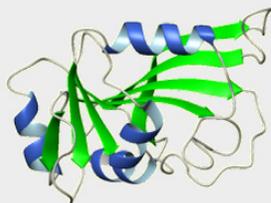
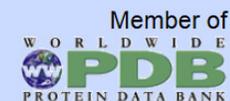


# http://www.bmrwisc.edu/



## Biological Magnetic Resonance Data Bank

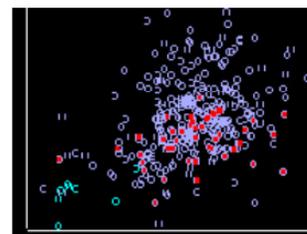
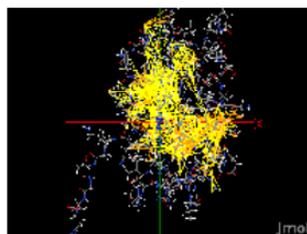
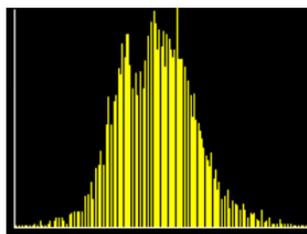
A Repository for Data from NMR Spectroscopy on Proteins, Peptides, Nucleic Acids, and other Biomolecules

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- ▶ Programmers' Corner
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  - Standard Compounds
  - NMR Peaks Query

### NMR Data Visualizations (see more)

Your data ?



Visualization options: [Single-entry](#), [Two-entry](#), [Restrains by PDB ID](#), [Chem shift histograms](#), [Visualization server](#)

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## BMRB Metabolomics search

Search for:

Enter search:

Experimental entries  Theoretical entries

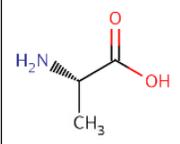
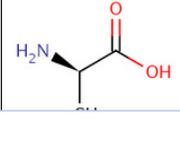
Select the top  matches

or upload batch file:

Search:

### 41 matches for alanine

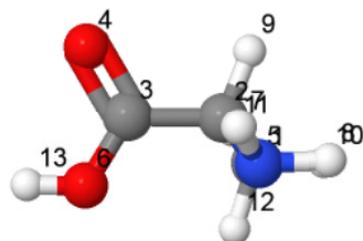
displaying 10

	Name	Image	BMRB ID	Synonym
<input type="button" value="ignore"/>	<u>L-Alanine</u>		<u>bmse000028</u>	Alanine
<input type="button" value="ignore"/>	<u>Alanine</u>		<u>bmse000282</u>	Alanine
<input type="button" value="ignore"/>	<u>L-Alanine</u>		<u>bmse000994</u>	Alanine
<input type="button" value="ignore"/>	<u>D-Alanine</u>		<u>bmse000236</u>	D-Alanine

# L-Alanine (C3 H7 N O2)

  
Synonym [Metabolomics home](#)[USDA standards](#)[All standards](#)[L-Alanine](#) [bmse000028 - data](#) [bmse000994 - data](#) [bmst000272 - theory](#)

Natural Isotopic formula weight: 89.093180000

[View large 3D structure](#)[JSmol](#)

L-Alanine

## bmse000028 Data

Entry STAR file: [bmse000028.str](#)Time Domain Data: [bmse000028.tar](#)

### L-alanine

Source: Sigma a7627

Solvent: 100 % D2O

Buffer: 50 mM sodium phosphate

Cytocide: 500  $\mu$ M sodium azideReference: 500  $\mu$ M DSS

Concentration and pH are given with spectrum

Spectrometer: Bruker DMX - 400 MHz

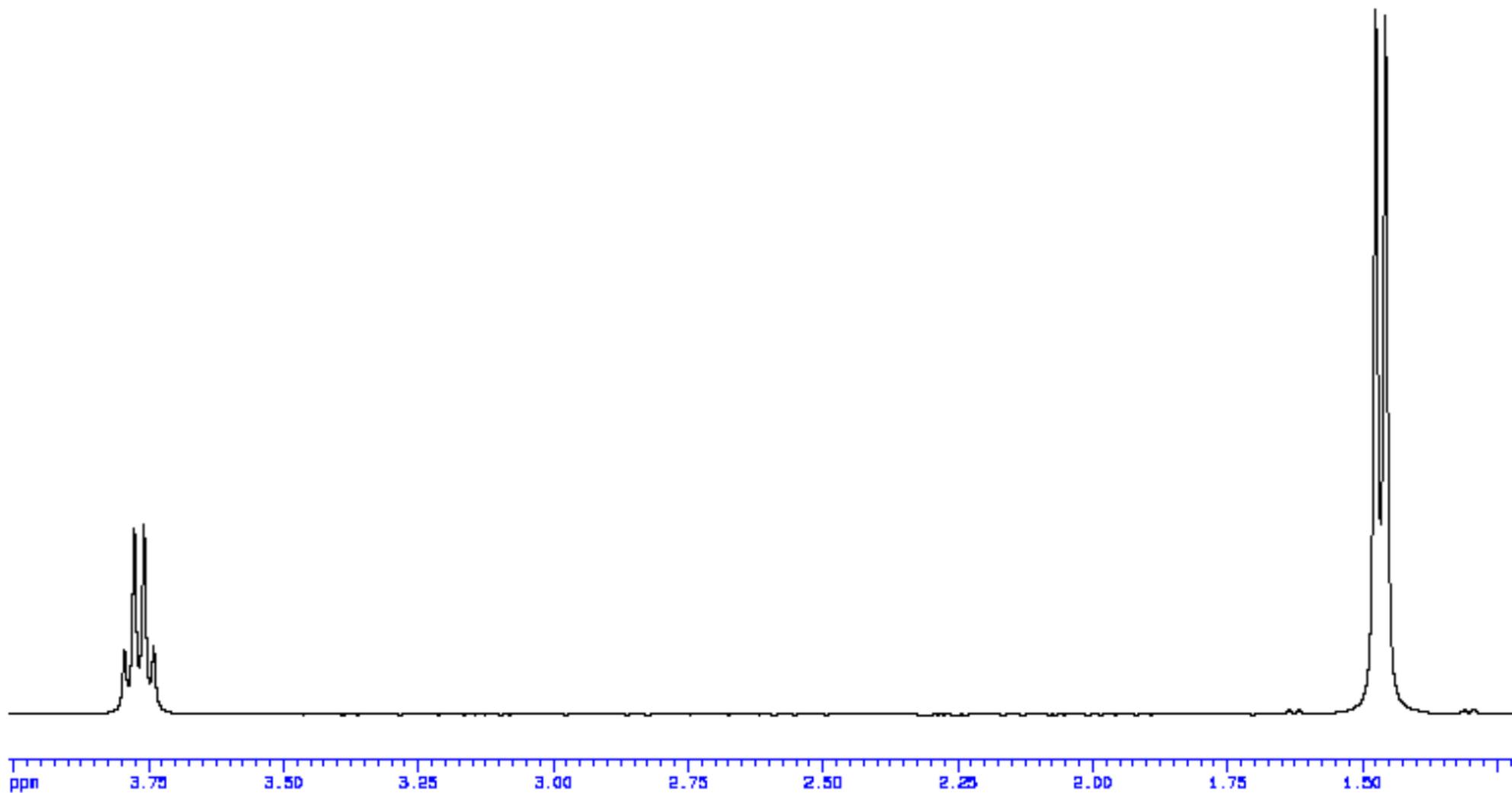
Data Source: Madison Metabolomics Consortium - Qiu Cui, Ian Lewis, Gareth Westler, Brendan Hodis, Mark E. Anderson, John L. Markley.

### Assigned Chemical Shifts

#### 1D 1H

Concentration: 100 mM, pH: 7.4, temperature: 298 K

[click on image for an expanded view](#)



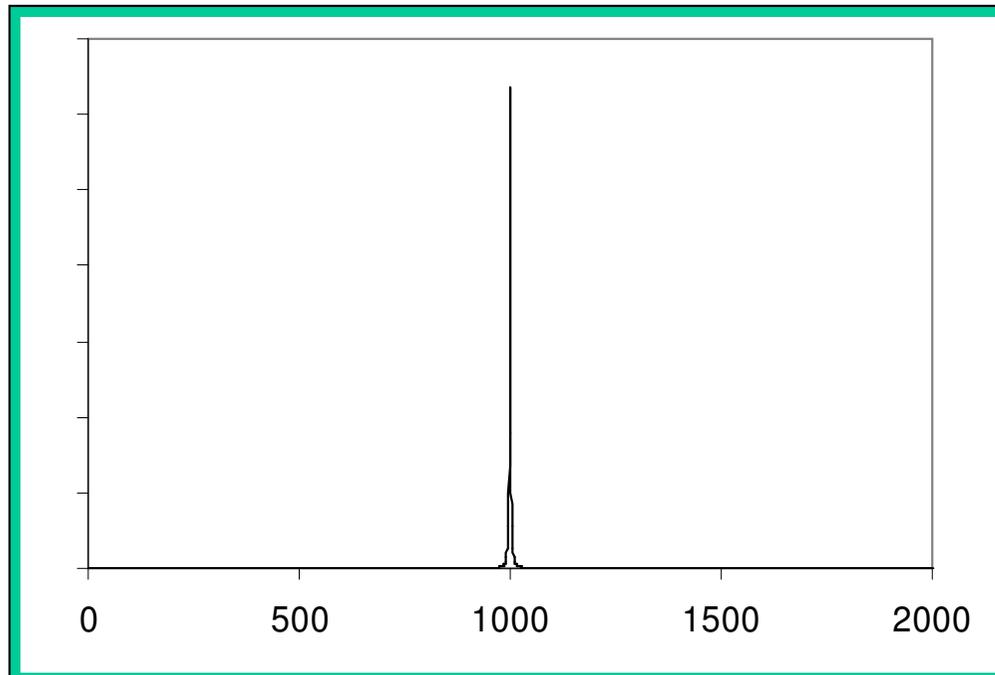
1D <sup>1</sup>H, pH 7.4 spectrum for L-Alanine, [bmse000028](#)

# Solvent Signal Suppression

***Calculation: 90% H<sub>2</sub>O, 1 mM solute***

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

$v_{\text{solute}}$ : 800 Hz,  $\Gamma_{\text{solute}}$ : 5.0 Hz



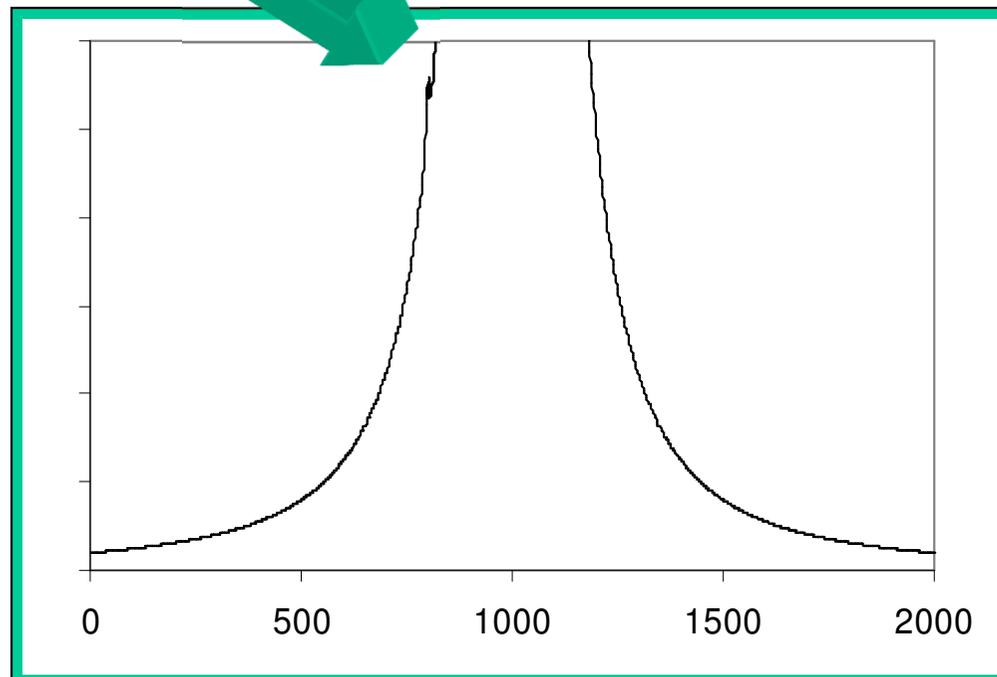
**Att.: 0**

# Solvent Signal Suppression

***Calculation: 90% H<sub>2</sub>O, 1 mM solute***

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

$v_{\text{solute}}$ : 800 Hz,  $\Gamma_{\text{solute}}$ : 5.0 Hz



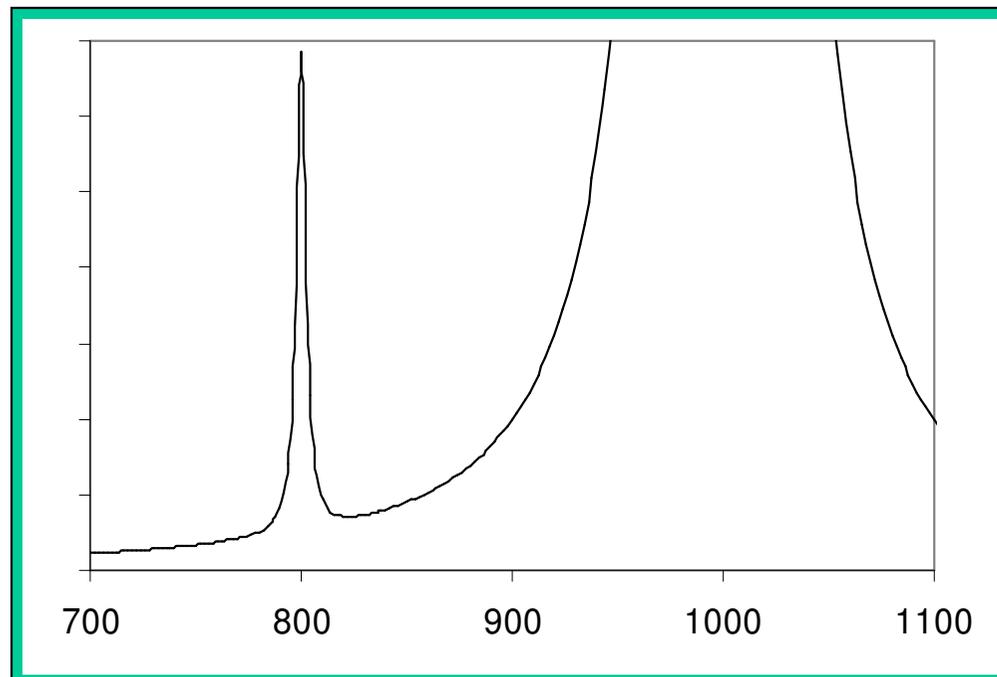
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# Solvent Signal Suppression

