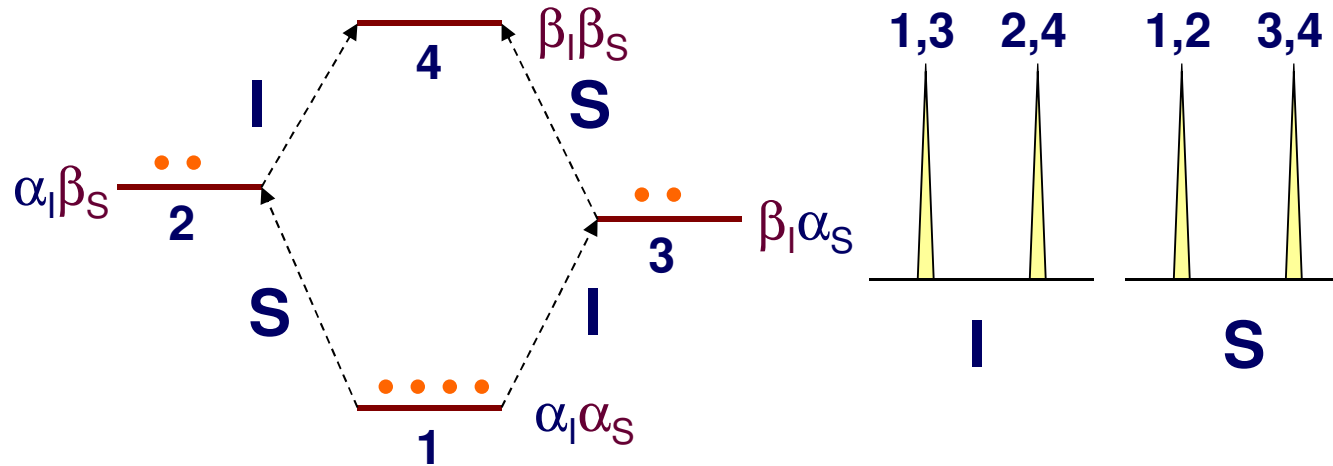
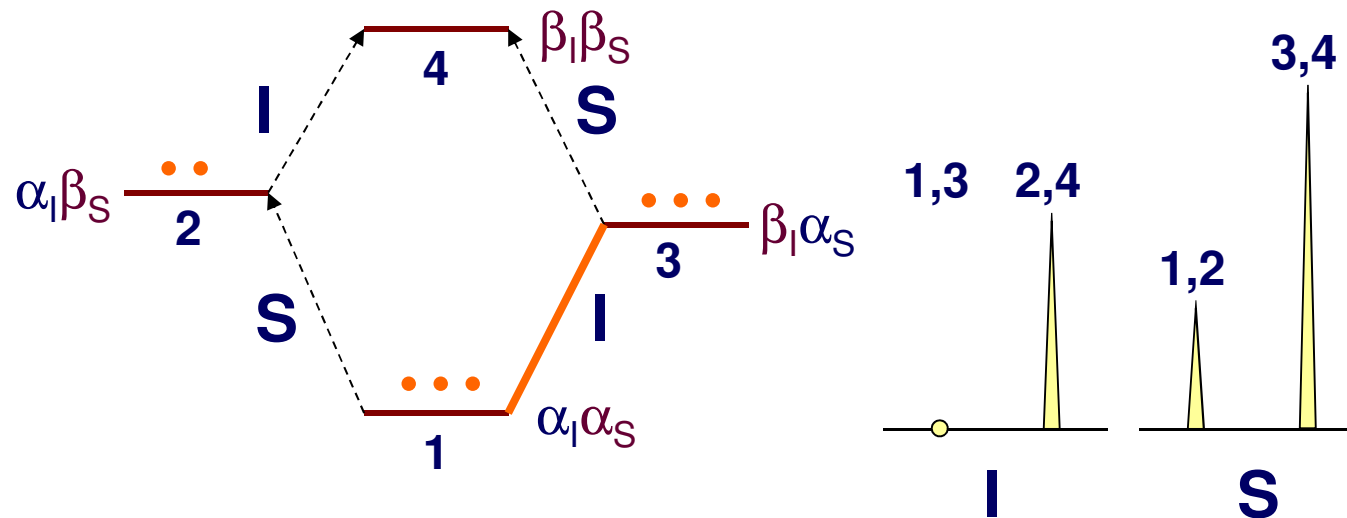


Polarization transfer

- For this diagram, we'll use two protons that are **J**-coupled weakly and have a large δ difference. We name them **I** and **S** to maintain I don't know which convention, and we indicate with a • the excess population from one state to the other:

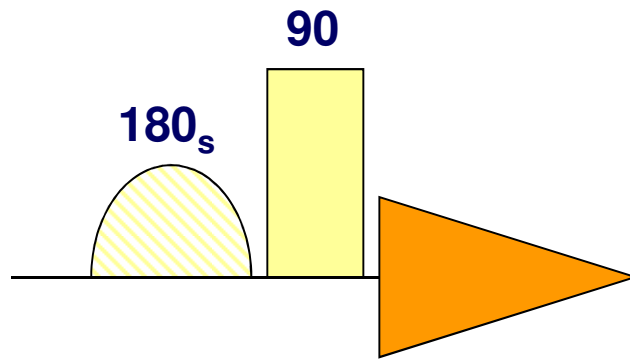


- Now we irradiate and saturate only one of the lines of one of the nuclei **selectively** (with CW...). After a certain time, the population differences for that transition become equalized.

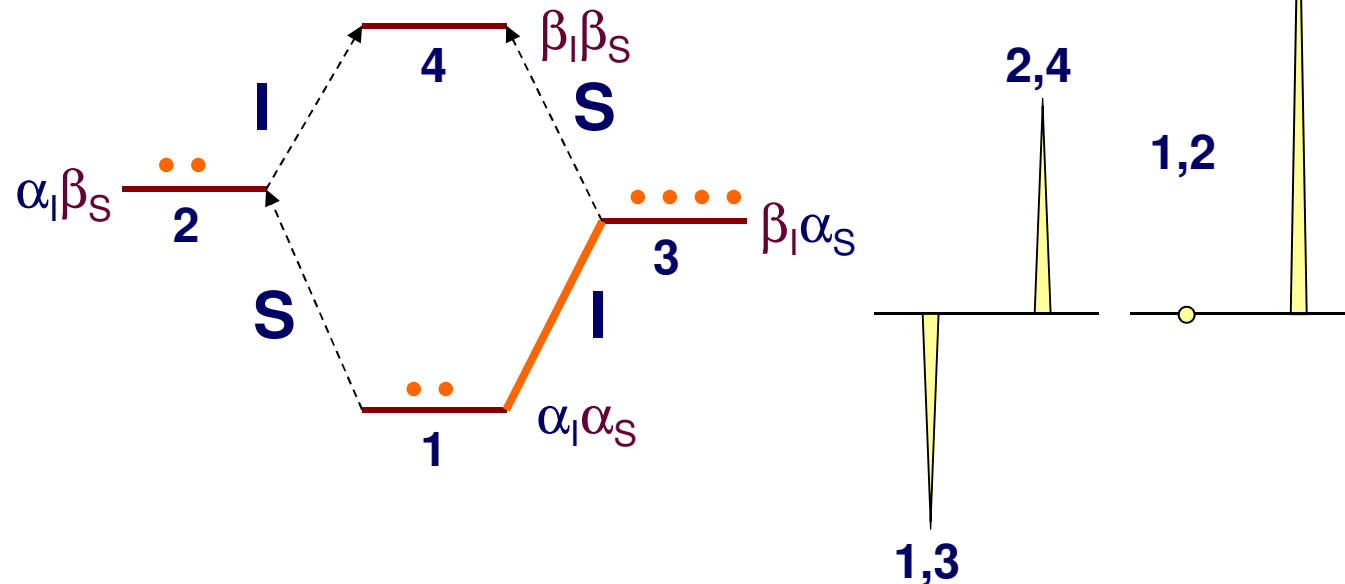


Polarization transfer. SPT and SPI

- Since we changed the populations of the spin system, the lines in the spectrum change intensity accordingly. What we did is transfer polarization from one nuclei to the other. This is called ***selective polarization transfer***, or ***SPT***.
- There is one variation of this technique. Think of the following pulse sequence:

