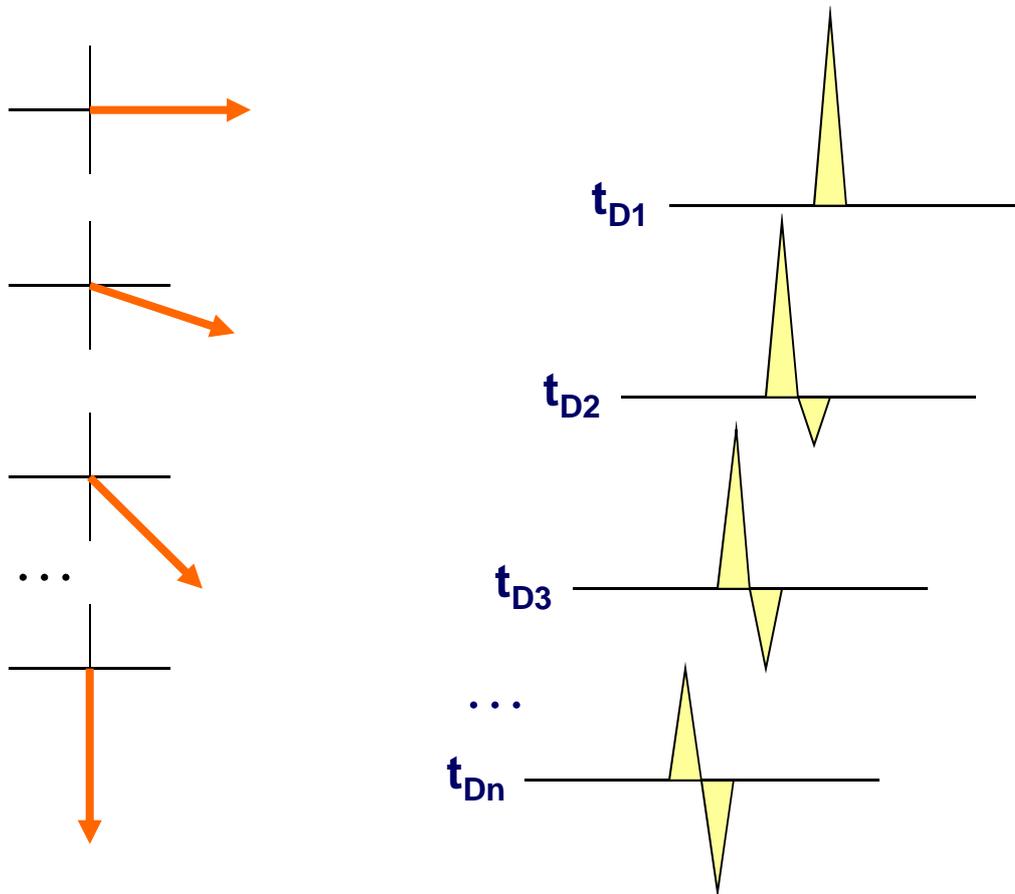


2D NMR spectroscopy

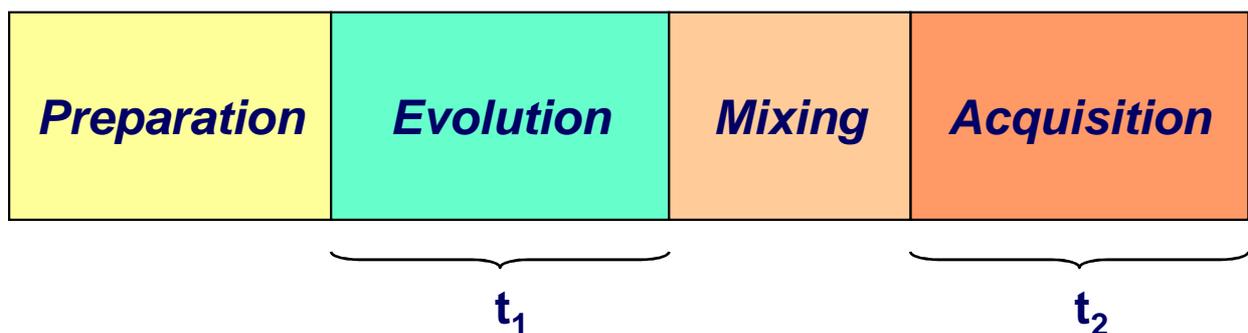
- So far we have been dealing with multiple pulses but a single dimension - that is, 1D spectra. We have seen, however, that a multiple pulse sequence can give different spectra which depend on the delay times we use.
- The 'basic' 2D spectrum would involve repeating a multiple pulse 1D sequence with 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 (**DE**):



- 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

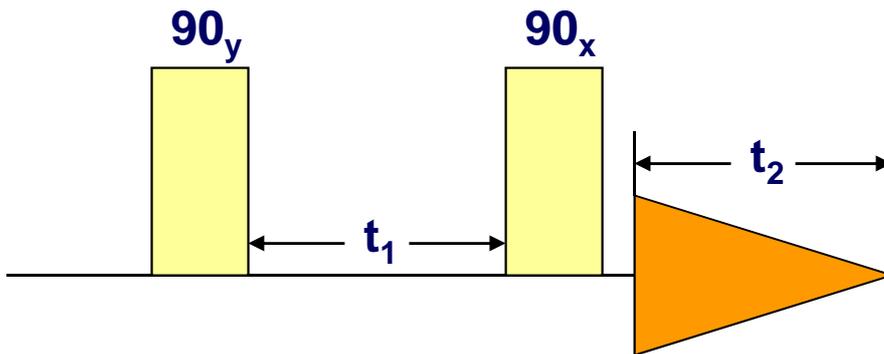
- There is some renaming that we need to do to be more in synch with the literature:
 - The first perturbation of the system (pulse) will now be called the **preparation** of the spin system.
 - The variable t_D is renamed the **evolution time**, t_1 .
 - We have a **mixing** event, in which information 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:



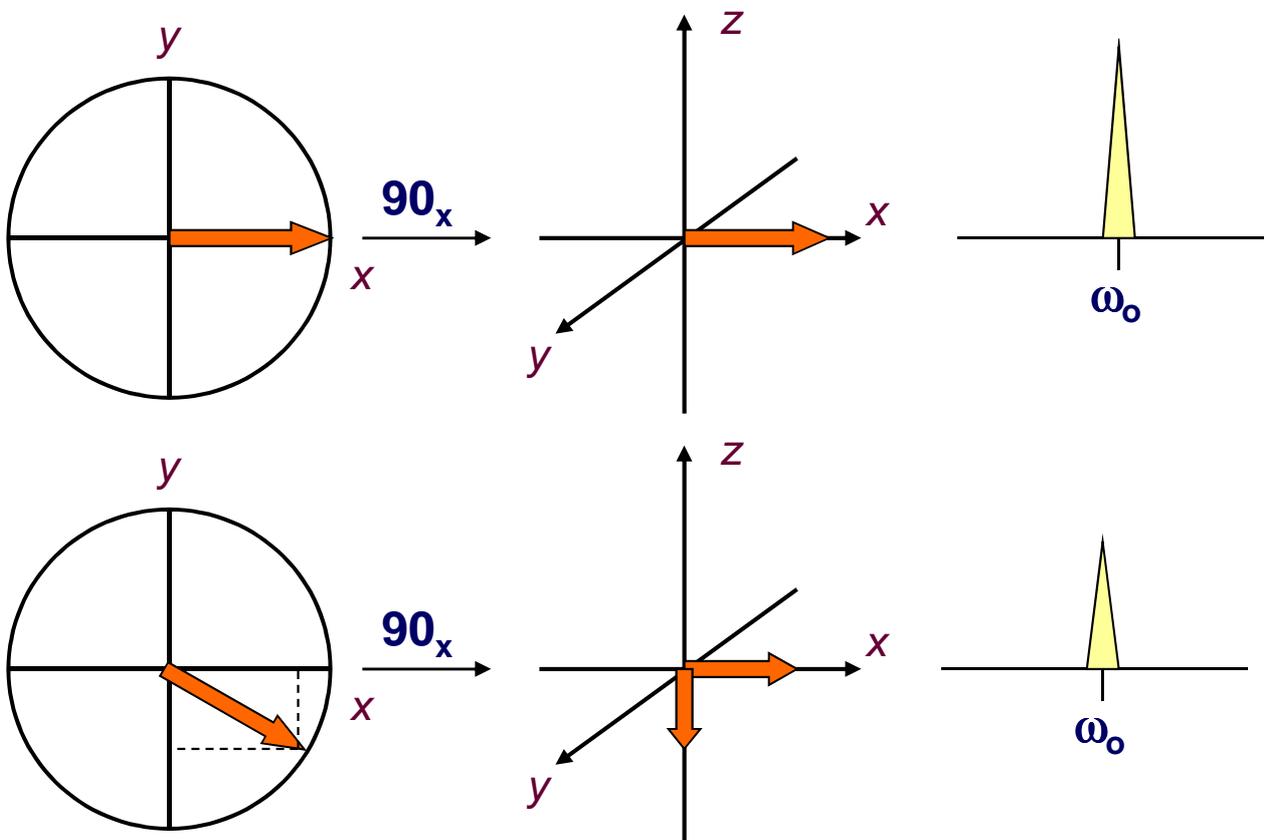
- t_1 is the variable delay time, and t_2 is the normal acquisition time. We can envision having f_1 and f_2 , for both frequencies...
- We'll see that this format is basically the same for all 2D experiments (and nD, for that matter...).

A rudimentary 2D experiment

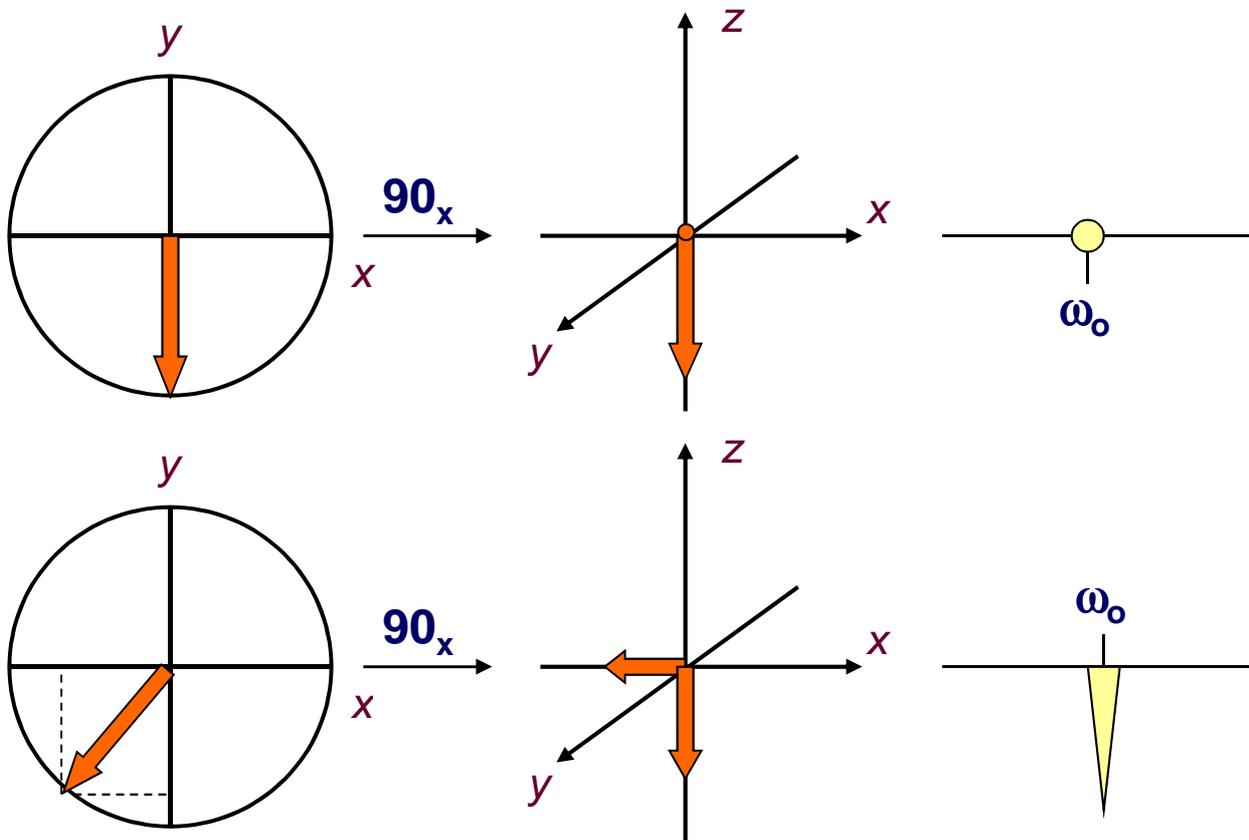
- We'll see how it works with the backbone of what will become the **COSY** pulse sequence. Think of this pulses, were t_1 is the preparation time:



- 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)

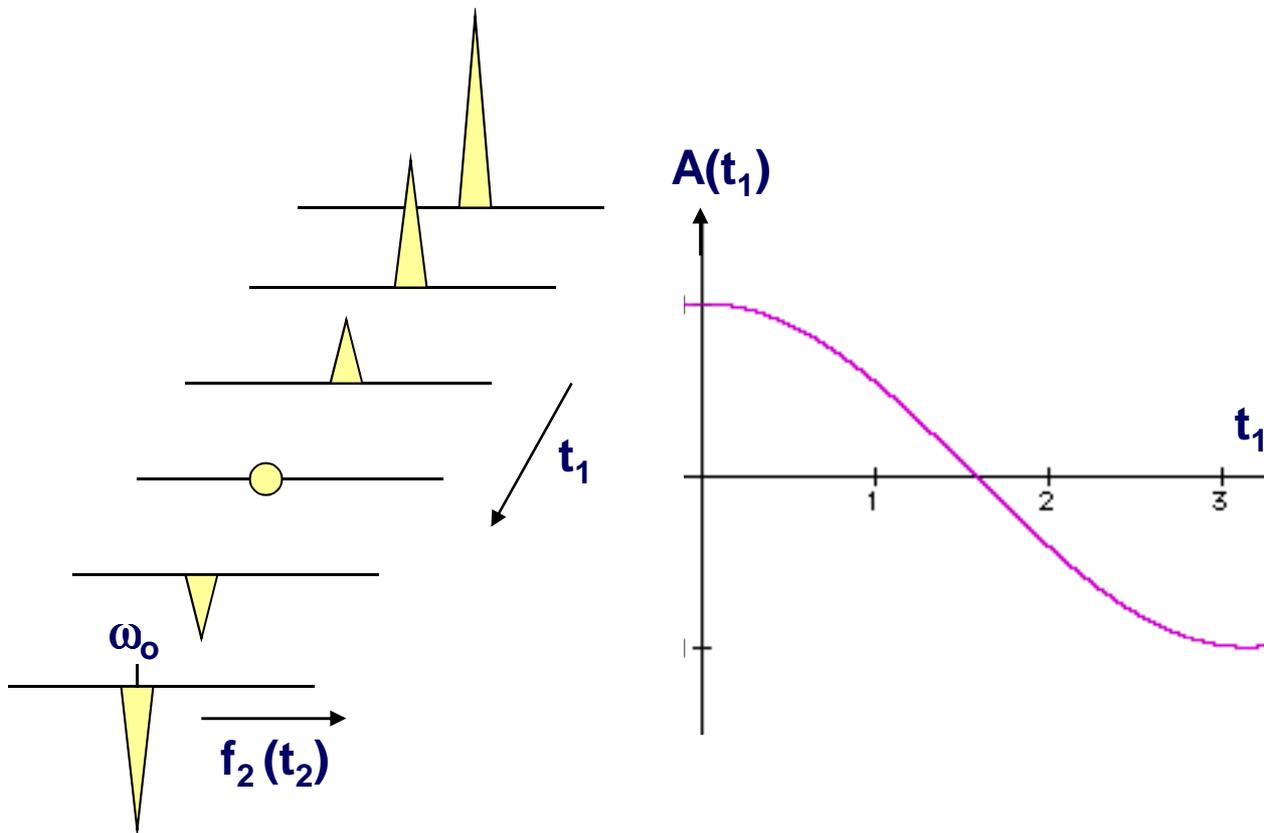


- The second $\pi/2$ pulse acts only on the y axis component of the magnetization of the $\langle xy \rangle$ plane.
- The x axis component is not affected, but its amplitude will depend on the frequency of the line.

$$A(t_1) = A_o * \cos(\omega_o * t_1)$$

The rudimentary 2D (...)

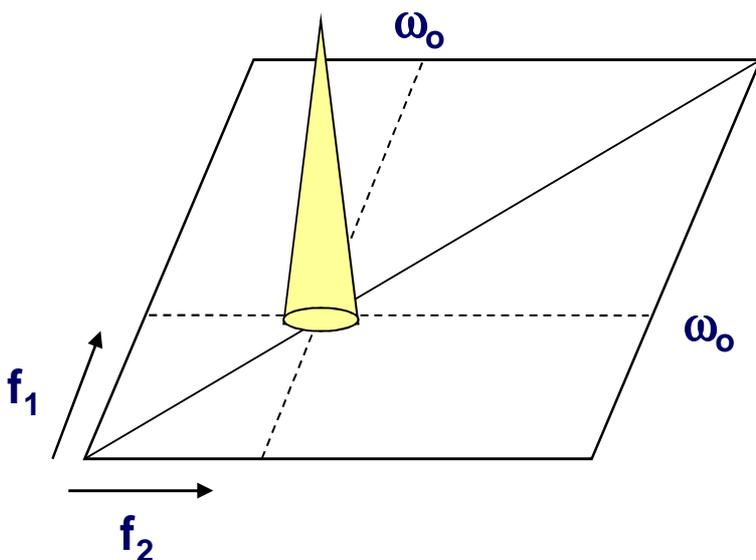
- If we plot all the spectra in a **stacked plot**, we get:



- 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.

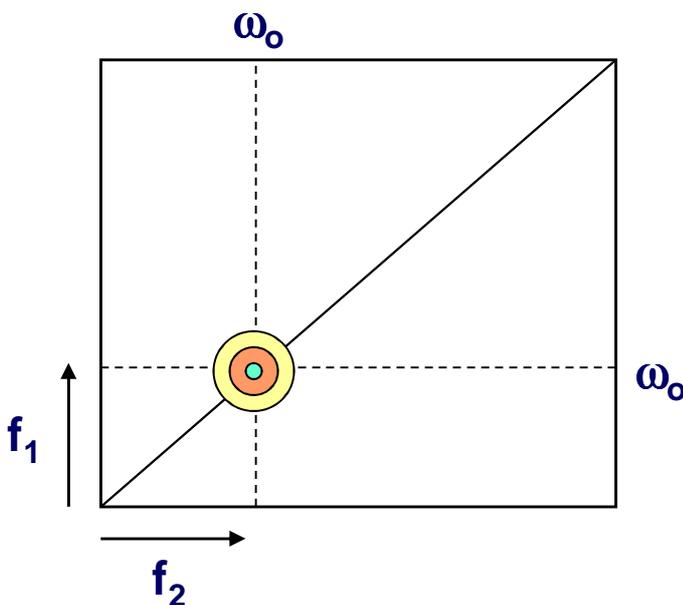
The rudimentary 2D (...)

- 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.

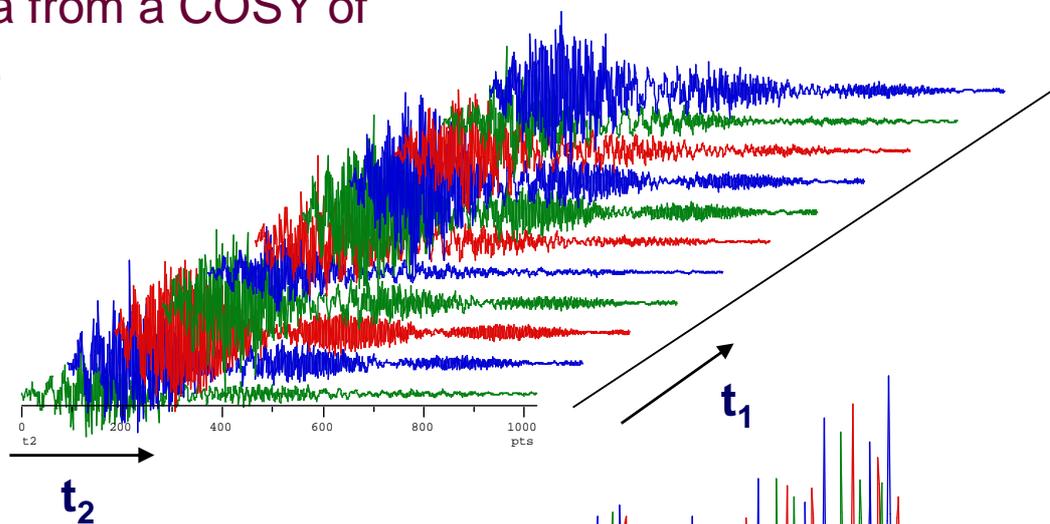


- Each slice is color-coded depending on the height of the peak.

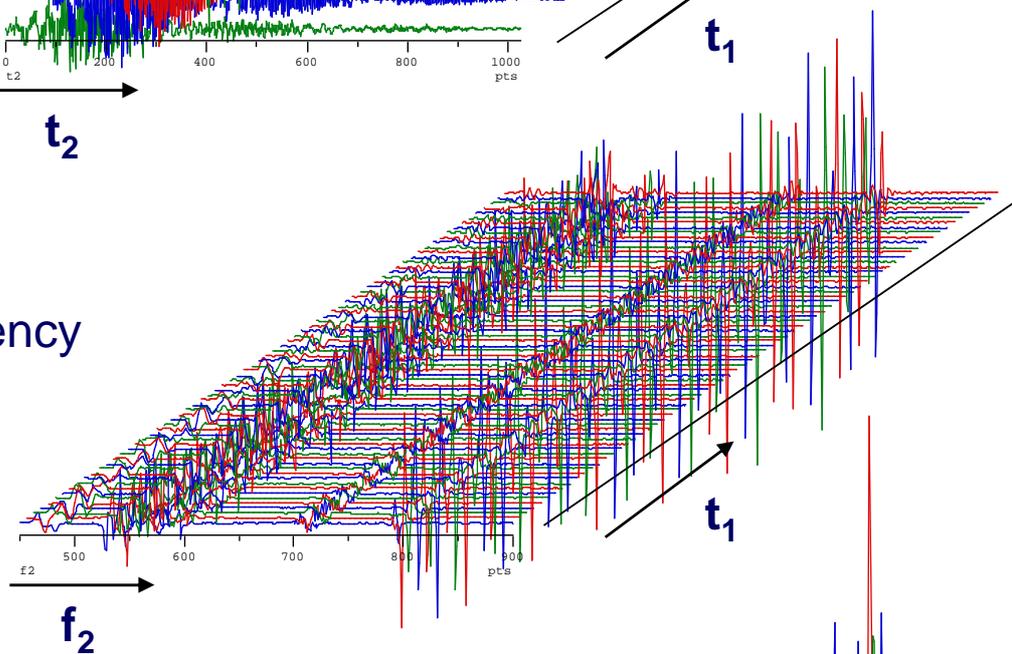
The same with some real data

- This is data from a COSY of pulegone...

