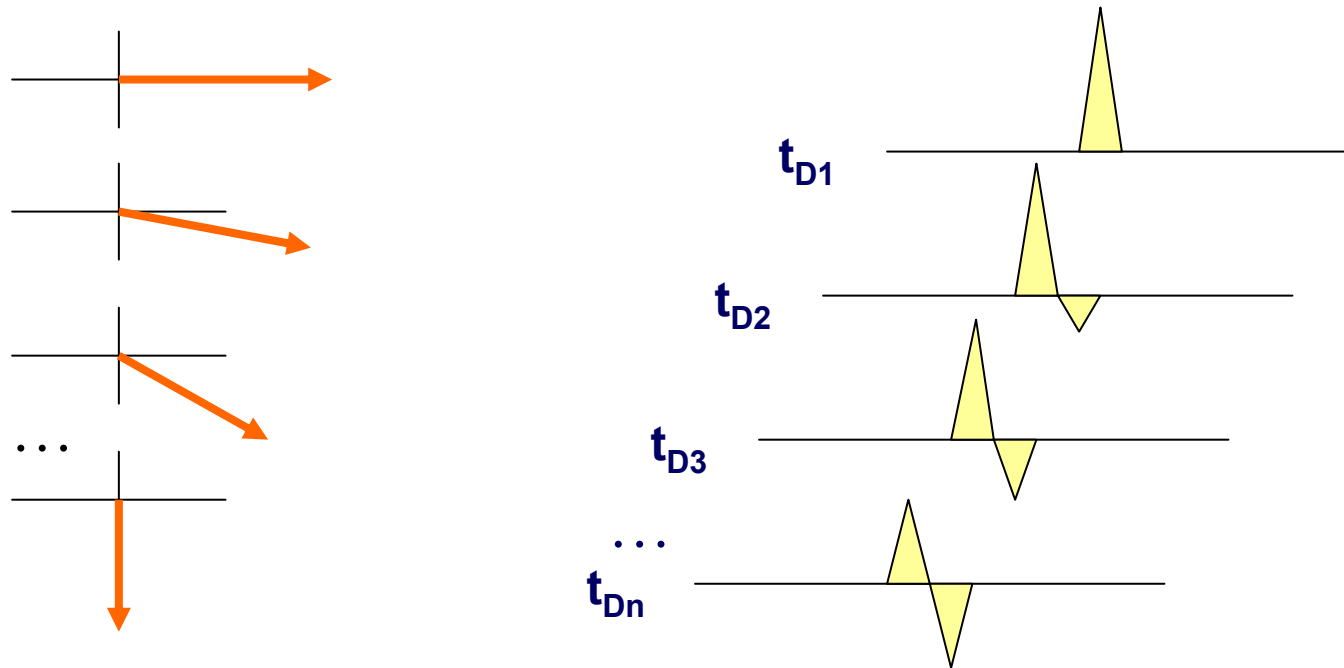


2D NMR spectroscopy

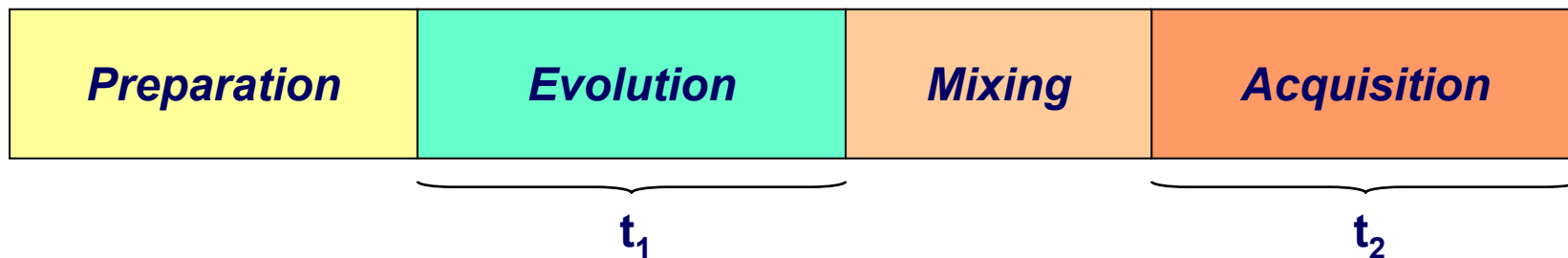
- The 'basic' 2D spectrum would involve repeating a multiple pulse 1D sequence with a systematic variation of the delay time t_D , and then plotting everything stacked. A very simple example would be varying the time before acquisition:



- We now have **two time domains**, one that appears during the acquisition as usual, and one that originates from the variable delay.

2D NMR basics

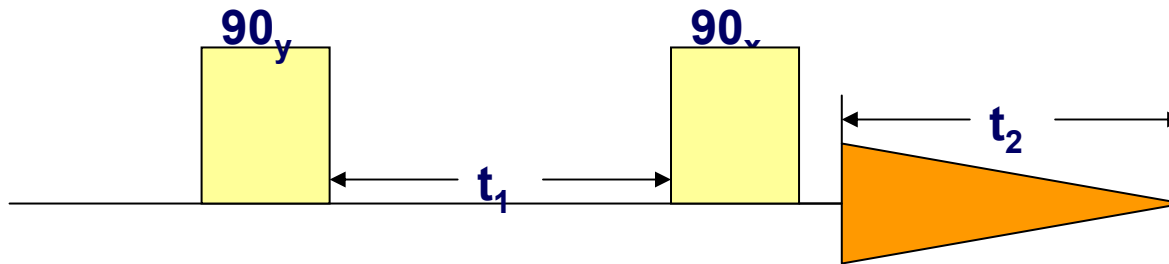
- The first perturbation of the system (pulse) will now be called the **preparation** of the spin system.
 - The variable t_D is renamed the **evolution time**, t_1 .
 - We have a **mixing** event, in which information from one part of the spin system is relayed to other parts.
 - Finally, we have an **acquisition period** (t_2) as with all 1D experiments.
- Schematically, we can draw it like this:



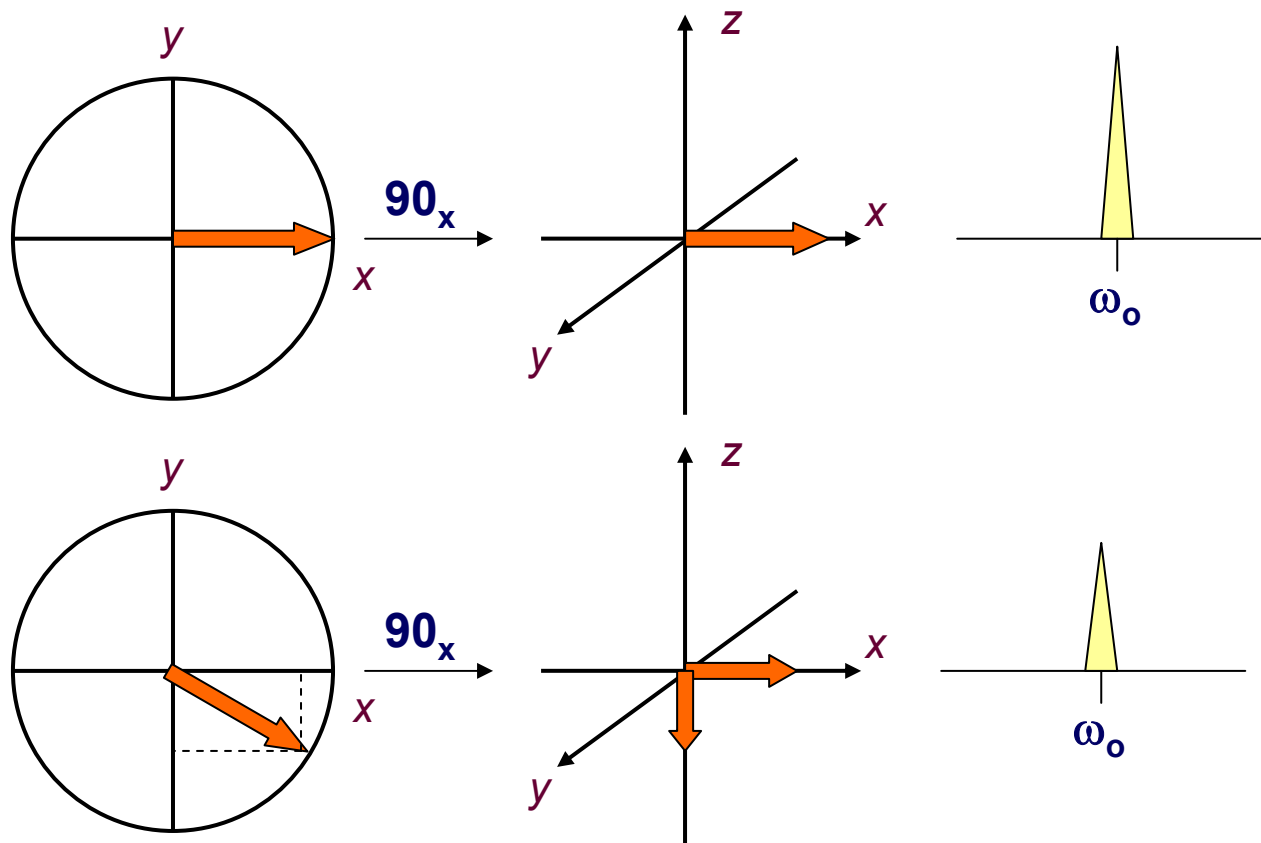
- t_1 is the variable delay time, and t_2 is the normal acquisition time. We can envision having f_1 and f_2 , for both frequencies...
- We'll see that this format is basically the same for all 2D pulse sequences and experiments.

