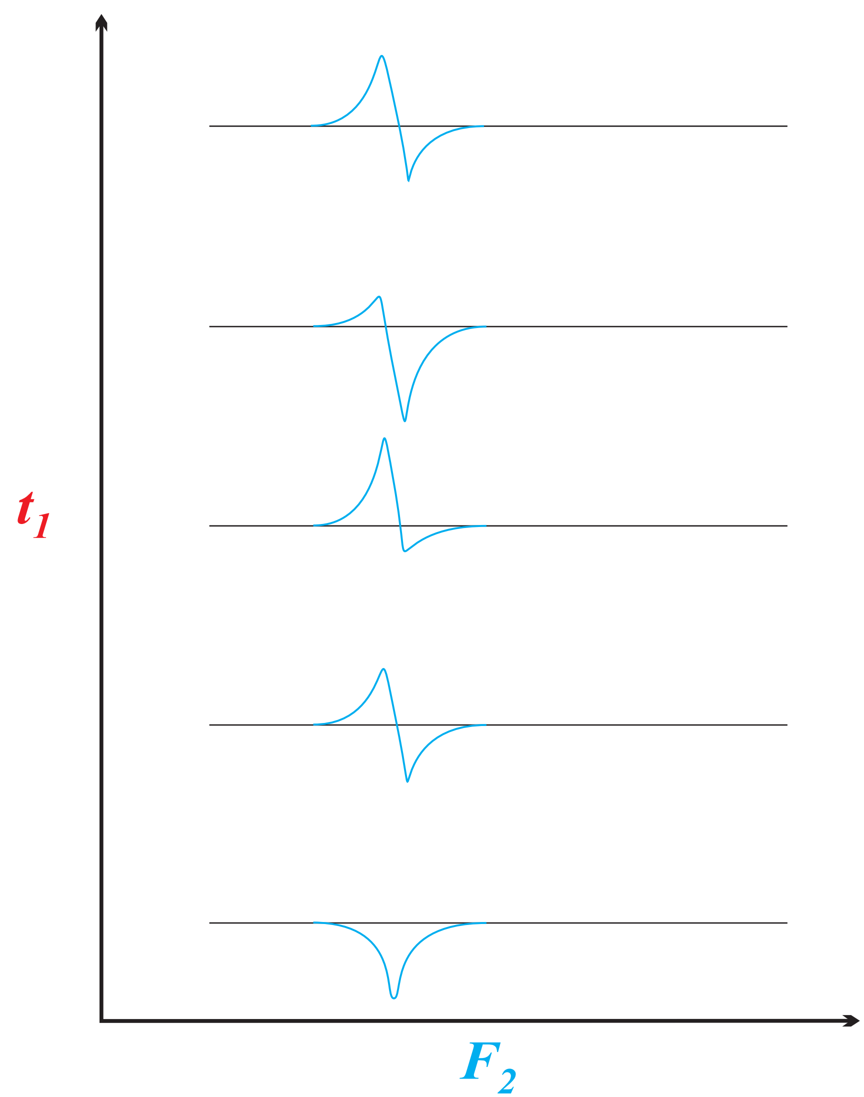
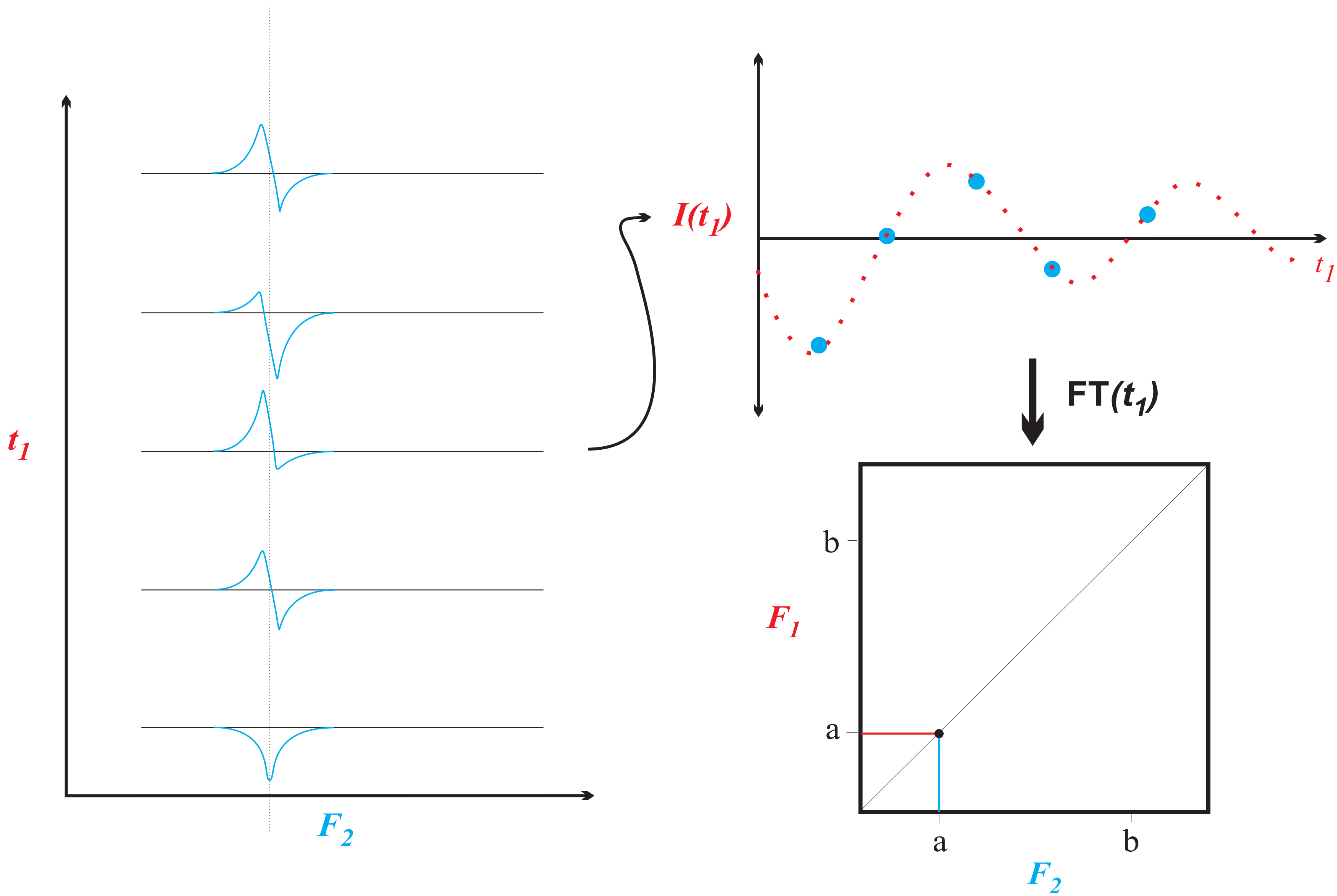