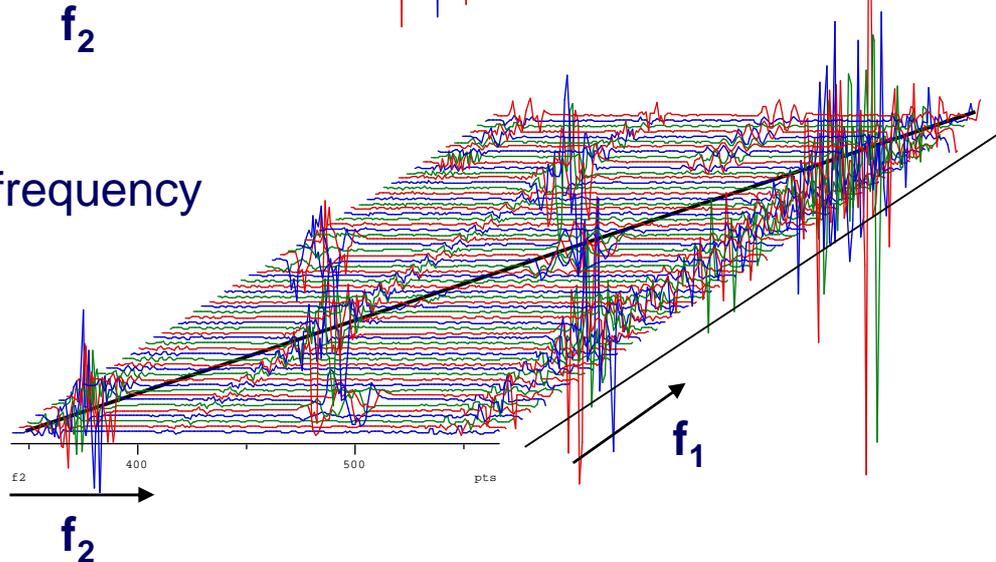
time - time



time - frequency

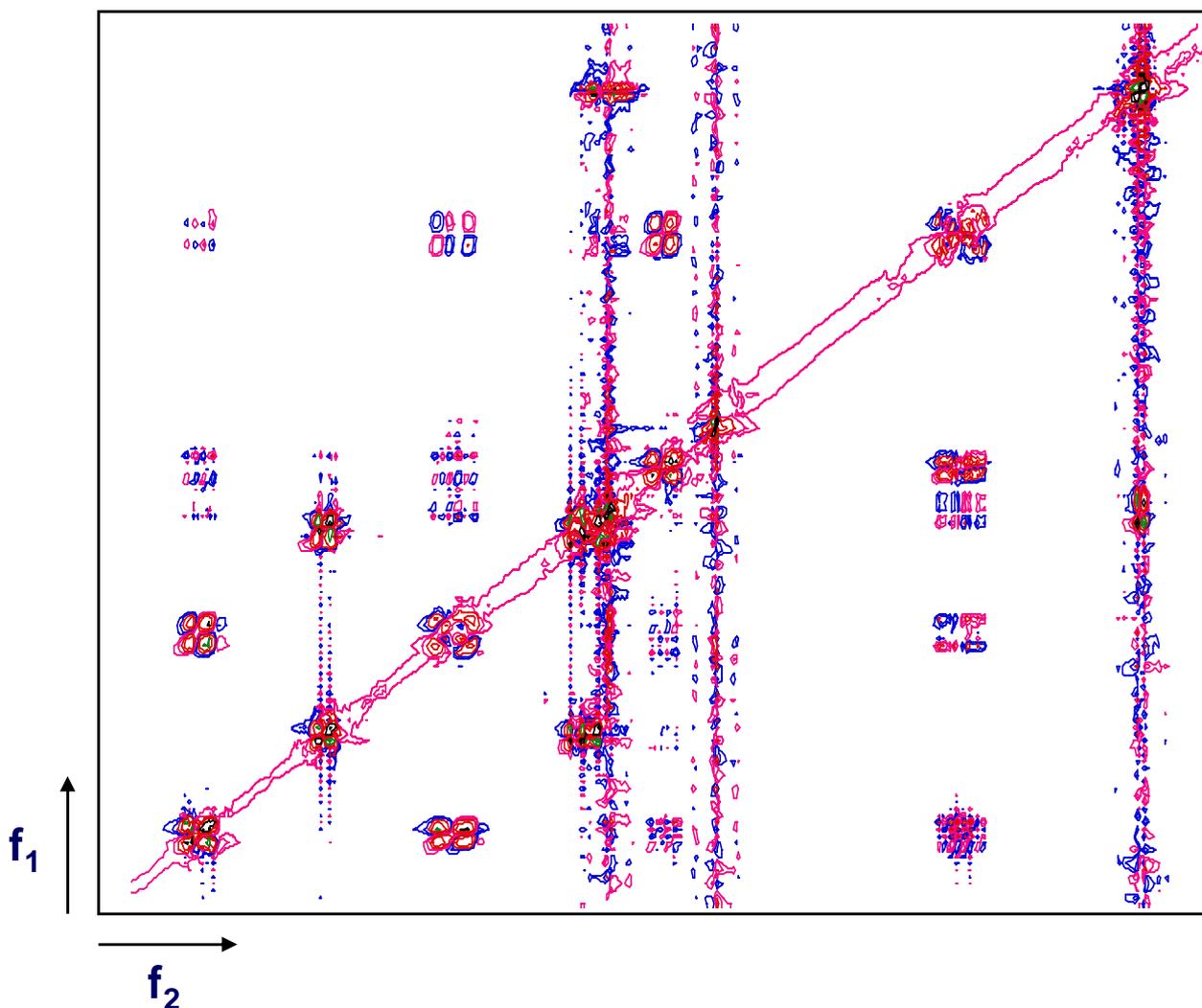


frequency - frequency



The same with some real data

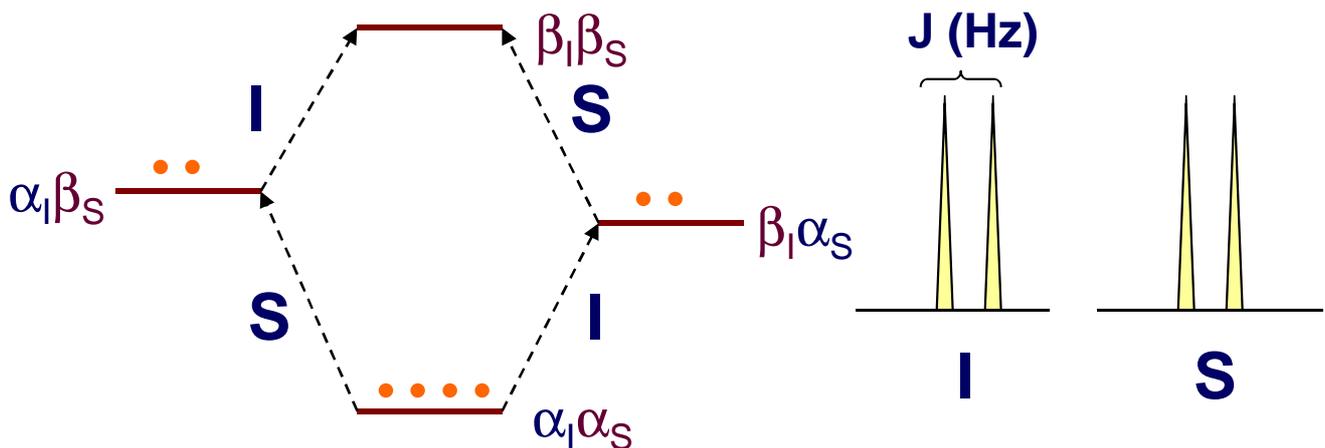
- Now the *contour-plot* showing all the *cross-peaks*:



- OK, where the heck did all the *off-diagonal* peaks come from, and what do they mean?
- I'll do the best I can to explain it, but again, there will be several black-box events. We really need a mathematical description to explain COSY rigorously.

Homonuclear correlation - COSY

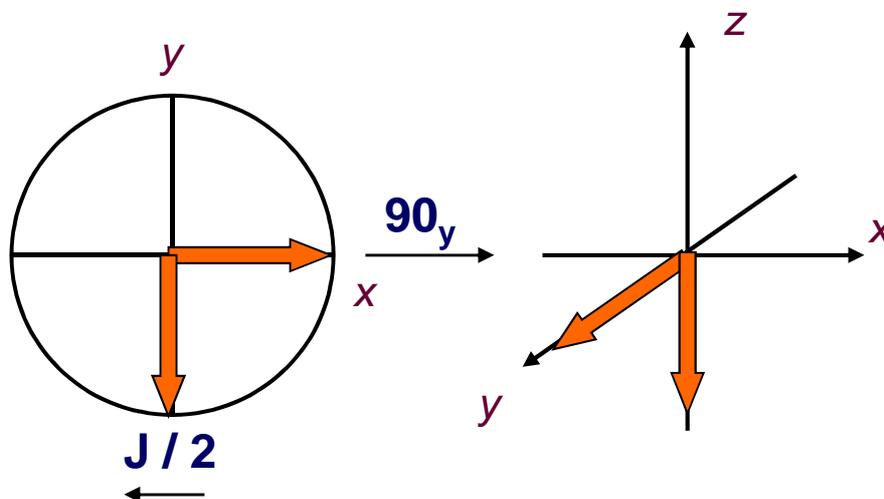
- **COSY** stands for **CORrelation SPECTROSCOPY**, and for this particular case in which we are dealing with homonuclear couplings, **homonuclear correlation spectroscopy**.
- In our development of the 2D idea we considered an isolated spin not coupled to any other spin. Obviously, this is not really 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.
- With this basic idea we'll try to see the effect of the COSY $90_y - t_1 - 90_y - t_1$ pulse sequence on a pair of coupled spins. If we recall the 2 spin-system energy diagram:



- We see that if we are looking at **I** and apply both $\pi / 2$ pulses, (a pseudo π pulse) we will invert some of the population of spin **S**, and this will have an effect on **I** (polarization transfer).