A rudimentary 2D experiment

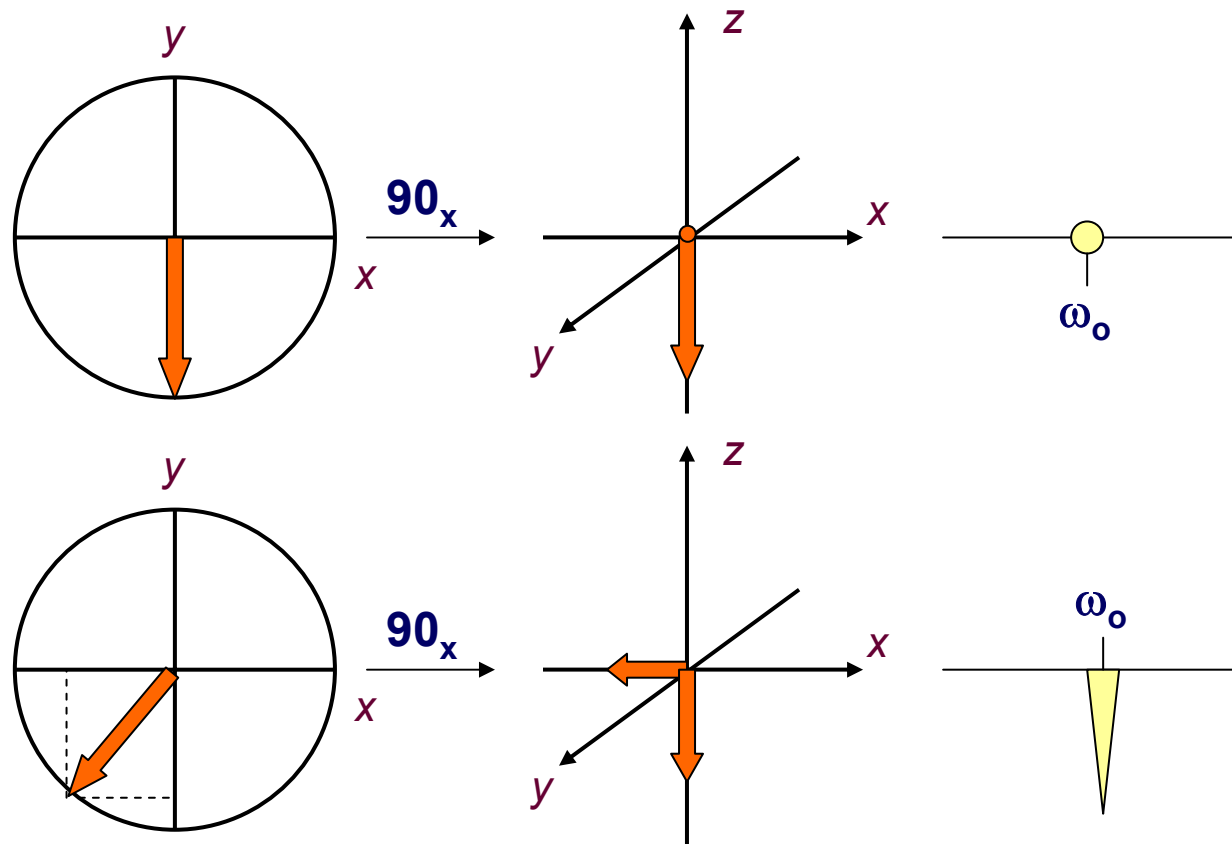
- We'll see how it works with the backbone of what will become the **COSY** pulse sequence. Think of these pulses, where t_1 is the preparation time:



- We'll analyze it for an off-resonance (ω_o) singlet for a bunch of different t_1 values. Starting after the first $\pi / 2$ pulse:



The rudimentary 2D (continued)

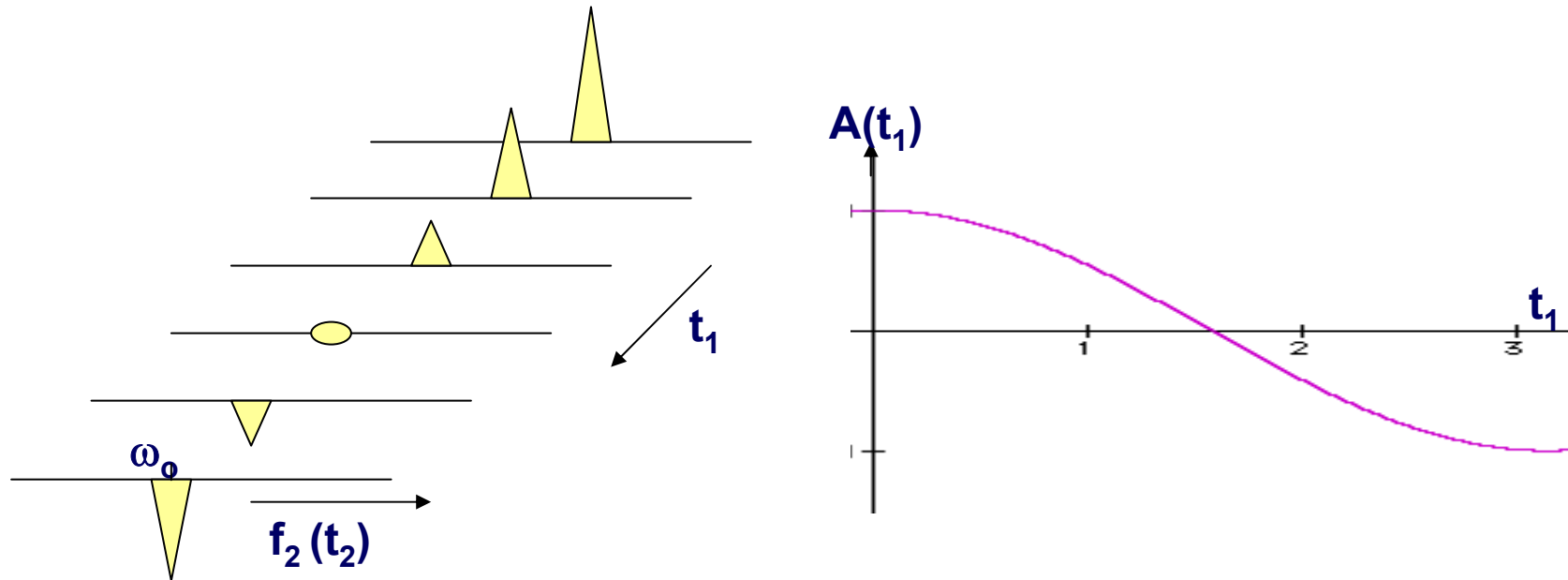


- The second $\pi / 2$ pulse acts only on the y axis component of the magnetization of the $\langle xy \rangle$ plane.
- The x axis component is not affected, but its amplitude will depend on the frequency of the line.

$$A(t_1) = A_0 * \cos(\omega_0 * t_1)$$

The rudimentary 2D (...)

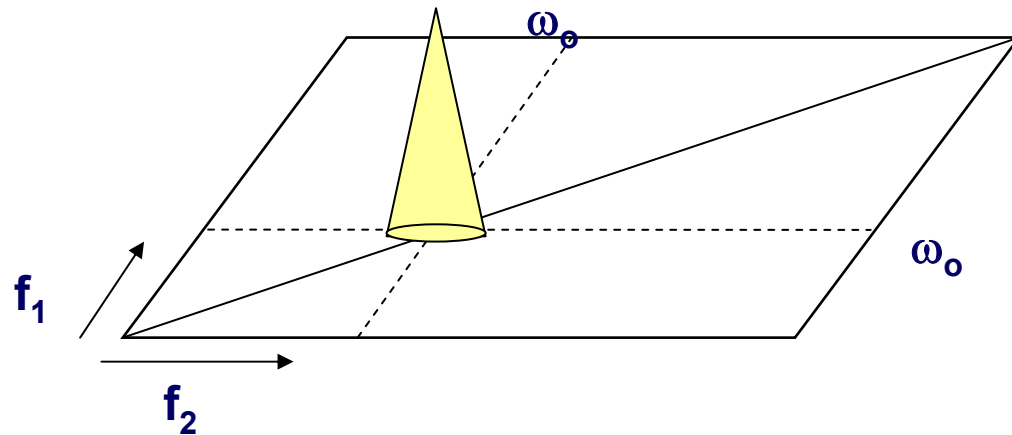
- If we plot all the spectra in a **stacked plot**, we get:



- 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 .
- One thing that we are overlooking here is that during all the pulsing and waiting and pulsing, the signal will also be affected by T_1 and T_2 relaxation.

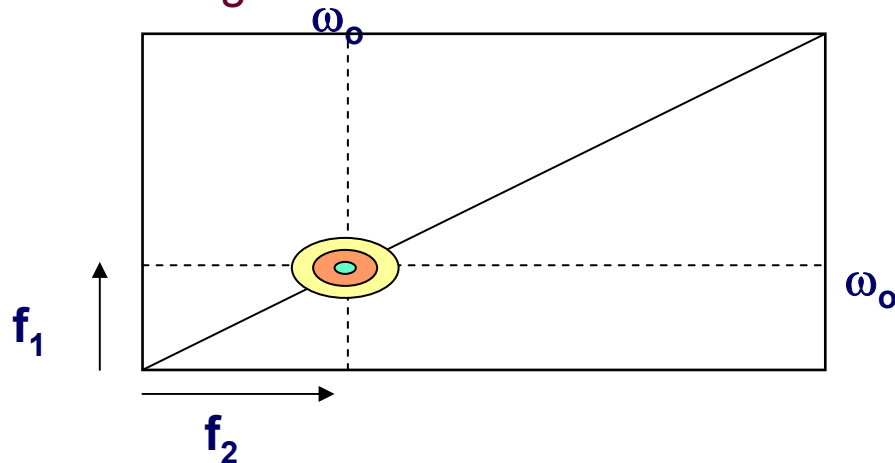
The rudimentary 2D (...)

- Now we have FIDs in t_1 , so we can do a **second Fourier transformation** in the t_1 domain (the first one was in the t_2 domain), and obtain a **two-dimensional spectrum**:



- We have a **cross-peak** where the two lines intercept in the 2D map, in this case on the **diagonal**.

- If we had a real spectrum with a lot of signals it would be a royal mess. We look it from above, and draw it as a **contour plot** - we chop all the peaks with planes at different heights.

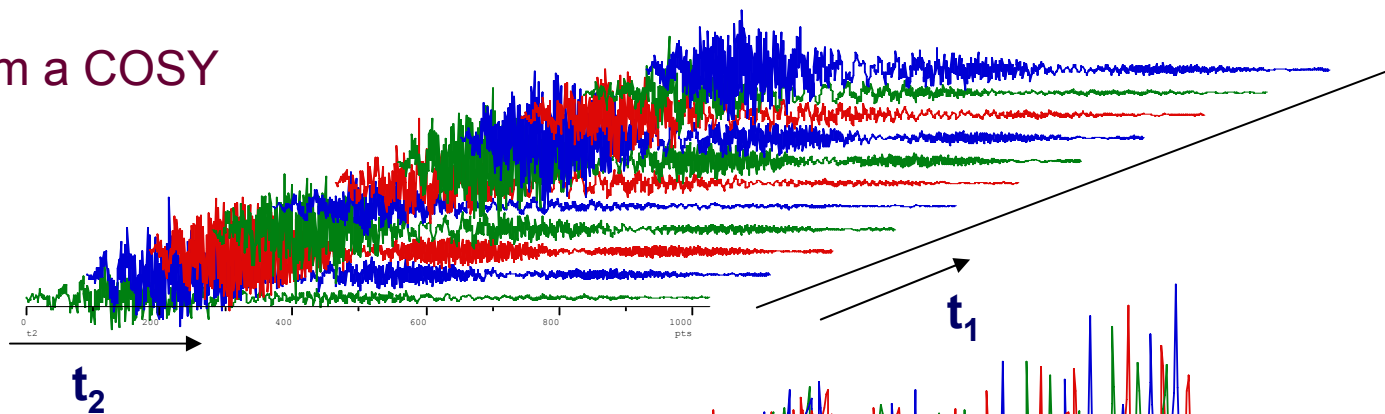


- Each slice is color-coded depending on the height of the peak.