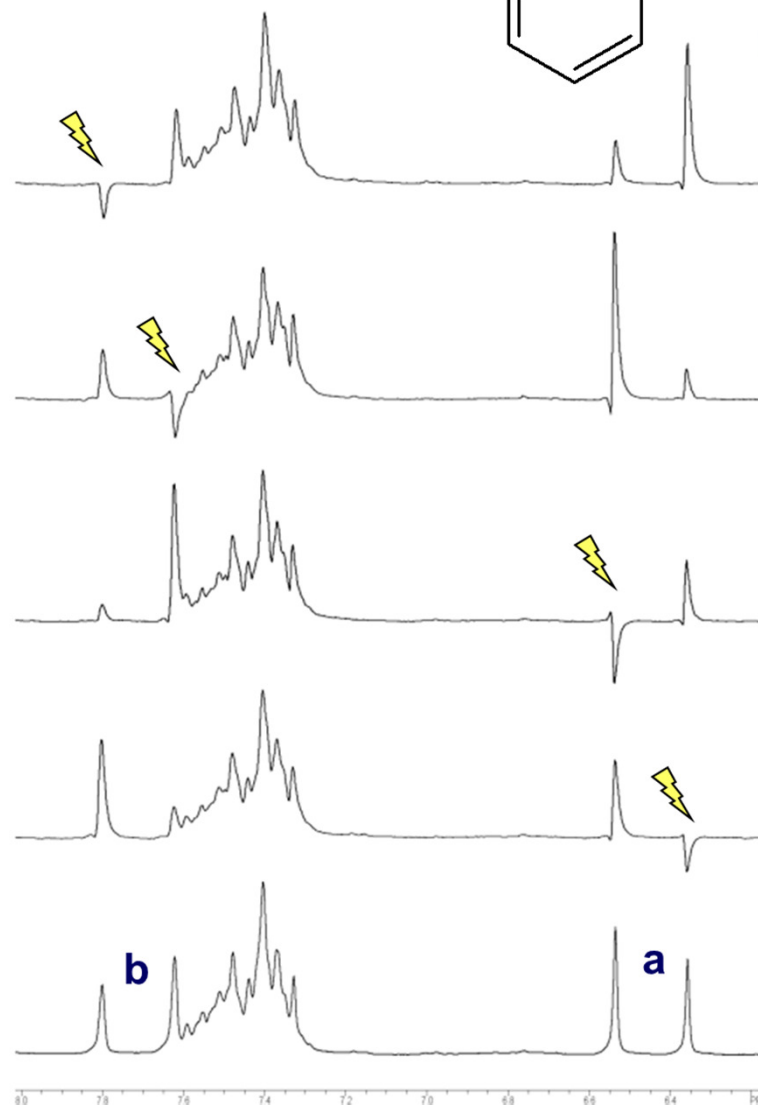
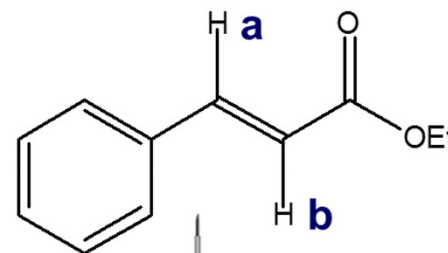


- The chubby pulse is a low power, selective π pulse. It inverts the populations of only one of the transitions in the spin system.



PT - SPT and SPI (continued)

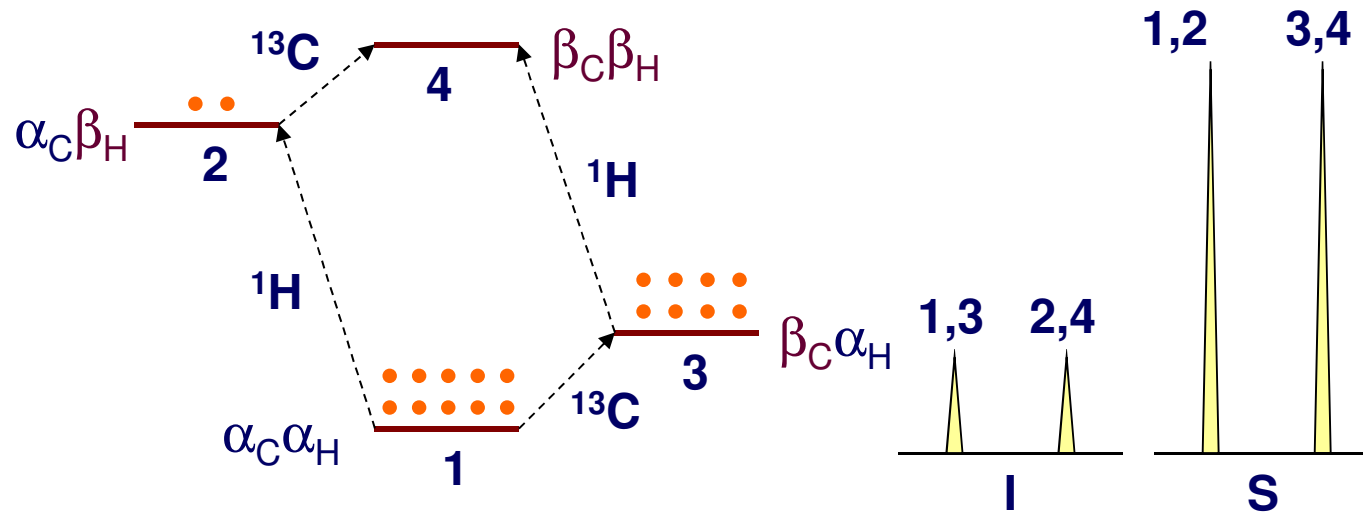
- A practical example using ethylcinnamate:



- In this case we invert selectively each line of the **a** and **b** olefinic ^1H 's and see how the intensities of the others vary.

Heteronuclear polarization transfer

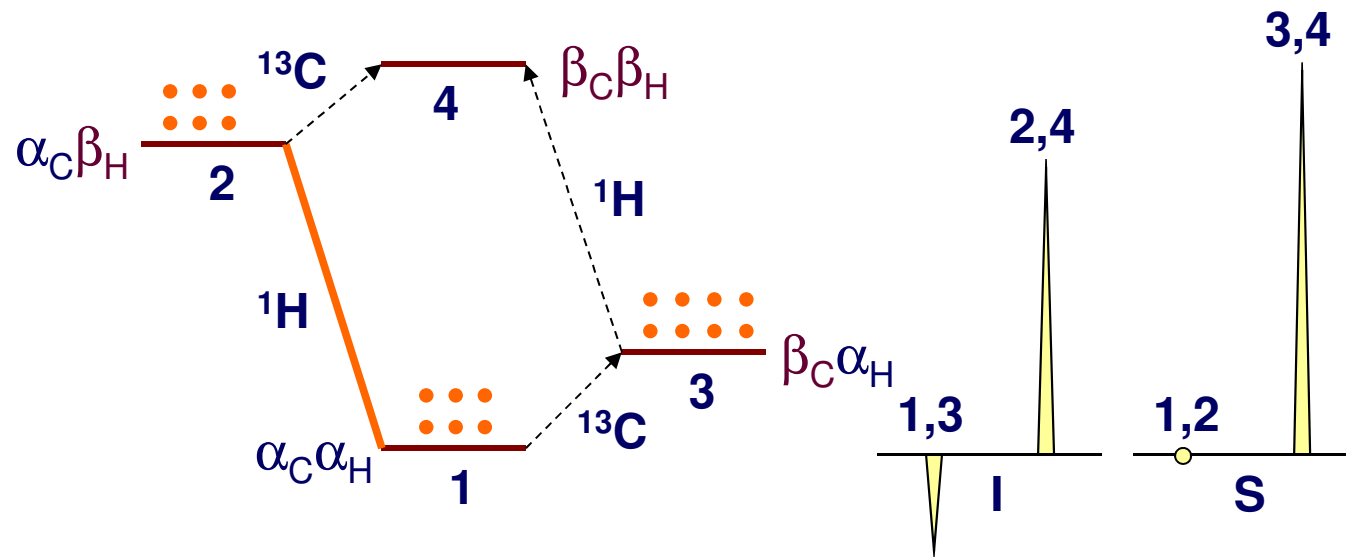
- In this case, we call the experiment ***selective population inversion***, or ***SPI***. Again, the intensities of the lines reflect what we've done to the populations of the spin system.
- Despite that we can use SPT and SPI to identify coupled spin systems in very crowded regions of the spectra, homonuclear PT is not as useful as heteronuclear PT. Let's think of the two experiments in a heteronuclear system:



- Here the population differences between the energy levels reflect that we have a **1** to **4** ratio between ^{13}C and ^1H due to the differences in the gyromagnetic ratios. Here is where we start seeing why it may be useful...
- One thing that we have wrong in the drawing is the relative intensities. Here we are drawing using only the ratios of the gyromagnetic ratio, not the abundance...