***Calculation: 90% H<sub>2</sub>O, 1 mM solute***

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

$v_{\text{solute}}$ : 800 Hz,  $\Gamma_{\text{solute}}$ : 5.0 Hz



**Att.: 10<sup>2</sup>**

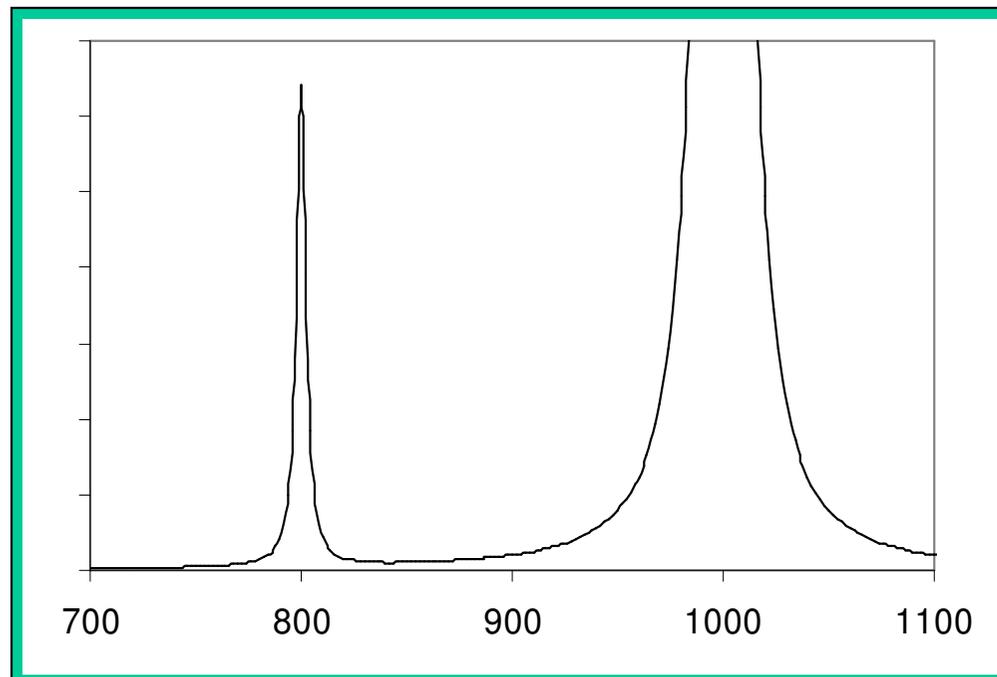
**(99% D<sub>2</sub>O)**

# Solvent Signal Suppression

***Calculation: 90% H<sub>2</sub>O, 1 mM solute***

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

$v_{\text{solute}}$ : 800 Hz,  $\Gamma_{\text{solute}}$ : 5.0 Hz



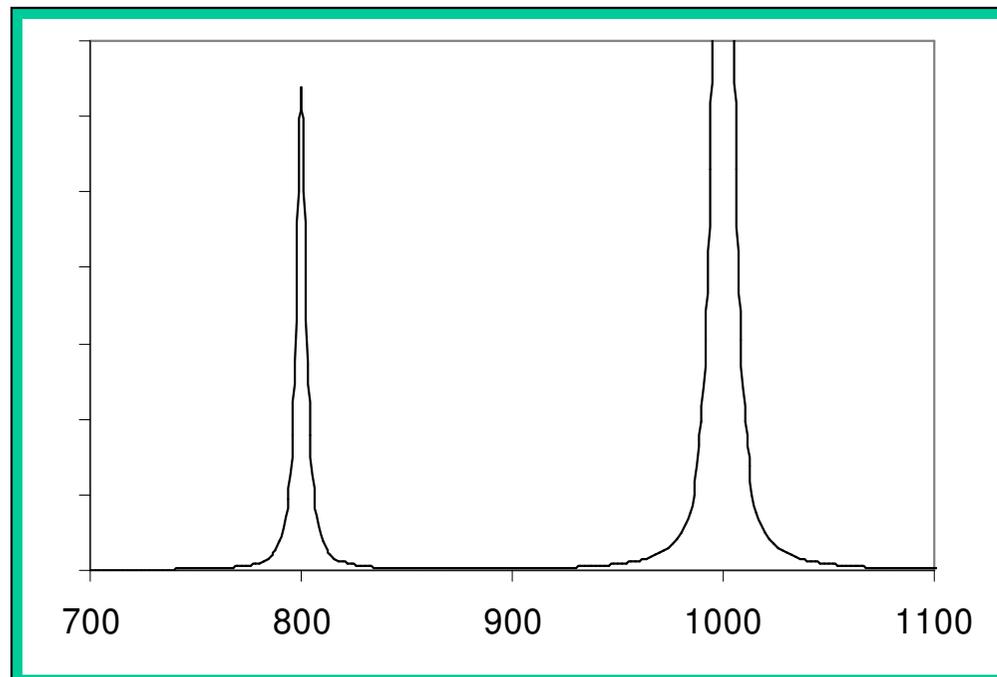
**Att.: 10<sup>3</sup>**

# Solvent Signal Suppression

***Calculation: 90% H<sub>2</sub>O, 1 mM solute***

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

$v_{\text{solute}}$ : 800 Hz,  $\Gamma_{\text{solute}}$ : 5.0 Hz



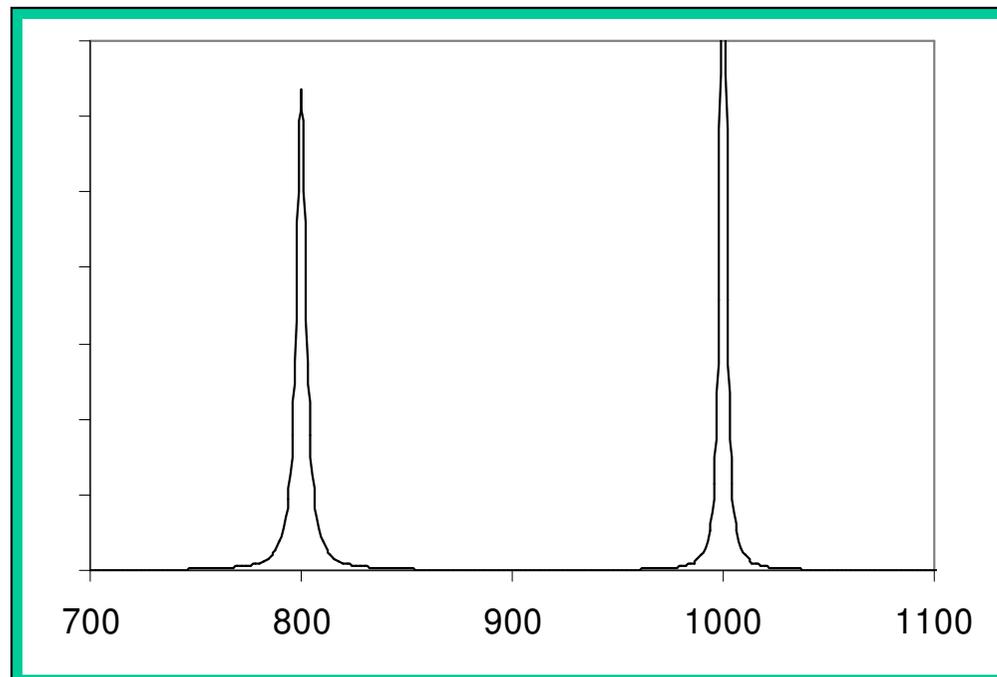
**Att.: 10<sup>4</sup>**

# Solvent Signal Suppression

***Calculation: 90% H<sub>2</sub>O, 1 mM solute***

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

$\nu_{\text{solute}}$ : 800 Hz,  $\Gamma_{\text{solute}}$ : 5.0 Hz



**Att.:  $10^5$**

# 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

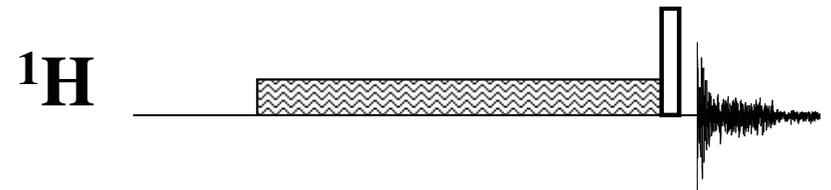
- 1) *Saturation Based Methods***
  - a) *Discrimination by Frequency***
  - b) *Discrimination by Relaxation Times***
- 2) *Methods Avoiding Solvent Saturation***
- 3) *Magnetization Destruction-Based Methods***
- 4) *Coherence Selection***
- 5) *Postacquisitional Methods***

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

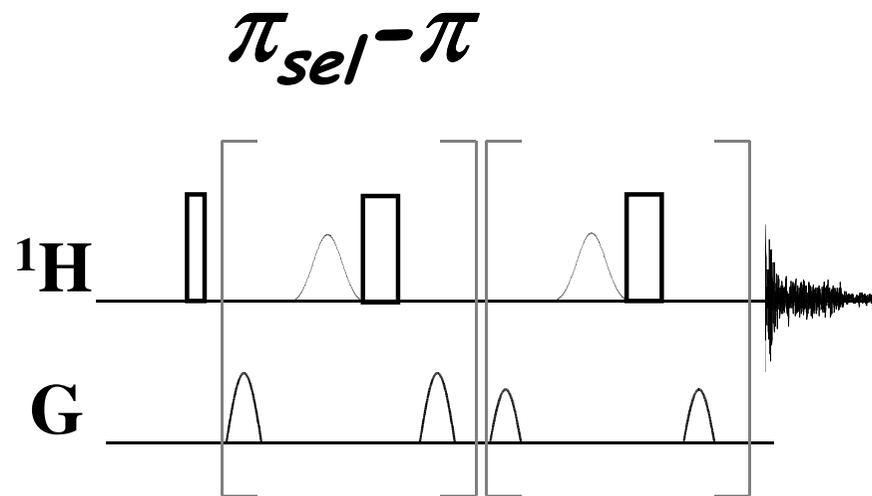
☹ bleaching out of resonances close to the solvent

☹ attenuation/suppression of resonances due to chemical exchange

### 3) Magnetization Destruction-Based Methods

*Double Pulsed Field Gradient Spin-Echo: DPFGE*

**"EXCITATION SCULPTING"**



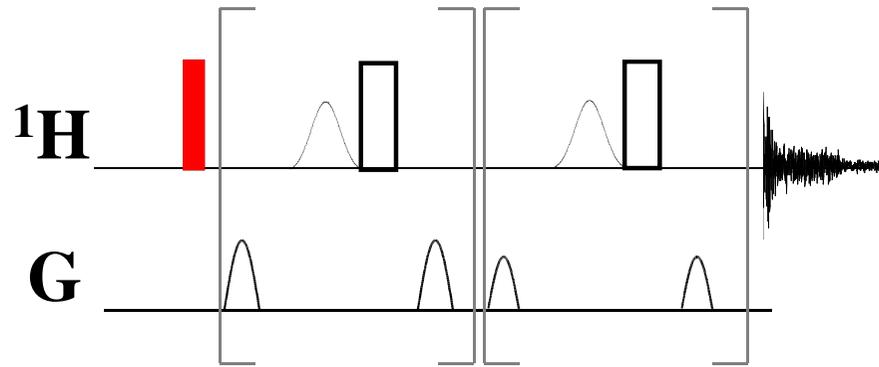
- ☺ multiple solvent suppression possible
- ☺ very efficient ( $>10^4$ )
- ☺ resonances undergoing exchange are observable
- ☹ delay of 4 - 12 ms after lecture pulse

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

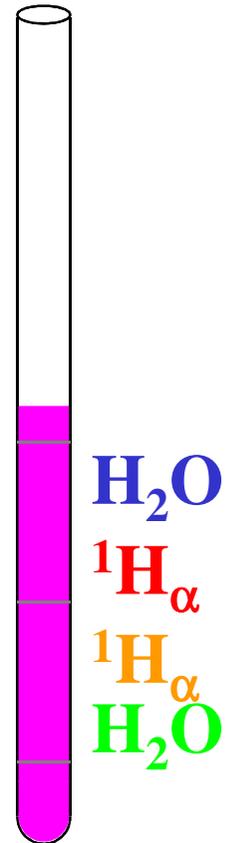
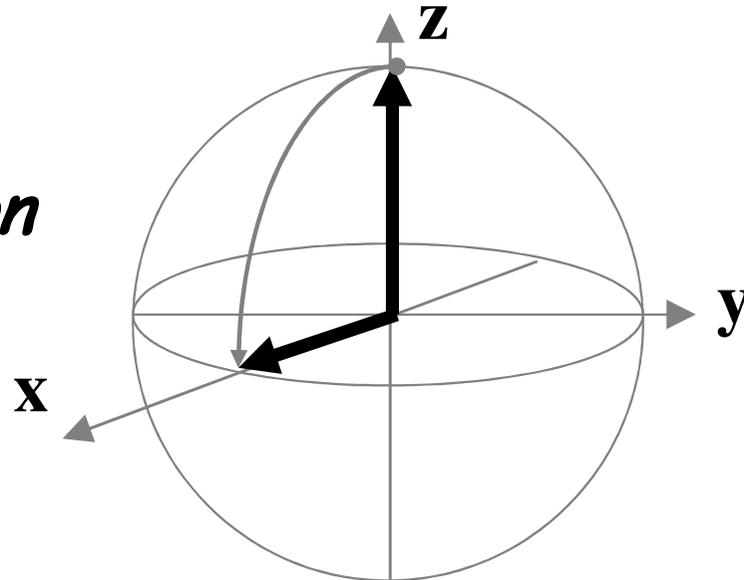
Shaka *et al.*, *J.A.C.S.*, 1995, 117, 4199

### 3) Magnetization Destruction-Based Methods

**"EXCITATION SCULPTING"**

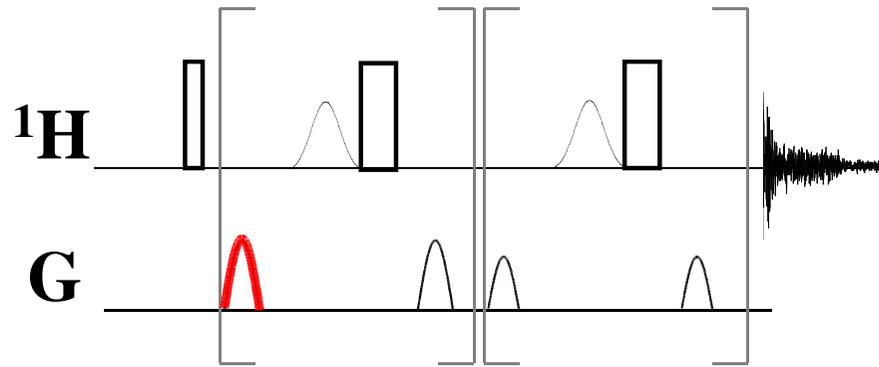


*90° excitation*

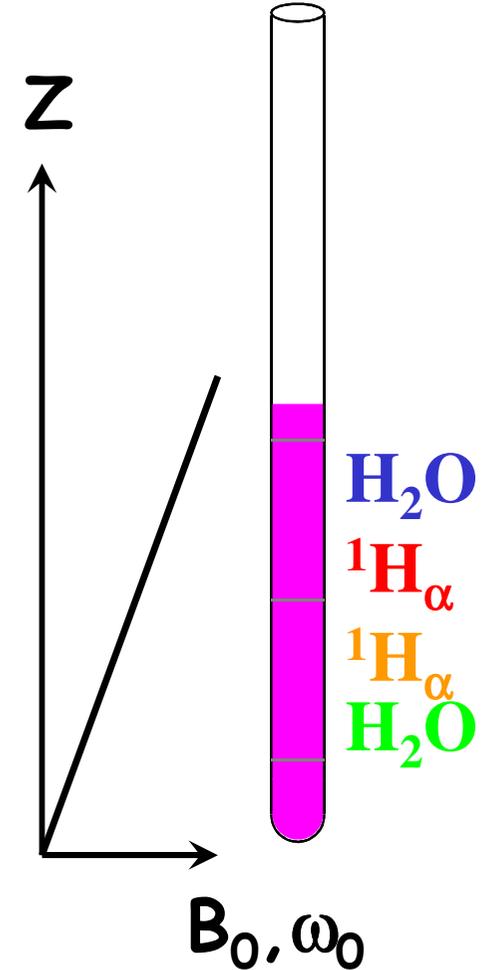
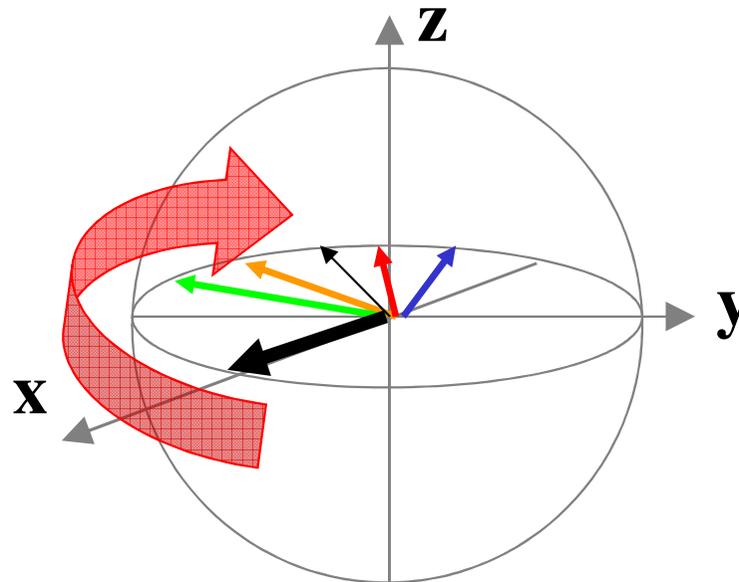