t_2 to F_2 in 2D NMR

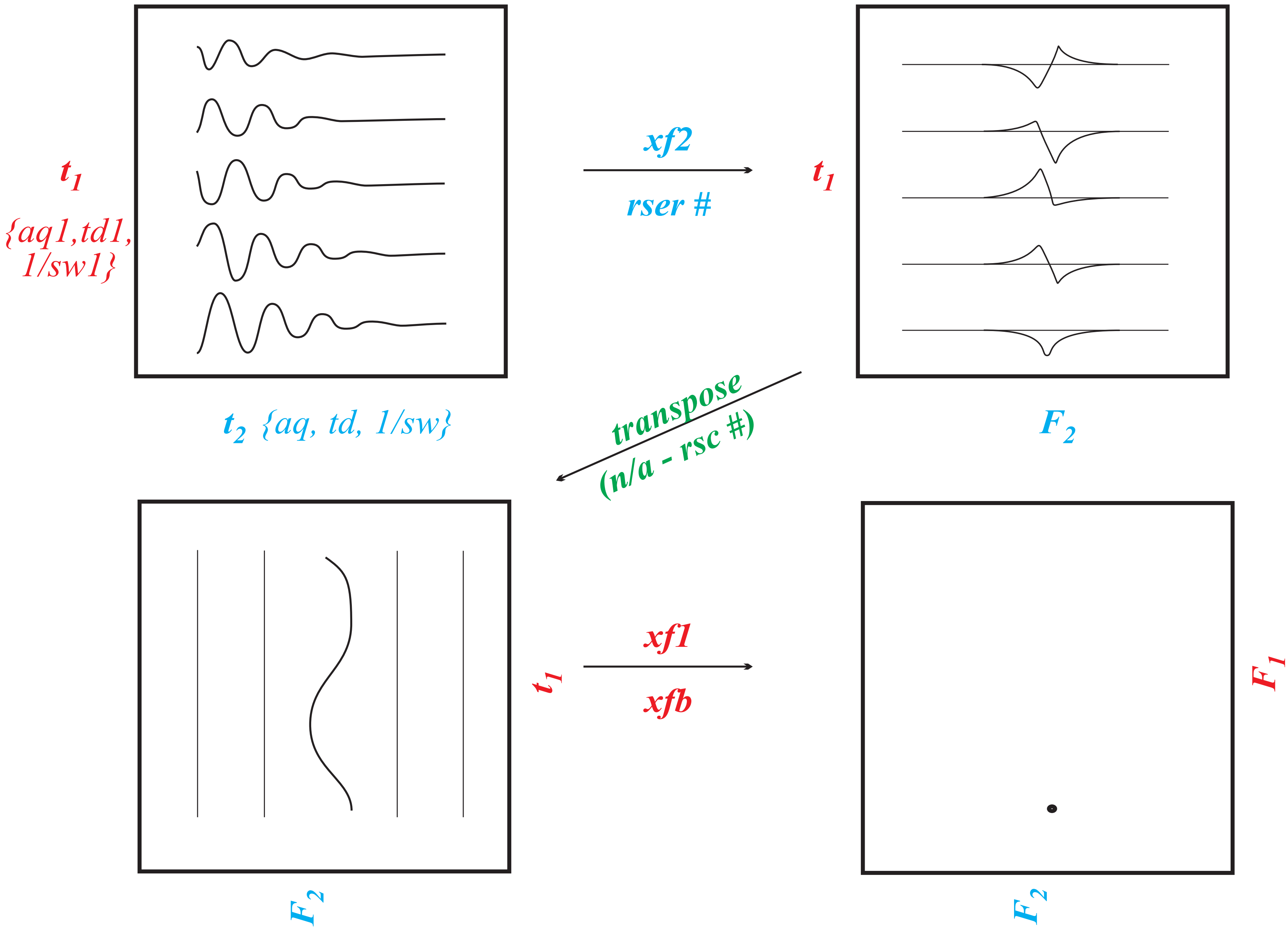




t_1 to F_1 in 2D NMR



Fourier Transforms in 2D Spectroscopy (topspin)

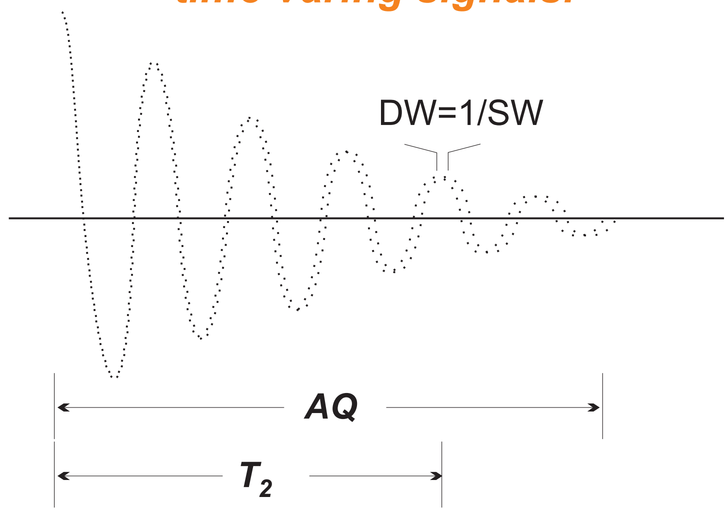


Resolution in 1D Spectroscopy

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

1d (true) resolution ~ 1/aq

Resolution depends on an ability to differentiate two very similar time-varing signals.



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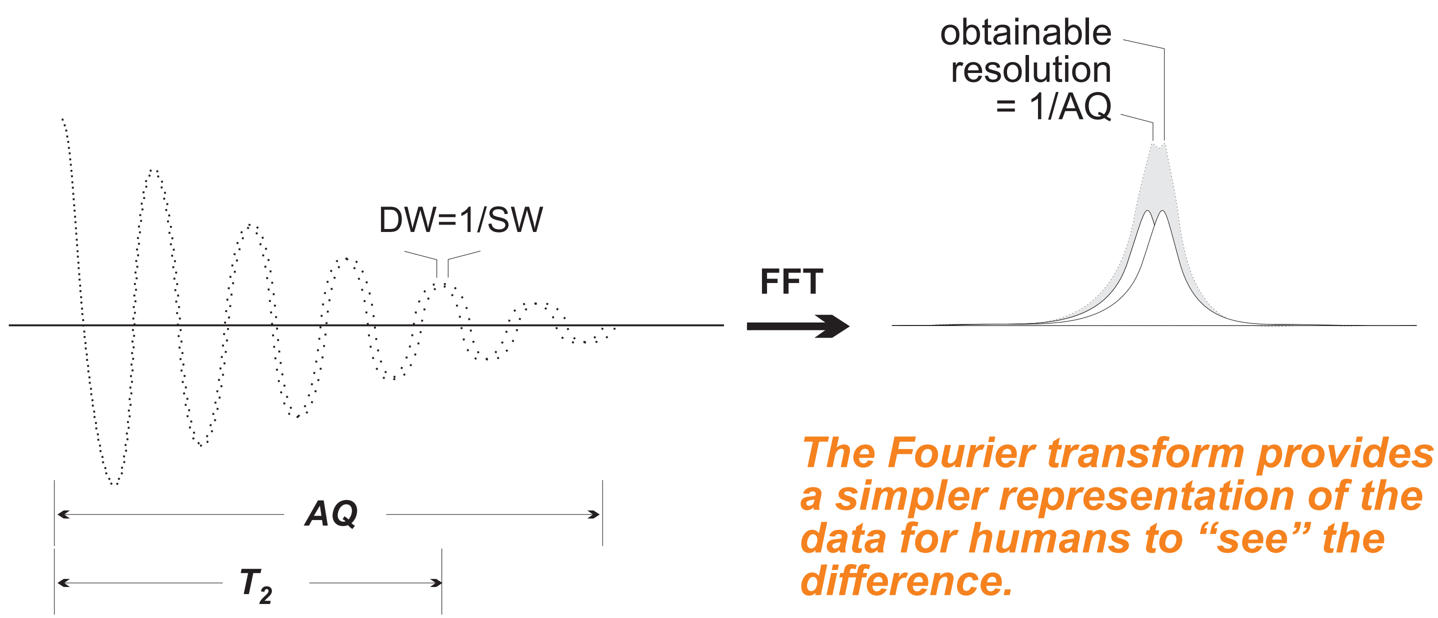