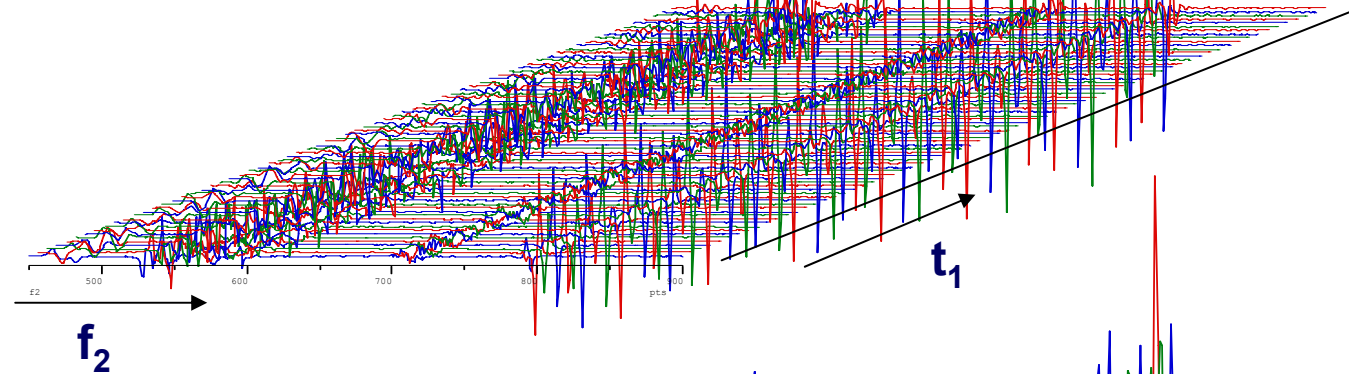
The same with some real data

- This is data from a COSY

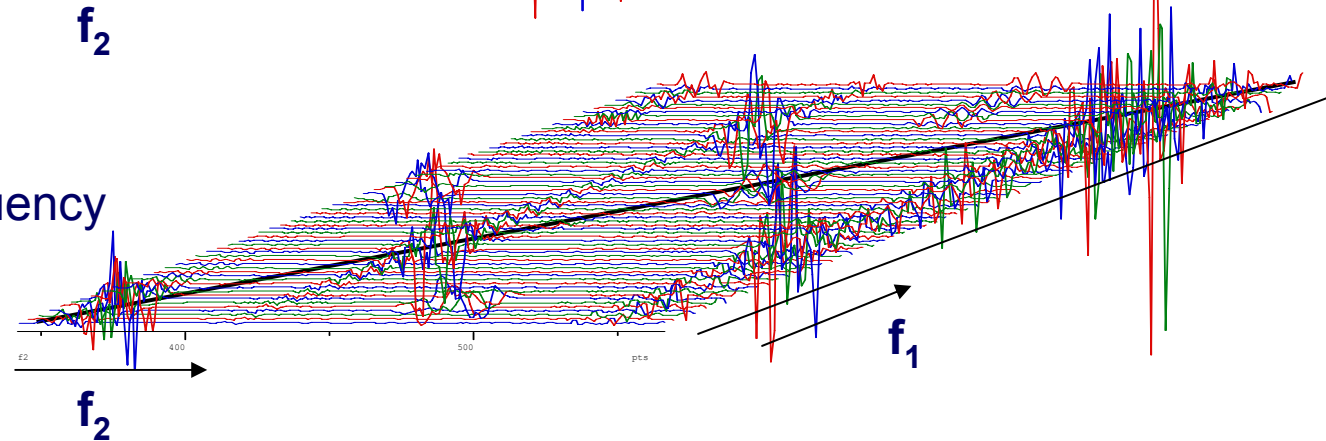
time - time



time - frequency

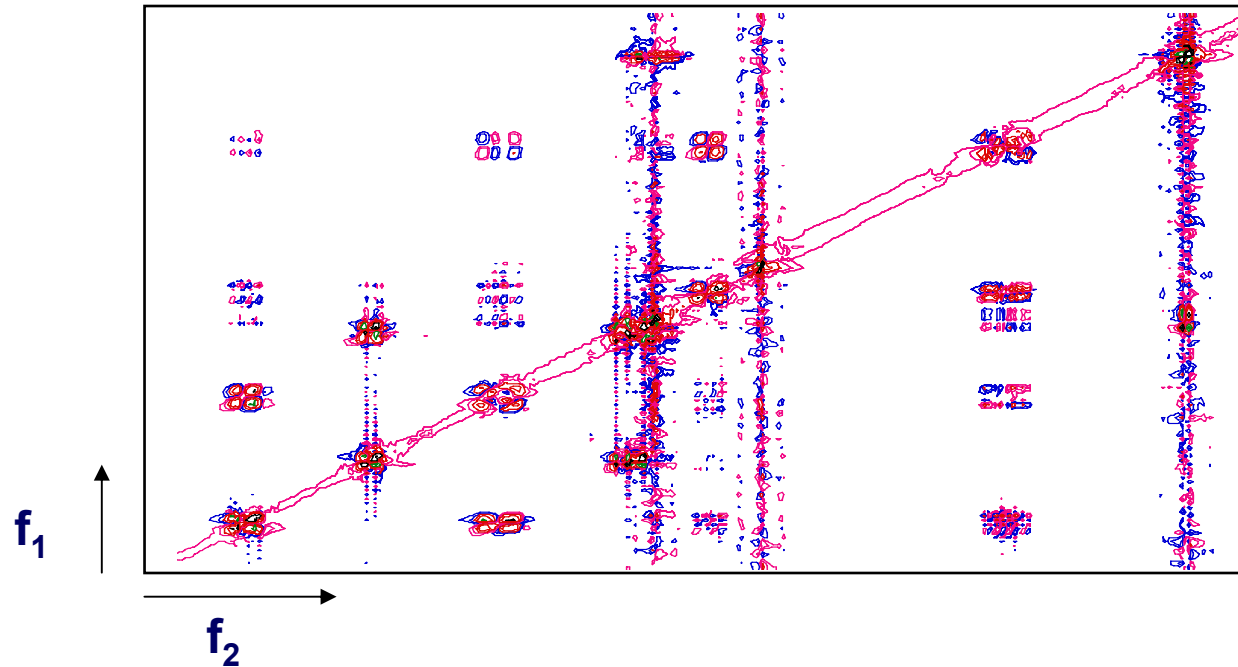


frequency - frequency



The same with some real data

- Now the *contour-plot* showing all the *cross-peaks*:



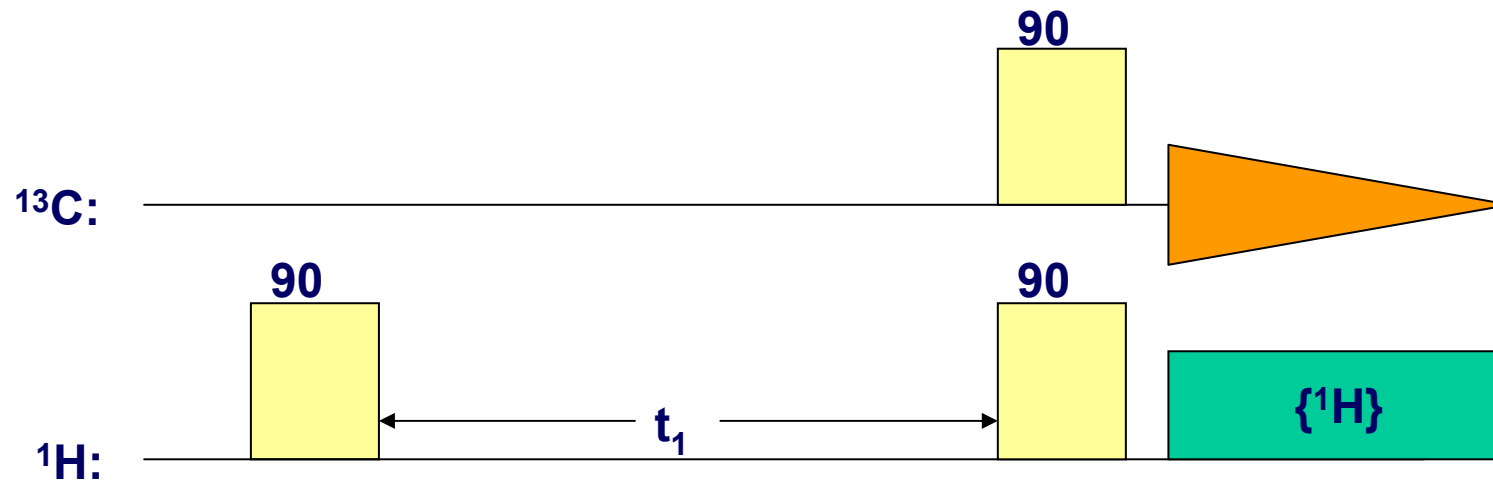
- OK, where did all the *off-diagonal* peaks come from, and what do they mean?

COSY stands for **CO**rrelation **S**pectroscopy, and for this particular case in which we are dealing with homonuclear couplings, **homonuclear correlation spectroscopy**.

In our development of the 2D idea we considered an isolated spin not coupled to any other spin. Obviously, this is not really useful. What COSY is good for is to tell which spin is connected to which other spin. The off-diagonal peaks are this, and they indicate that those two peaks in the diagonal are coupled.