# 3) Magnetization Destruction-Based Methods

*"EXCITATION SCULPTING"*

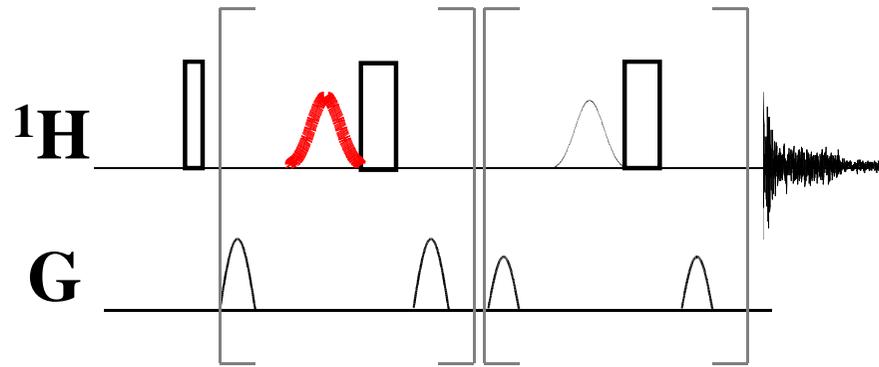


*defocussing  
gradient*

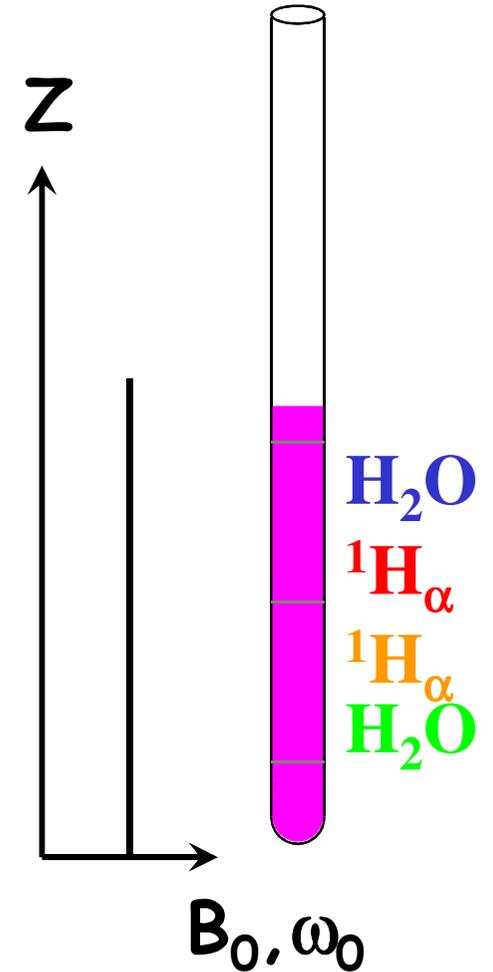
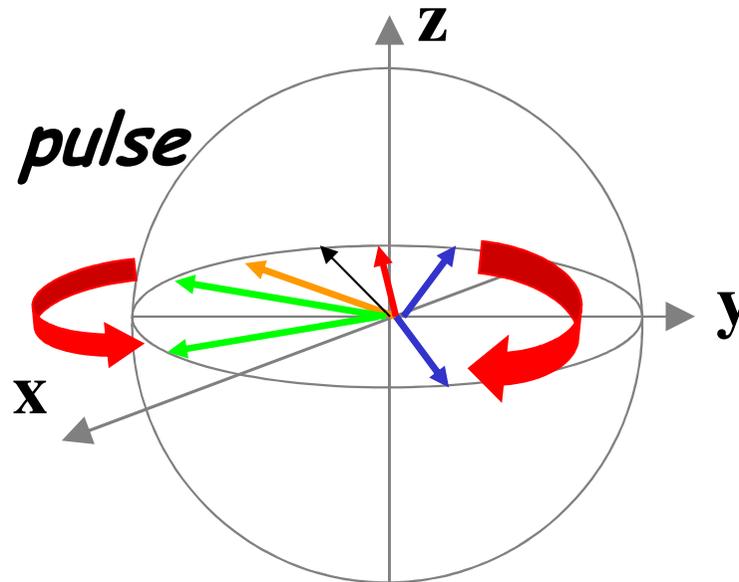


### 3) Magnetization Destruction-Based Methods

*"EXCITATION SCULPTING"*

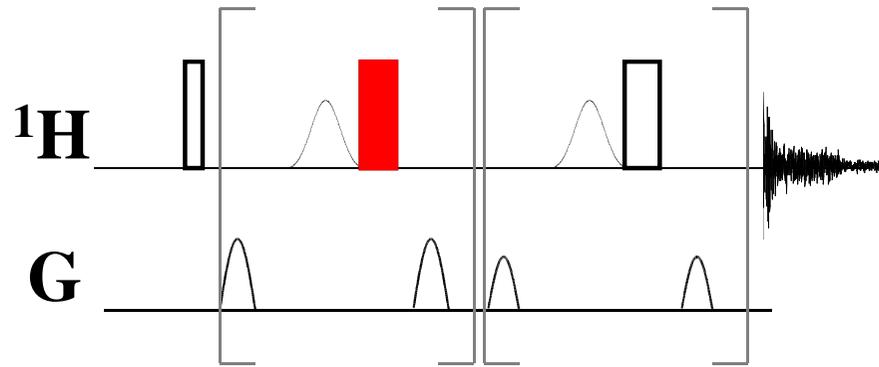


*180° selective pulse*

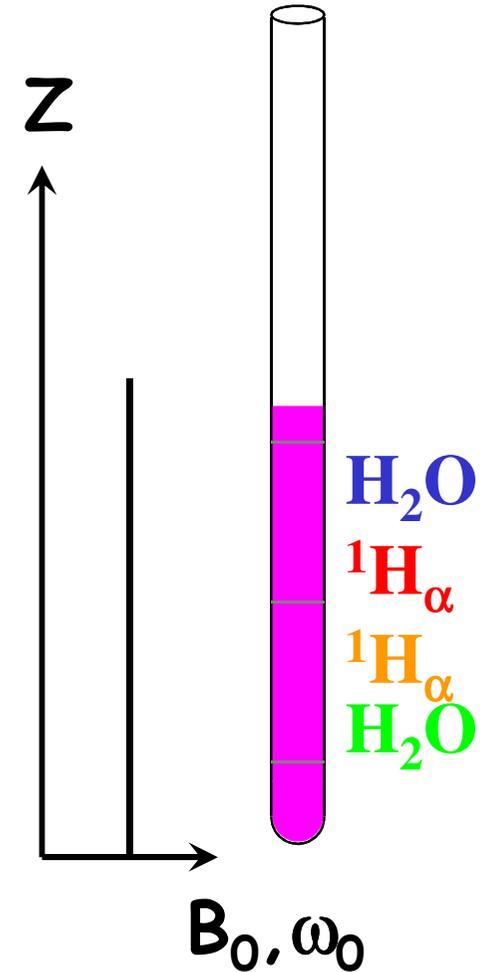
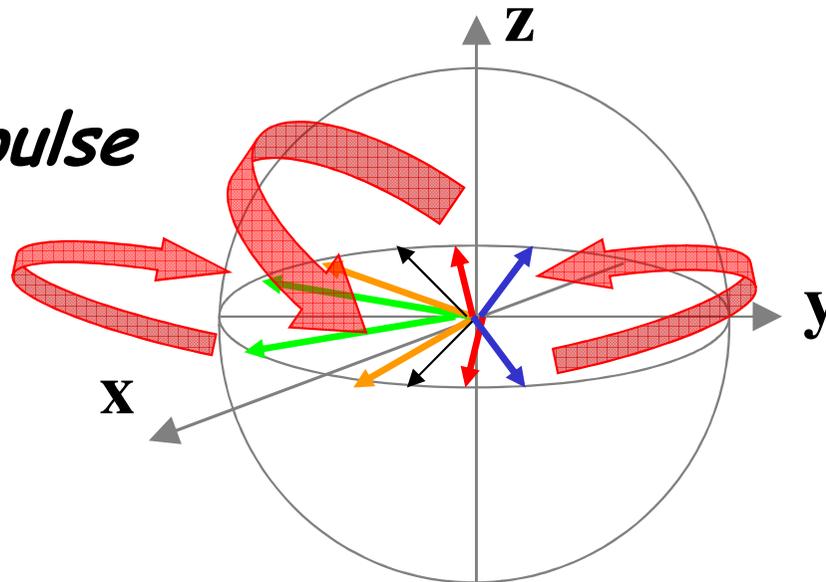


# 3) Magnetization Destruction-Based Methods

*"EXCITATION SCULPTING"*

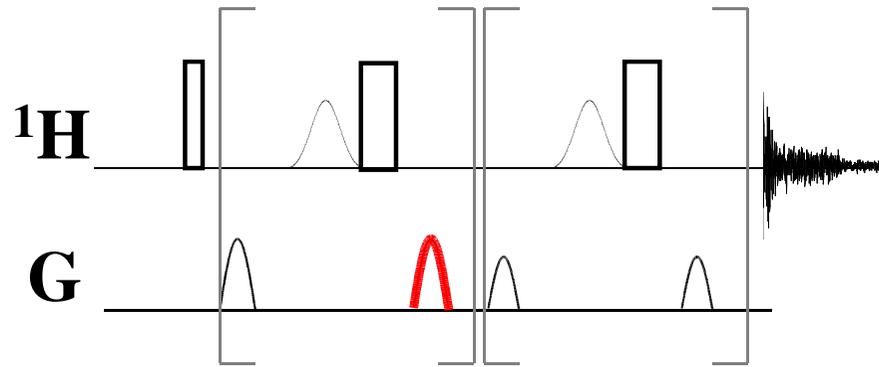


*180° hard pulse*



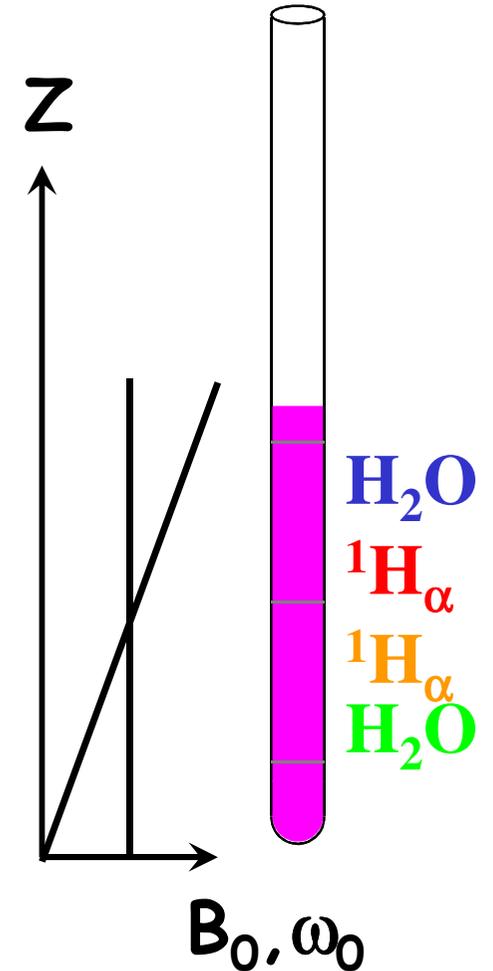
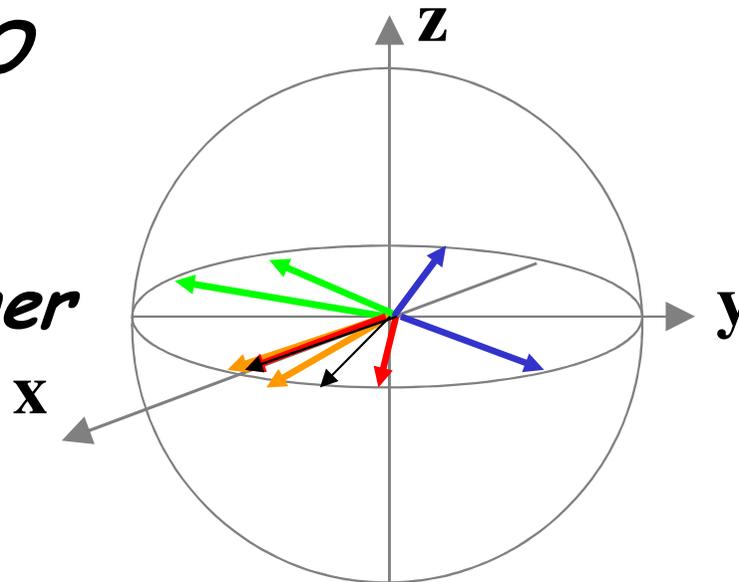
### 3) Magnetization Destruction-Based Methods

*"EXCITATION SCULPTING"*



*defocussing  
gradient for  $\text{H}_2\text{O}$*

*refocussing  
gradient for other  
frequencies*



# Water Suppression techniques

**2 mM Sucrose 90% H<sub>2</sub>O**

**d1: 1.5s**

**pl9: 58 dB**

**ds: 8**

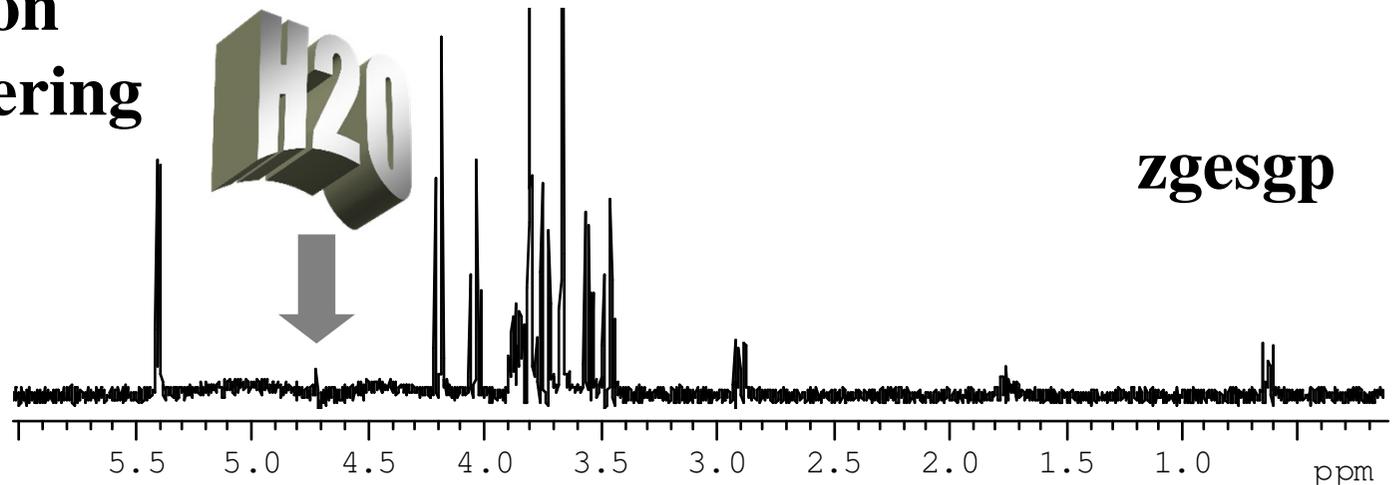
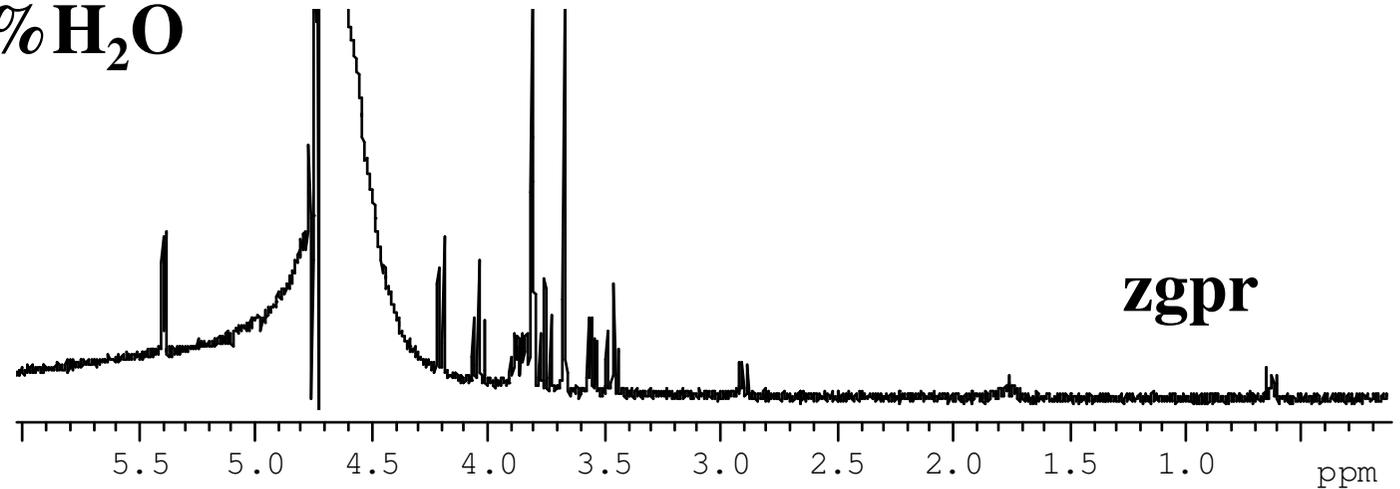
**ns: 8**