The Fourier transform provides a simpler representation of the data for humans to "see" the difference.

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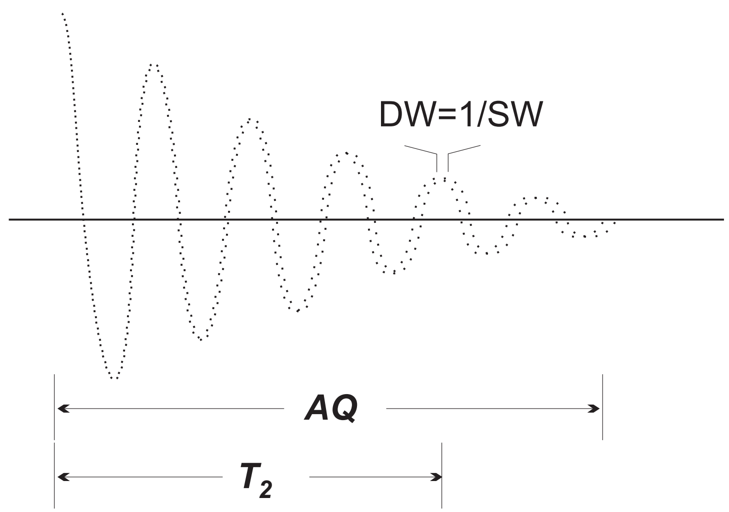
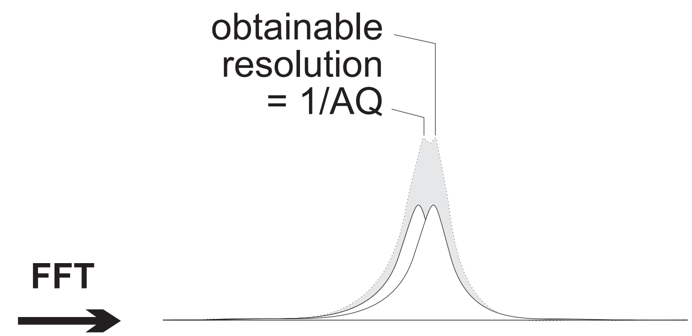
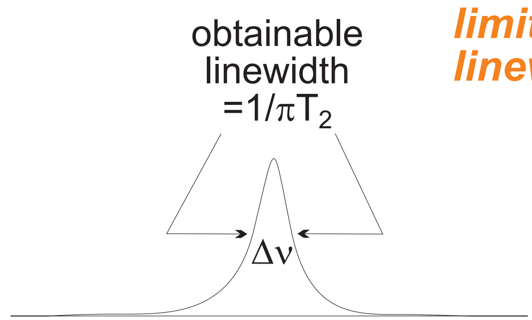




The fundamental limitation of natural linewidth remains.



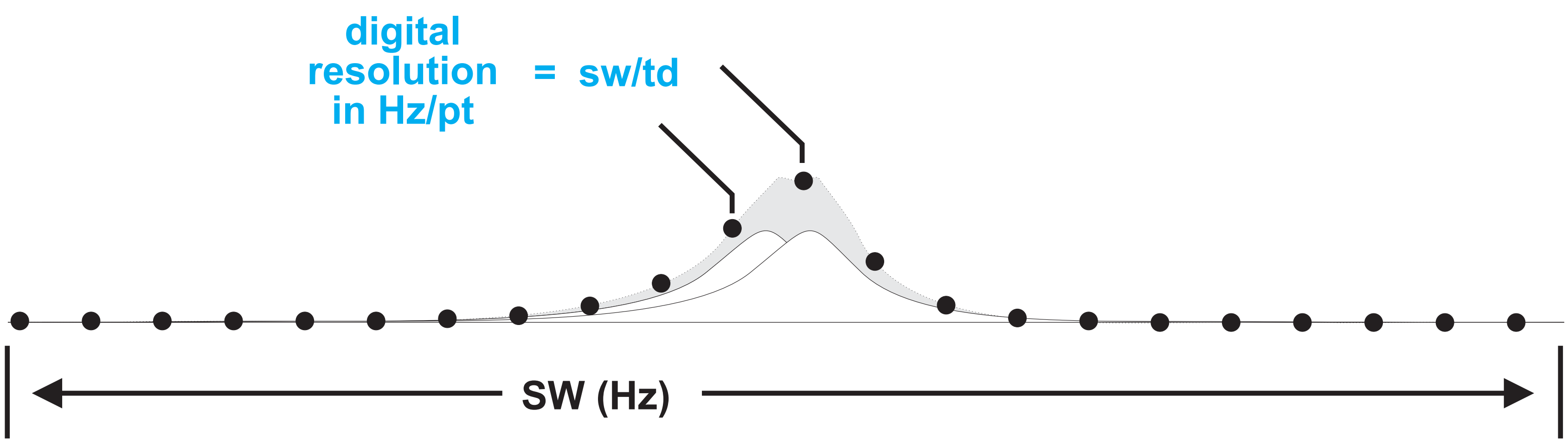
Resolution in 2D Correlation Spectroscopy