Heteronuclear polarization transfer - SPT

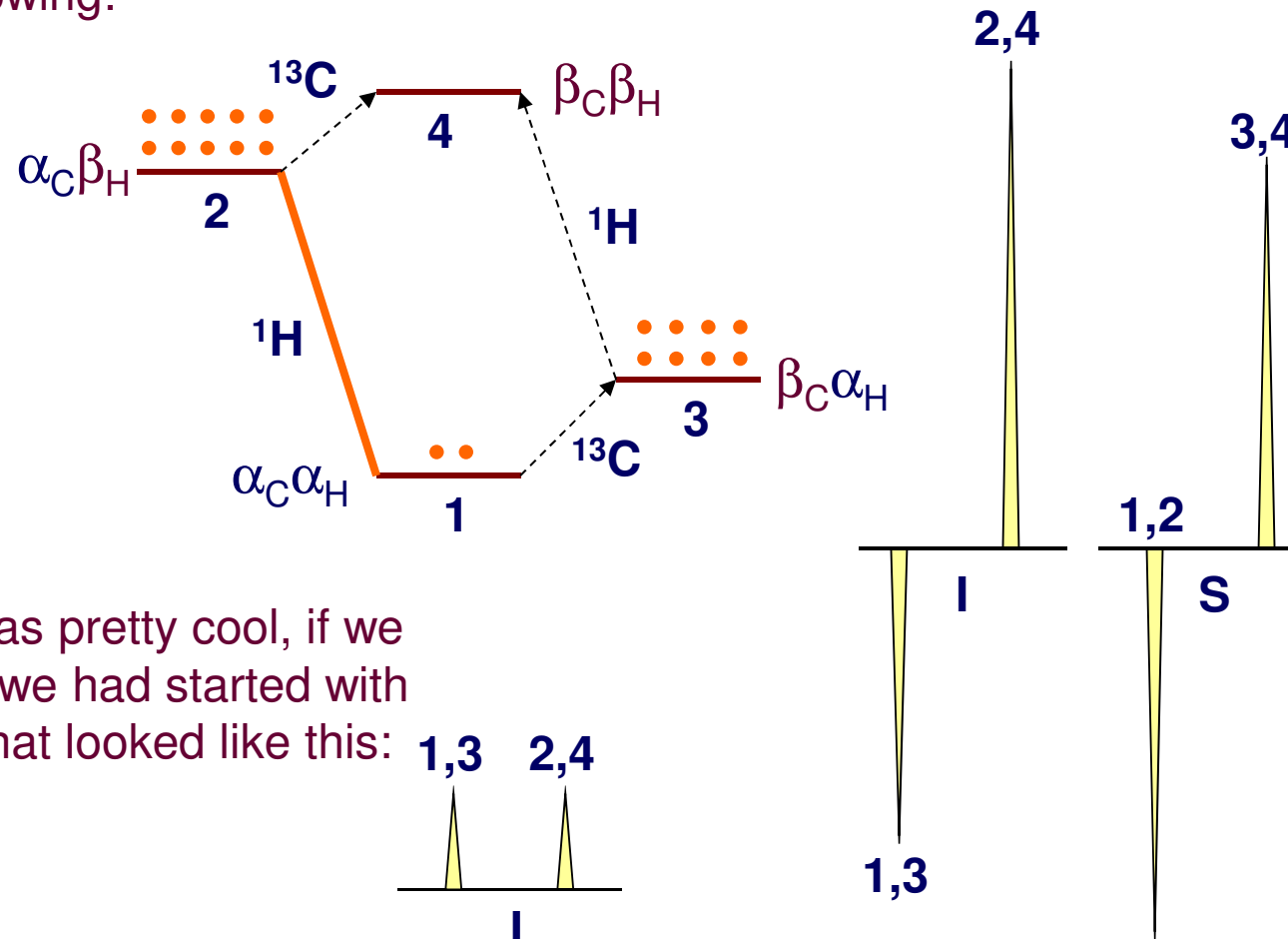
- Now we'll apply SPT and SPI on this spin system, and see what happens. First SPT...
- After we saturate, say, the **1,2** transition we get the following populations in the energy diagram:



- The signals of both spins change accordingly, but now we have a **3-fold** increase for one of the ^{13}C transitions. Now we are talking
- If we consider the absolute values of the signal, we have twice as much signal as in the original spectrum...

Heteronuclear polarization transfer - SPI

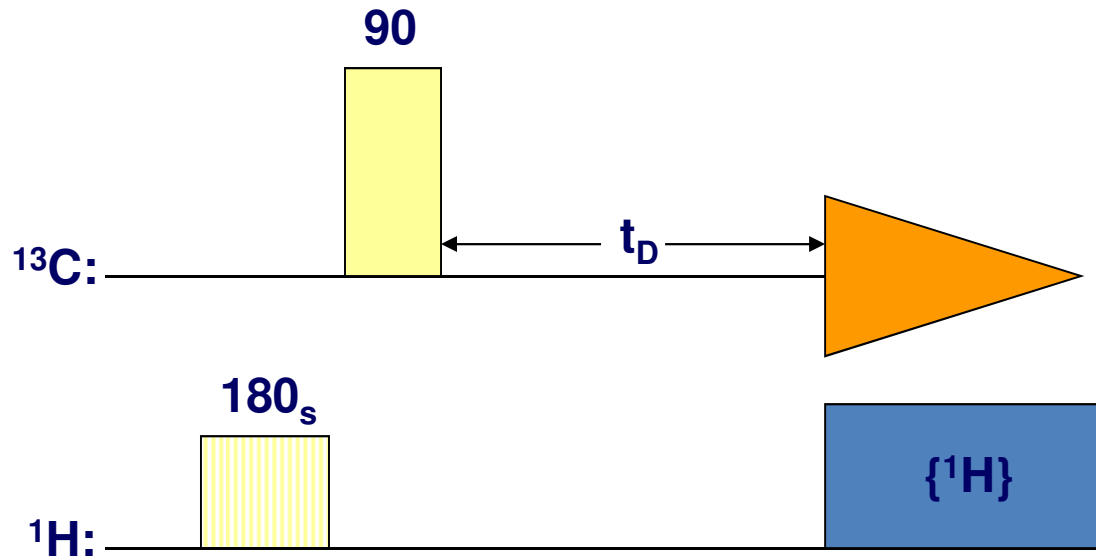
- Now we do the same analysis for SPI. If we invert selectively the populations of **1,2**, we get the following:



- Now **THAT** was pretty cool, if we consider that we had started with a ^{13}C signal that looked like this:
- By manipulating the polarization of the protons, we obtain an enhancement of **4** in the ^{13}C signal (considering positive and negative signals).

J-modulation and polarization transfer

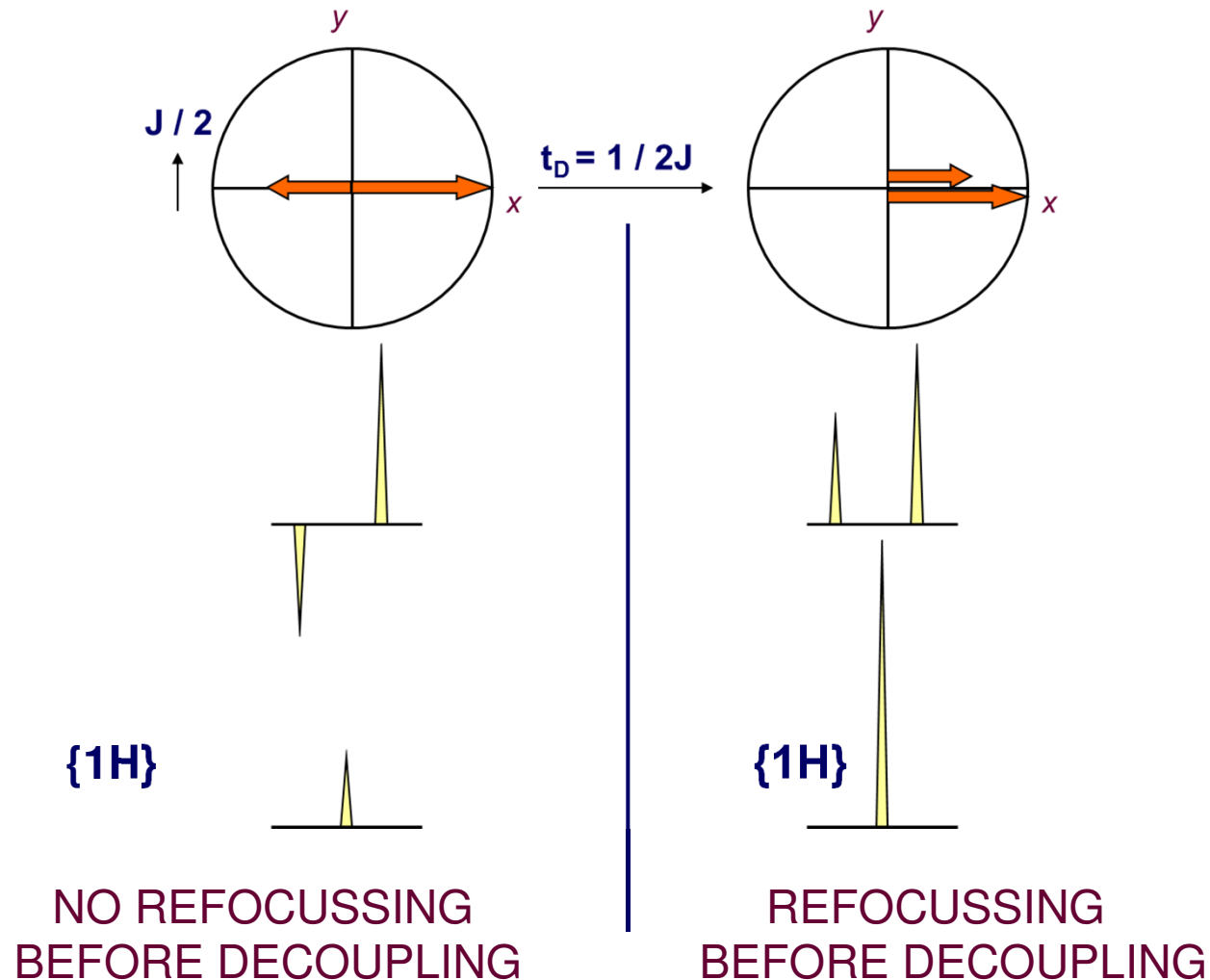
- The increase of the ^{13}C signal is good and all that, but we still have to deal with a spectrum that is proton-coupled and has up and down peaks. We cannot decouple to do this, because the enhancement is there due the ^1H levels, which would be gone if we decouple...
- What we do is combine it with ***J-modulation***. Consider that we use the following pulse sequence:



- We set t_D to $1 / 2J$. This means that after the $\pi / 2$ pulse on ^{13}C and the t_D , the ^{13}C magnetization will have ***refocussing*** couplings. We'll see it with vectors...