**td: 8k**

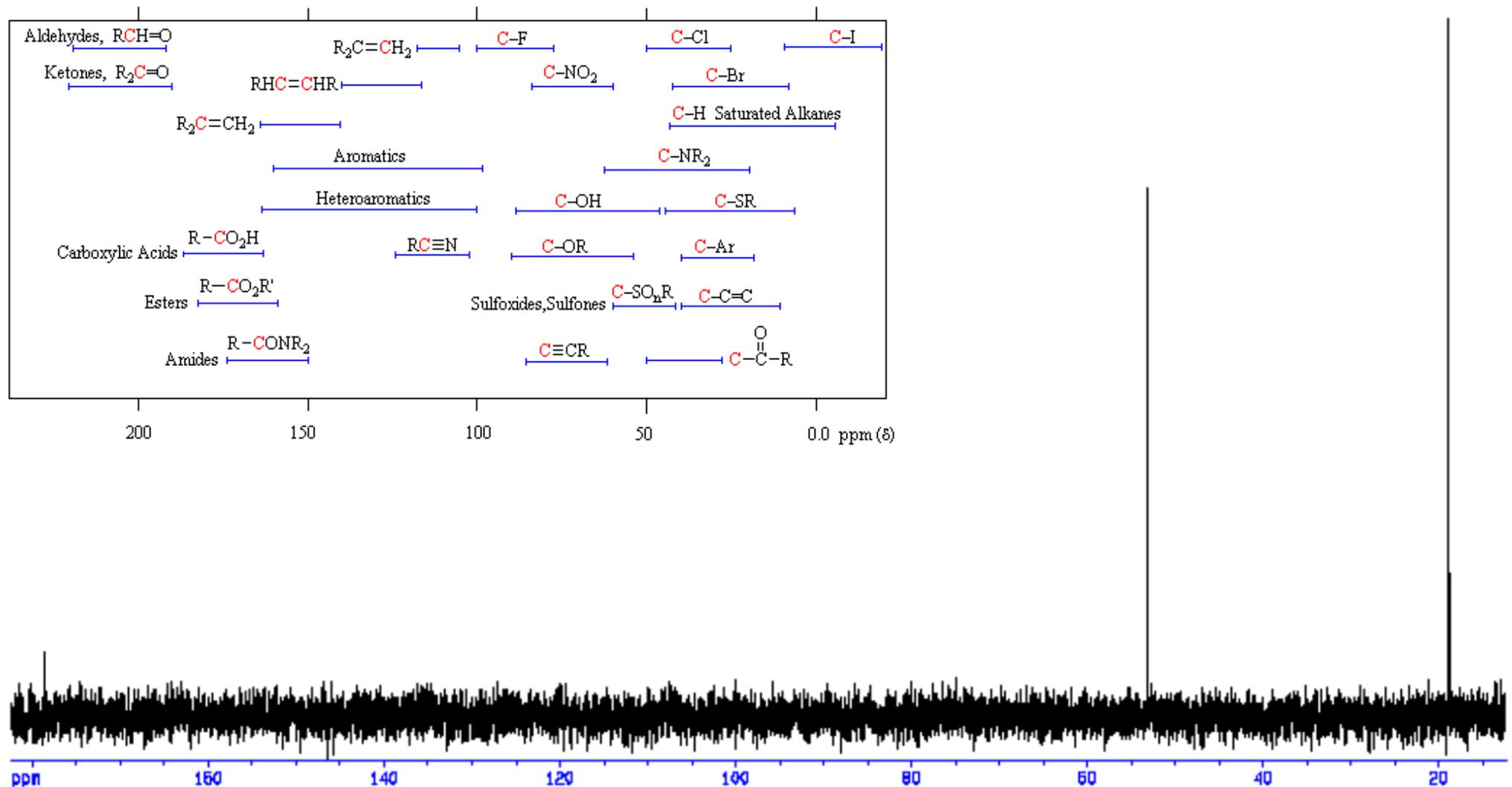
**si: 16k**

**no window function**

**no H<sub>2</sub>O signal filtering**



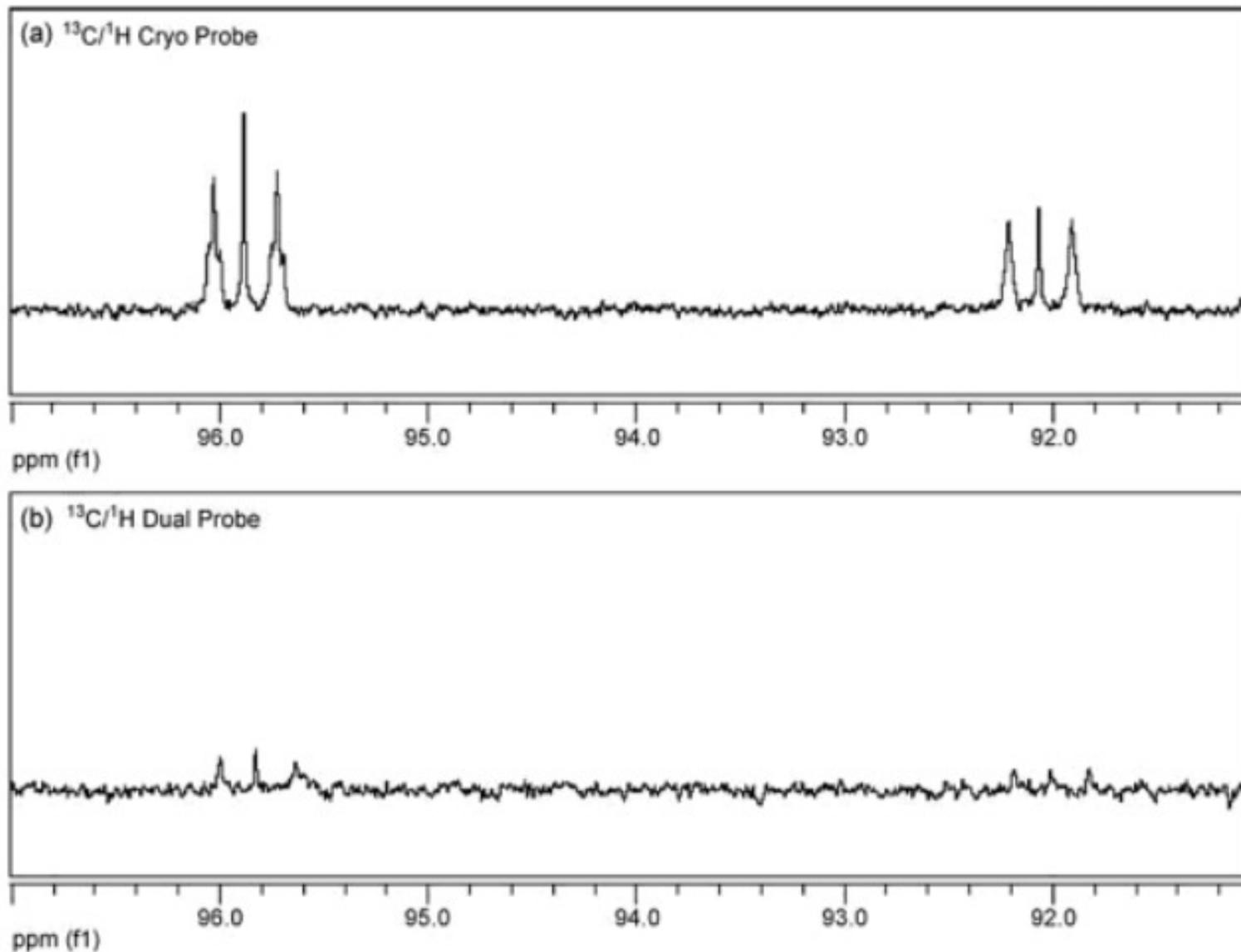
# $^{13}\text{C}$ NMR



1D  $^{13}\text{C}$ , pH 7.4 spectrum for L-Alanine, [bmse000028](#)

Increase number of scans  
to increase sensitivity

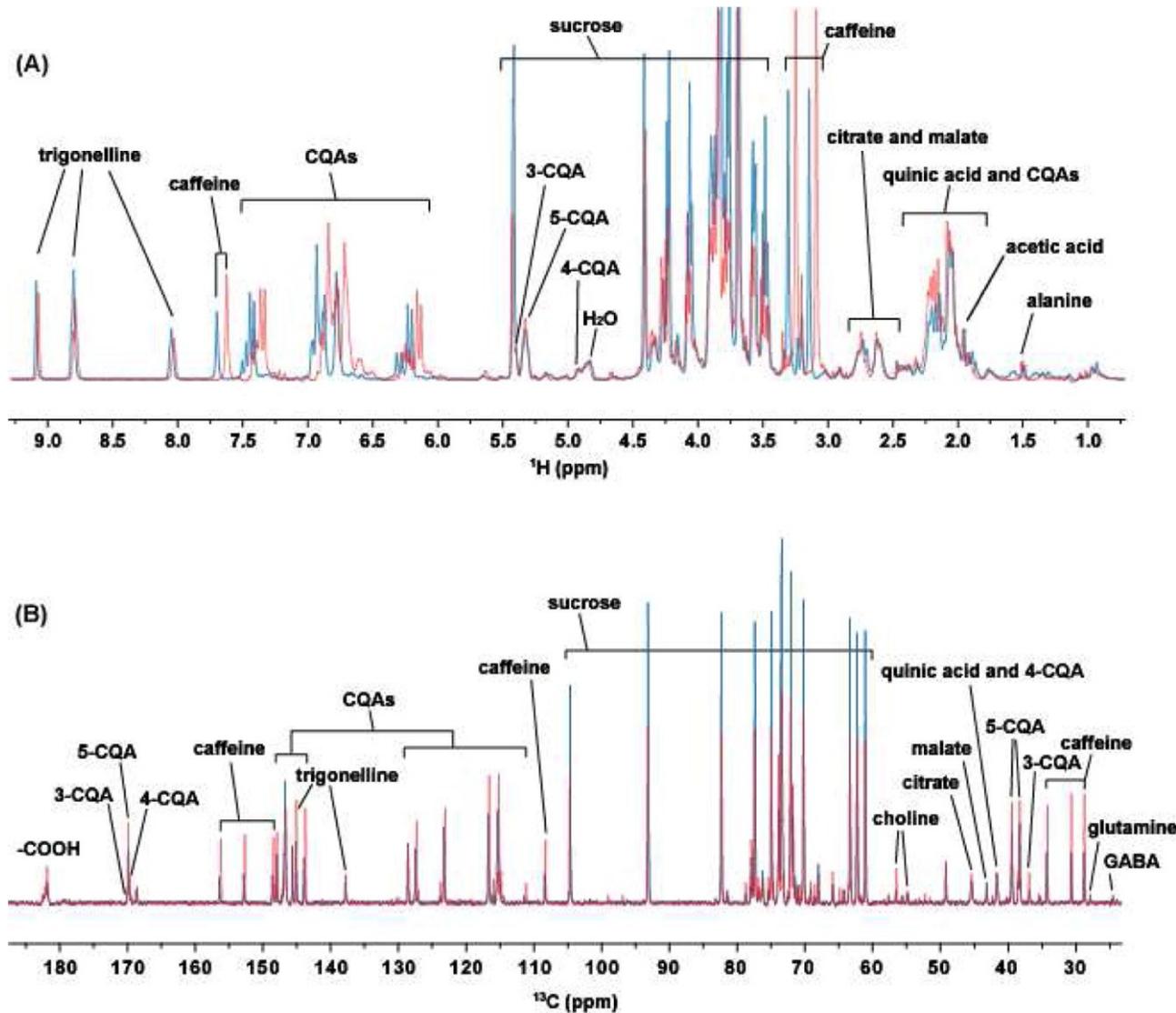
# $^{13}\text{C}$ NMR: sensitivity improvement



**Figure 2.5**  $^{13}\text{C}$ - $1\beta$  and  $^{13}\text{C}$ - $1\alpha$  NMR signals of a mixture of 5  $\mu\text{g}$  of  $[\text{U-}^{13}\text{C}_6]$ glucose and 95  $\mu\text{g}$  of unlabeled glucose in 0.5 ml of  $\text{D}_2\text{O}$ . Eight

scans,  $90^\circ$  pulses, 2 s relaxation delay, experiment time 1 min, 1 Hz line broadening. (a) CryoProbe; (b) conventional probe.

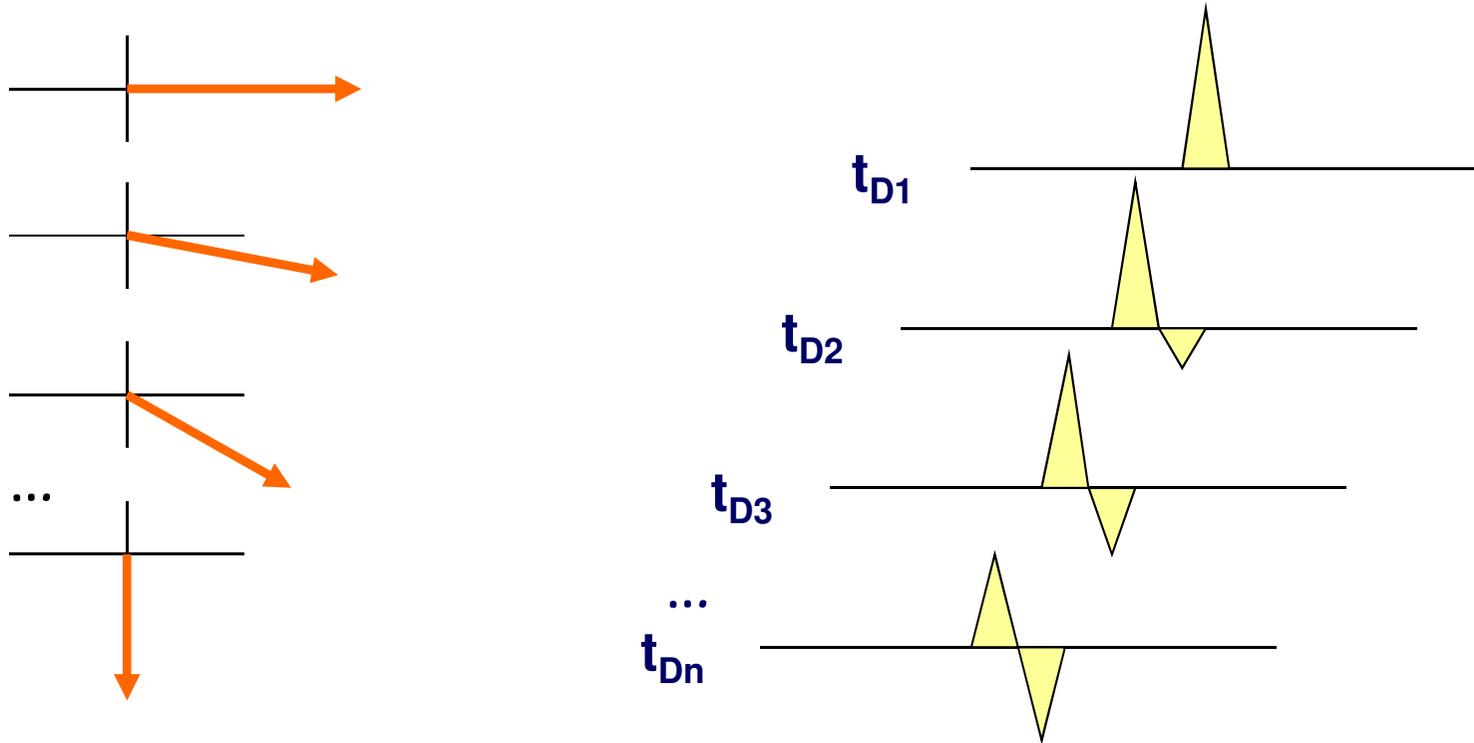
# $^{13}\text{C}$ NMR



Assigned (A)  $^1\text{H}$  and (B)  $^{13}\text{C}$  NMR spectra of two typical green coffee bean extracts of the arabica from Colombia (blue line) and the robusta from Indonesia (red line).