1d (true) resolution ~ 1/aq

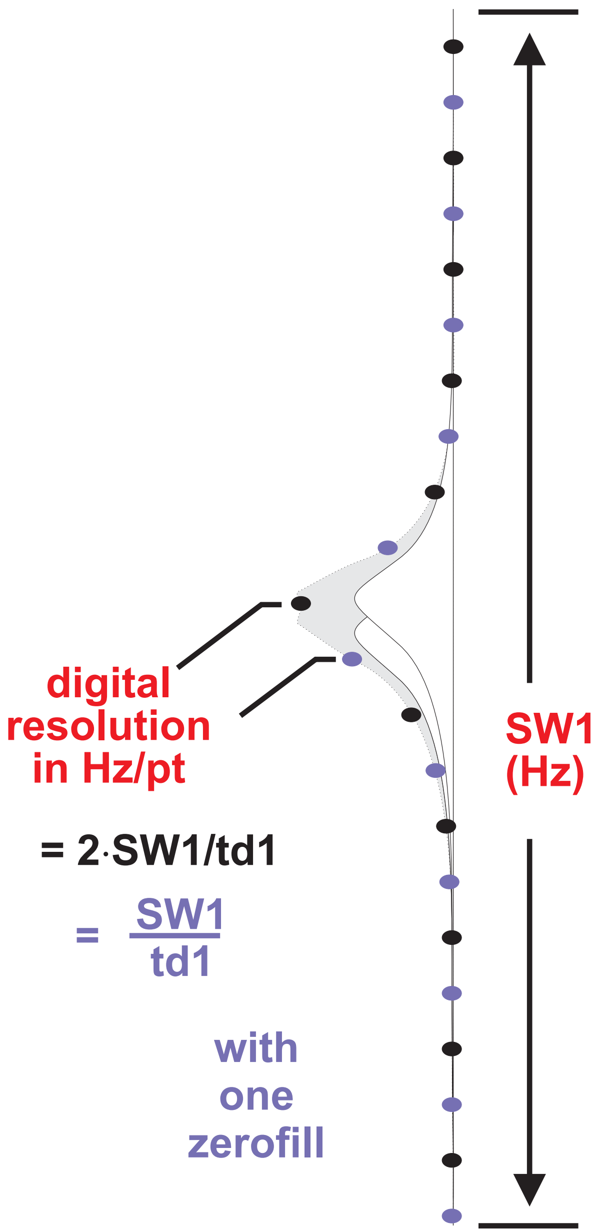
2D NMR works identically, but limitations on data set sizes (td and td1) make the digital resolution a key factor. It may appear that:

dig. res. F2 ~ sw/(td/2) = **1/aq** ≡ **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

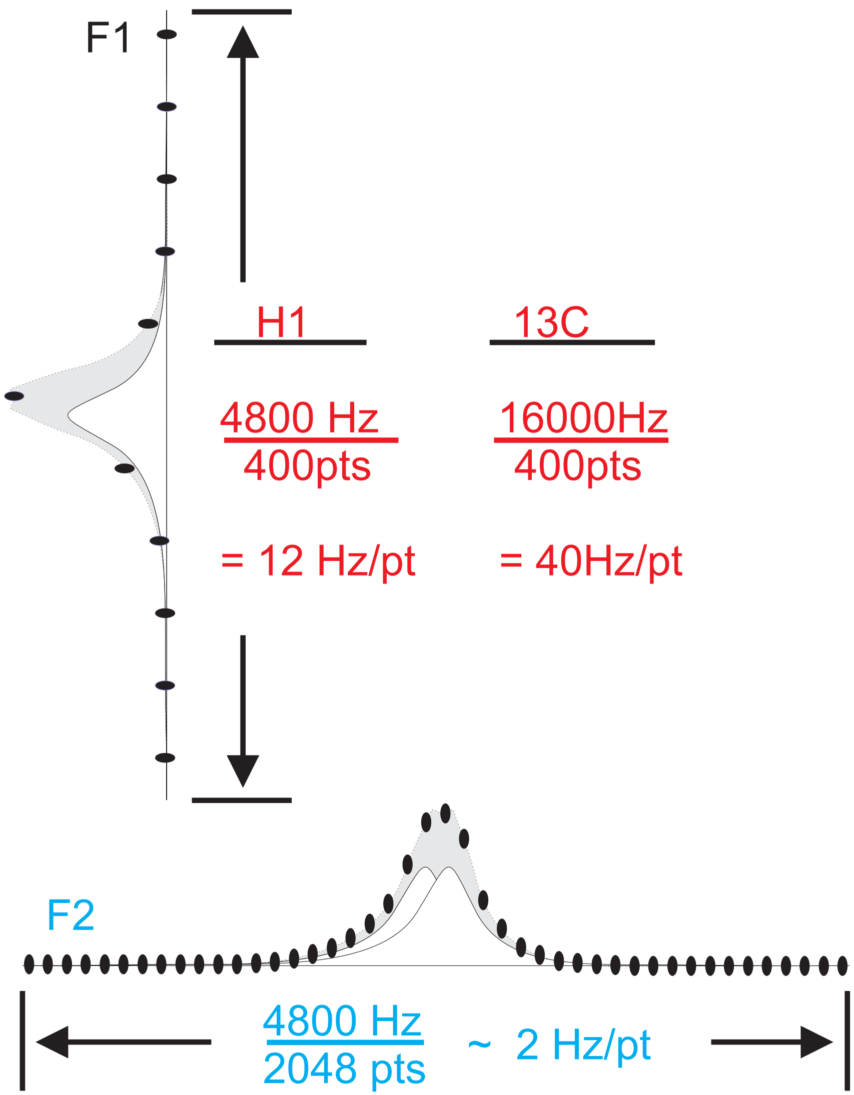


A minimum of one zerofill is always performed in $\boldsymbol{t_1}$(F1), however:

$$\text{dig. res. F1} \sim \text{sw1}/(2\times\text{td1}/2) =$$

$$1/(2\times\text{at1}) \equiv \mathbf{dresF1} = \frac{\mathbf{sw1}}{\mathbf{td1}}$$