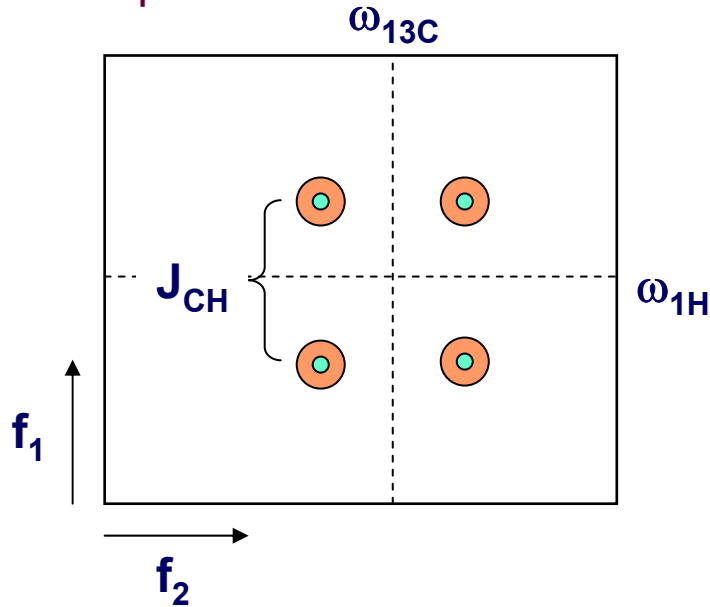
Heteronuclear correlation - HETCOR

- In a similar fashion we can perform a 2D experiment in which we analyze heteronuclear connectivity, that is, which ^1H is connected to which ^{13}C . This is called **HETCOR**, for **HET**ero-nuclear **COR**relation spectroscopy.
- The pulse sequence in this case involves both ^{13}C and ^1H , because we have to somehow label the intensities of the ^{13}C with what we do to the populations of ^1H . The basic sequence is as follows:



HETCOR (...)

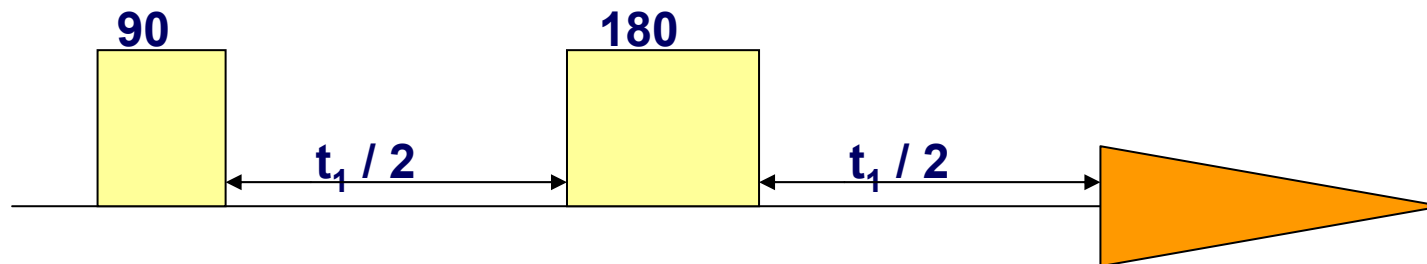
- Again, Fourier transformation on both time domains gives us the 2D correlation spectrum, in this case as a contour plot:



- The main difference in this case is that the 2D spectrum is not symmetrical, because one axis has ^{13}C frequencies and the other ^1H frequencies.
- Now, we still have the J_{CH} coupling splitting all the signals of the 2D spectrum in little squares. The J_{CH} are in the 50 - 250 Hz range, so we can start having overlap of cross-peaks from different **CH** spin systems.

Homonuclear 2D J spectroscopy - HOMO2DJ

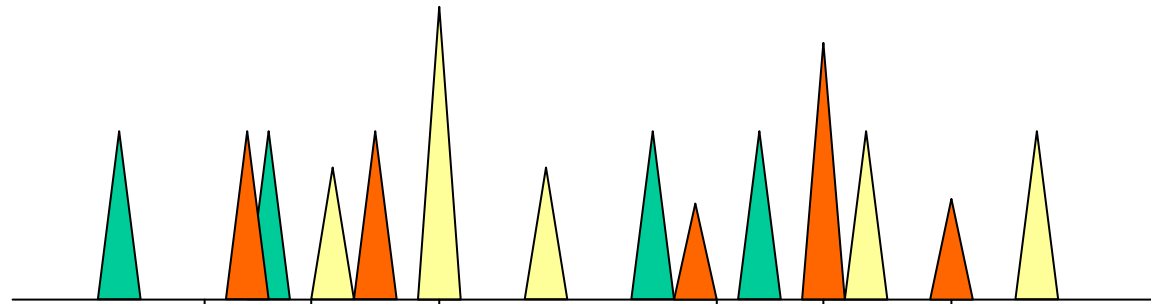
- The 2D experiments we've analyzed so far are used to find out correlations or connections between spin systems. There are many other things that we can extract from 2D experiments in which we take advantage of the spreading-out of signals.
- One of the most annoying things is to have a cool sample full of peaks with nice multiplicity patterns which is all overlapped. We can exploit the higher dimensionality to dodge this.
- This is what **HOMO2DJ** can be used for. The idea behind it is to put δ information in one axis and **J** information in the other.
- The pulse sequence is a variation of the spin-echo sequence in which the delays are varied between each experiment:



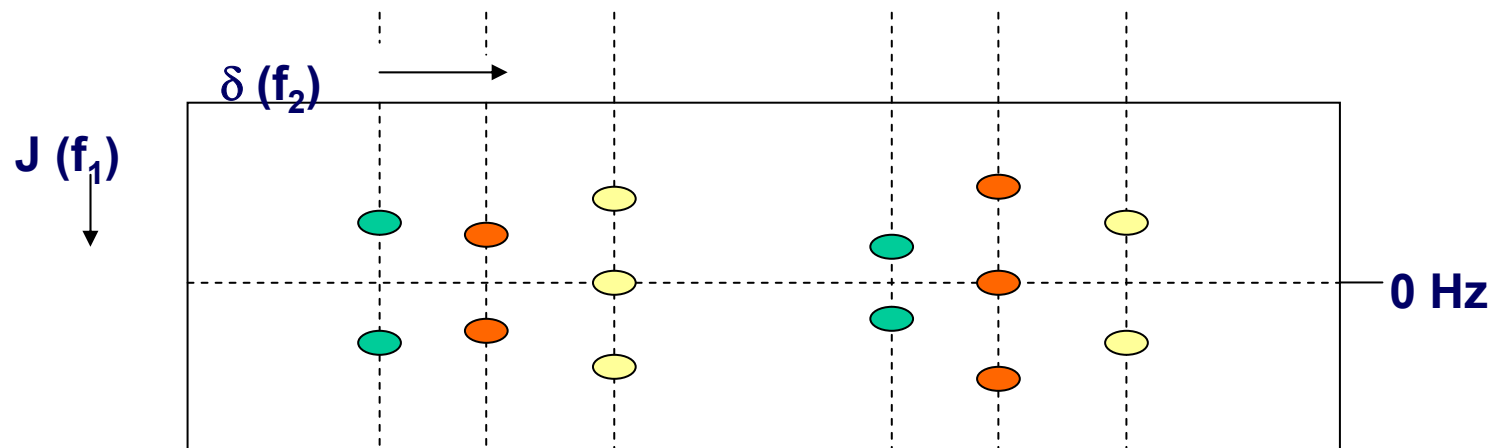
- We'll analyze it for a triplet and a doublet.

HOMO2DJ - Many signals

- For a really complicated pattern we see the advantage. For a ^1H -1D that looks like this:



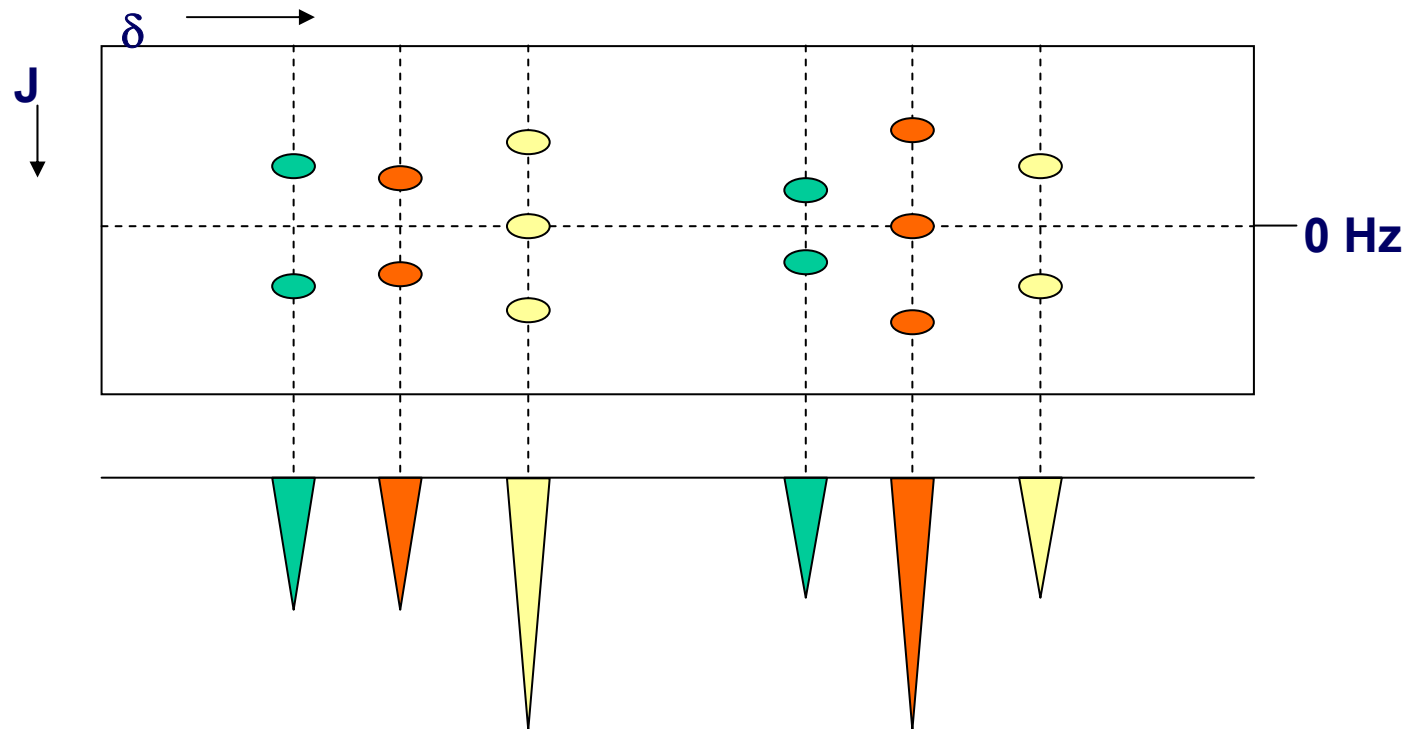
- We get an **HOMO2DJ** that has everything resolved in δ s and **J**s:



- We have all the δ information on the f_2 axis and the **J** data on the f_1 axis.