# 2D NMR spectroscopy

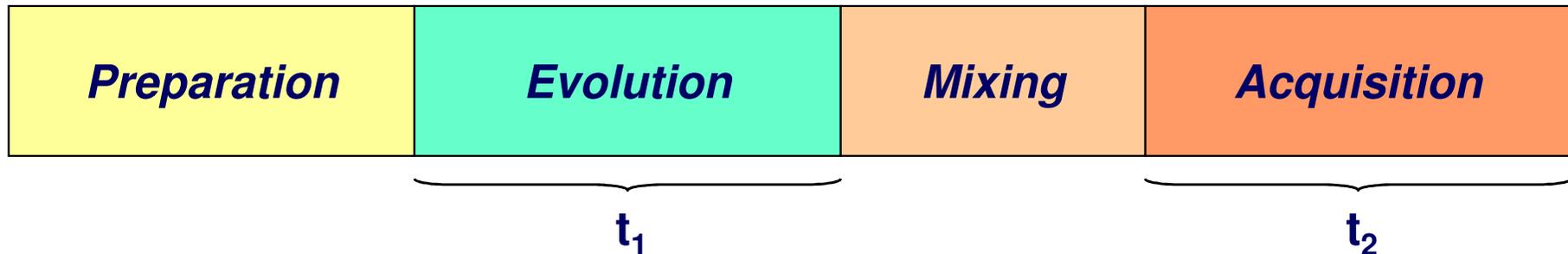
- The 'basic' 2D spectrum would involve repeating a multiple pulse 1D sequence with a systematic variation of the delay time  $t_D$ , and then plotting everything stacked. A very simple example would be varying the time before acquisition:



- 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

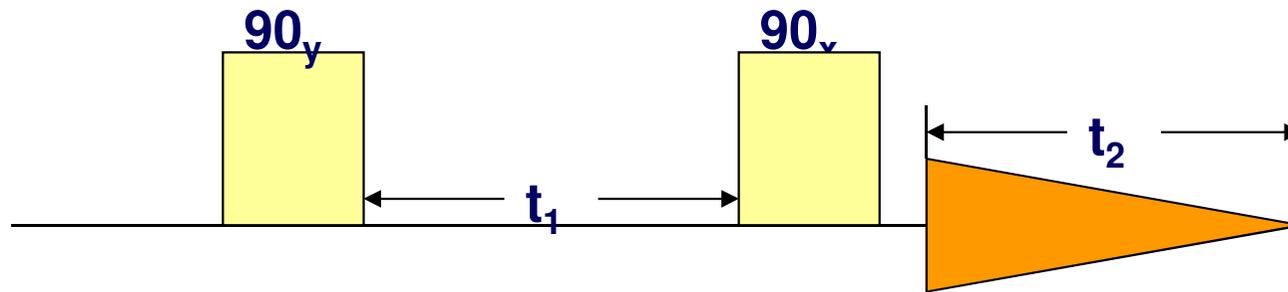
- The first perturbation of the system (pulse) will now be called the **preparation** of the spin system.
  - The variable  $t_D$  is renamed the **evolution time**,  $t_1$ .
  - We have a **mixing** event, in which information from one part of the spin system is relayed to other parts.
  - Finally,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



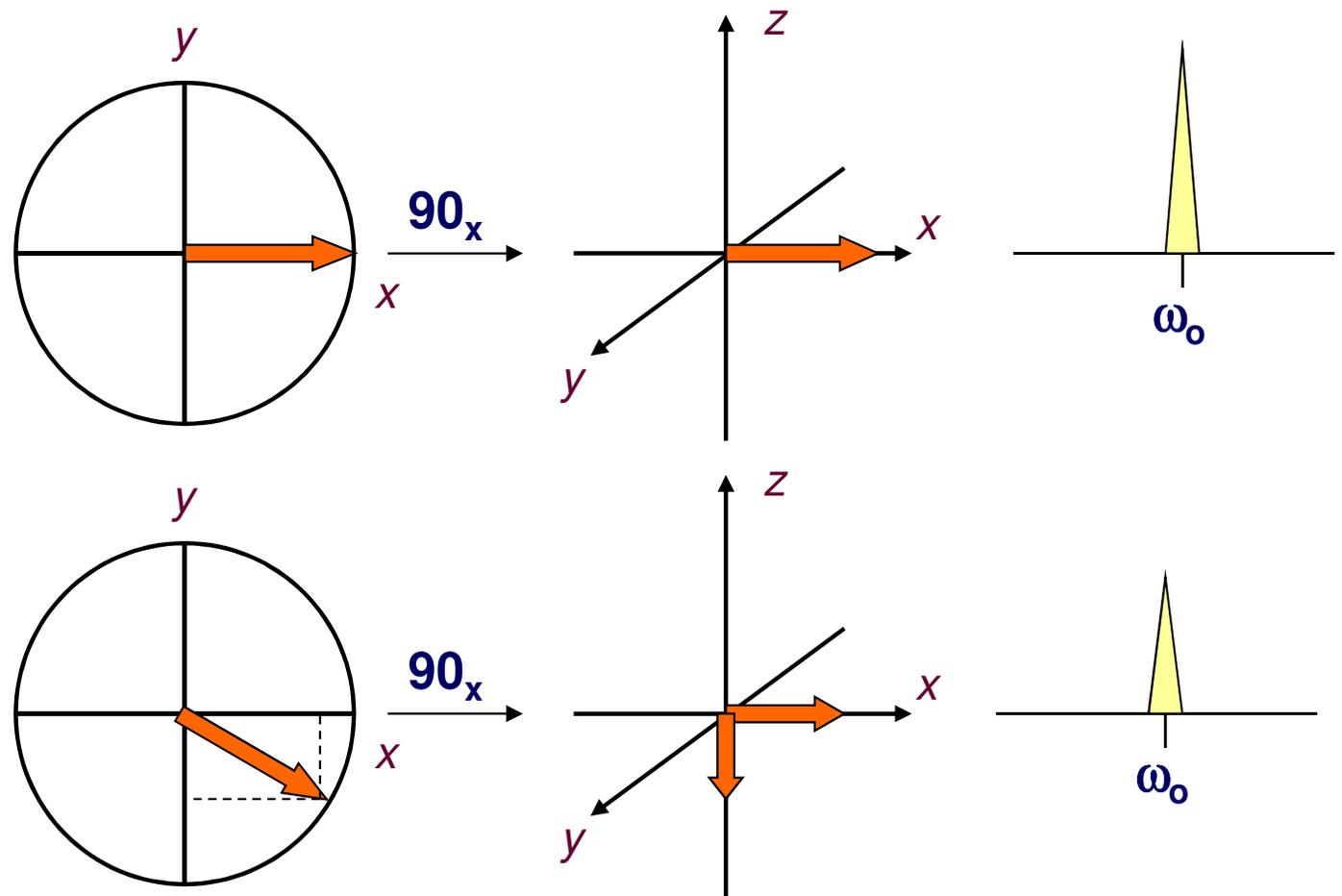
- $t_1$  is the variable delay time, and  $t_2$  is the normal acquisition time. We can envision having  $f_1$  and  $f_2$ , for both frequencies...
- We'll see that this format is basically the same for all 2D pulse sequences and experiments.

# A rudimentary 2D experiment

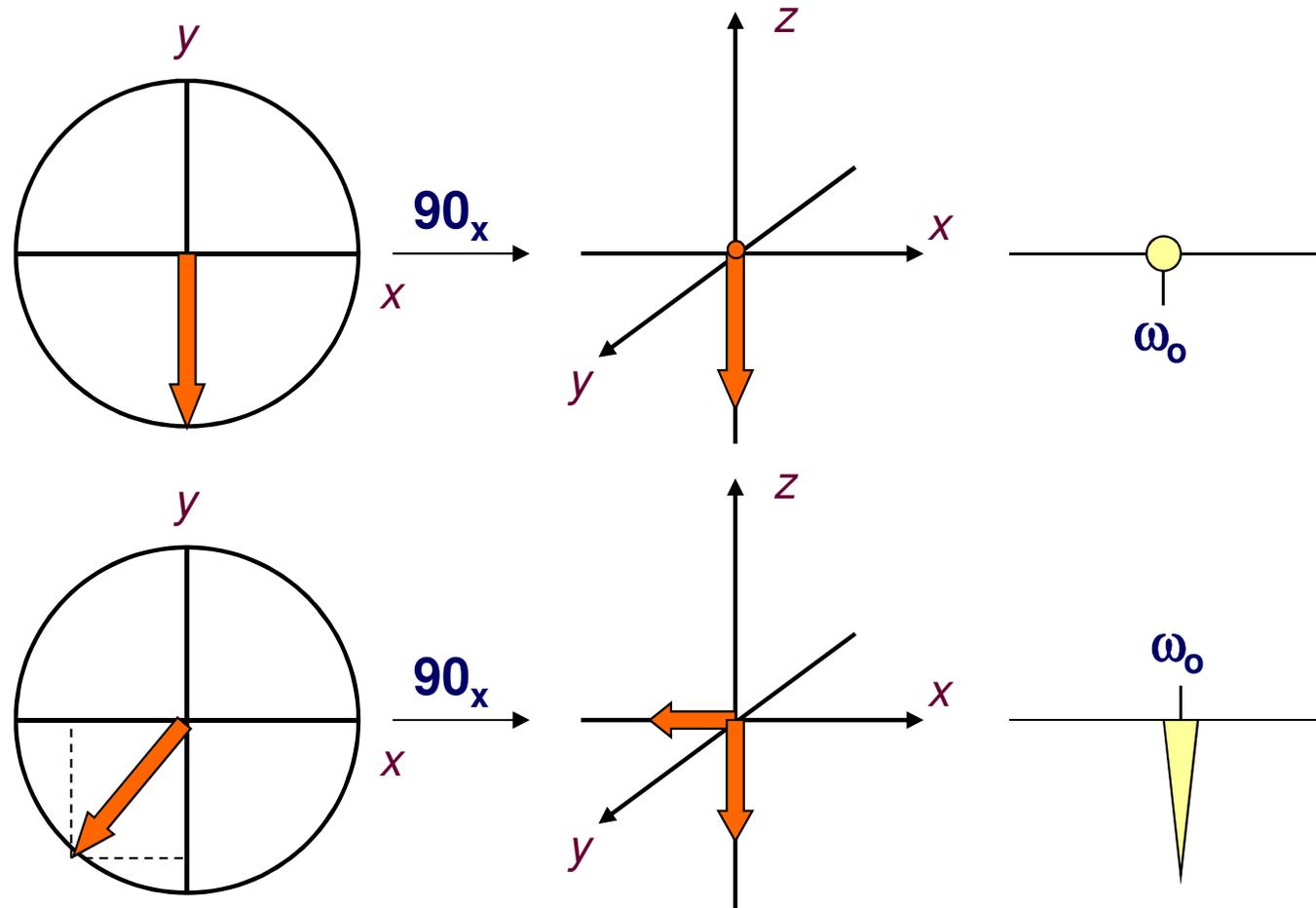
- We'll see how it works with the backbone of what will become the **COSY** pulse sequence. Think of these pulses, where  $t_1$  is the preparation time:



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



# The rudimentary 2D (continued)

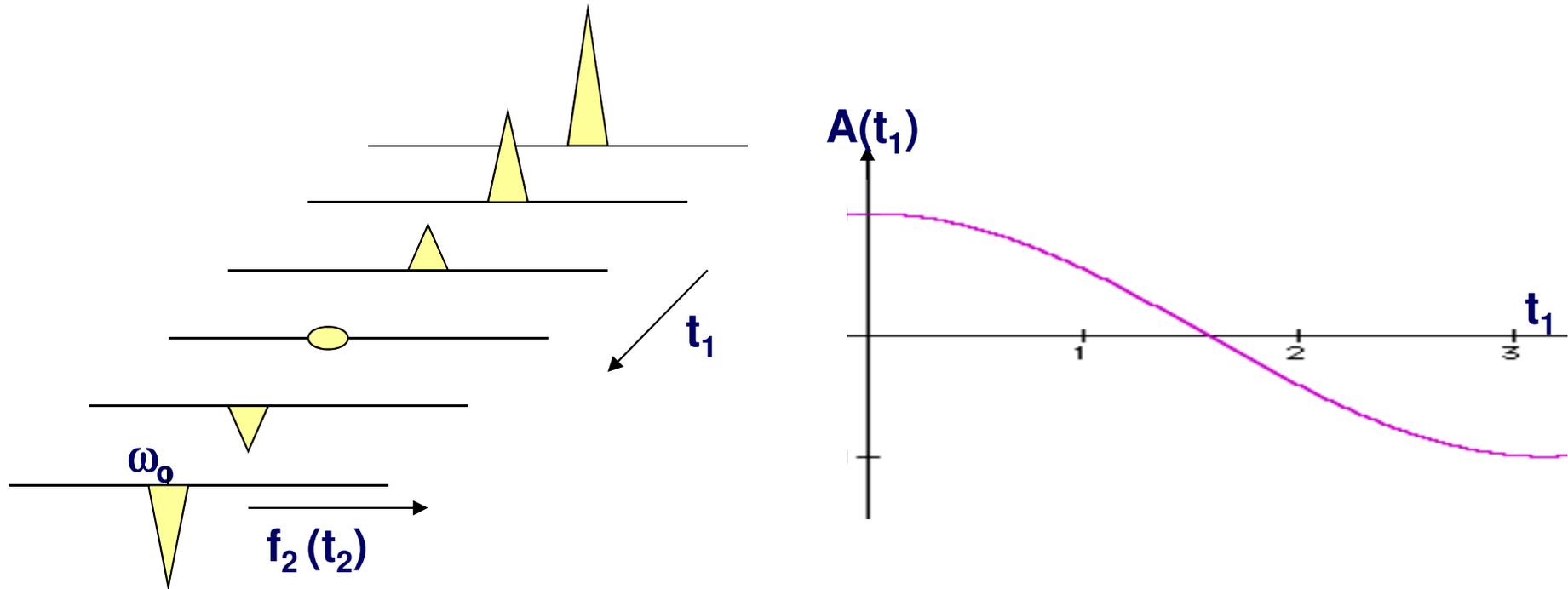


- The second  $\pi / 2$  pulse acts only on the  $y$  axis component of the magnetization of the  $\langle xy \rangle$  plane.
- The  $x$  axis component is not affected, but its amplitude will depend on the frequency of the line.

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

# The rudimentary 2D (...)

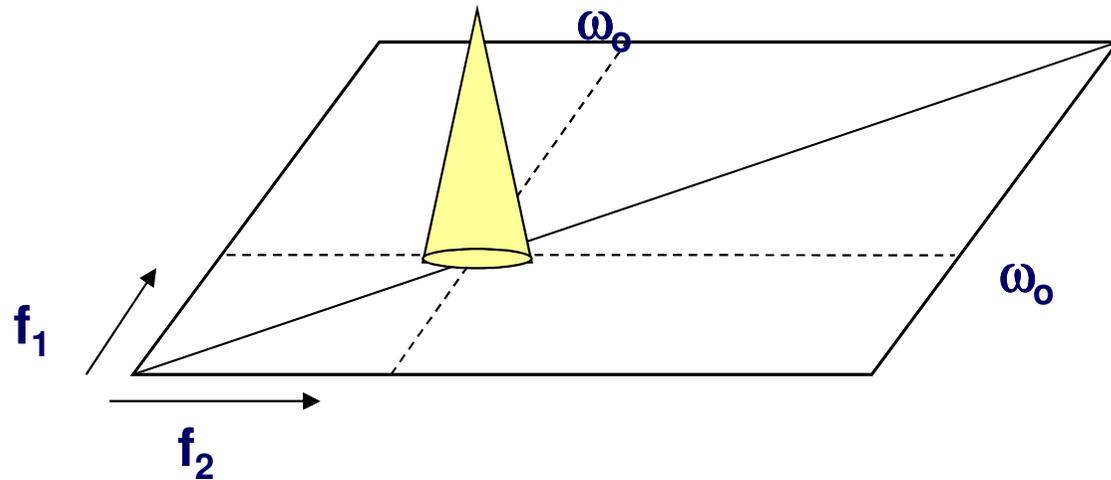
- If we plot all the spectra in a **stacked plot**, we get:



- 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.