Resolution in 2D Correlation Spectroscopy



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

dig. res. F1 ~ sw1/(2×td1/2) =

1/(2×aq1) ≡ **dresF1** $= \dfrac{\mathbf{sw1}}{\mathbf{td1}}$

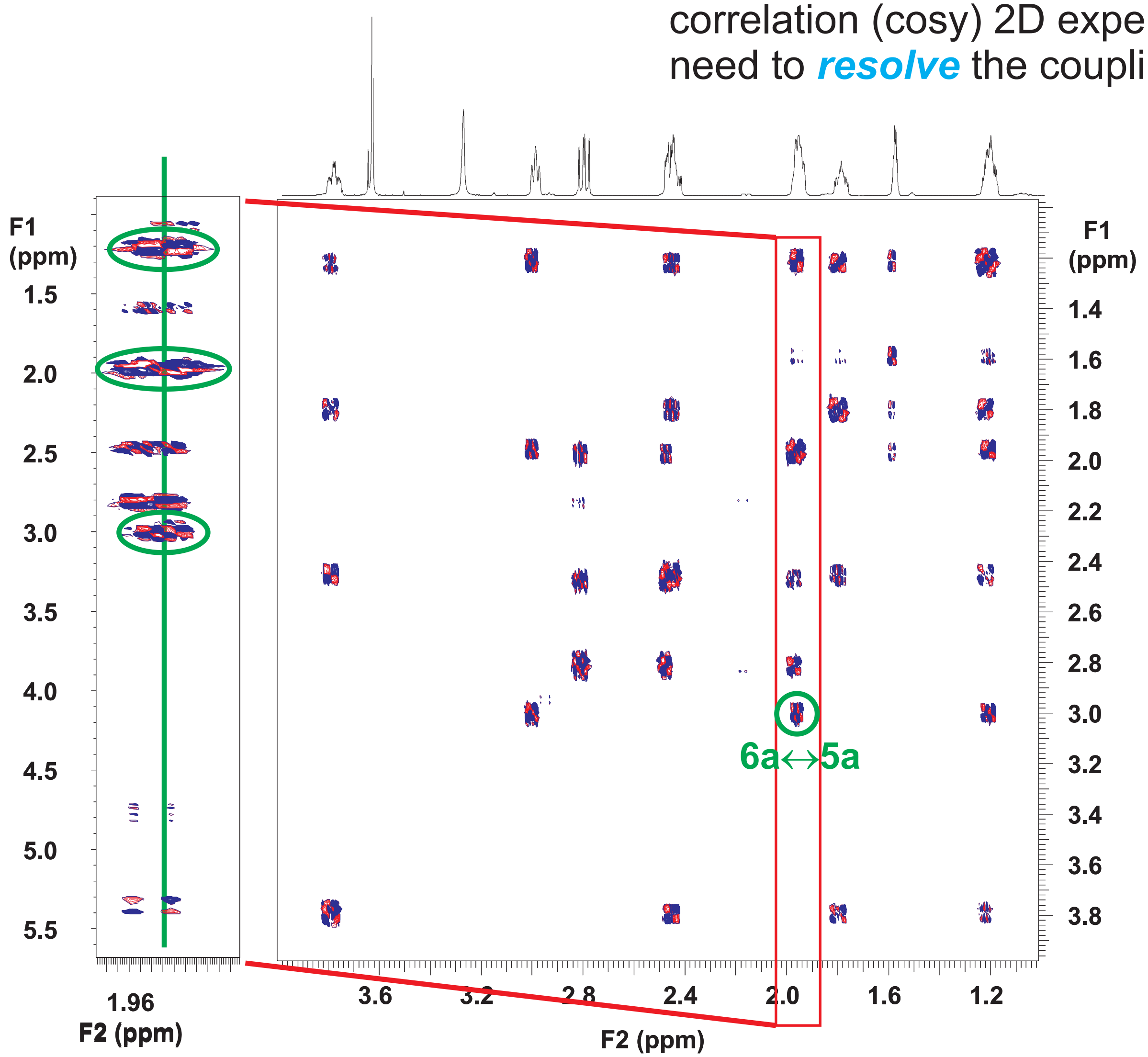
Typical values for 1H cosy:

sw = sw1 = 8ppm
td1 ~ 256@300MHz; td1 ~ 512@500MHz

dresF1 ~ 9 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.



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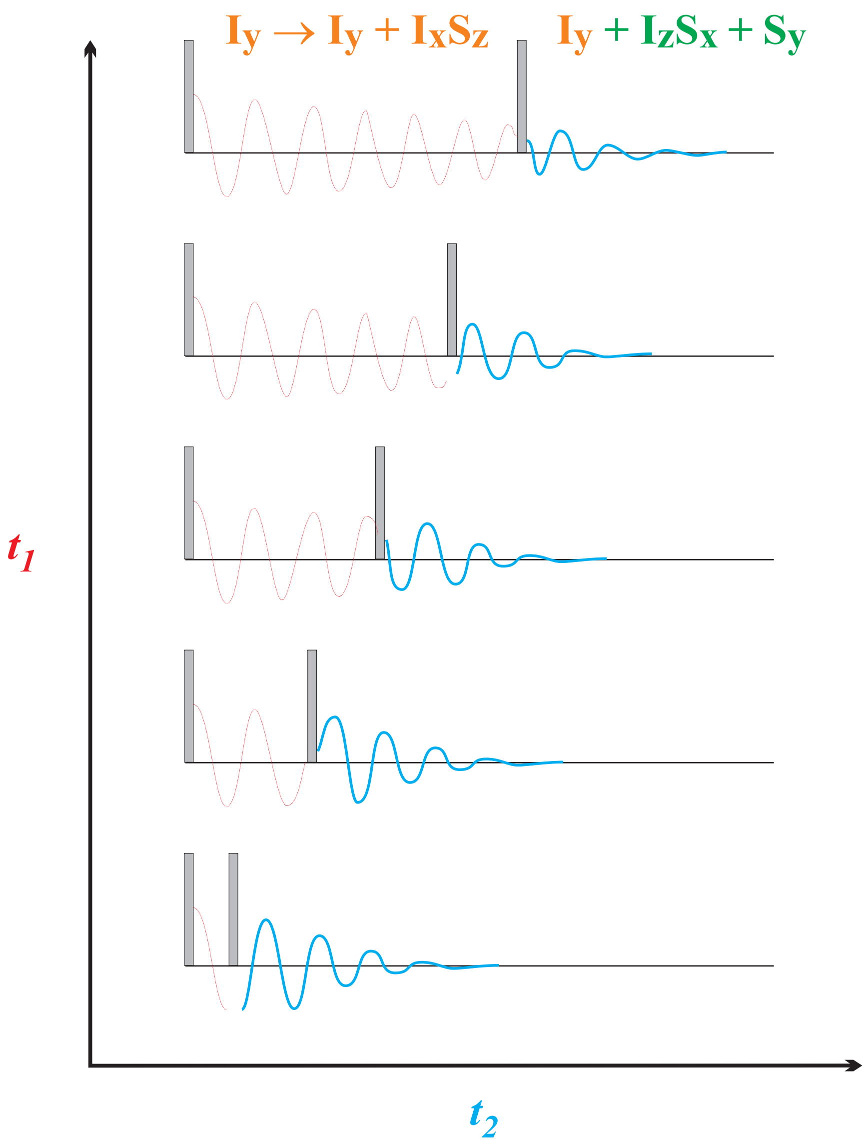


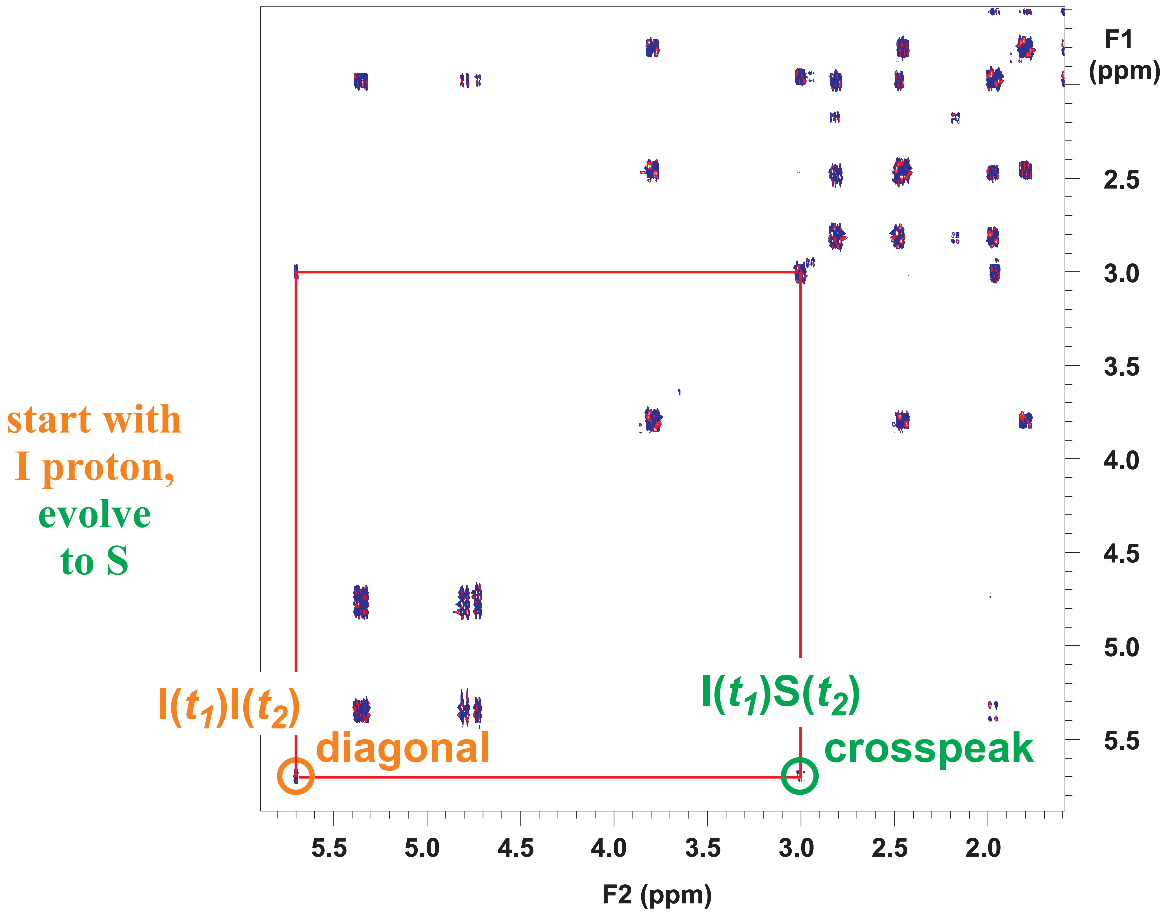
Although we are using J-couplings in a correlation (cosy) 2D experiment, we do not need to ***resolve*** the coupling.