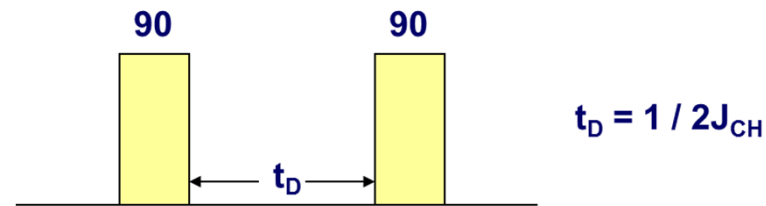
J -modulation and polarization transfer (...)

- We will only consider the ^{13}C magnetization, because for the ^1H we only inverted selectively the populations (the chubby π pulse). After the $\pi/2$ ^{13}C pulse, we have the **+5** and **-3** components of the magnetization in the $\langle xy \rangle$ plane:

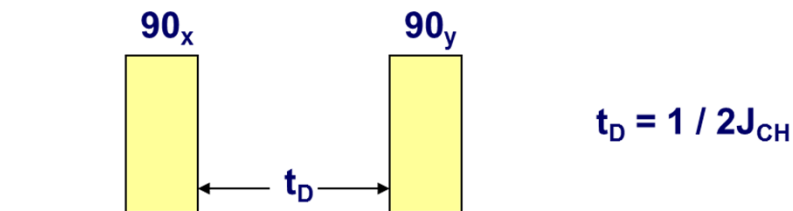


Selective polarization transfer with hard pulses

- So far, so good. One of the drawbacks of SPI and SPT is that we use selective pulses, which many times are hard to come by. It would be good if we could use hard pulses to do the same thing. The following ^1H pulse sequences do this.
- The first one is selective for ^1H lines that are on-resonance with both $\pi / 2$ pulses. Note that the pulses are applied on the same axis:



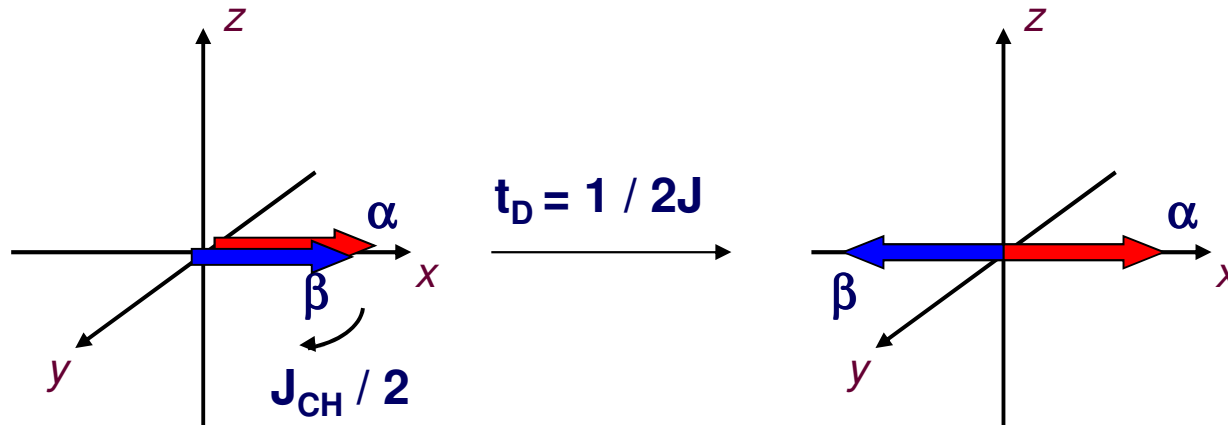
- The other one will invert the population of a single proton if the pulse is on resonance with the chemical shift of the doublet - It is at the center of the doublet:



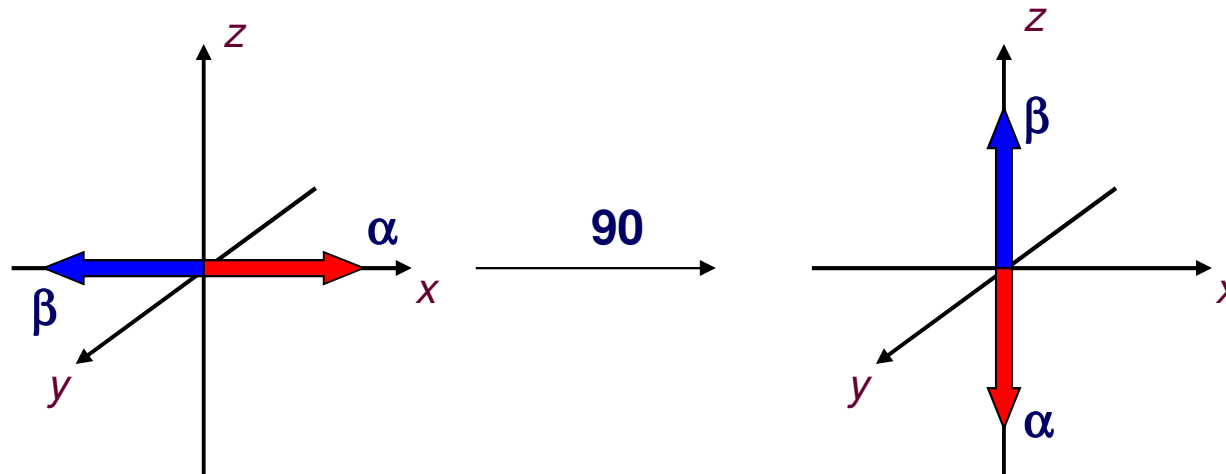
- In both cases, $t_D = 1 / 2J_{\text{CH}}$. We'll analyze the first one, and the other one will be part of homework or something...

SPT with hard pulses (continued)

- After the $\pi / 2$ pulse, both α and β vectors lie in the $+x$ axis:



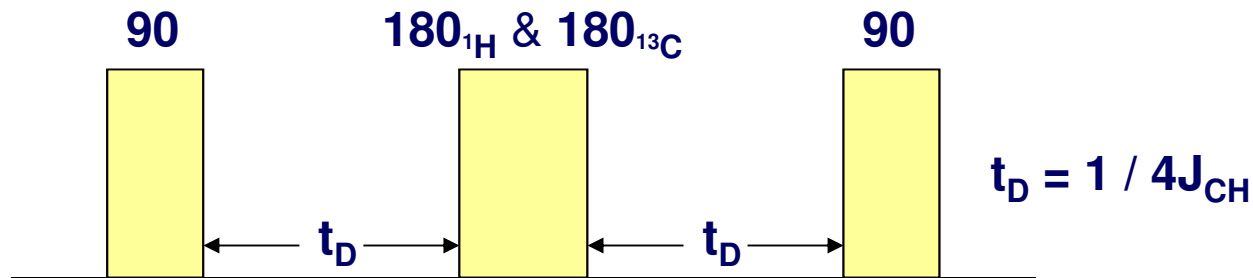
- If we wait $1 / 2J_{CH}$ seconds, we have that the faster vector (α) moves away from β by π radians. If at this point we apply the second $\pi / 2$ pulse, we invert the populations (α and β states will change location).



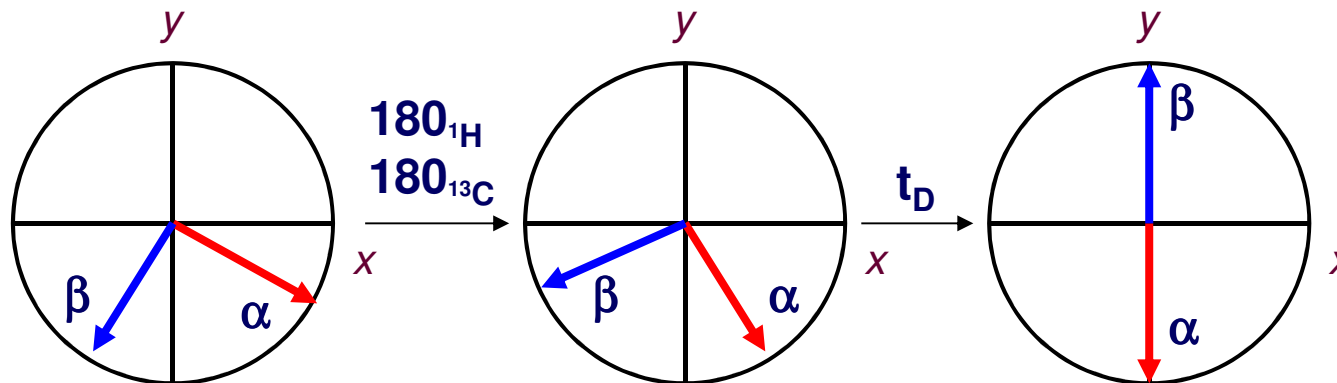
- This sequence can now be used together with ^{13}C excitation to see enhancement of ^{13}C nuclei attached to this proton.