Homonuclear correlation (continued)

- Since the **I** to **S** or **S** to **I** polarization transfers are the same, we'll explain it for **I** to **S** and assume we get the same for **S** to **I**. We first perturb **I** and analyze what happens to **S**.
- After the first $\pi/2$, we have the two **I** vectors in the x axis, one moving at $\omega_1 + J/2$ and the other at $\omega_1 - J/2$. The effect of the second pulse is that it will put the components of the magnetization aligned with **y** on the **-z** axis, which means a partial inversion of the **I** populations.
- For $t_1 = 0$, we have complete inversion of the **I** spins (it is a π pulse and the signal intensity of **S** does not change. For all other times we will have a change on the **S** intensity that depends periodically on the resonance frequency of **I**).
- The variation of the population inversion for **I** depends on the cosine (or sine) of its resonance frequency. Considering that we are on-resonance with one of the lines and if $t_1 = 1/4 J$:

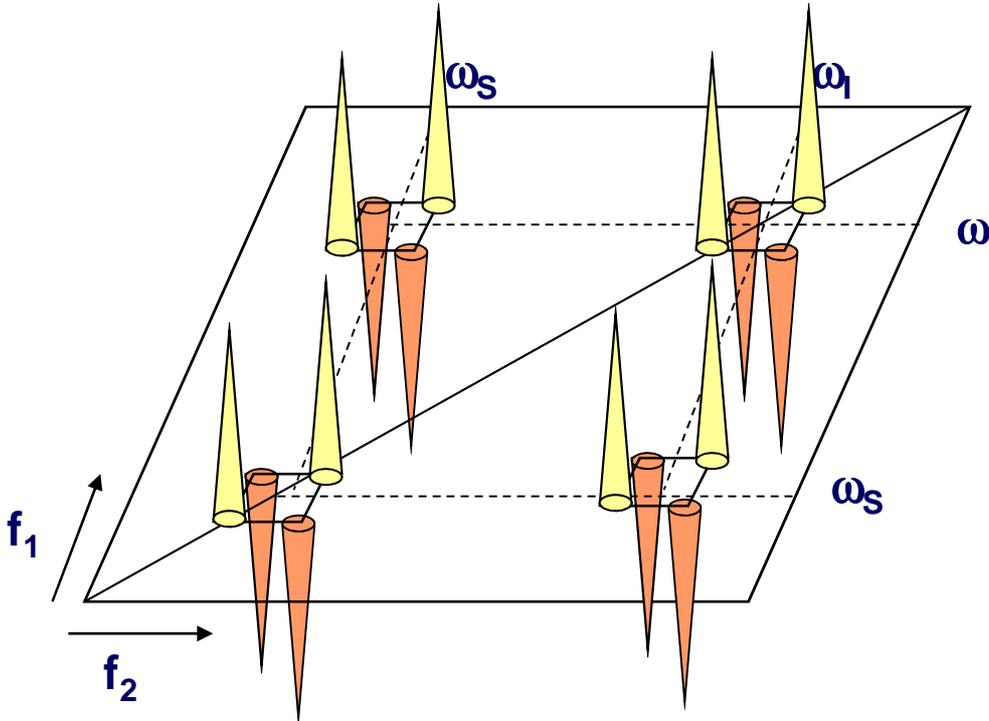


Homonuclear correlation (...)

- If we do it really general (nothing on-resonance), we would come to this relationship for the change of the **S** signal (after the $\pi / 2$ pulse) as a function of the **I** resonance frequency and J_{IS} coupling:

$$A_S(t_1, t_2) = A_0 * \sin(\omega_I * t_1) * \sin(J_{IS} * t_1) * \sin(\omega_S * t_2) * \sin(J_{IS} * t_2)$$

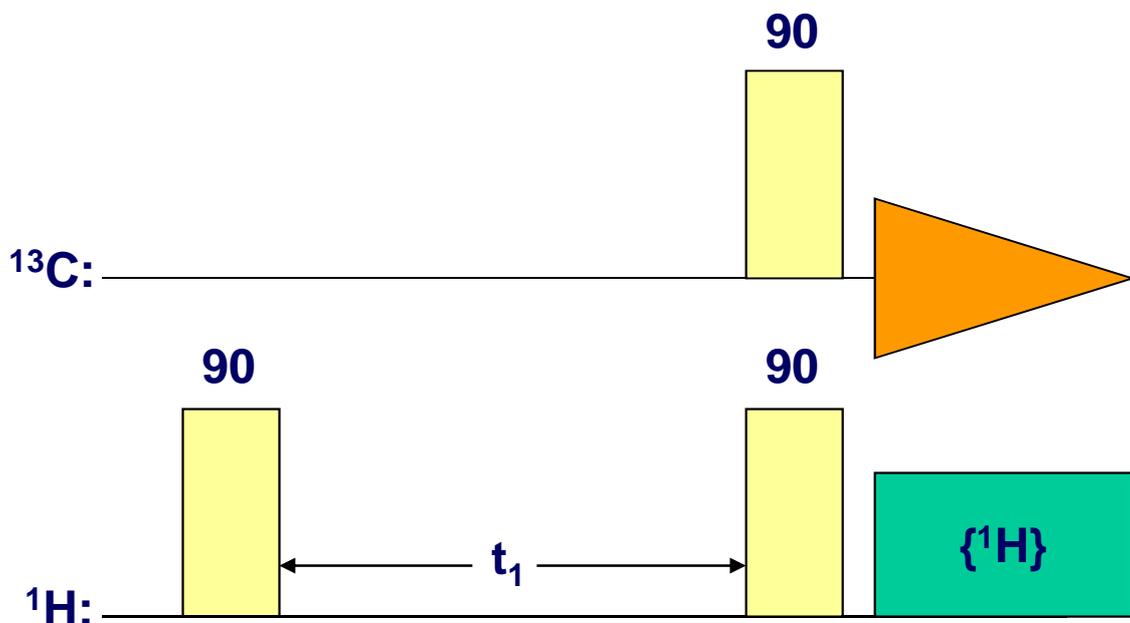
- After Fourier transformation on t_1 and t_2 , and considering also the **I** spin, we get:



- This is the typical pattern for a doublet in a **phase-sensitive COSY**. The sines make the signals dispersive in f_1 and f_2 .

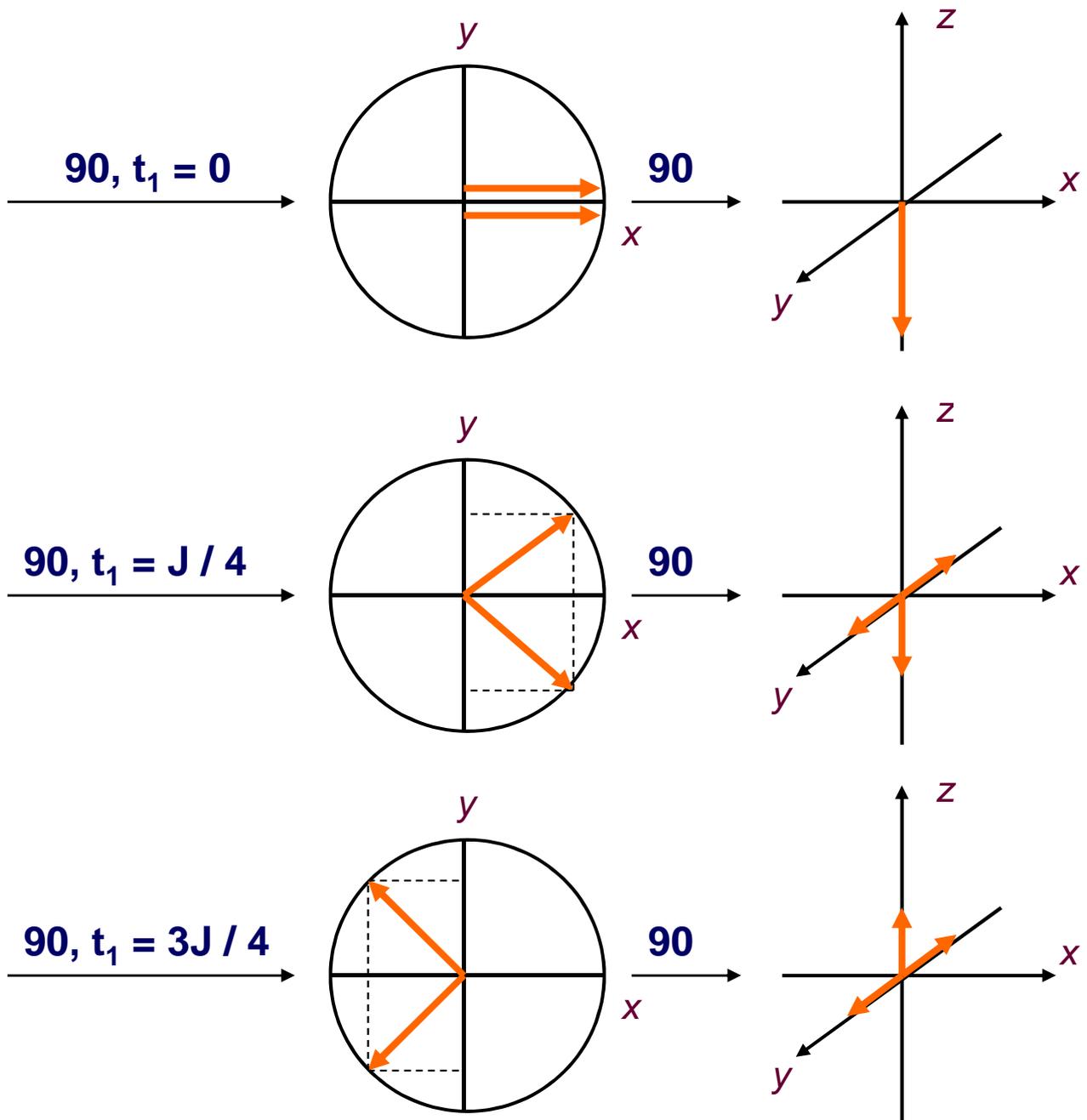
Heteronuclear correlation - HETCOR

- The COSY (or *Jenner experiment*) was one of the first 2D experiments developed (1971), and is one of the most useful 2D sequences for structural elucidation. There are thousands of variants and improvements (*DQF-COSY*, *E-COSY*, etc.).
- 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 *HETero-nuclear CORrelation 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:



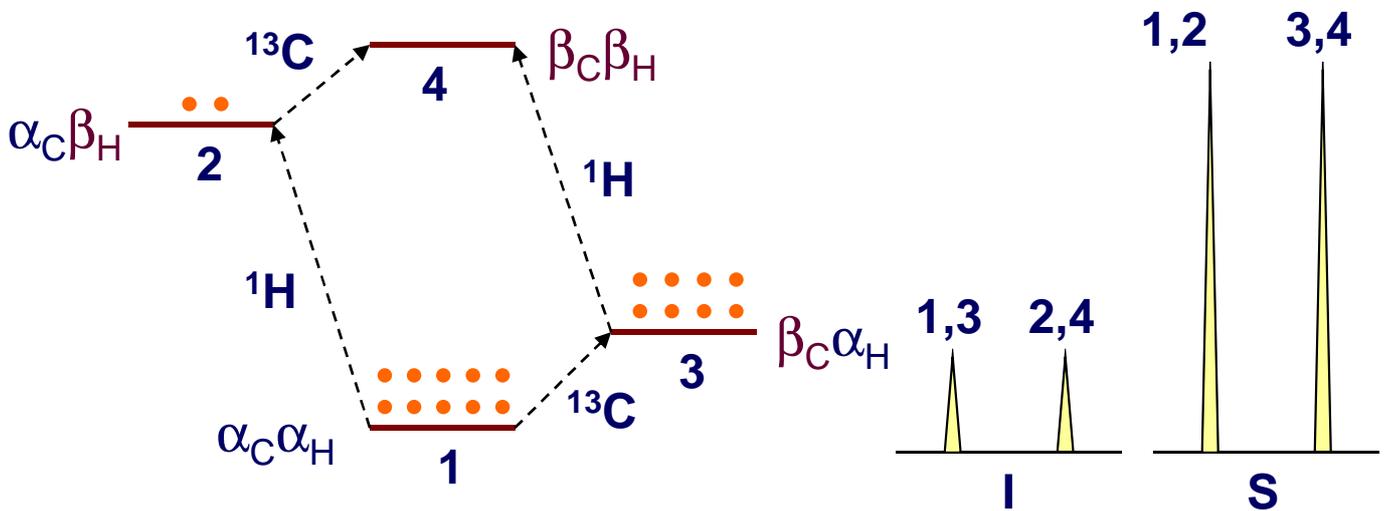
HETCOR (continued)

- We first analyze what happens to the ^1H proton (that is, we'll see how the ^1H populations are affected), and then see how the ^{13}C signal is affected. For different t_1 values we have:



HETCOR (...)