HOMO2DJ - Conclusion

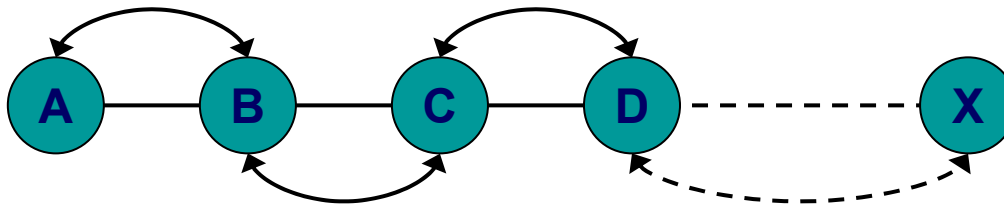
- Another advantage is that if we project the 2D spectrum on its δ axis, we basically get a fully decoupled ^1H spectrum:



- Finally, since we take ~ 256 or 512 t_1 experiments, we have that many points defining the J couplings which are between 1 and 20 Hz.
- For 50 Hz and 512 t_1 experiments, 0.09 Hz / point. We can measure J_{HH} with great accuracy on the f_1 dimension.

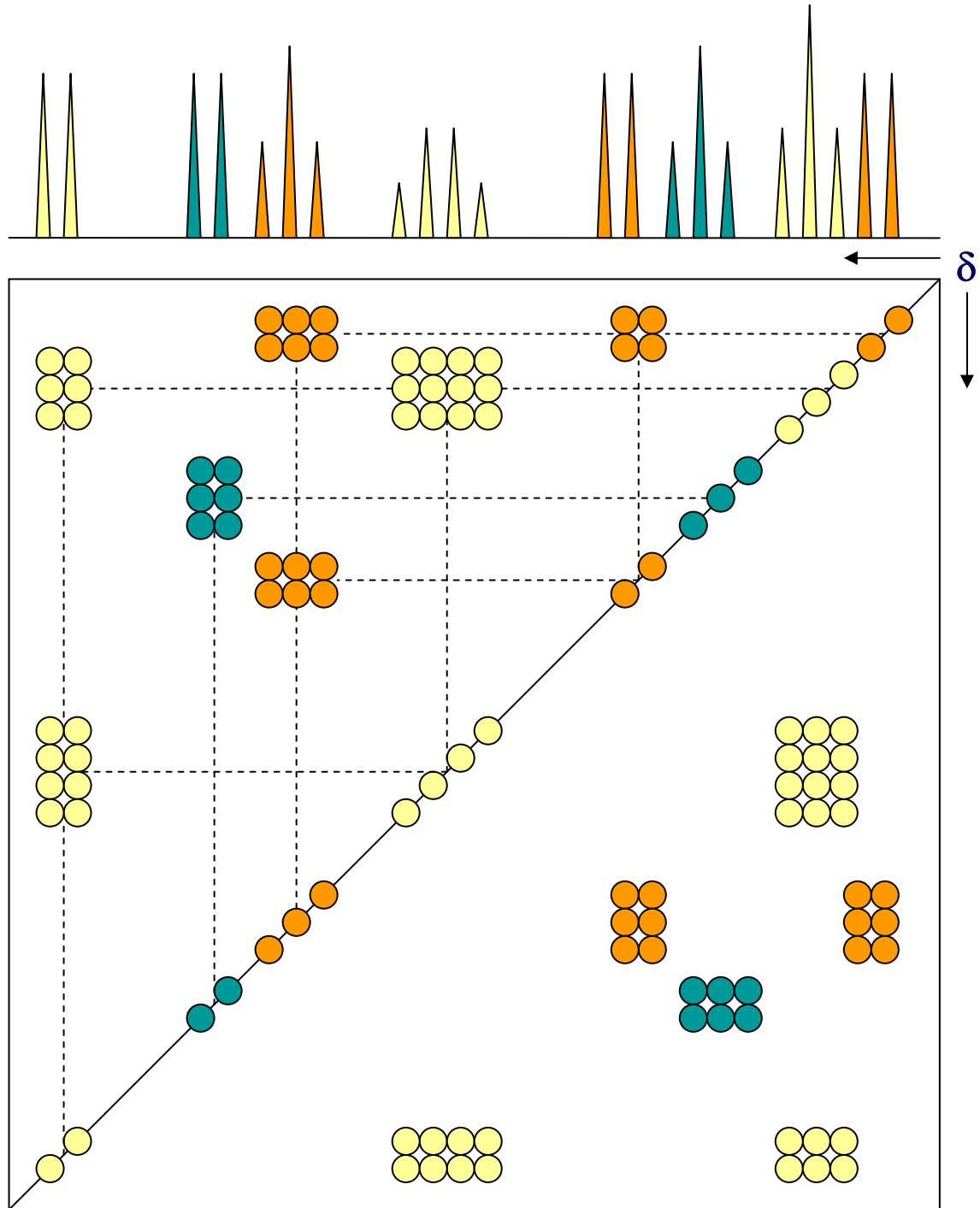
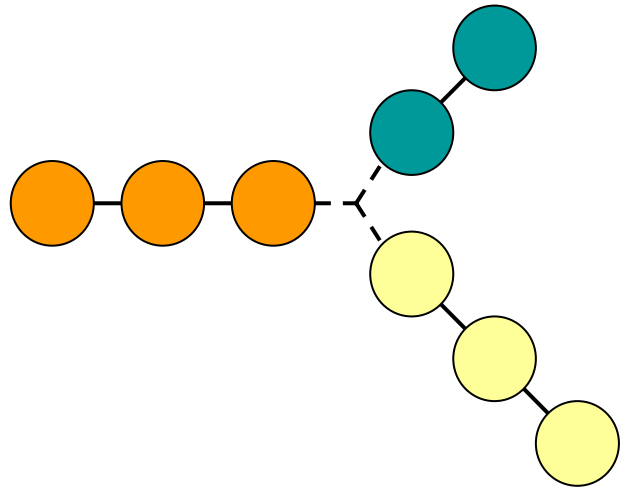
TOCSY

- **HOHAHA** (*HOmo-nuclear HArtmann-HAhn experiment*) or **TOCSY** (*Total Correlation Spectroscopy*).
- Its purpose is to identify a complete system of coupled spins. To make a very long story short, we have thorough mixing of all states in the system, and coherence from a certain spin in a coupled system will be transferred to all other spins in it. In other words, this spin **correlates** to all others in the system:



TOCSY (...)

- In the 2D plot we get all spins from a particular spin system in the same line.