Non-selective polarization transfer

- Another big pain of SPT and SPI is that it is selective, and we have to go one proton at a time. It would be nice if we could do all at once, so we transfer polarization from all protons to all the insensitive nuclei attached to them (^{13}C or ^{15}N) One way of doing this is combining the last pulse sequence with a spin-echo with a $t_D = 1 / 4J_{\text{CH}}$



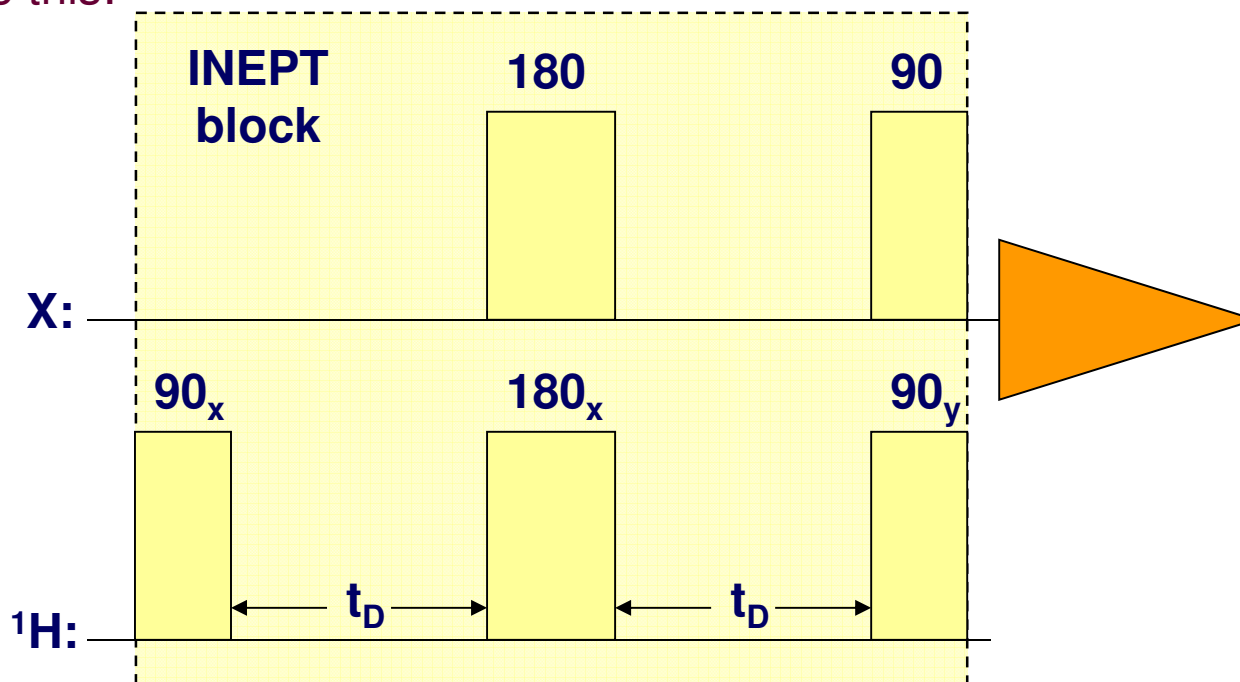
- The π pulse and the 2 t_D delays refocus chemical shift, so the populations of all protons in the molecule will be inverted. The π pulse on the X nucleus flips the α and β labels:



- Now the $\pi / 2$ will flip the α and β vectors back to the z axis, and we have inversion of the ^1H populations.

Non-selective polarization transfer - INEPT

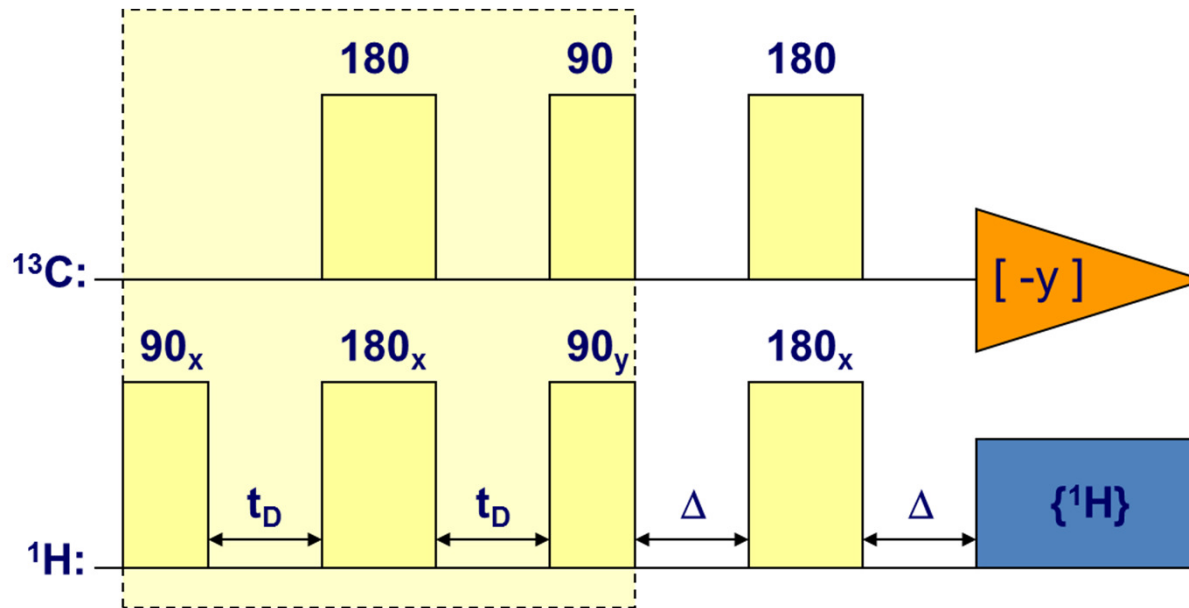
- If we expand this last sequence a little bit more we get **INEPT** (***I**nsensitive **N**uclei **E**nhancement by **P**olarization **T**ransfer*). It is an important pulse sequence building block found throughout multiple pulse sequences.
- It is used to increase the sensitivity (polarization) of nuclei such as ^{13}C and ^{15}N . It looks like this:



- Here **X** is either ^{13}C or ^{15}N . The analysis is the same as what we saw for the protons, plus the 'read' $\pi / 2$ pulse for the **X** nuclei to create (and be able to detect) transverse magnetization.

Refocused INEPT

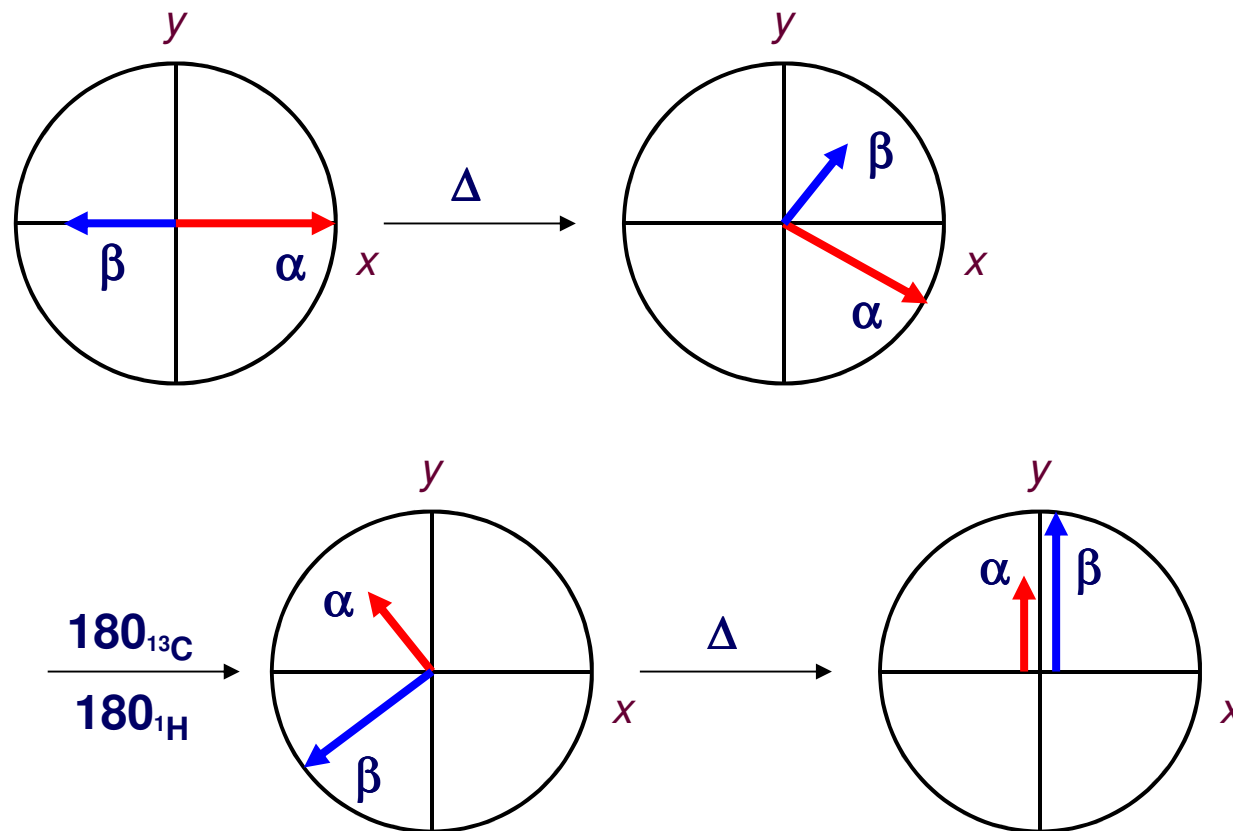
- With the regular INEPT we still have the '+5 up' and '-3 down' problem. We would like to have the two lines refocused into a single line, and we already know normal decoupling is not an option.
- We simply combine the INEPT sequence with a refocusing chunk at the end, and detect in the -y axis:



- Depending on the type of carbon we use different Δ delays:
 - CH - $\Delta = 1 / 4J$
 - CH_2 - $\Delta = 1 / 8J$
- For all types of carbons to have more or less the same enhancement, we use $\Delta \approx 1 / 7J$.

Refocused INEPT (continued)

- After the $\pi / 2$ ^{13}C pulse, we have the enhanced (+5 & -3) ^{13}C magnetization on the $\langle xy \rangle$ plane.

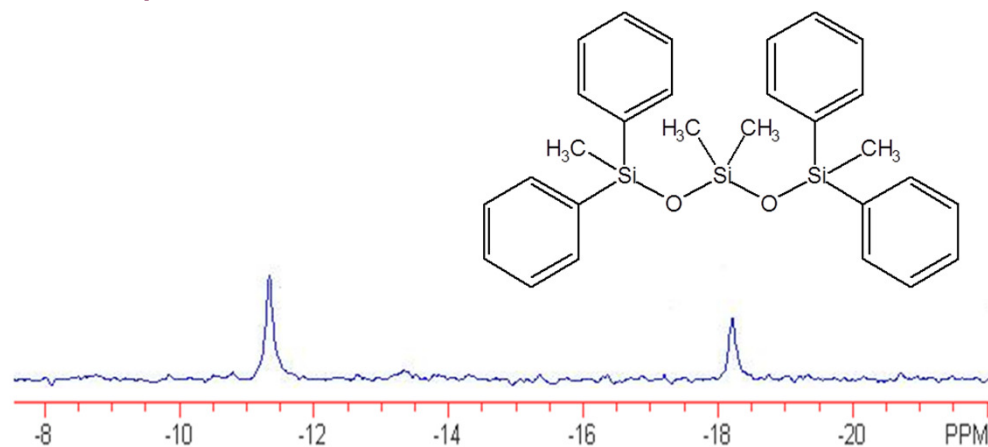


- Variations of this sequence is all over the place. With it we can transfer polarization to and from insensitive nuclei (^{13}C , ^{15}N , ^{29}Si , etc., etc.). We can also use it to **edit** spectra as well as to **label** a certain type of nuclei in the sample with information from others (δ 's, \mathbf{J} 's).

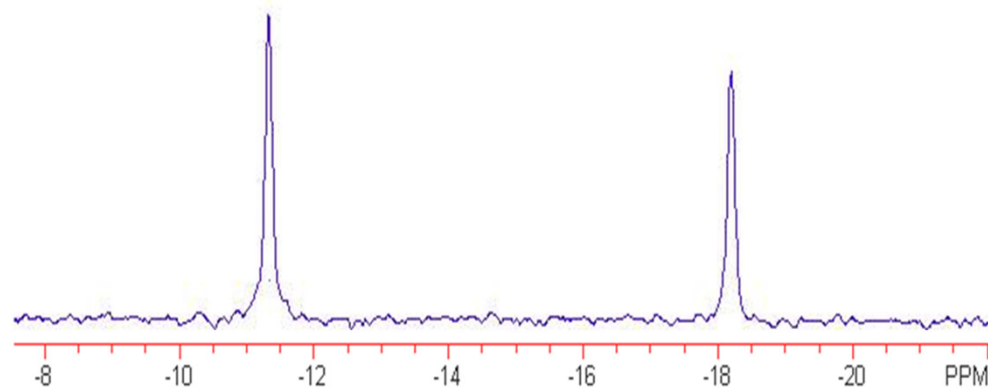
- The block is used in almost all protein NMR pulse sequences involving ^1H , ^{15}N , and ^{13}C .

INEPT (continued)

- Example of INEPT from ^1H to ^{29}Si .
- Normal ^{29}Si 1D spectrum:



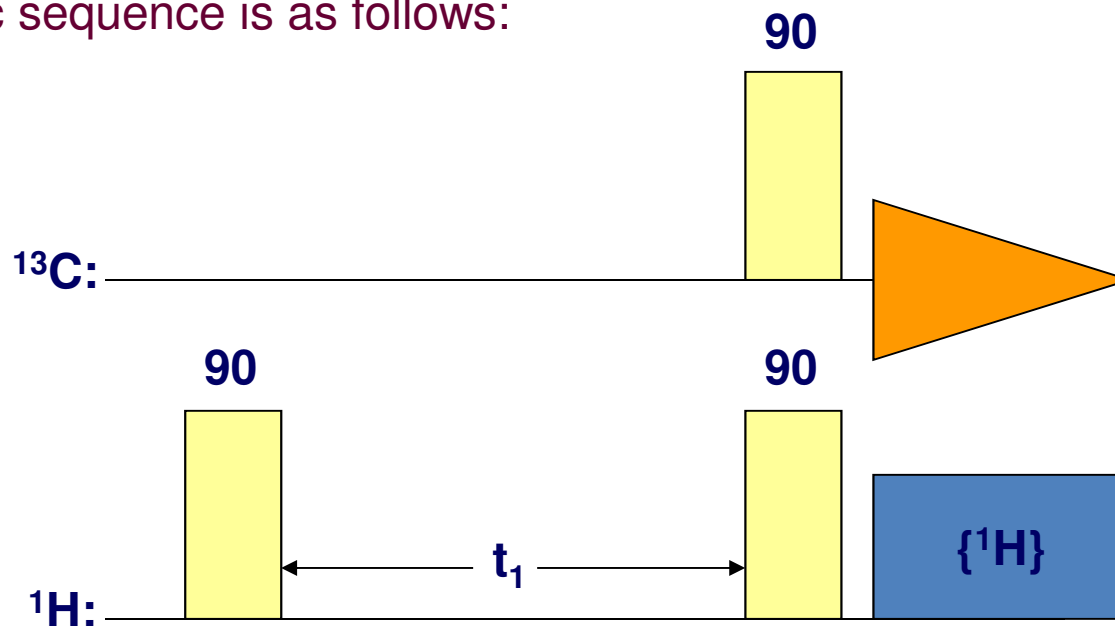
- refocused ^{29}Si INEPT spectrum:



- The $^2J_{^1\text{H}-^{29}\text{Si}}$ coupling is ~ 7 Hz, and the $\gamma_{^1\text{H}} / \gamma_{^{29}\text{Si}}$ ratio is 5.