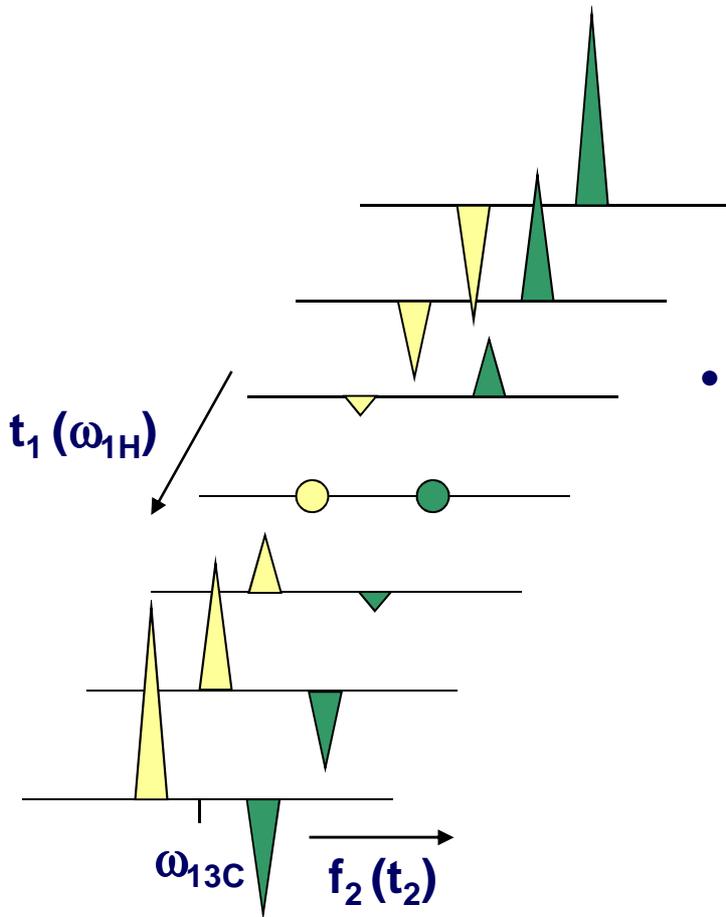
- As was the case for COSY, we see that depending on the t_1 time we use, we have a variation of the population inversion of the proton. We can clearly see that the amount of inversion depends on the J_{CH} coupling.
- Although we did it on-resonance for simplicity, we can easily show that it will also depend on the 1H frequency (δ).
- From what we know from SPI and INEPT, we can tell that the periodic variation on the 1H population inversion will have the same periodic effect on the polarization transfer to the ^{13}C . In this case, the two-spin energy diagram is for 1H and ^{13}C :



- Now, since the intensity of the ^{13}C signal that we detect on t_2 is modulated by the frequency of the proton coupled to it, the ^{13}C FID will have information on the ^{13}C **and** 1H frequencies.

HETCOR (...)

- Again, the intensity of the ^{13}C lines will depend on the ^1H population inversion, thus on $\omega_{1\text{H}}$. If we use a stacked plot for different t_1 times, we get:



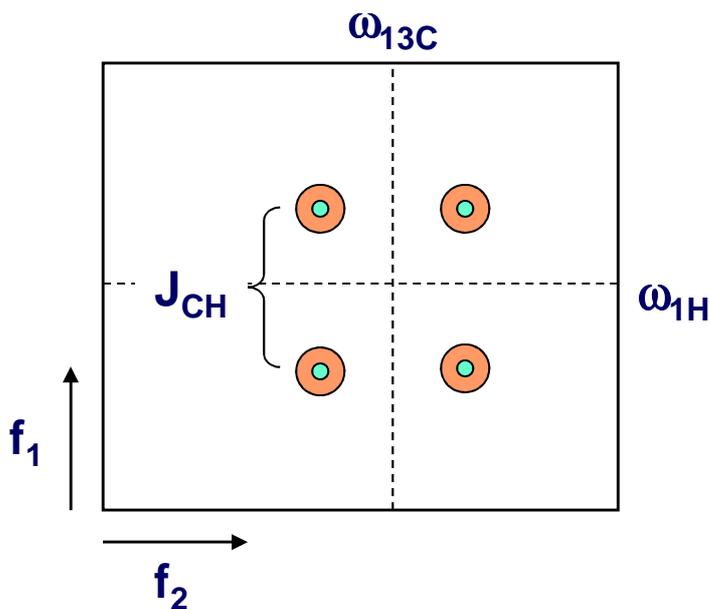
- The intensity of the two ^{13}C lines will vary with the $\omega_{1\text{H}}$ and J_{CH} between +5 and -3 as it did in the INEPT sequence.

- Mathematically, the intensity of one of the ^{13}C lines from the multiplet will be an equation that depends on $\omega_{13\text{C}}$ on t_2 and $\omega_{1\text{H}}$ on t_1 , as well as J_{CH} on both time domains:

$$A_{13\text{C}}(t_1, t_2) \propto \text{trig}(\omega_{1\text{H}}t_1) * \text{trig}(\omega_{13\text{C}}t_2) * \text{trig}(J_{\text{CH}}t_1) * \text{trig}(J_{\text{CH}}t_2)$$

HETCOR (...)

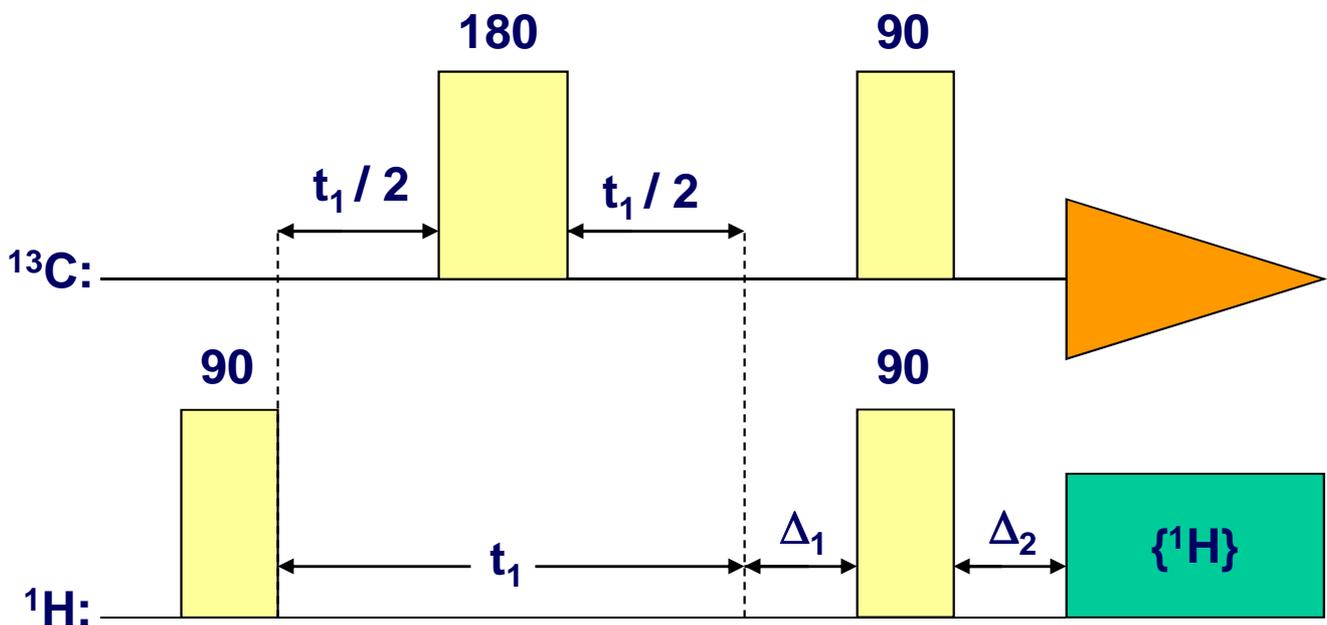
- Again, Fourier transformation on both time domains gives us the 2D correlation spectrum, in this case as a contour plot:



- 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.
- Pretty cool. 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.
- We'll see how we can get rid of them without decoupling (if we decouple we won't see ^1H to ^{13}C polarization transfer...).

HETCOR with no J_{CH} coupling

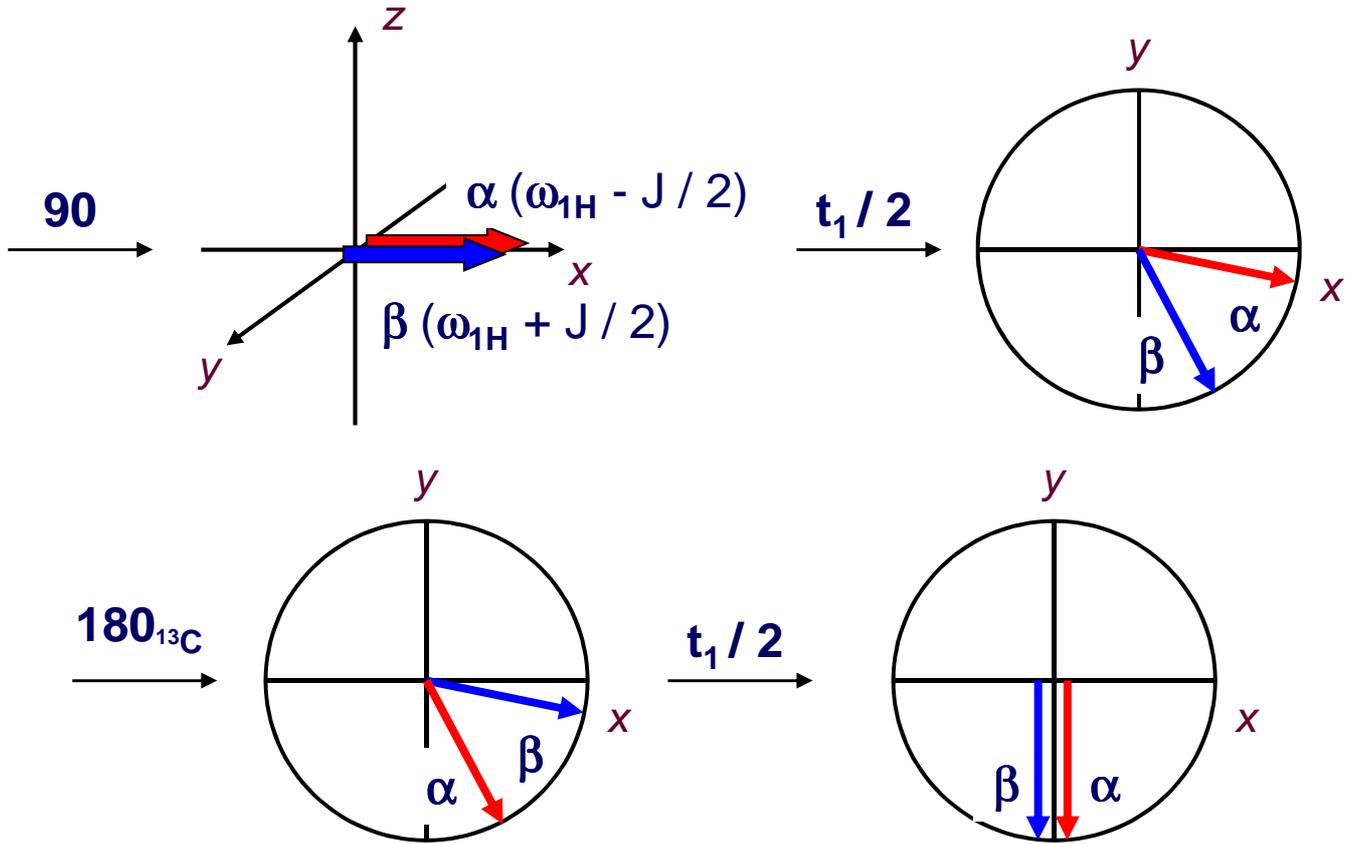
- The idea behind it is pretty much the same stuff we did with the refocused INEPT experiment.