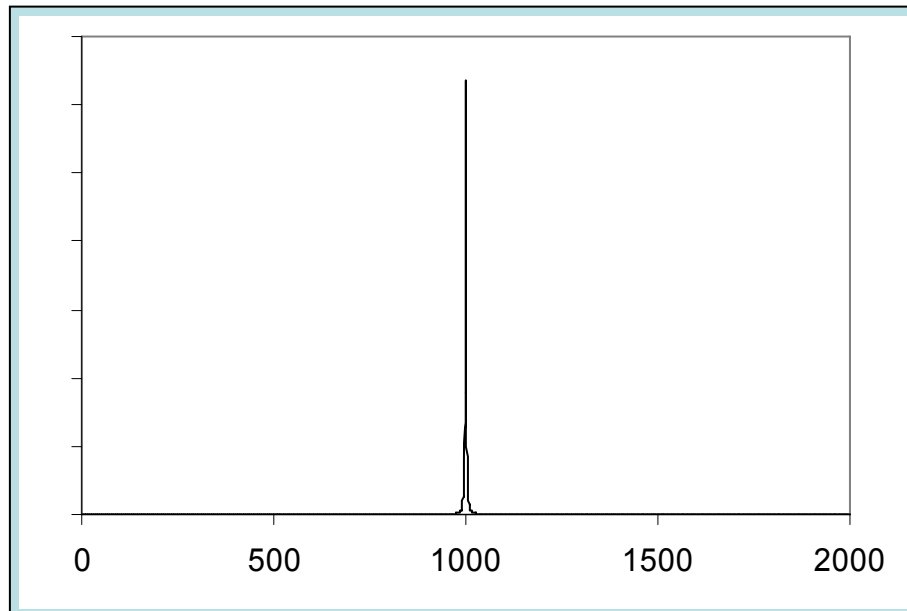


Solvent Signal Suppression

Calculation: 90% H₂O, 1 mM solute

$\nu_{\text{H}_2\text{O}}$: 1000 Hz, $\Gamma_{\text{H}_2\text{O}}$: 2.5 Hz

ν_{solute} : 800 Hz, Γ_{solute} : 5.0 Hz



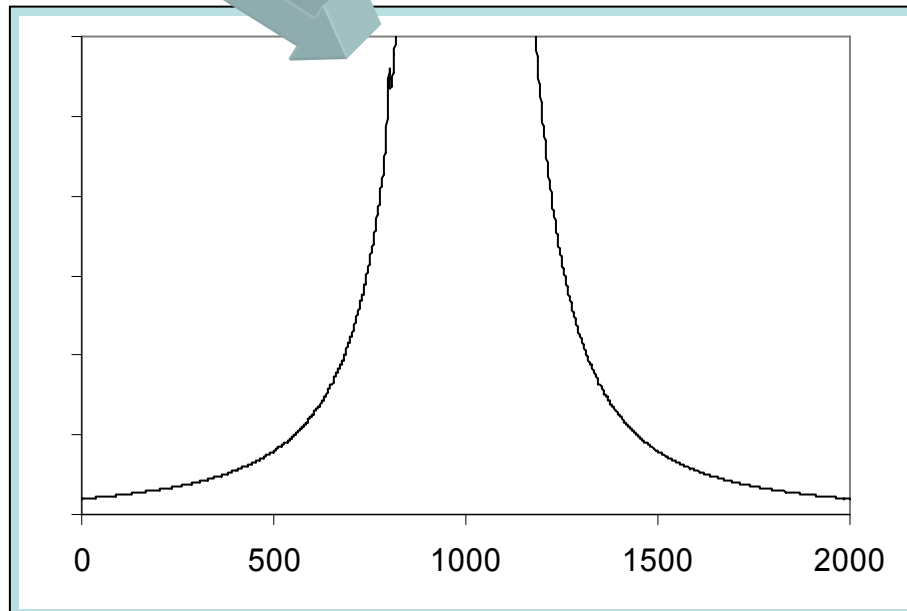
Att.: 0

Solvent Signal Suppression

Calculation: 90% H₂O, 1 mM solute

$\nu_{\text{H}_2\text{O}}$: 1000 Hz, $\Gamma_{\text{H}_2\text{O}}$: 2.5 Hz

ν_{solute} : 800 Hz, Γ_{solute} : 5.0 Hz



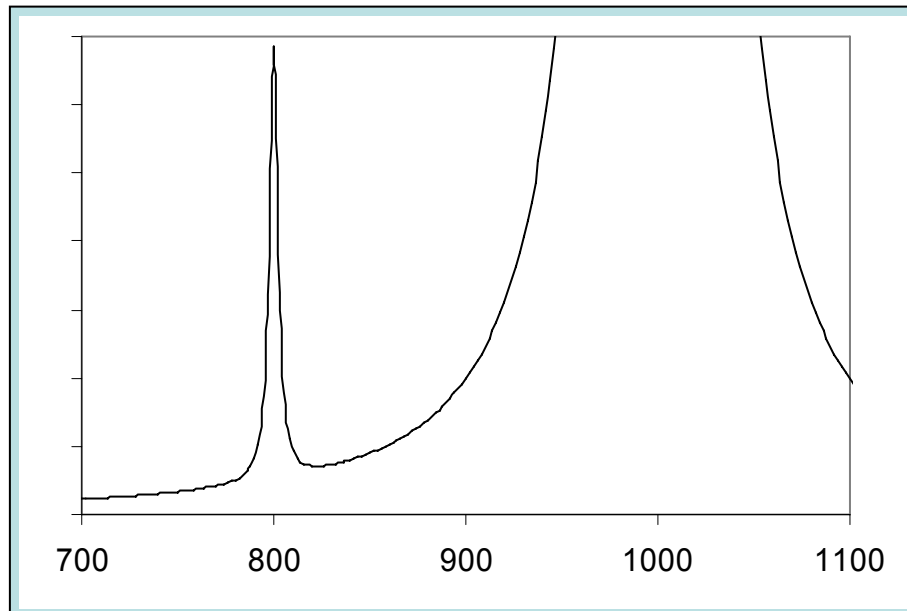
Att.: 0

Solvent Signal Suppression

Calculation: 90% H₂O, 1 mM solute

$v_{\text{H}_2\text{O}}$: 1000 Hz, $\Gamma_{\text{H}_2\text{O}}$: 2.5 Hz

v_{solute} : 800 Hz, Γ_{solute} : 5.0 Hz



Att.: 10²

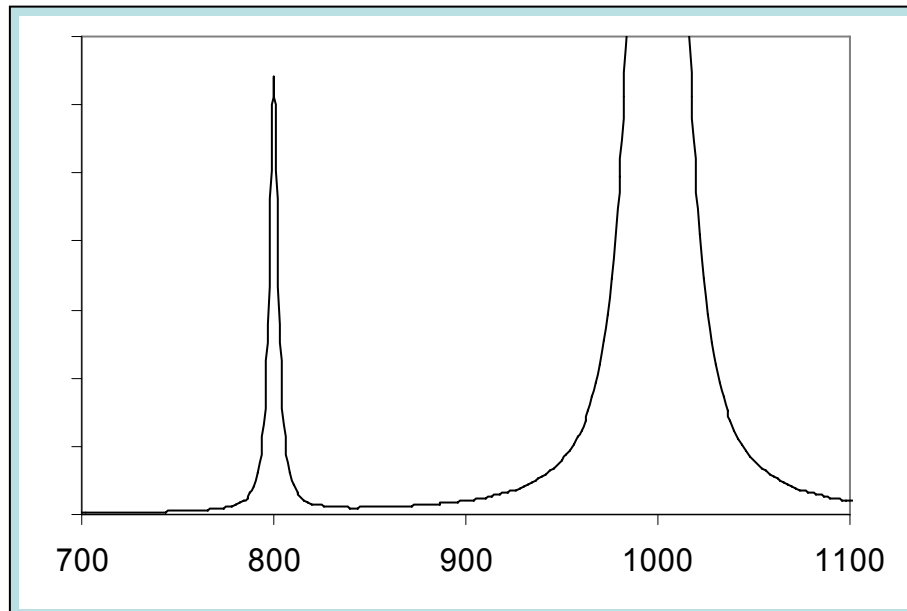
(99% D₂O)

Solvent Signal Suppression

Calculation: 90% H₂O, 1 mM solute

$\nu_{\text{H}_2\text{O}}$: 1000 Hz, $\Gamma_{\text{H}_2\text{O}}$: 2.5 Hz

ν_{solute} : 800 Hz, Γ_{solute} : 5.0 Hz



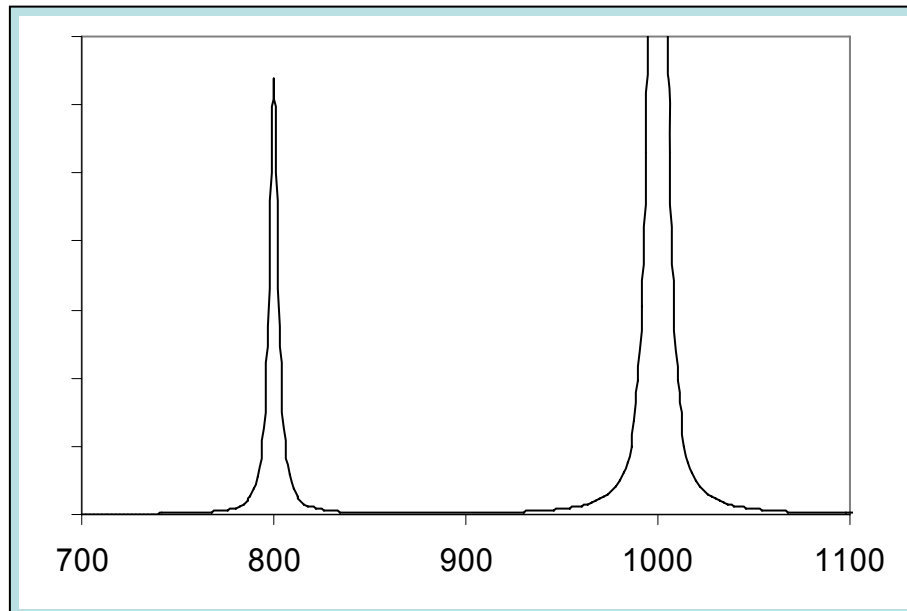
Att.: 10³

Solvent Signal Suppression

Calculation: 90% H₂O, 1 mM solute

$\nu_{\text{H}_2\text{O}}$: 1000 Hz, $\Gamma_{\text{H}_2\text{O}}$: 2.5 Hz

ν_{solute} : 800 Hz, Γ_{solute} : 5.0 Hz



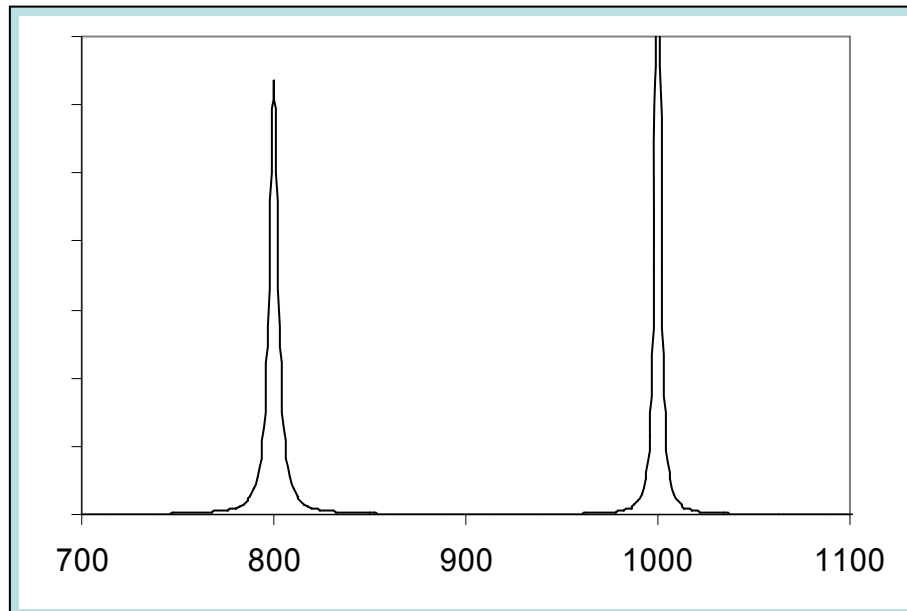
Att.: 10⁴

Solvent Signal Suppression

Calculation: 90% H₂O, 1 mM solute

$\nu_{\text{H}_2\text{O}}$: 1000 Hz, $\Gamma_{\text{H}_2\text{O}}$: 2.5 Hz

ν_{solute} : 800 Hz, Γ_{solute} : 5.0 Hz



Att.: 10⁵

Solvent Signal Suppression

Ideal Solvent Signal Suppression:

- *takes no time*
- *affects only solvent resonance and not solute resonances*
- *does not interfere with the pulse sequence*
- *simple to set up*

Solvent Signal Suppression

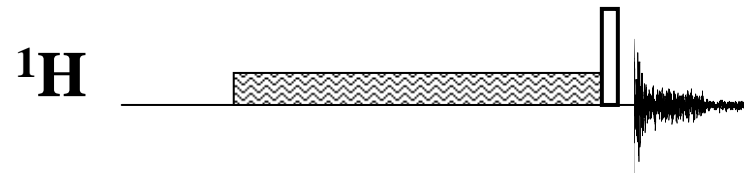
- 1) *Saturation Based Methods*
 - a) *Discrimination by Frequency*
 - b) *Discrimination by Relaxation Times*
- 2) *Methods Avoiding Solvent Saturation*
- 3) *Magnetization Destruction-Based Methods*
- 4) *Coherence Selection*
- 5) *Postacquisitional Methods*

Price, *Annual Reports on NMR Spectroscopy*,
Academic Press, 1999, 38, 289

1) Saturation Based Methods

→ *discrimination by chemical shift*

- *presaturation*



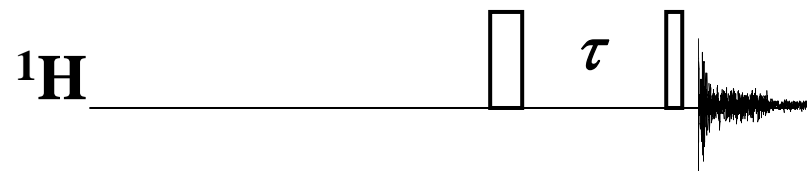
- ☺ flexible & easy to set up
- ☹ bleaching out of resonances close to the solvent
- ☹ attenuation/suppression of resonances due to chemical exchange

1) Saturation Based Methods

→ *Discrimination by Relaxation Times*

WEFT (180- τ -90)

$$\tau = \ln(2) T_1^{H_2O}$$



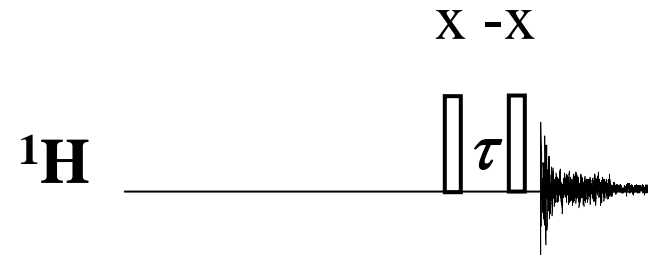
Benz *et al.*, J.Mag.Reson., 1972, 8, 114