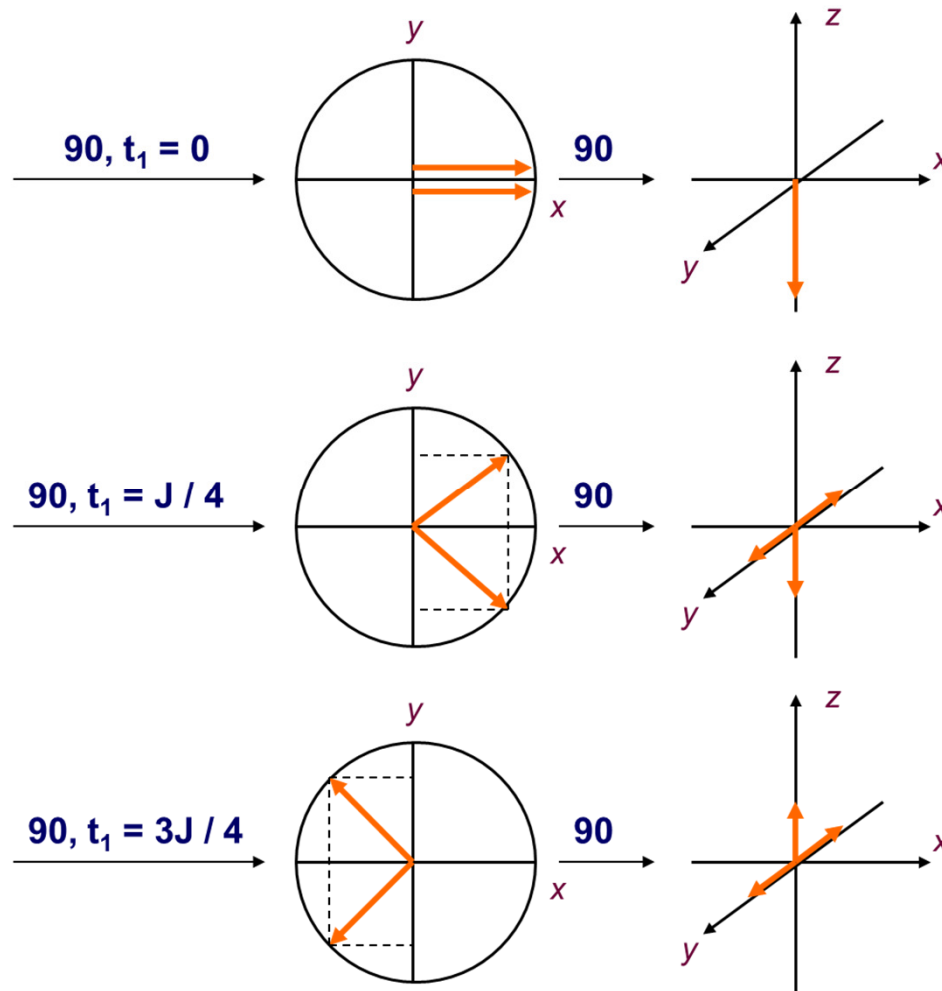
Heteronuclear correlation - HETCOR

- 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:



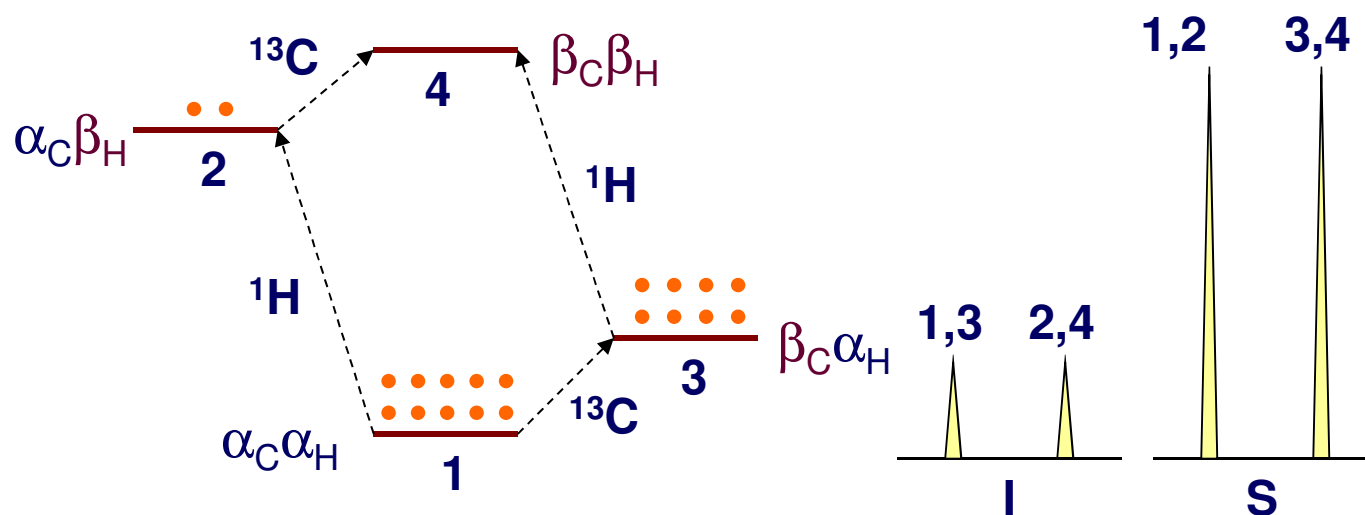
HETCOR (continued)

- 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 (...)

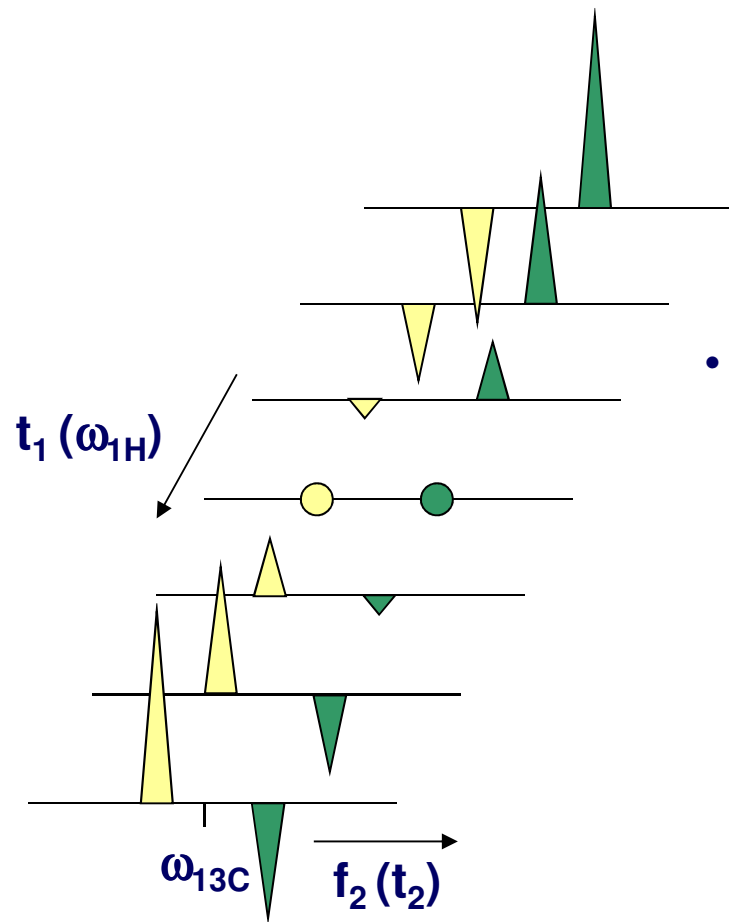
- As was the case for COSY, we see that depending on the t_1 time we use, we have a variation of the population inversion of the proton. We can clearly see that the amount of inversion depends on the J_{CH} coupling.
- Although we did it on-resonance for simplicity, we can easily show that it will also 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 (...)

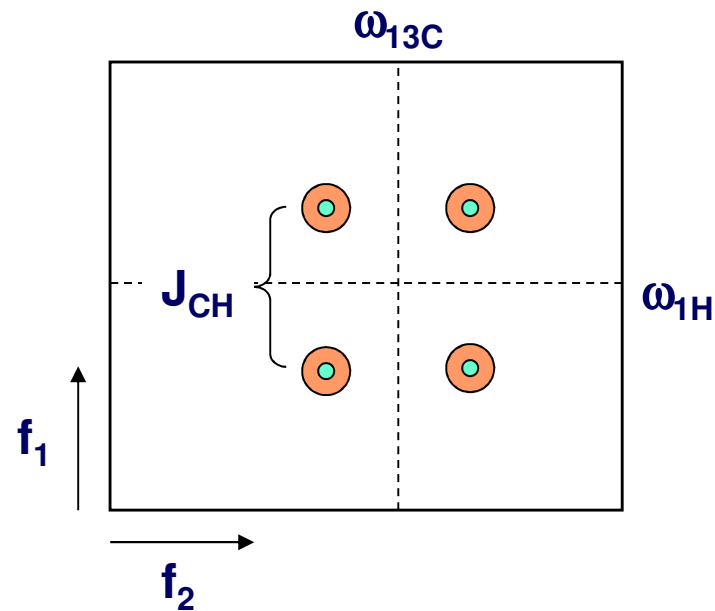
- Again, the intensity of the ^{13}C lines will depend on the ^1H population inversion, thus on $\omega_{1\text{H}}$. If we use a stacked plot for different t_1 times, we get:



- 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.

HETCOR (...)