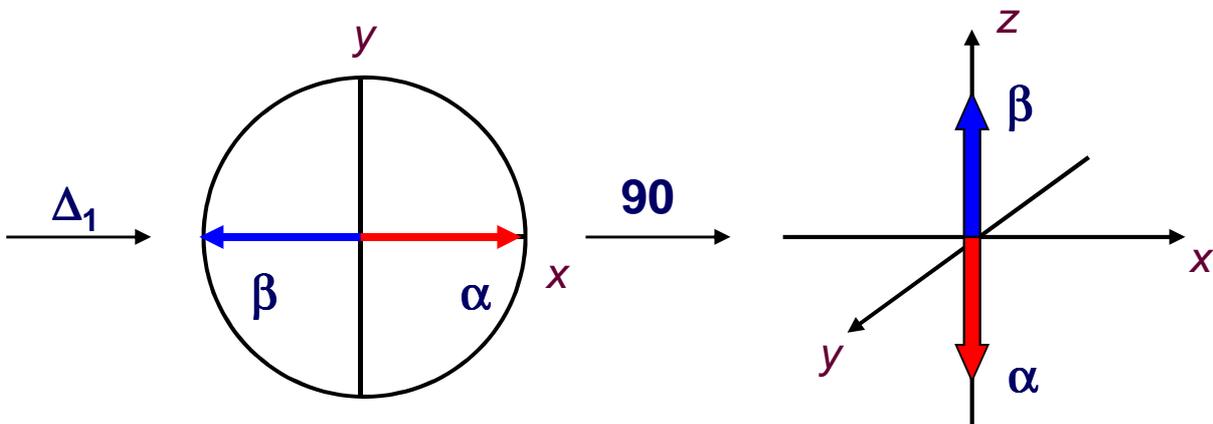
- We use a ^{13}C π pulse to refocus ^1H magnetization, and two delays to maximize polarization transfer from ^1H to ^{13}C and to get refocusing of ^{13}C vectors before decoupling.
- As in INEPT, the effectiveness of the transfer will depend on the delay Δ and the carbon type. We use an average value.
- We'll analyze the case of a methine (CH) carbon...

HETCOR with no J_{CH} coupling (continued)

- For a certain t_1 value, the 1H magnetization behavior is:

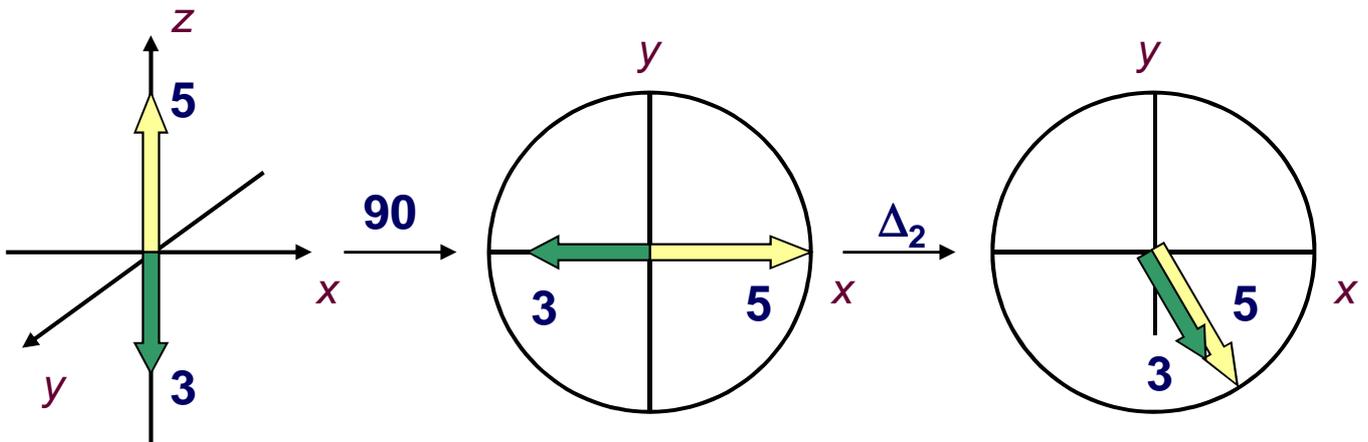


- Now, if we set Δ_1 to $1/2J$ both 1H vectors will dephase by exactly 180 degrees in this period. This is when we have maximum population inversion for this particular t_1 , and no J_{CH} effects:

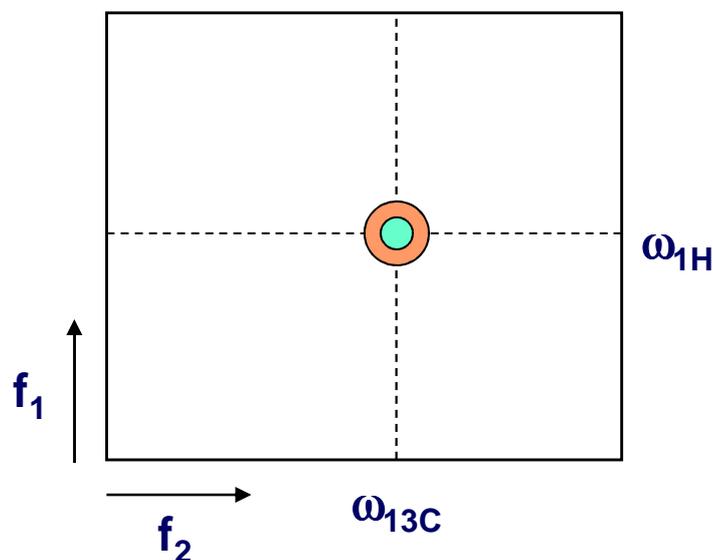


HETCOR with no J_{CH} coupling (...)

- Now we look at the ^{13}C magnetization. After the proton $\pi/2$ we will have the two ^{13}C vectors separated in a 5/3 ratio on the $\langle z \rangle$ axis. After the second delay Δ_2 (set to $1/2J$) they will refocus and come together:

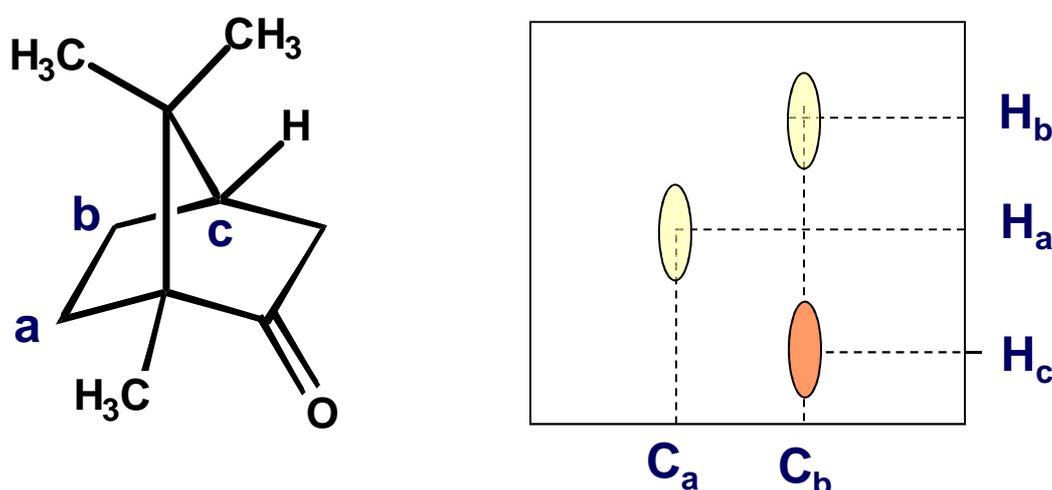


- We can now decouple 1H because the ^{13}C magnetization is refocused. The 2D spectrum now has no J_{CH} couplings (but it still has the chemical shift information), and we just see a single cross-peak where formed by the two chemical shifts:



Long range HETCOR (continued)

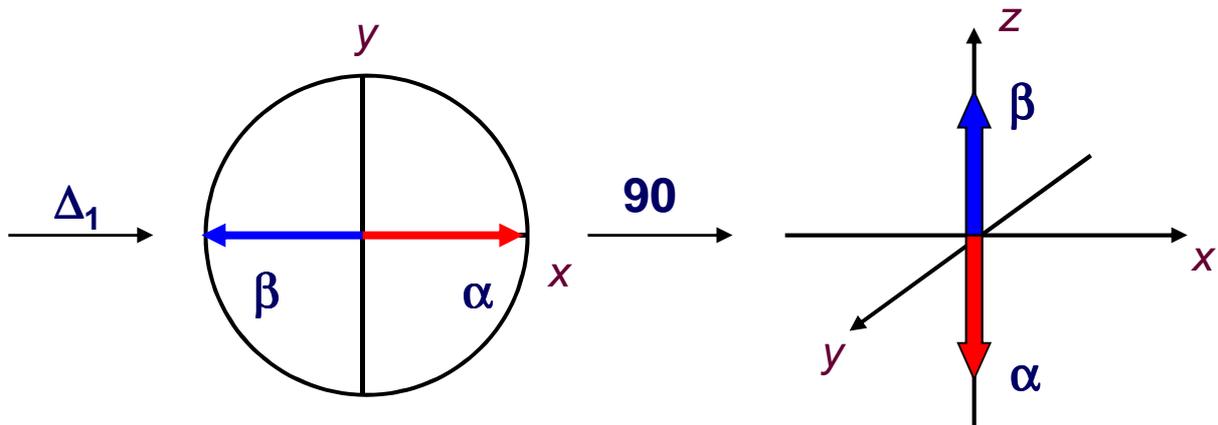
- The problem here is that both carbons **a** and **b** are pretty similar chemically and magnetically: From this data alone we would not be able to determine which one is which.
- It would be nice if we could somehow determine which of the two carbons is the one closer to the proton at **C_c**, because we would unambiguously assign these carbons in camphor:



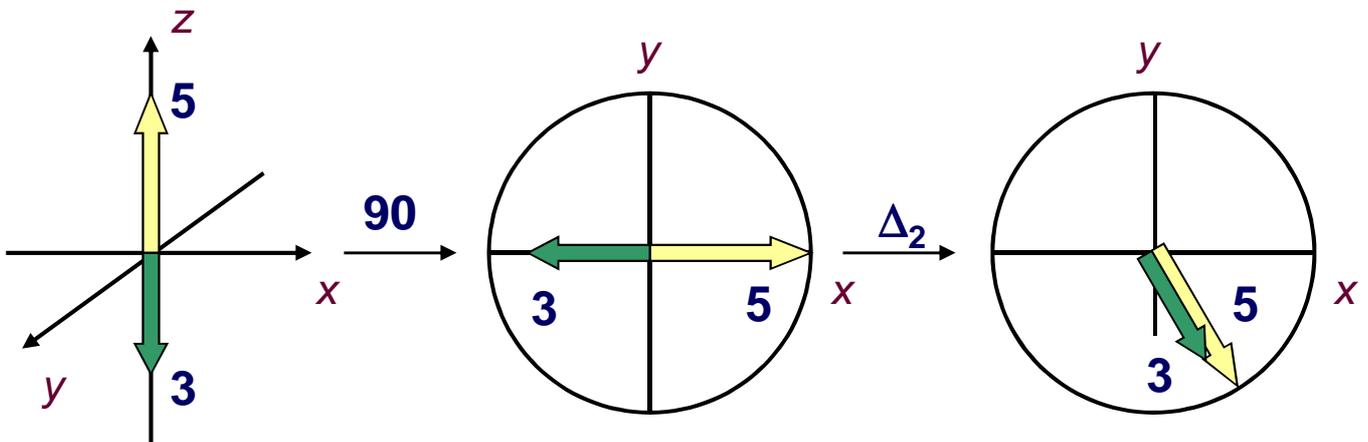
- How can we do this? There is, in principle, a very simple experiment that relies on long-range CH couplings.
- Apart from $^1J_{\text{CH}}$ couplings, carbons and protons will show long-range couplings, which can be across two or three bonds (either $^2J_{\text{CH}}$ or $^3J_{\text{CH}}$). Their values are a lot smaller than the direct couplings, but are still considerably large, in the order of 5 to 20 Hz.
- Now, how can we twitch the HETCOR pulse sequence to show us nuclei correlated through long-range couplings?

Long range HETCOR (...)

- The key is to understand what the different delays in the pulse sequence do, particularly the Δ_1 and Δ_2 delays. These were used to refocus antiphase ^{13}C magnetization. For the ^1H part of the sequence:



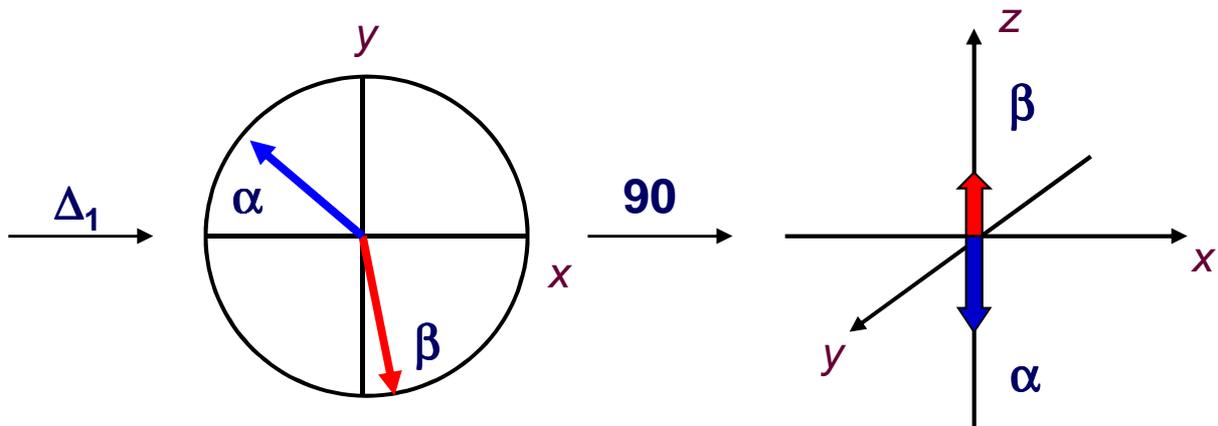
- For the ^{13}C part:



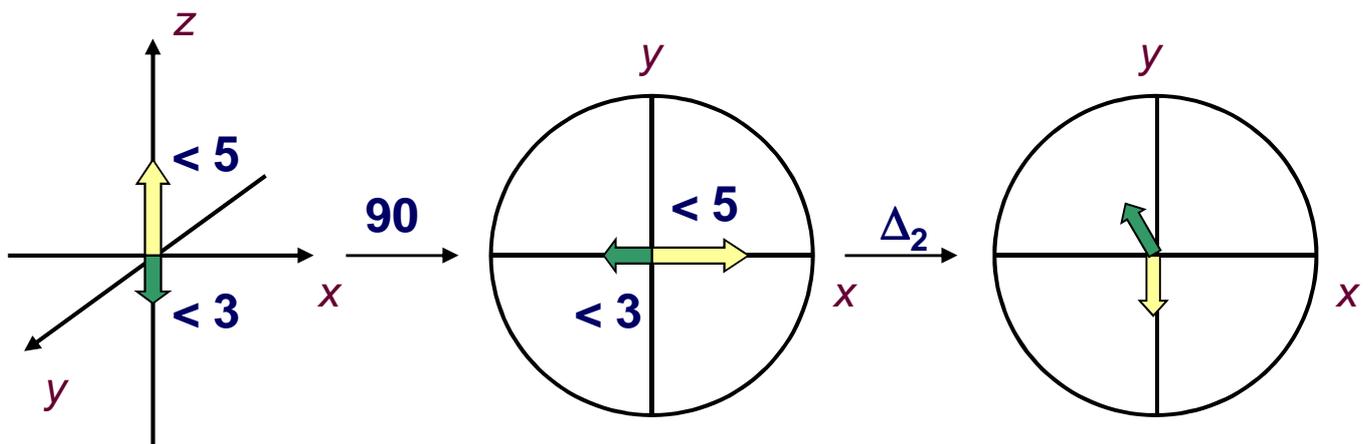
- In order to get refocusing, i.e., to get the '-3' and the '+5' vectors aligned, and in the case of a methine (CH), the Δ_1 and Δ_2 delays have to be $1/2 * ^1J_{\text{CH}}$. So, what would happen if we set the Δ_1 and Δ_2 delays to $1/2 * ^2J_{\text{CH}}$?

Long range HETCOR (...)

- To begin with, Δ_1 and Δ_2 will be in the order of 50 ms instead of 5 ms, which is much longer than before. What will happen now is that antiphase ^{13}C magnetization due to $^1\text{J}_{\text{CH}}$ couplings will not refocus, and will tend to cancel out. For the ^1H part of the refocusing:



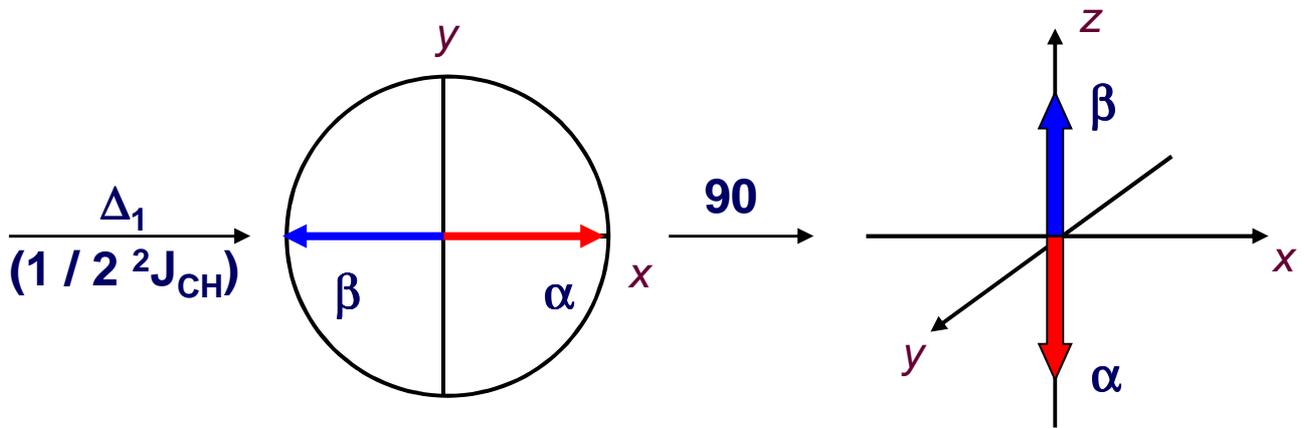
- The delay values are now way off the mark for $^1\text{J}_{\text{CH}}$, and we do not have complete inversion of the ^1H populations. Now, for the ^{13}C part:



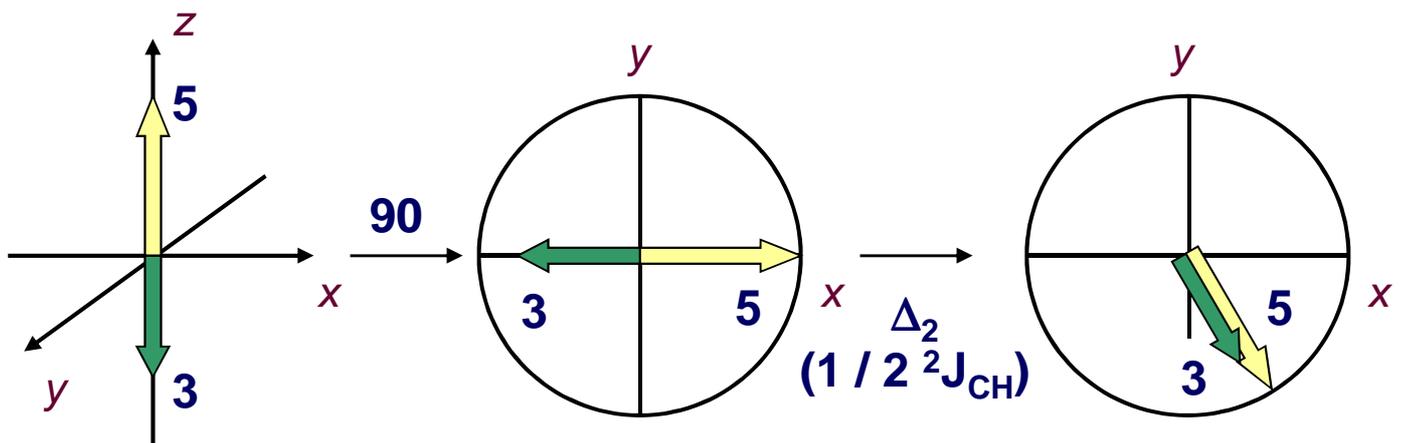
- At the time we decouple ^1H , we will almost kill all the ^{13}C signal that evolved under the effect of $^1\text{J}_{\text{CH}} \dots$

Long range HETCOR (...)