- ☺ **no presaturation**
- ☺ **fast recycle times are possible**
- ☹ **weak suppression capacity**

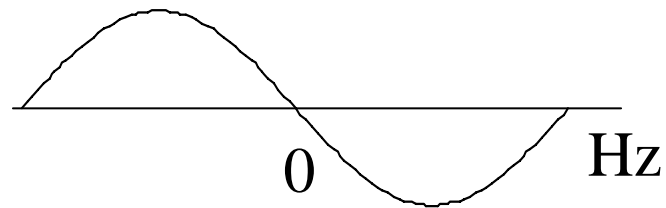
2) Methods avoiding Solvent Saturation

→ *Binomial Lecture Pulse*

Jump-Return (1₁, 90- τ -90)



Excitation Profile:

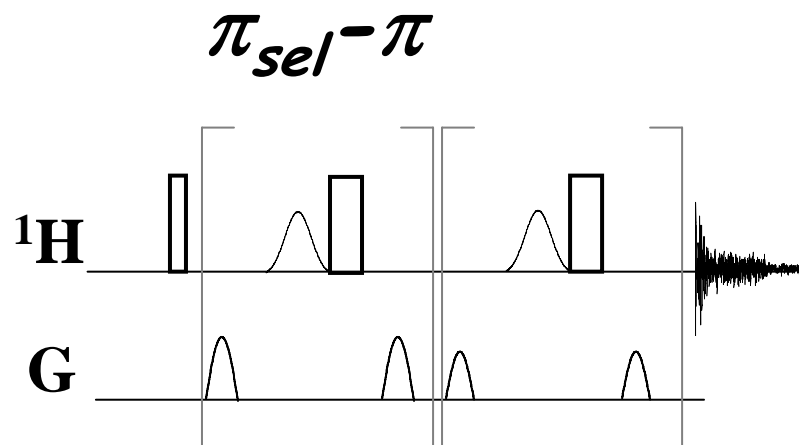


- ☺ exchangeable protons are observable
- ☺ fast
- ☹ uneven excitation

3) Magnetization Destruction-Based Methods

Double Pulsed Field Gradient Spin-Echo: DPFGESE

"EXCITATION SCULPTING"



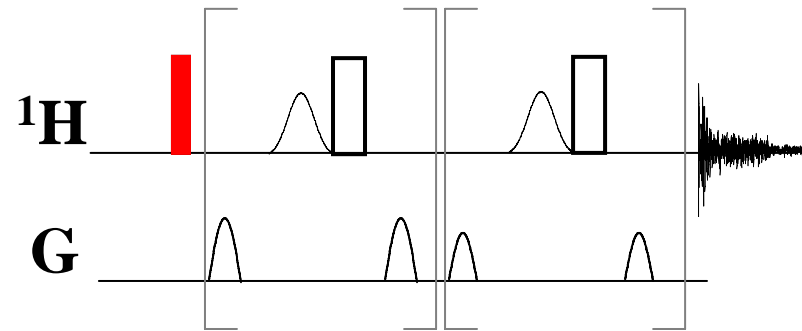
- ☺ multiple solvent suppression possible
- ☺ very efficient ($>10^4$)
- ☺ resonances undergoing exchange are observable
- ☹ delay of 4 - 12 ms after lecture pulse

Shaka *et al.*, J.Magn.Res., 1995, A112, 275

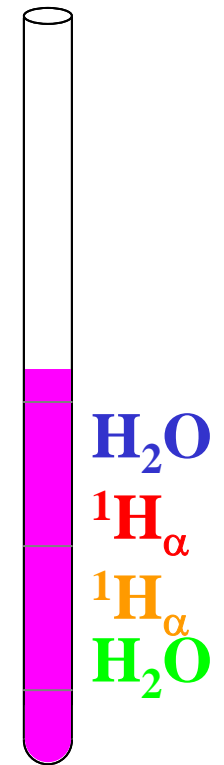
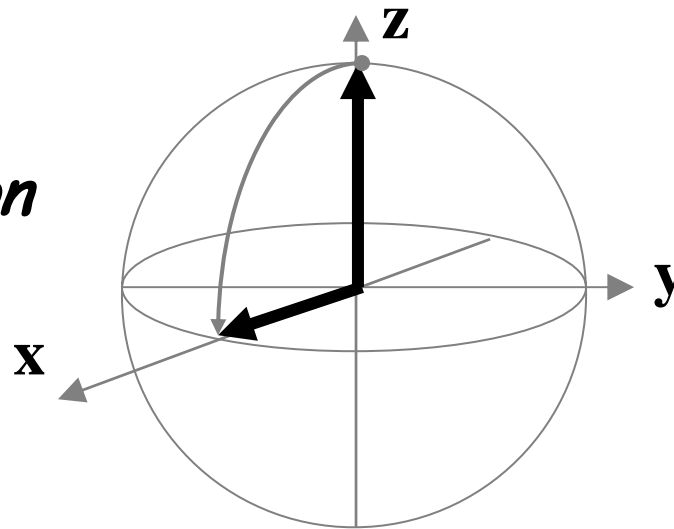
Shaka *et al.*, J.A.C.S., 1995, 117, 4199

3) Magnetization Destruction-Based Methods

"EXCITATION SCULPTING"

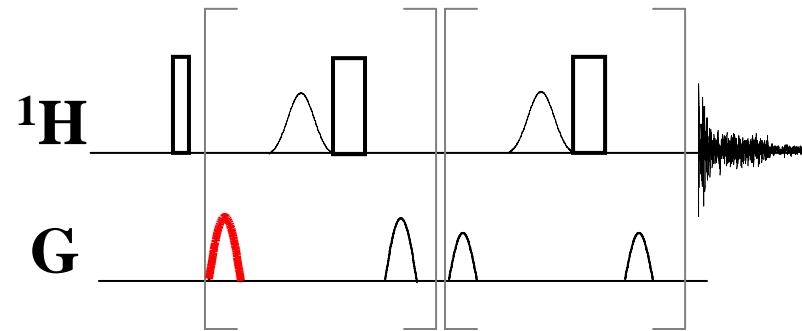


90° excitation

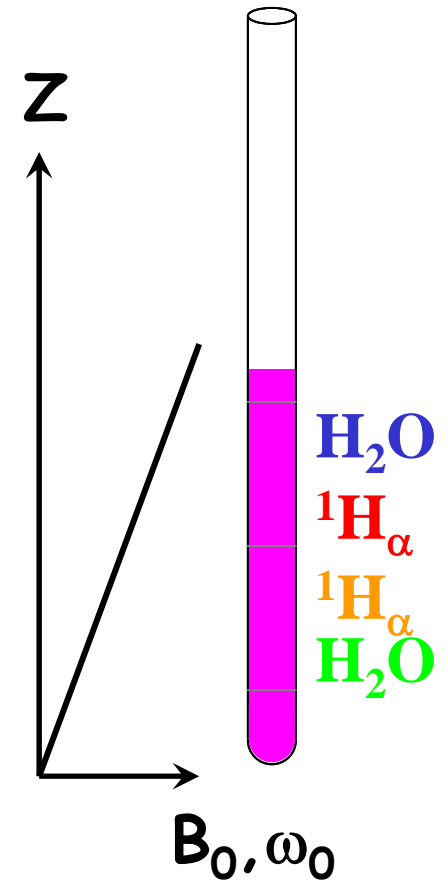
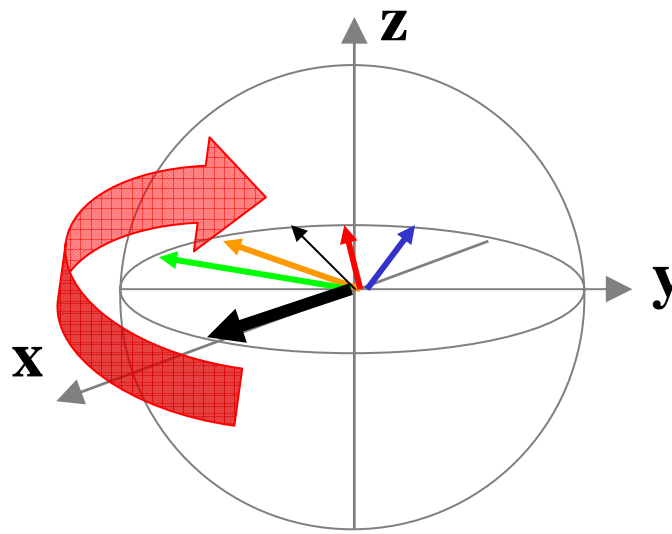


3) Magnetization Destruction-Based Methods

"EXCITATION SCULPTING"

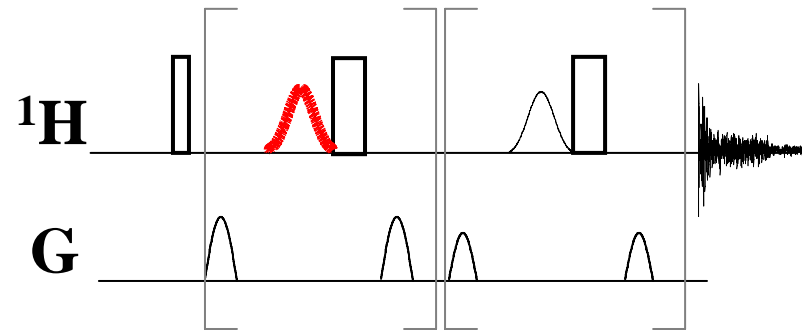


*defocussing
gradient*

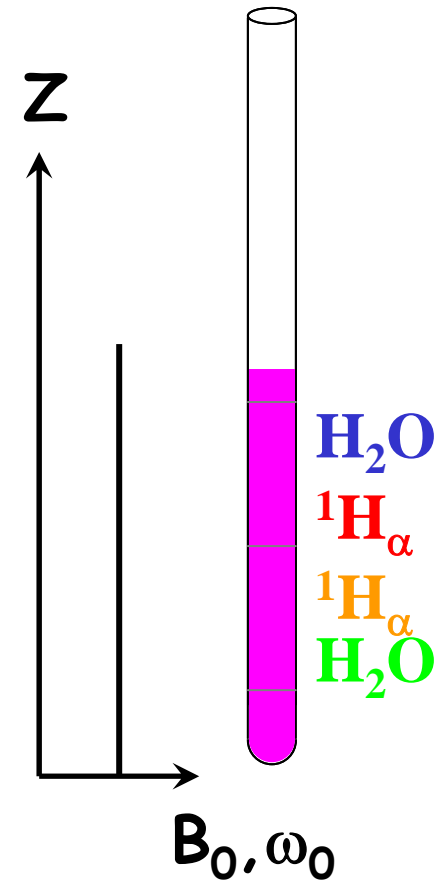
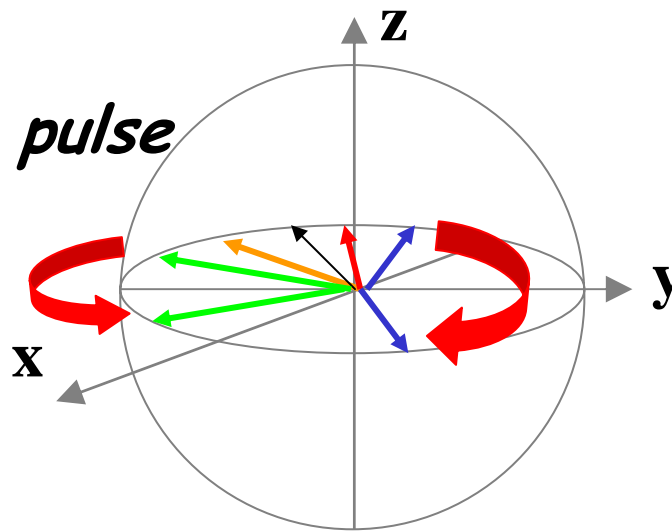