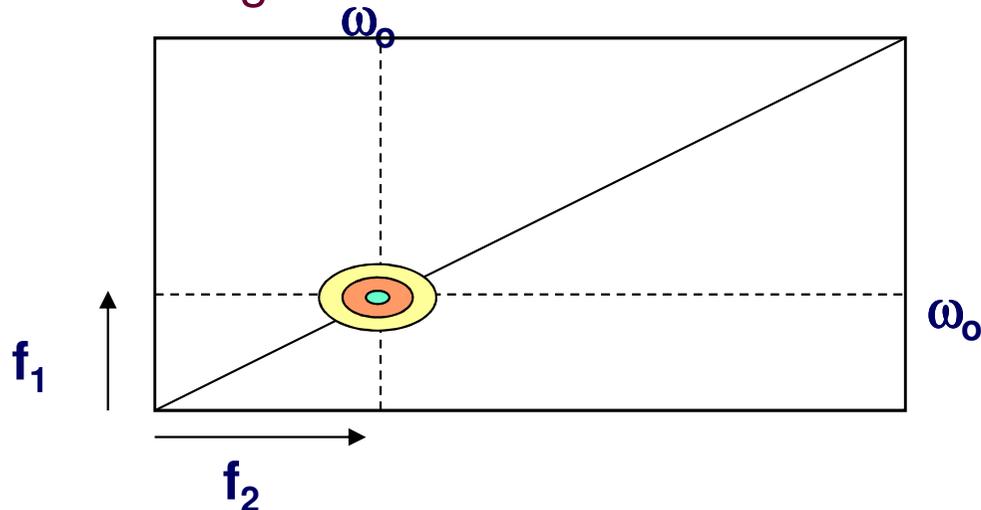
# The rudimentary 2D (...)

- 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.

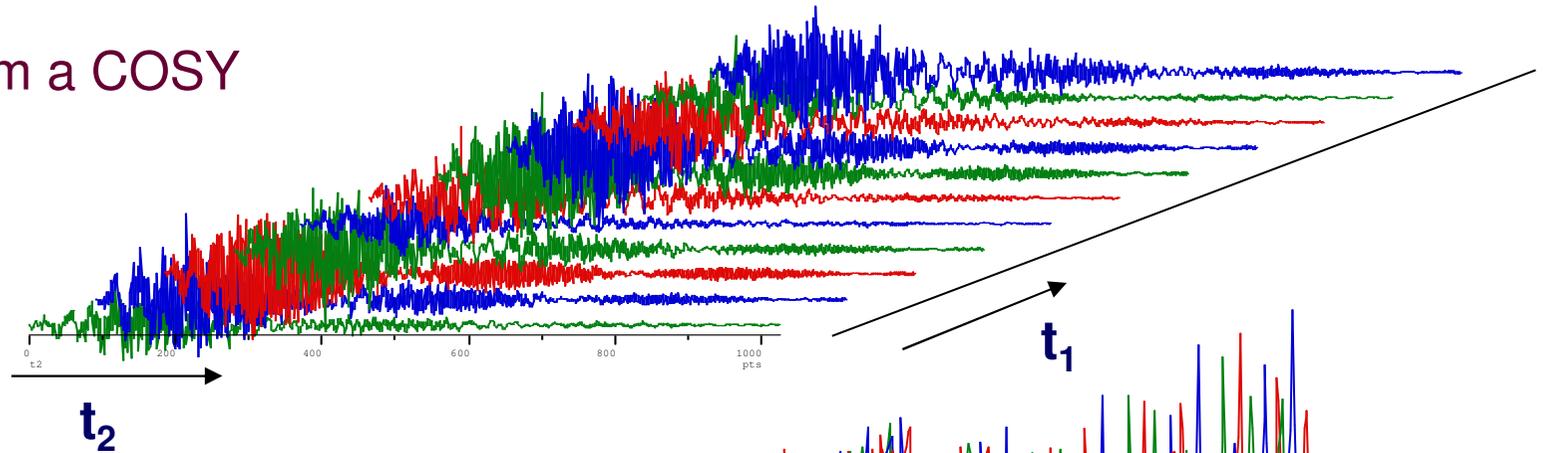


- Each slice is color-coded depending on the height of the peak.

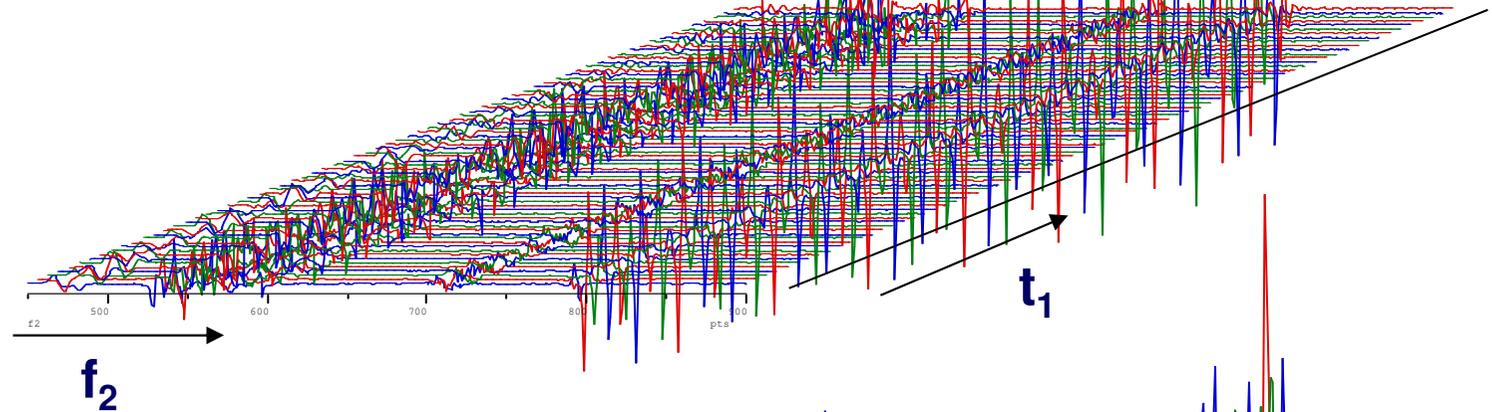
# The same with some real data

- This is data from a COSY

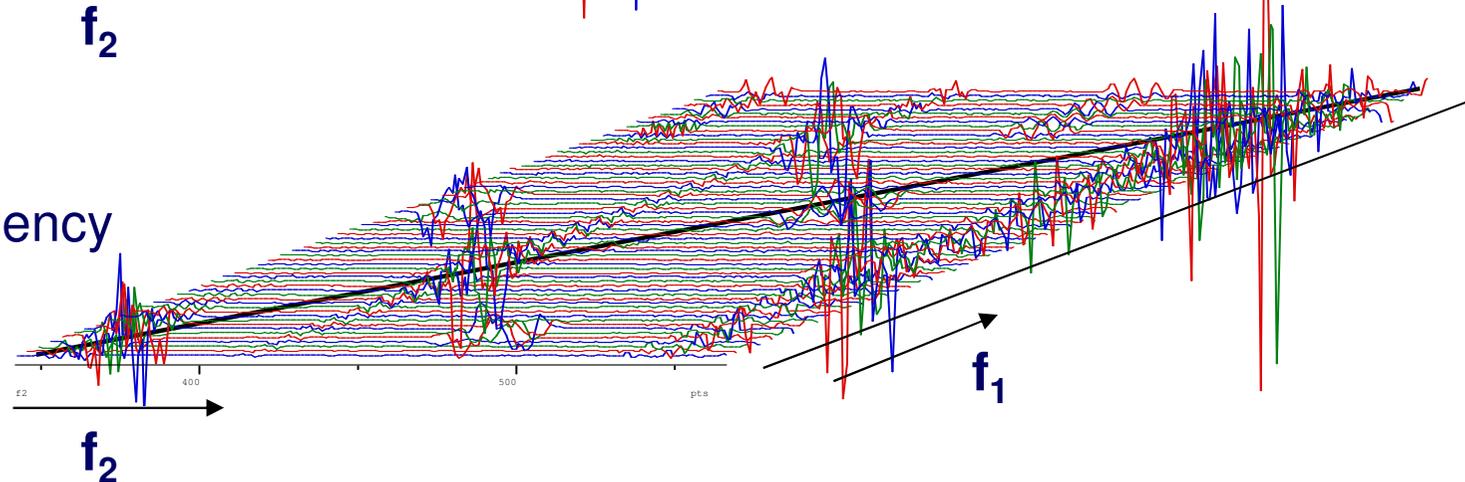
time - time



time - frequency

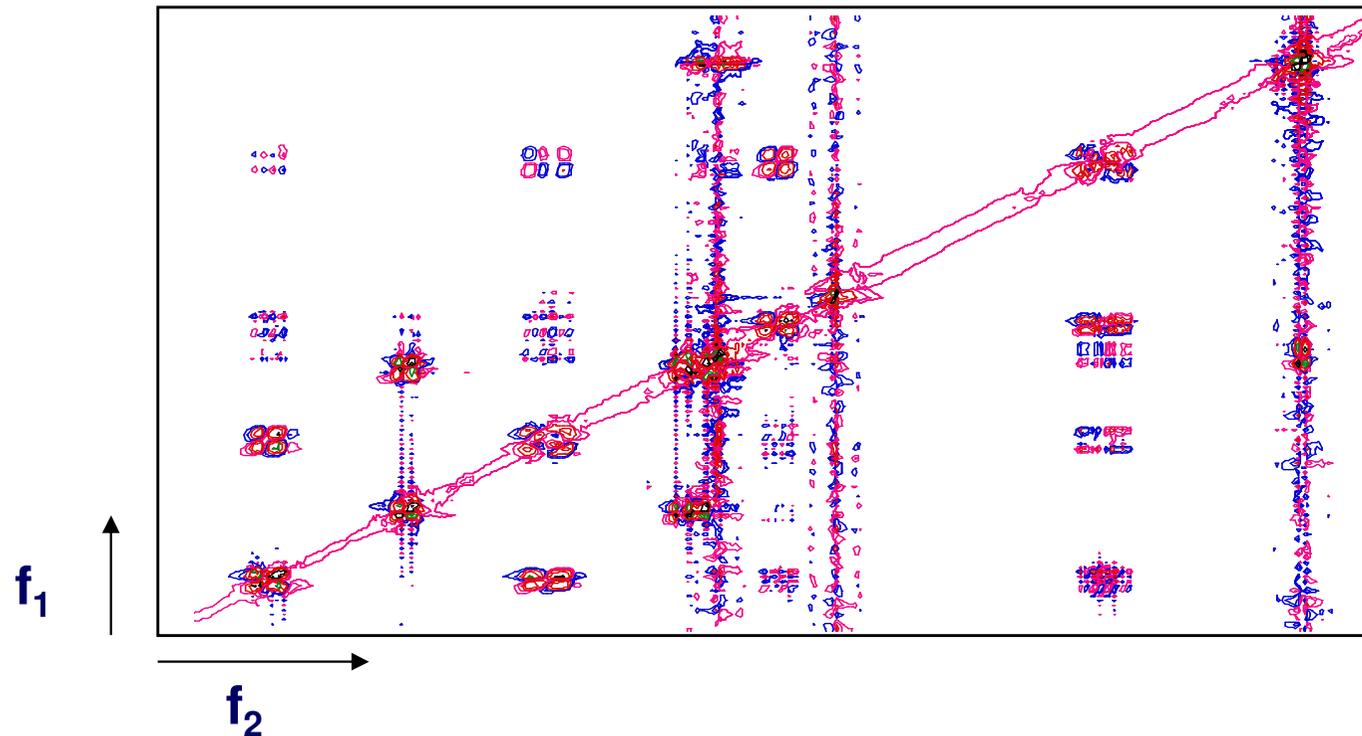


frequency - frequency



# The same with some real data

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



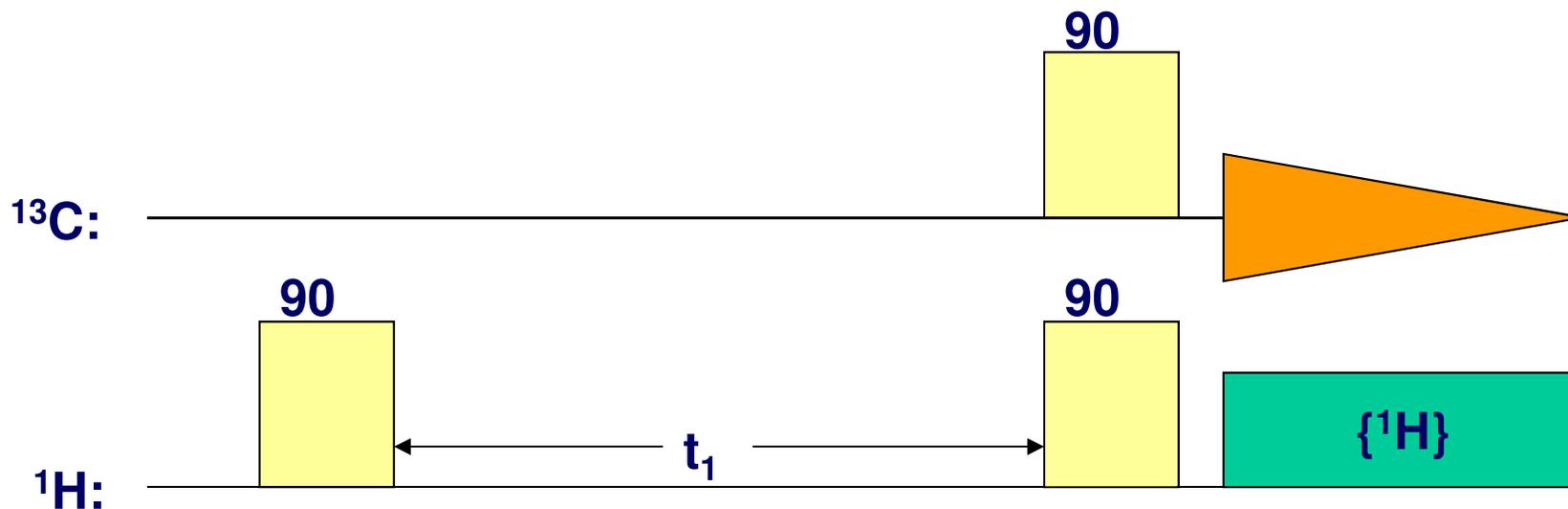
- OK, where did all the *off-diagonal* peaks come from, and what do they mean?