- In the end, we'll see that most of the magnetization that evolved under the effect of different $^1J_{CH}$ s will be wiped out. On the other hand, ^{13}C antiphase magnetization that originated due to $^2J_{CH}$ will have the right Δ_1 and Δ_2 delays, so it will behave as we saw before. For 1H :



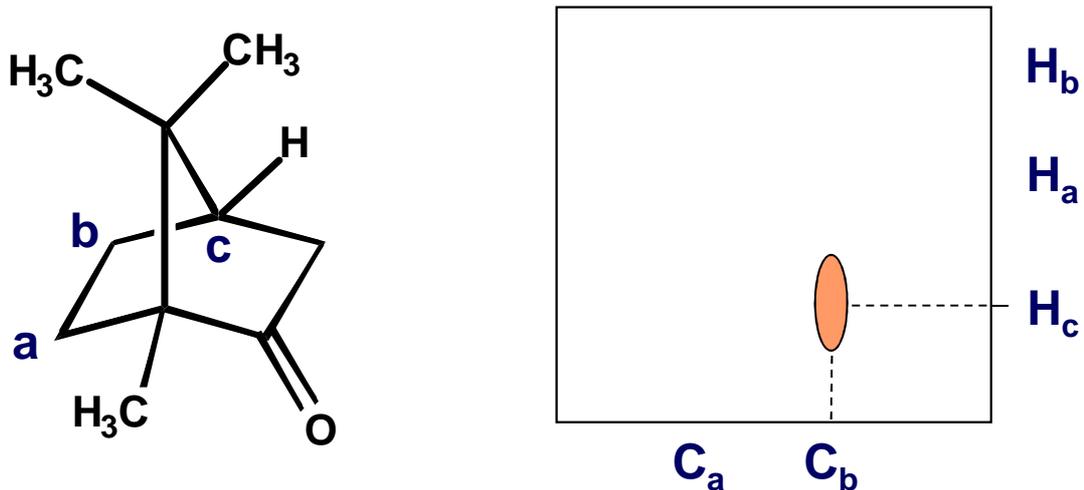
- For ^{13}C :



- So in the end, only ^{13}C that have $2J_{CH}$ couplings will give rise to correlations in our HETCOR and we will be able to achieve what we wanted

Long range HETCOR (...)

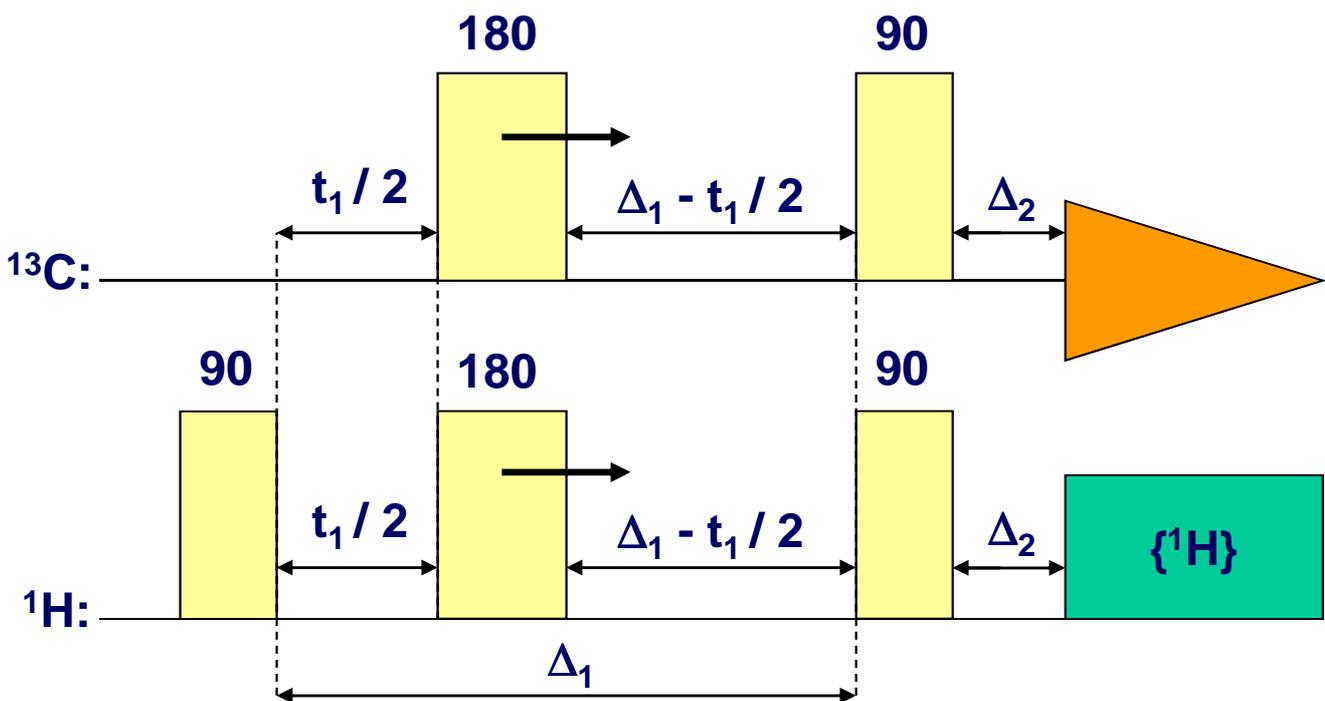
- If we take our HETCOR using these values for Δ_1 and Δ_2 , and if we consider everything working in our favor, we get:



- Great. We can now see our long-range ¹H-¹³C coupling, and we can now determine which CH₂ carbon is which in camphor. Note that we did the whole explanation for CHs for simplicity, but the picture is pretty much the same for CH₂s.
- As usual, things never go the way we want. This sequence has several drawbacks. First, selecting the right Δ_1 and Δ_2 to see ${}^2J_{\text{CH}}$ over ${}^1J_{\text{CH}}$ is kind of a crap-shot.
- Second, we are now talking of pretty long delays Δ_1 and Δ_2 on top of the variable evolution delay (which is usually in the order of 10 to 20 ms). We will have a lot of relaxation, not only of the ¹³C but of the ¹H, during this time, and our signal will be pretty weak.
- Furthermore, since ¹H relaxes considerably, the inversion will vanish away and we don't get strong correlations.

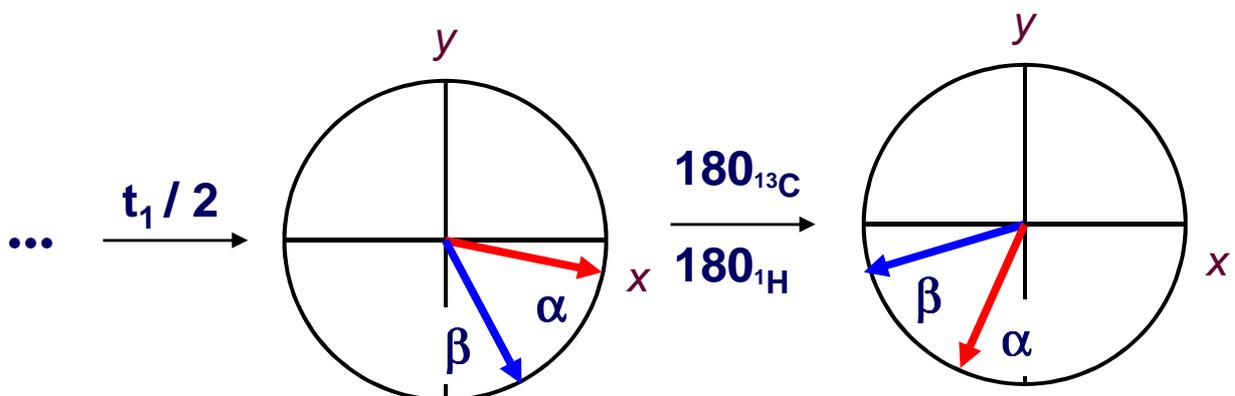
COLOC-HETCOR

- How can we avoid these problems? If we want to keep the idea we have been using, i.e., to refocus ^{13}C magnetization associated with $^2J_{\text{CH}}$, we need to keep the Δ_1 and Δ_2 delays.
- Then the only delay that we could, in principle, make shorter is the variable evolution delay, t_1 . How do we do this, if we need this delay to vary from experiment to experiment to get the second dimension?
- The solution is to perform a **constant time** experiment. This involves to have an evolution time t_1 that is overall constant, and equal to Δ_1 , but have the pulses inside the evolution progress during this time. The best example of such a pulse sequence is called **CORrelations via LONG-range COUplings**, or **COLOC**. The pulse sequence is:



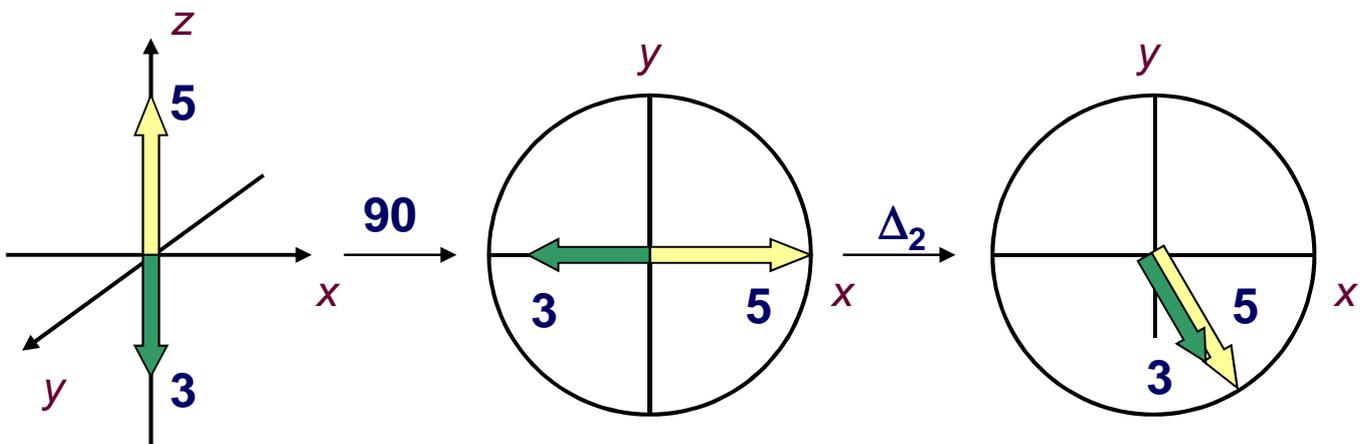
COLOC-HETCOR (continued)

- As you see from the pulse sequence, the Δ_1 period remains the same, as so does the total t_1 period. However, we achieve the evolution in t_1 by shifting the two 180 pulses through the t_1 period constantly from one experiment to the other.
- We can analyze how this pulse sequence works in the same way we saw how the regular HETCOR works. We'll see the analysis for a C-C-H. The first ^1H 90 puts ^1H magnetization in the $\langle xy \rangle$ plane, where it evolves under the effect of J_{CH} ($^2J_{\text{CH}}$ in the case of C-C-H) for a period $t_1/2$, which is variable.
- The combination of 180 pulses in ^1H and ^{13}C inverts the ^1H magnetization and flips the labels of the ^1H vectors:



COLOC-HETCOR (...)

- Now, after $\Delta_1 - t_1 / 2$, the magnetization continues to dephase. However, since the total time is Δ_1 , we will get the complete inversion of ^1H magnetization, we have the maximum polarization transfer from ^1H to ^{13}C , and we tag the ^{13}C magnetization with the ^1H frequency (which gives us the correlation...).
- Since we always have complete inversion of ^1H magnetization and refocusing, we won't have $^2J_{\text{CH}}$ splitting in the ^{13}C dimension (f_1).
- Finally, over the Δ_2 delay we have refocusing of the ^{13}C antiphase magnetization, just as in the refocused HETCOR, and we can decouple protons during acquisition:



- The main advantage of this pulse sequence over HETCOR is that we accomplish the same but in a much shorter time, because the Δ_1 period is included in the t_1 evolution. Furthermore, instead of increasing t_1 from experiment to experiment, we change the relative position of the 180 pulses to achieve the polarization transfer and frequency labeling.