3) Magnetization Destruction-Based Methods

"EXCITATION SCULPTING"

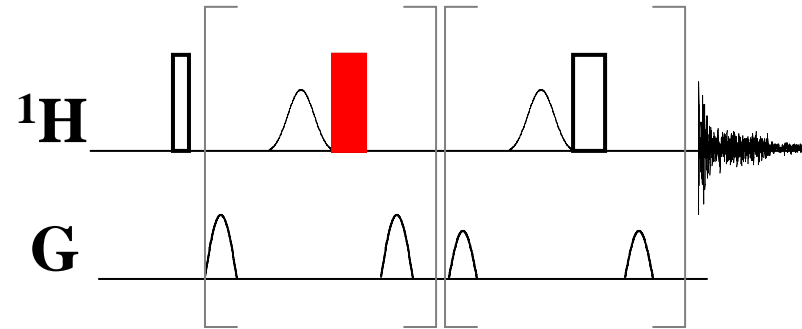


180° selective pulse

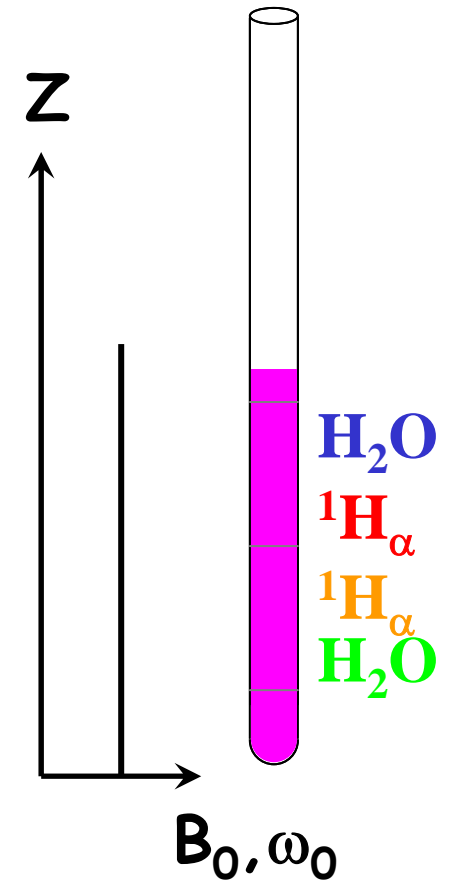
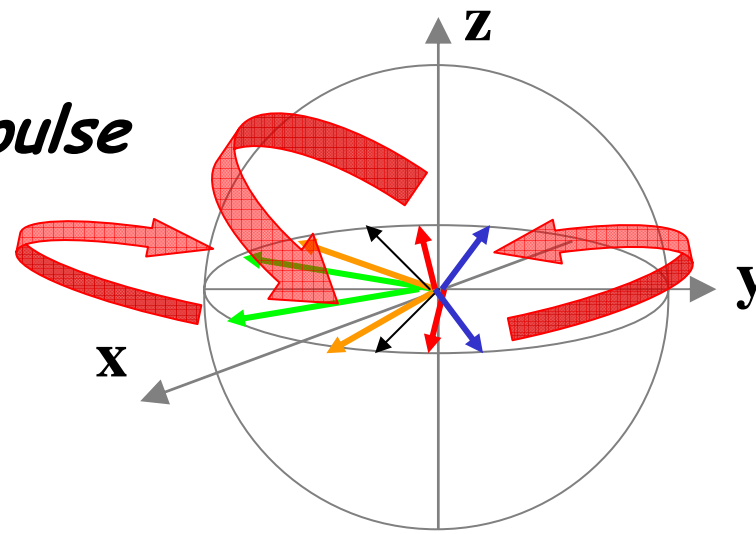


3) Magnetization Destruction-Based Methods

"EXCITATION SCULPTING"

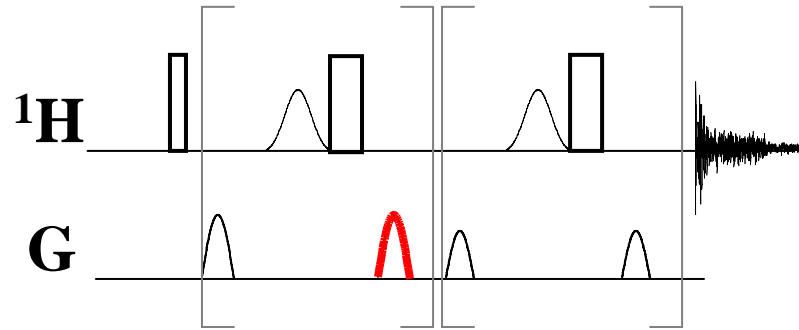


180° hard pulse



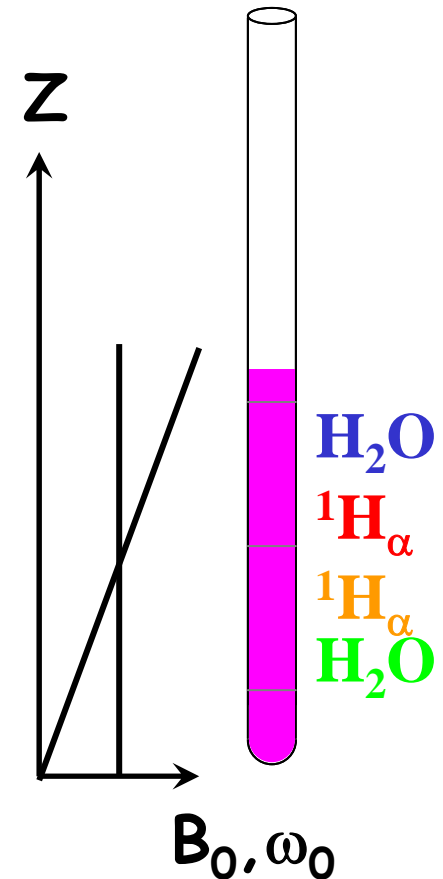
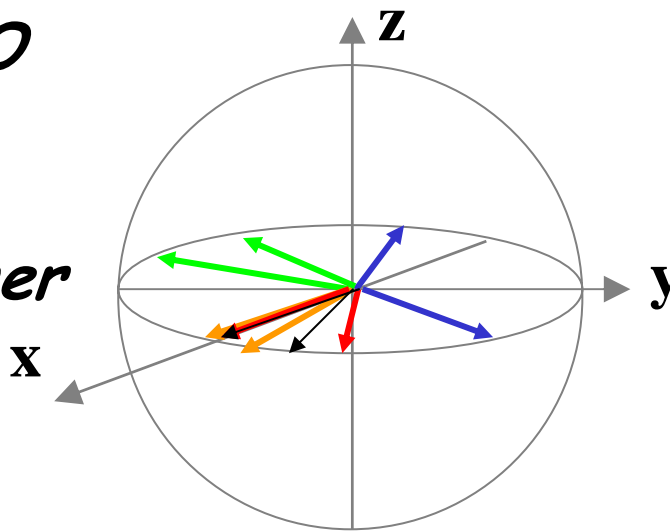
3) Magnetization Destruction-Based Methods

"EXCITATION SCULPTING"



*defocussing
gradient for H_2O*

*refocussing
gradient for other
frequencies*



Water Suppression techniques

2 mM Sucrose 90% H₂O

d1: 1.5s

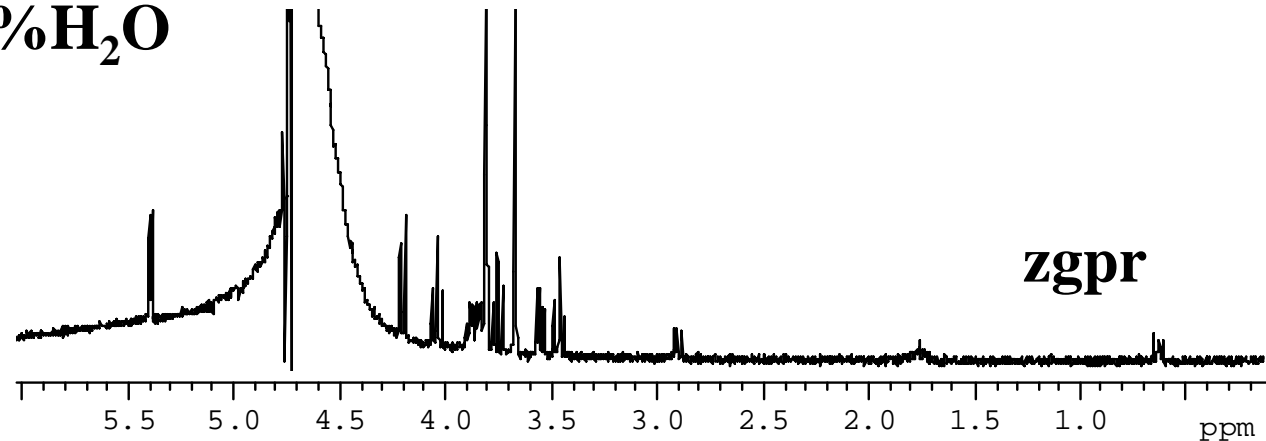
pl9: 58 dB

ds: 8

ns: 8

td: 8k

si: 16k



no window function

no H₂O signal filtering