**COSY** stands for **CO**rrelation **S**pectroscop**Y**, 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.

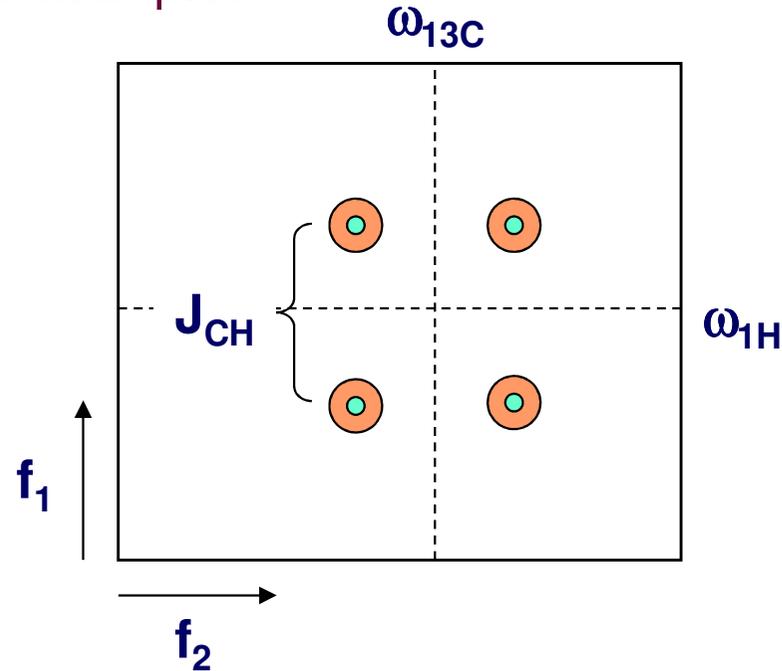
# Heteronuclear correlation - HETCOR

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

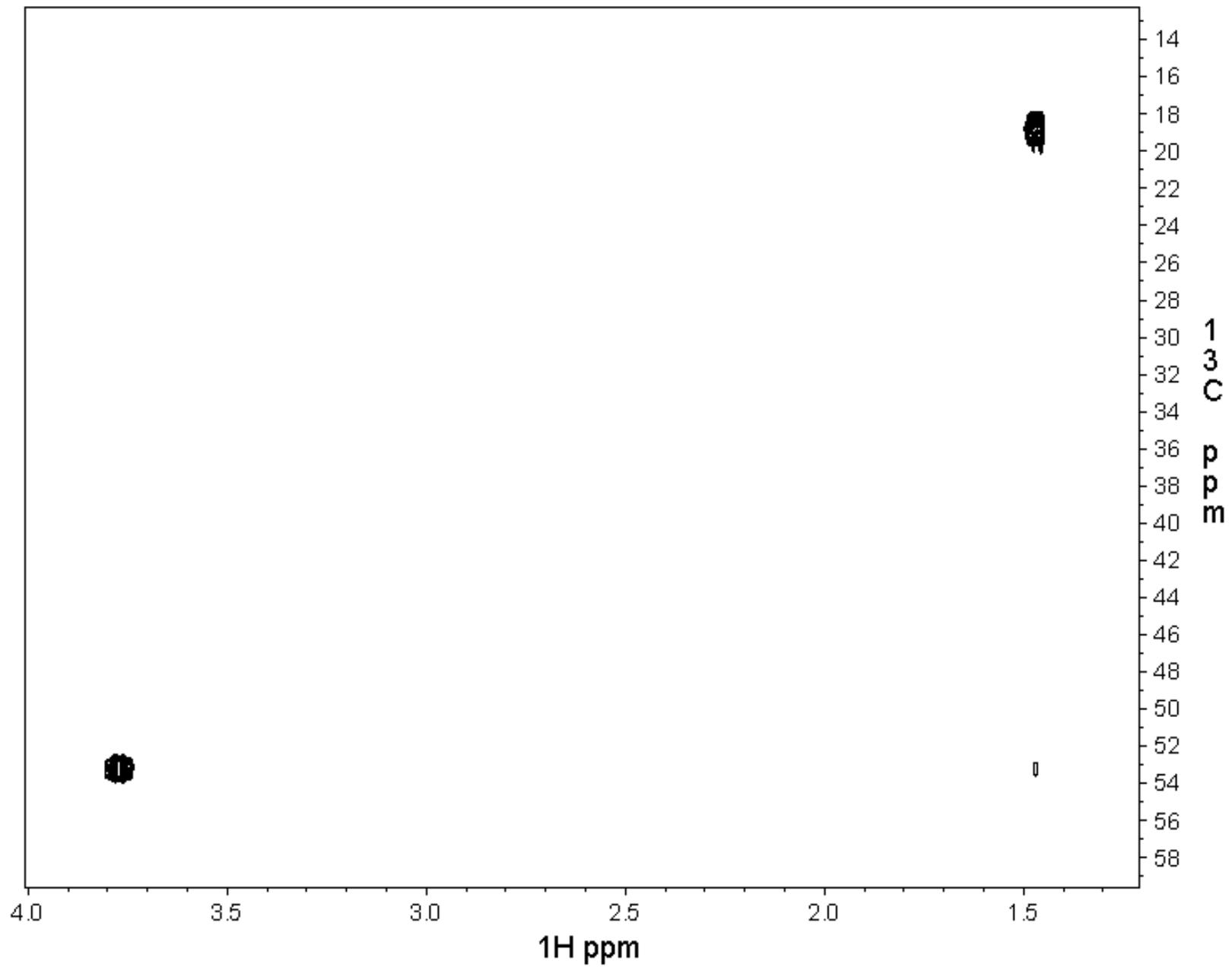


# HETCOR (...)

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



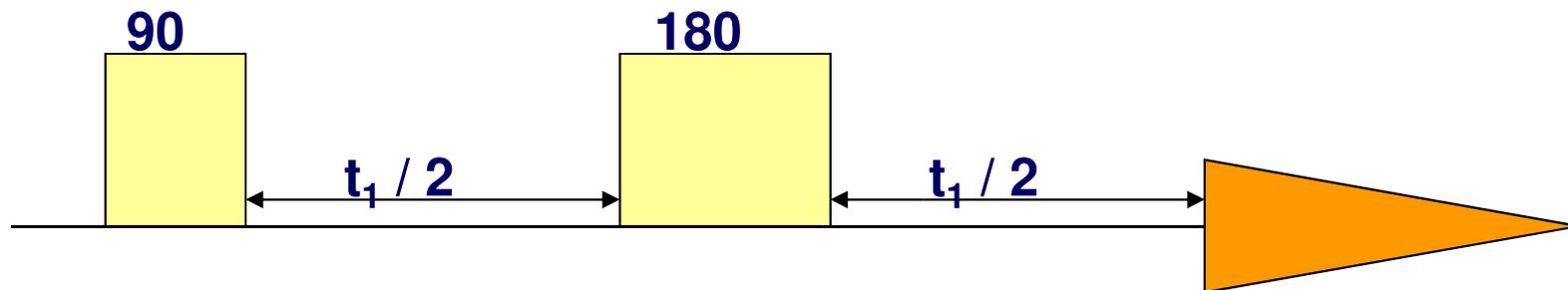
- The main difference in this case is that the 2D spectrum is not symmetrical, because one axis has  $^{13}\text{C}$  frequencies and the other  $^1\text{H}$  frequencies.
- Now, we still have the  $\mathbf{J}_{\text{CH}}$  coupling splitting all the signals of the 2D spectrum in little squares. The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



L-alanine

# Homonuclear 2D J spectroscopy - HOMO2DJ

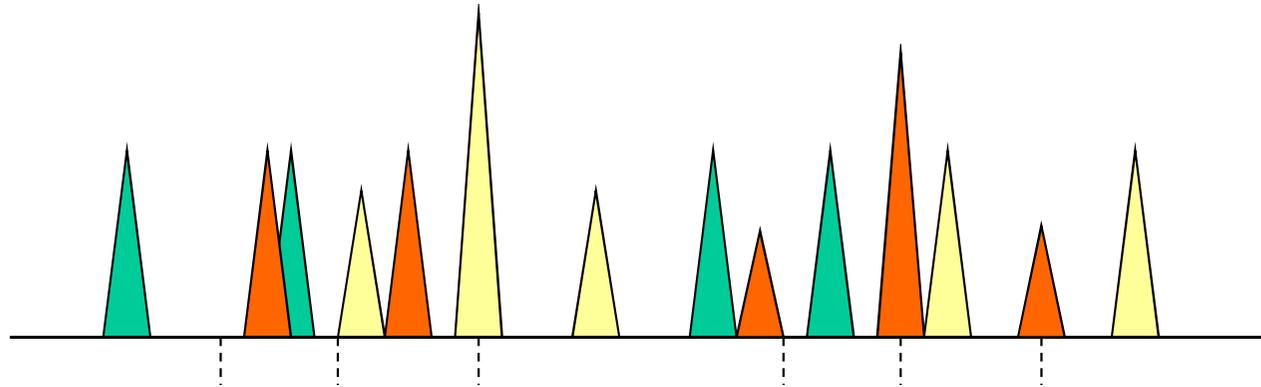
- The 2D experiments we've analyzed so far are used to find out correlations or connections between spin systems. There are many other things that we can extract from 2D experiments in which we take advantage of the spreading-out of signals.
- One of the most annoying things is to have a cool sample 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  $\delta$  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:



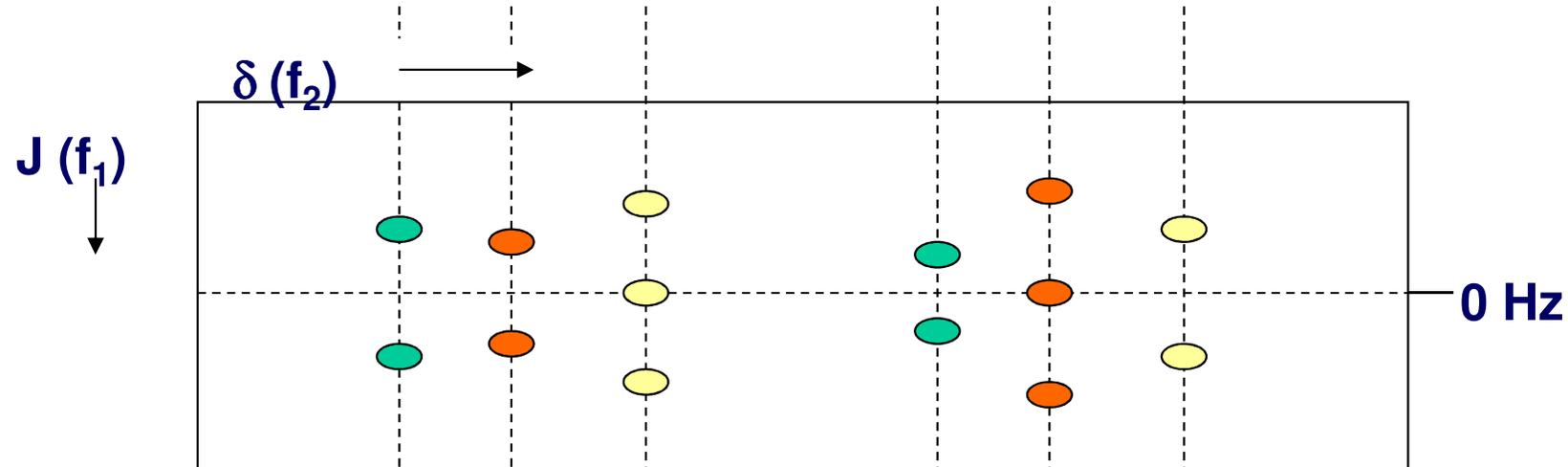
- We'll analyze it for a triplet and a doublet.

# HOMO2DJ - Many signals

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



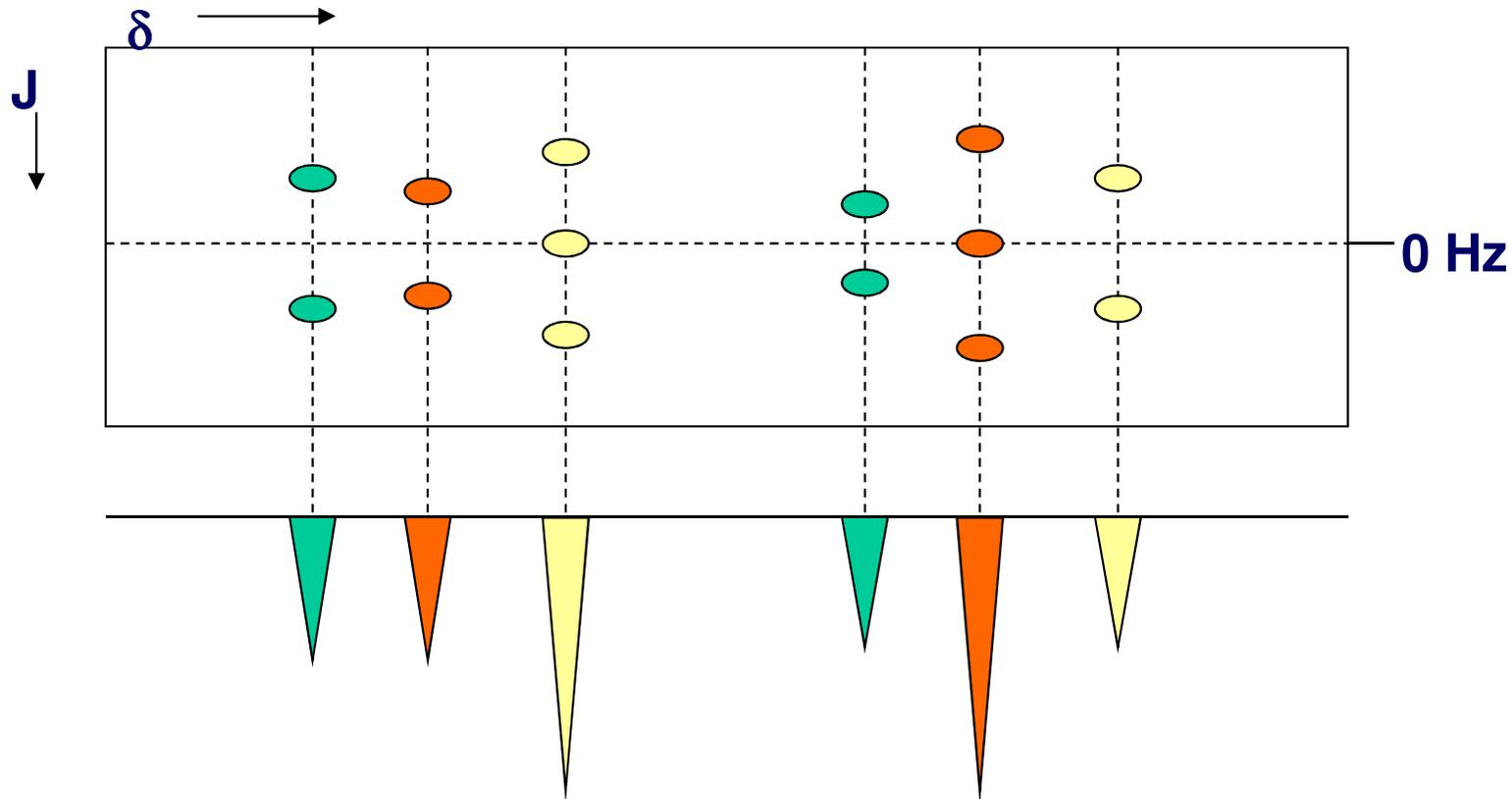
- We get an **HOMO2DJ** that has everything resolved in  $\delta$ s and **J**s:



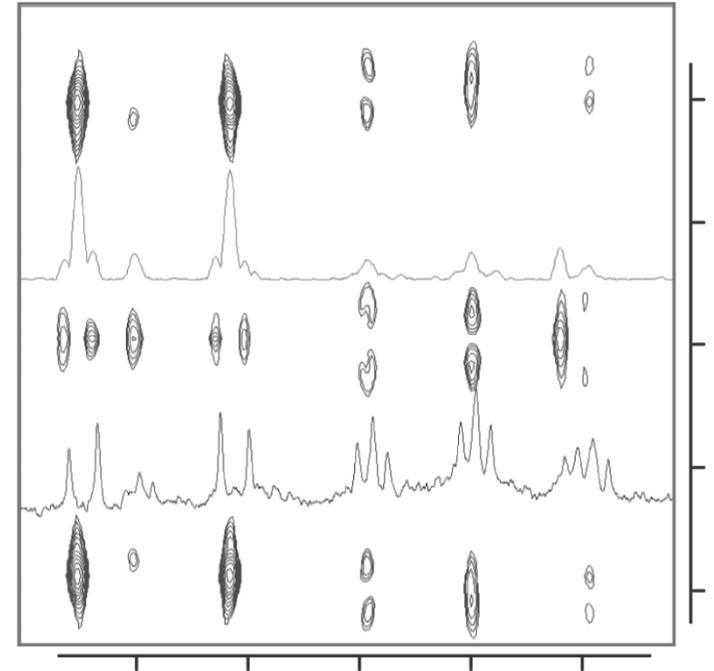
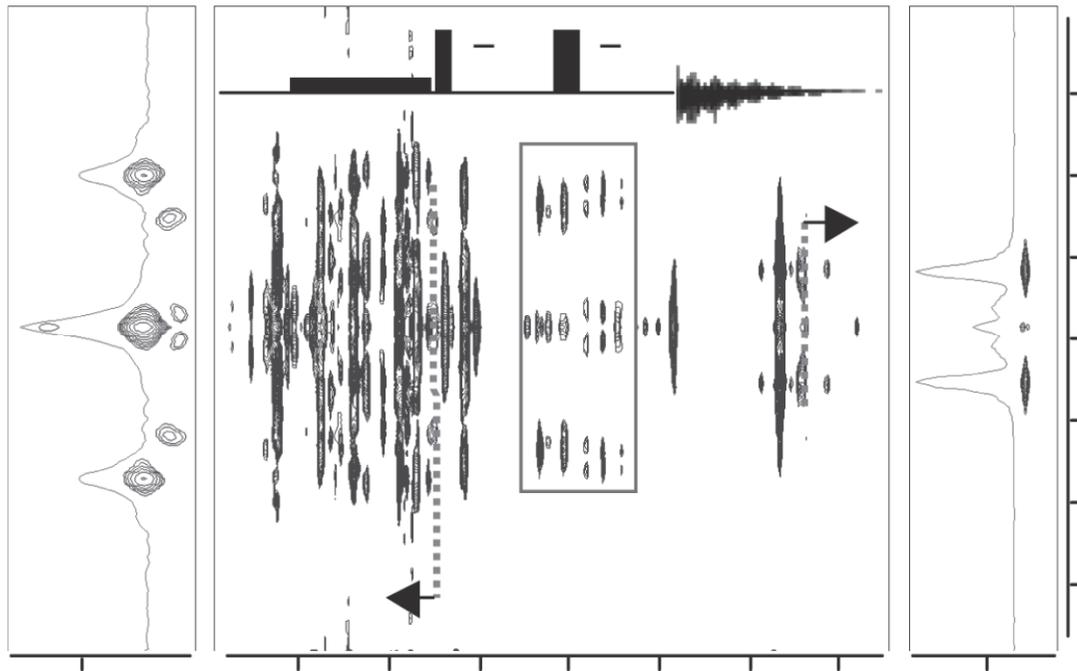
- We have all the  $\delta$  information on the  $f_2$  axis and the **J** data on the  $f_1$  axis.