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

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

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

Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy

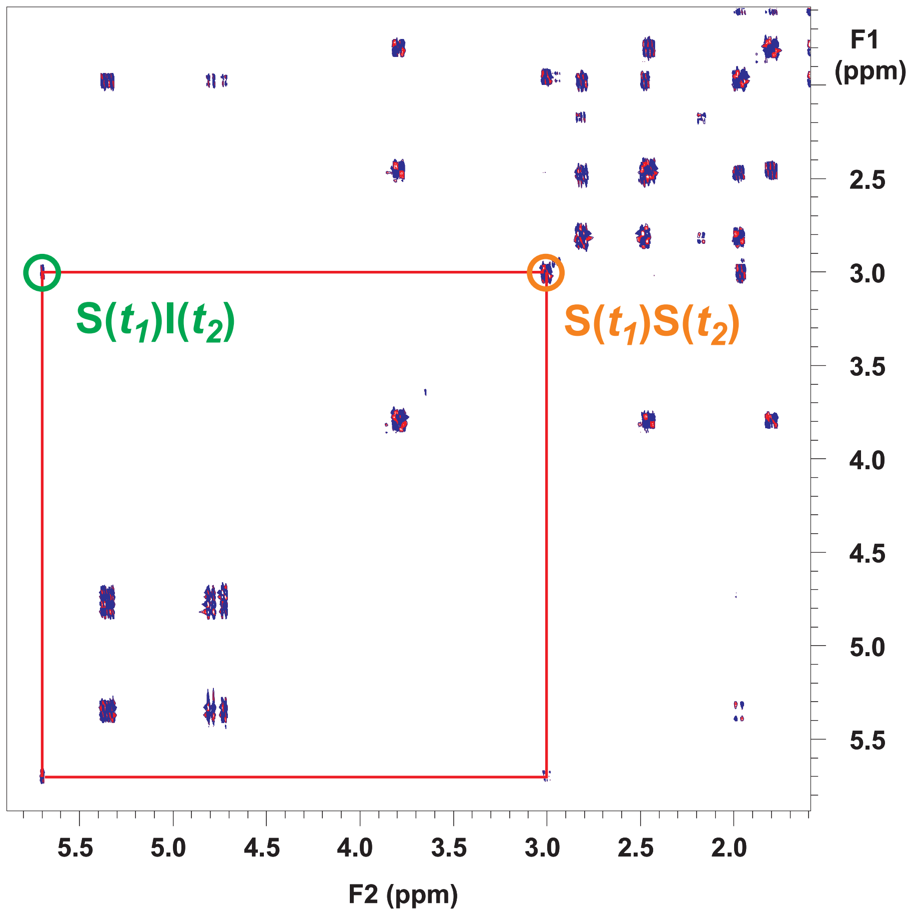


5.7ppm proton in t_1

5.7ppm proton in t_2 or 3.0ppm proton in t_2

Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy

start with
S proton,
evolve
to I



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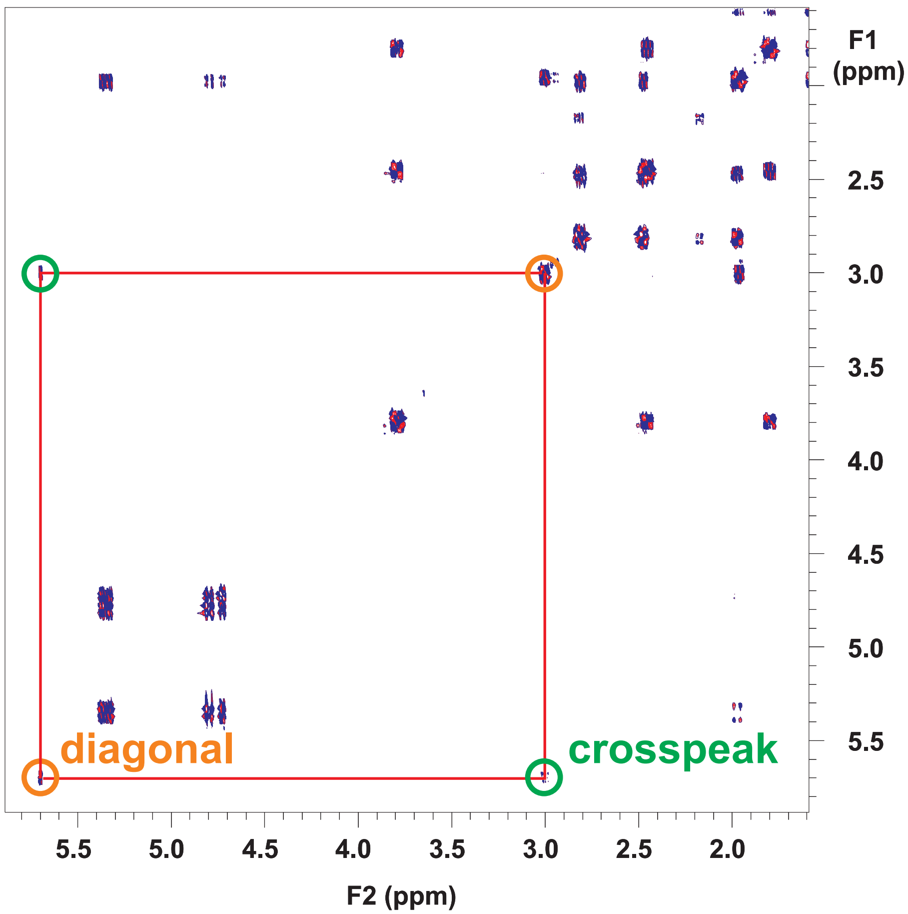