# HOMO2DJ - Conclusion

- Another advantage is that if we project the 2D spectrum on its  $\delta$  axis, we basically get a fully decoupled  $^1\text{H}$  spectrum:

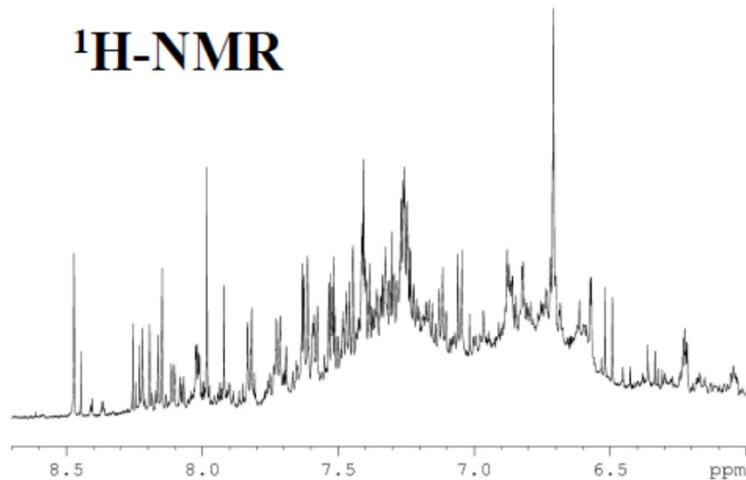


- Finally, since we take  $\sim 256$  or  $512$   $t_1$  experiments, we have that many points defining the  $J$  couplings which are between 1 and 20 Hz.
- For 50 Hz and 512  $t_1$  experiments, 0.09 Hz / point. We can measure  $J_{\text{HH}}$  with great accuracy on the  $f_1$  dimension.

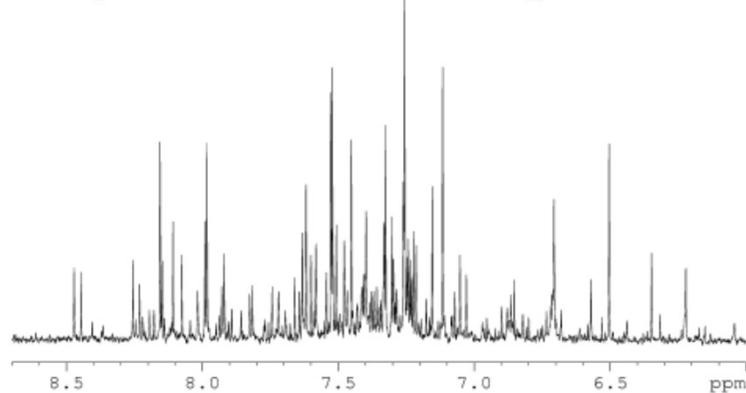


J-coupling resolved spectroscopy of rat CSF

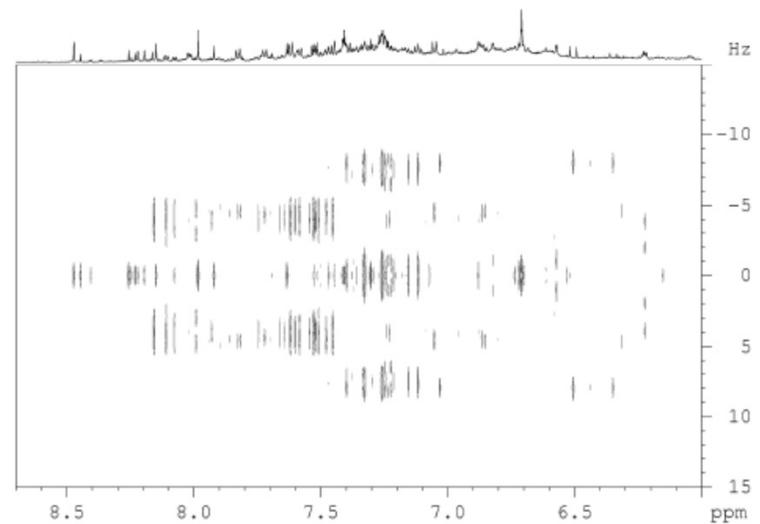
## $^1\text{H}$ -NMR



## Projected J-resolved spectrum



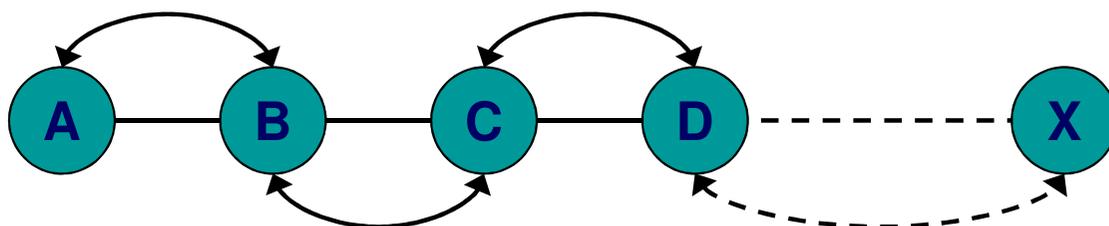
## 2D-J-resolved spectrum



*Arabidopsis* Root Exudate

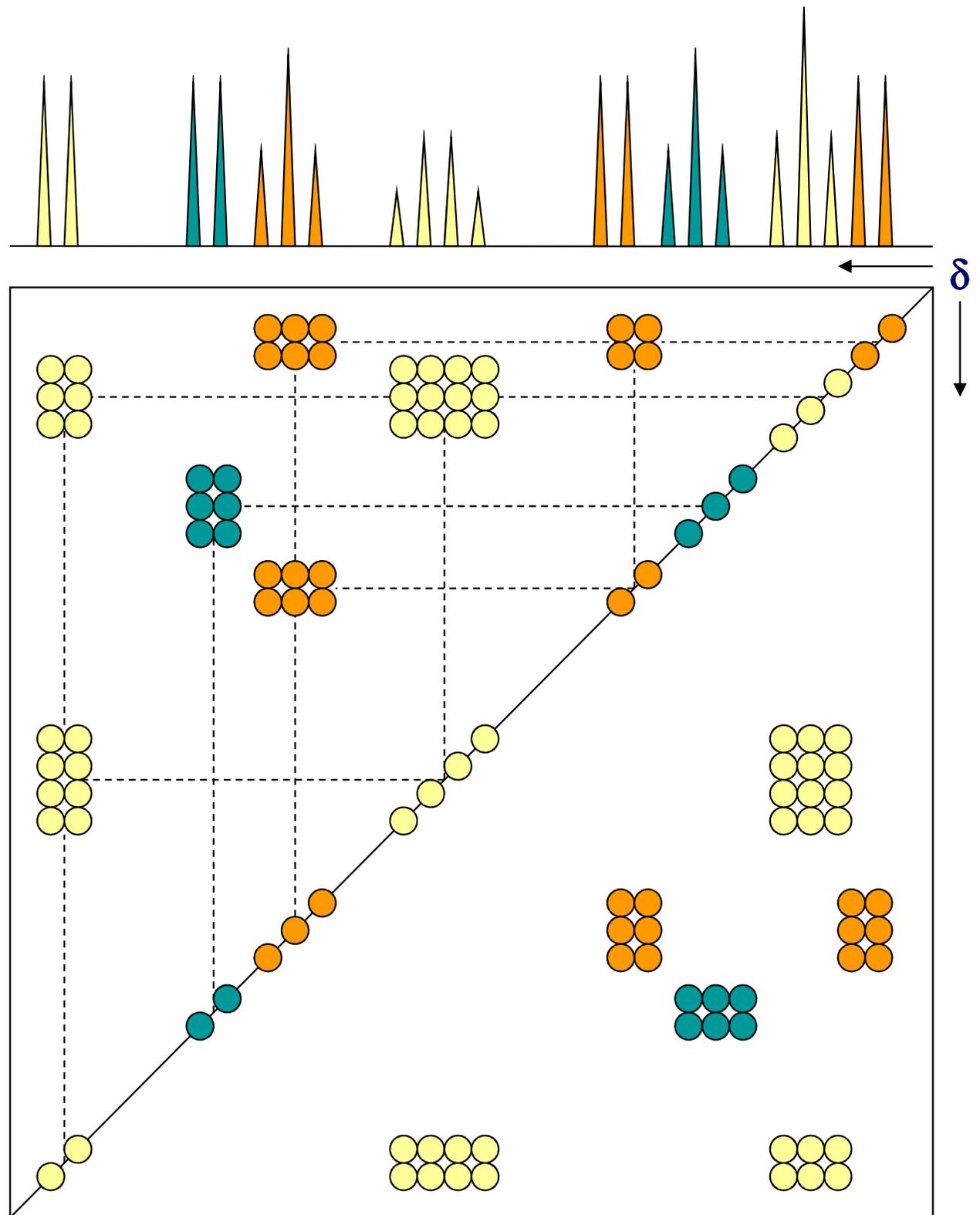
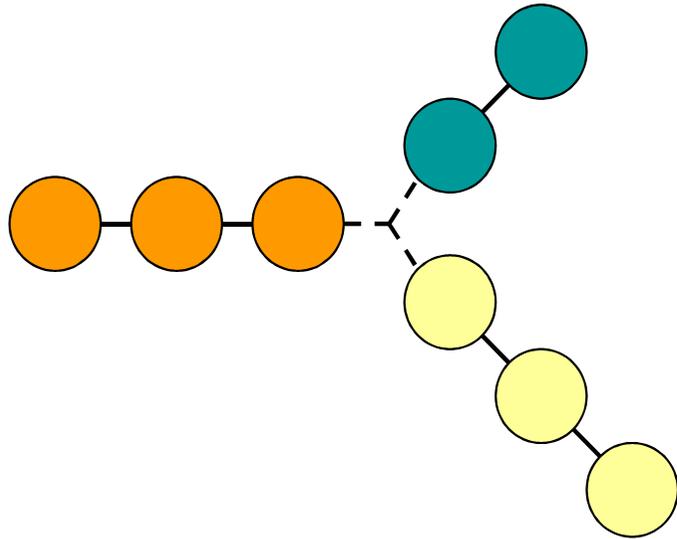
# TOCSY

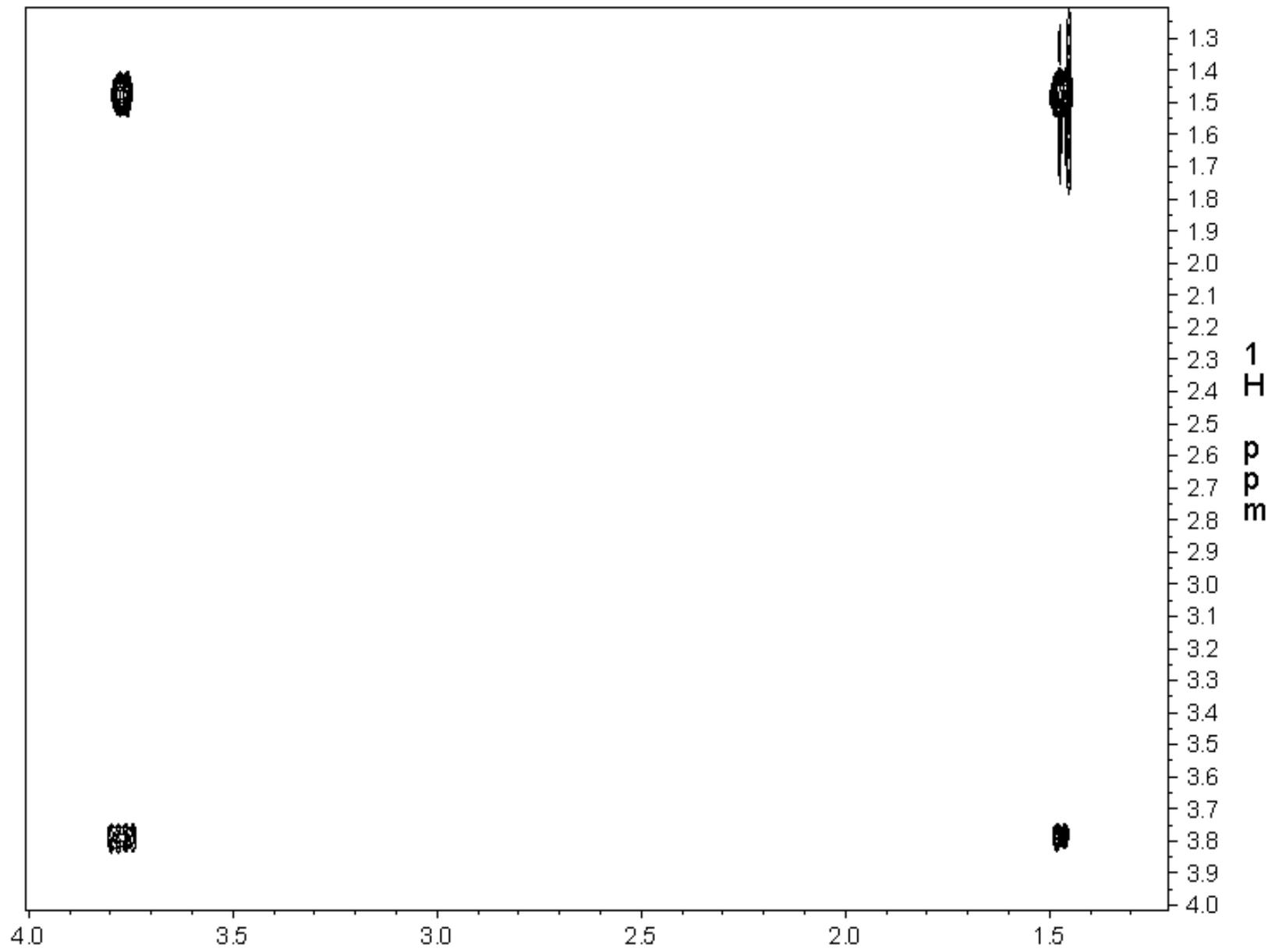
- **HOHAHA** (*HOmo-nuclear HArtmann-HAhn experiment*) or **TOCSY** (*Total Correlation Spectroscopy*).
- Its purpose is to identify a complete system of coupled spins. To make a very long story short, we have thorough mixing of all states in the system, and coherence from a certain spin in a coupled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





2D alanine

$^1\text{H}$  ppm

$^{13}\text{C}$  ppm