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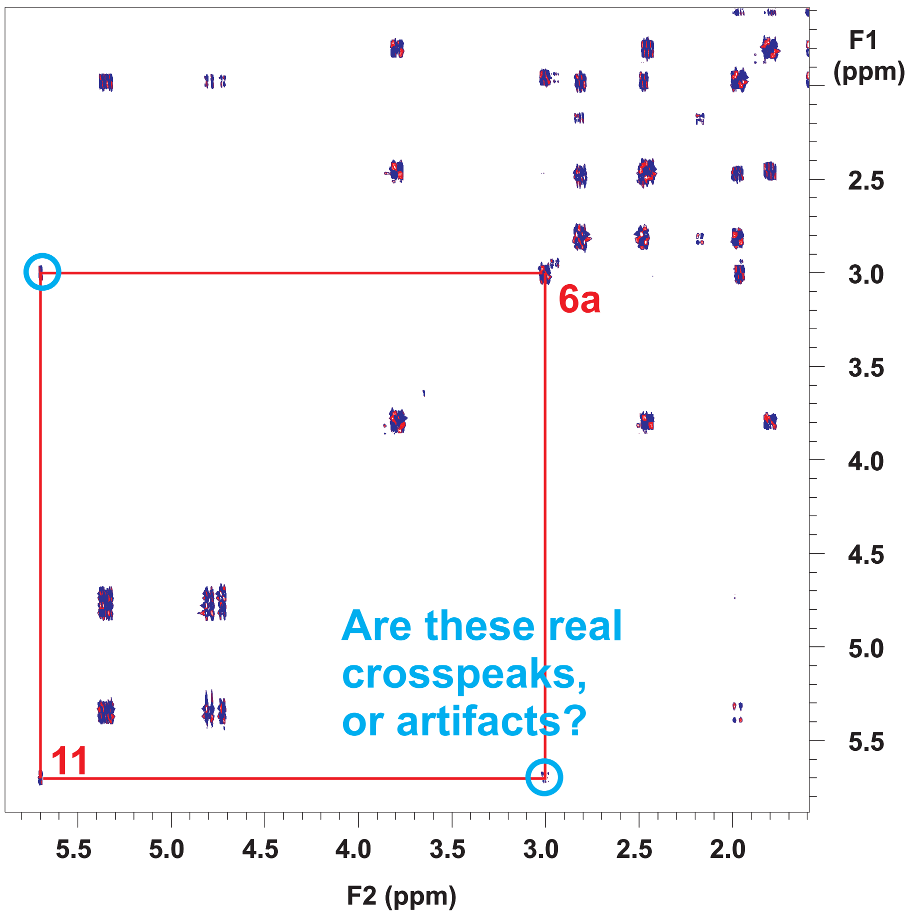
$$I_y(t_1) = I_y\cos(\pi J t_1) + I_xS_z\sin(\pi J t_1)$$

The trignometric 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.

Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy



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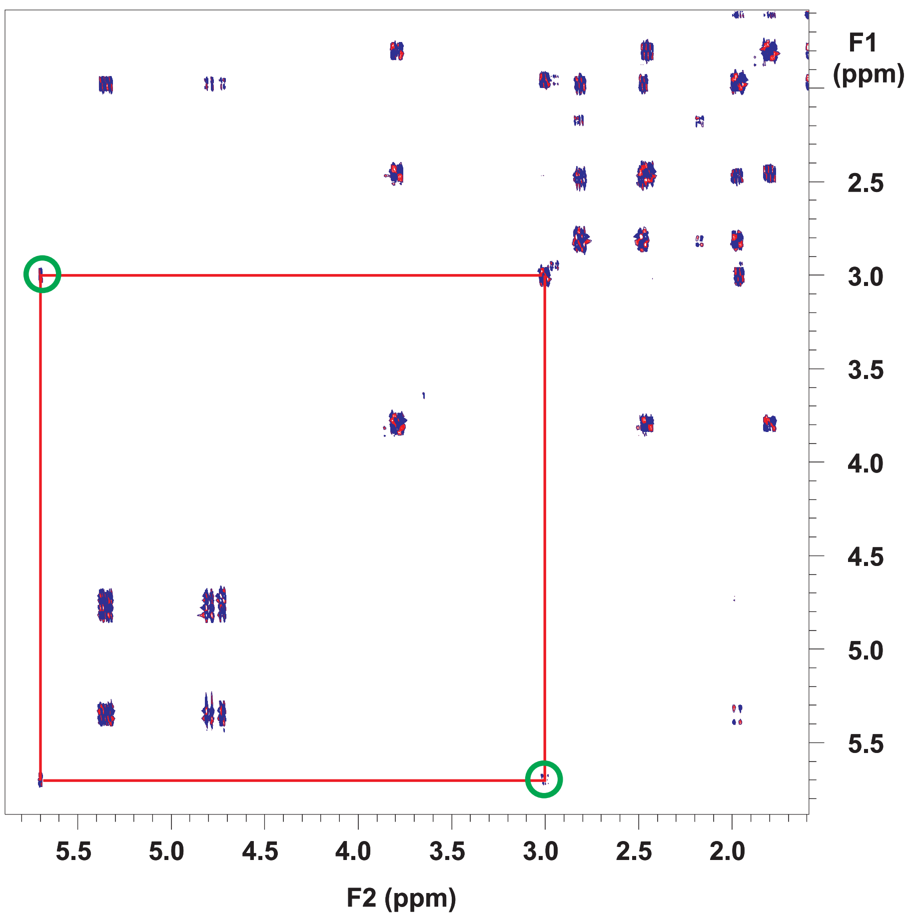
It is not necessary to achieve full antiphase creation with t_1=1/2J, but only that the t_1 evolution be sufficiently long to produce observable crosspeak intensity (i.e., crosspeaks unambiguously bigger than the noise).

⇨ $\sin(\pi J t_1) \ll 1$ **is OK.**

Evolution, not Resolution, is Crucial in 2D Correlation Spectroscopy



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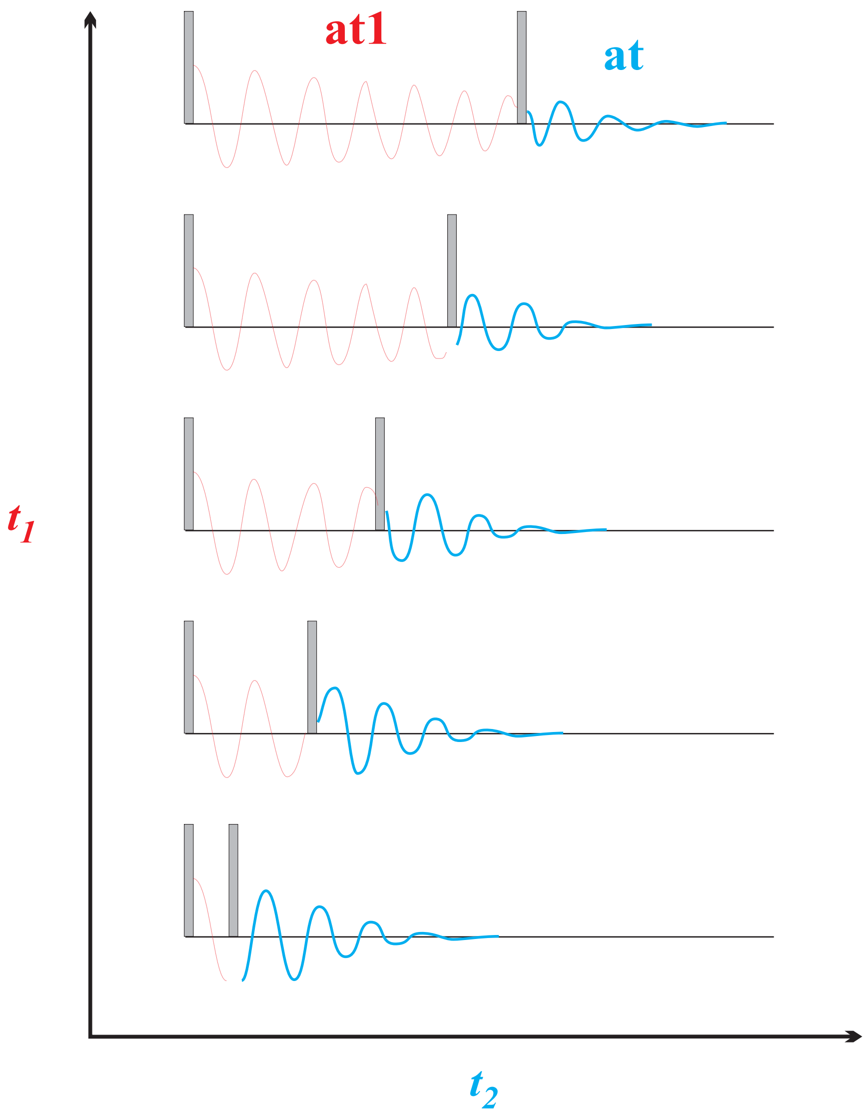
But how much < 1 is OK? Crosspeaks will always be observed (on any properly functioning, high-field spectrometer) when:

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

in proton-proton COSYs, and within t_1 = aq1/2 because of the sinebell apodization.

Size of J-Couplings Observed in 1H COSY



From the considerations stated on the previous page, we can arrive at an important empirically verified rule-of-thumb for 1H cosy. Start with:

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

make some substitutions:

$$\sin(\pi J t_1) = \sin(\pi J \times at1/2) = \sin(\pi J \times td1/4sw1)$$

and now solve for J:

$$J \gtrsim \frac{4\ sw1}{\pi \times td1} \times \arcsin(0.25) \sim \frac{sw1}{3 \times td1}$$

Thus crosspeaks will be observed when:

$$\boxed{J_{observed} \gtrsim \frac{sw1}{3 \times td1} = \frac{1}{6 \times aq1}}$$

Better quality data (reduced artifacts, superior sequence, more ns) will allow less-intense crosspeaks to be observed (e.g., those ≥ 10%), increasing the factor of 6 to perhaps 15). Smaller J's can then be observed for the same **td1** and **sw1**.

Summary of Evolution for 2D NMR Experiments

For 1H COSY:

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

Increasing **td1** decreases J_{obs}.

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

For heteronuclear 1-bond cosy, HSQC:

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

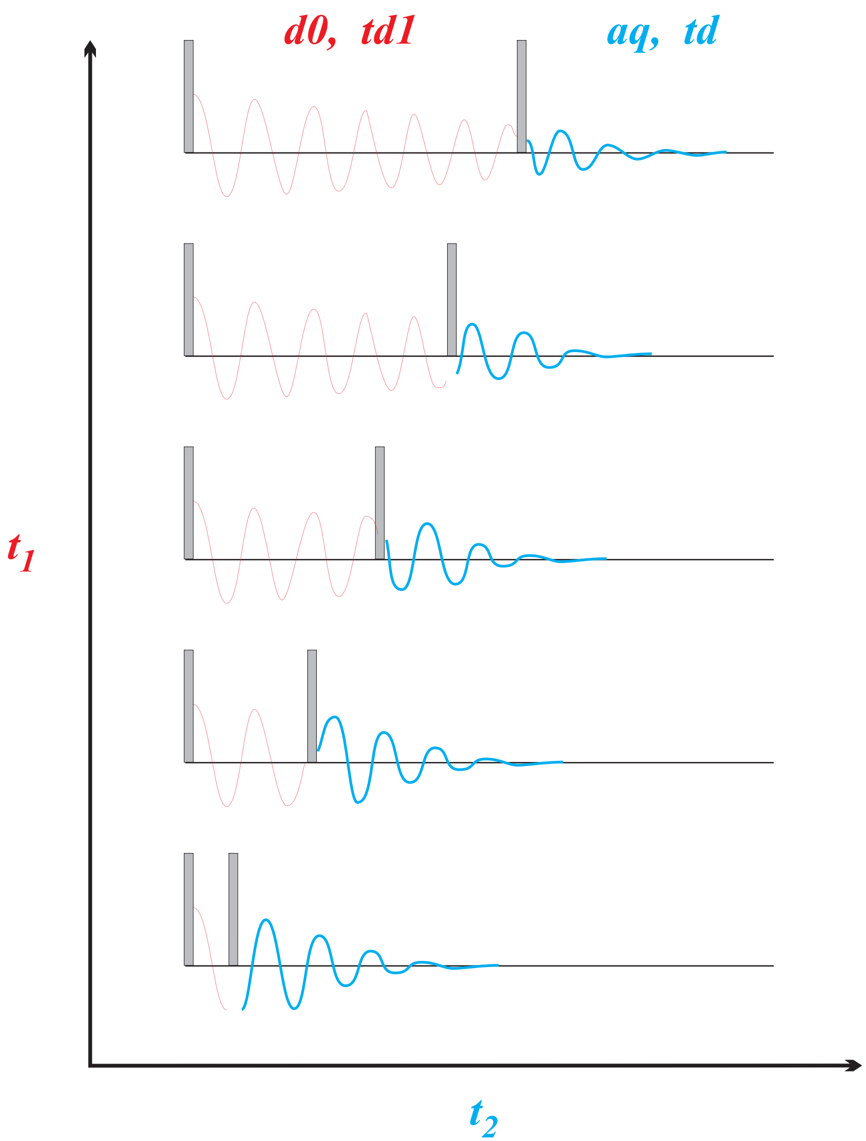
td1 is set as a compromise between experiment time and signal-to-noise.

For heteronuclear n-bond cosy, gHMBC:

Delays become long, so experiment is modified from HSQC.

Still fixed delays at approx $^{1}/_{2}\,{}^{n}J_{CH}$, so **td1** is a compromise between exp. time and s/n.

S/N, *ns* and *td1*: Time of 2D Experiments



1D: $S/N \propto \sqrt{ns}$

2D: $S/N \propto \sqrt{(td1 \cdot ns)}$

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

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

Time 2D: $\approx \left(aq + d1 + \frac{td1}{2 \times sw1}\right) \cdot td1 \cdot ns$

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.