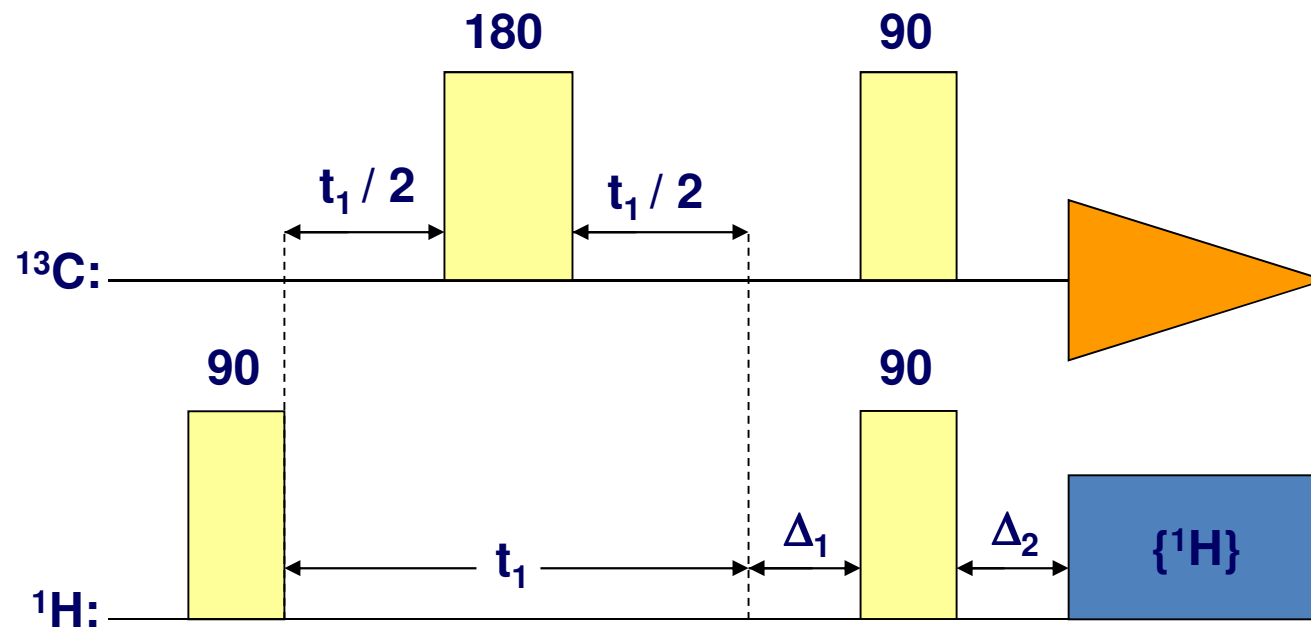
- Again, Fourier transformation on both time domains gives us the 2D correlation spectrum, in this case as a contour plot:



- 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

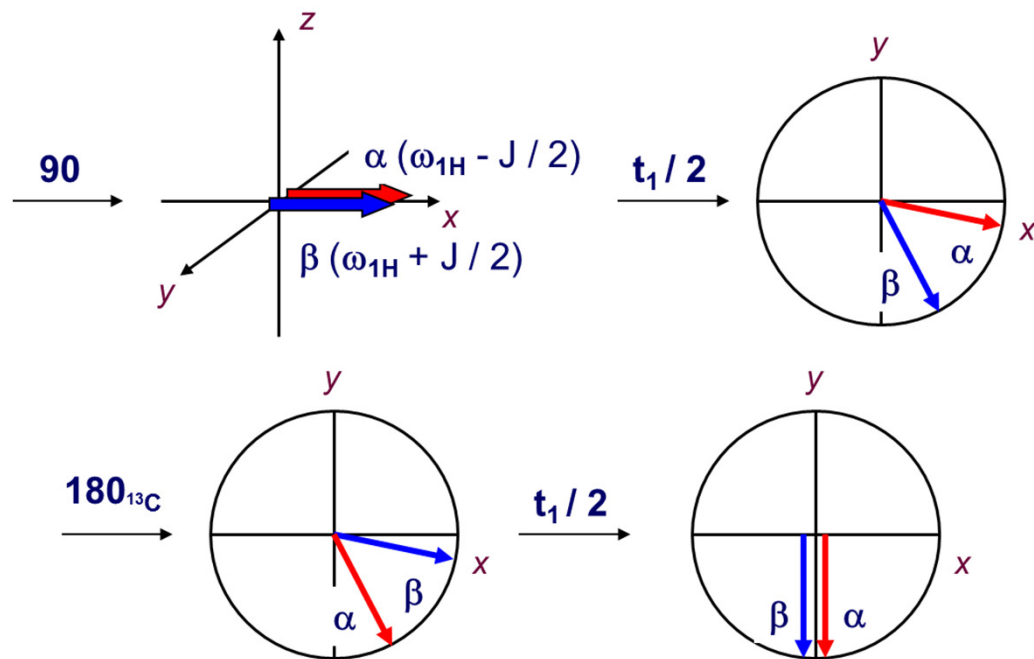
- The idea behind it is pretty much the same stuff we did with the refocused INEPT experiment.



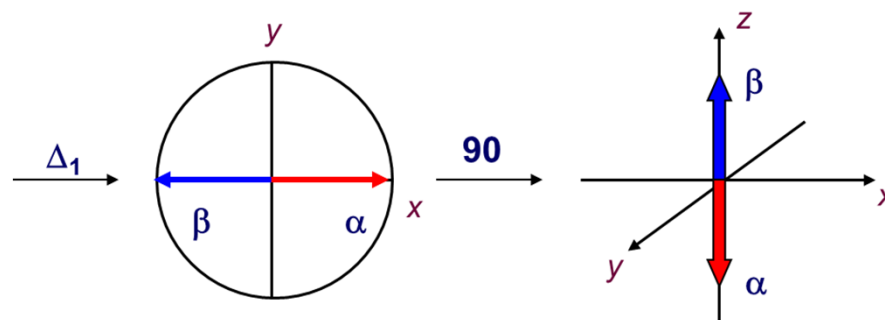
- We use a ^{13}C π pulse to refocus 1H magnetization, and two delays to maximize polarization transfer from 1H to ^{13}C and to get refocusing of ^{13}C vectors before decoupling.
- As in INEPT, the effectiveness of the transfer will depend on the delay Δ and the carbon type. We use an average value.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

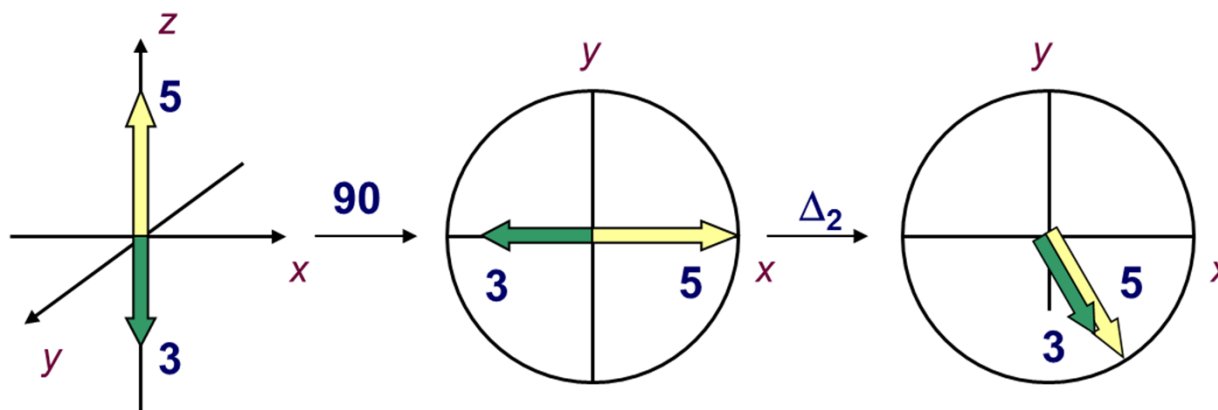


- Now, if we set Δ_1 to $1/2J$ both 1H vectors will dephase by exactly 180 degrees in this period. This is when we have maximum population inversion for this particular t_1 , and no J_{CH} effects:

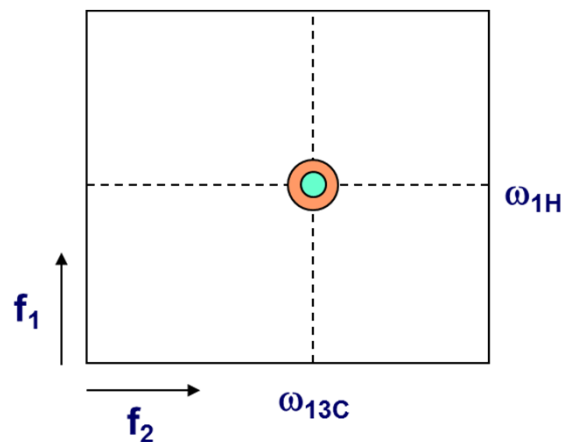


HETCOR with no J_{CH} coupling (...)

- 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:



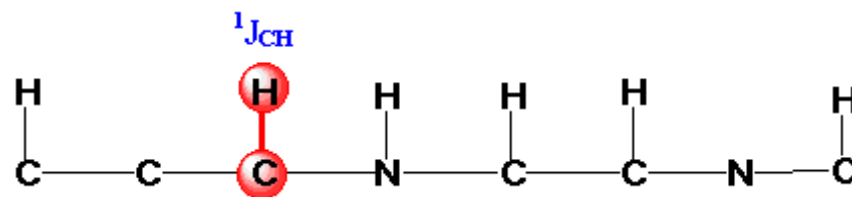
2D HETCOR Experiment

Eccito (impulso a 90°) ^1H

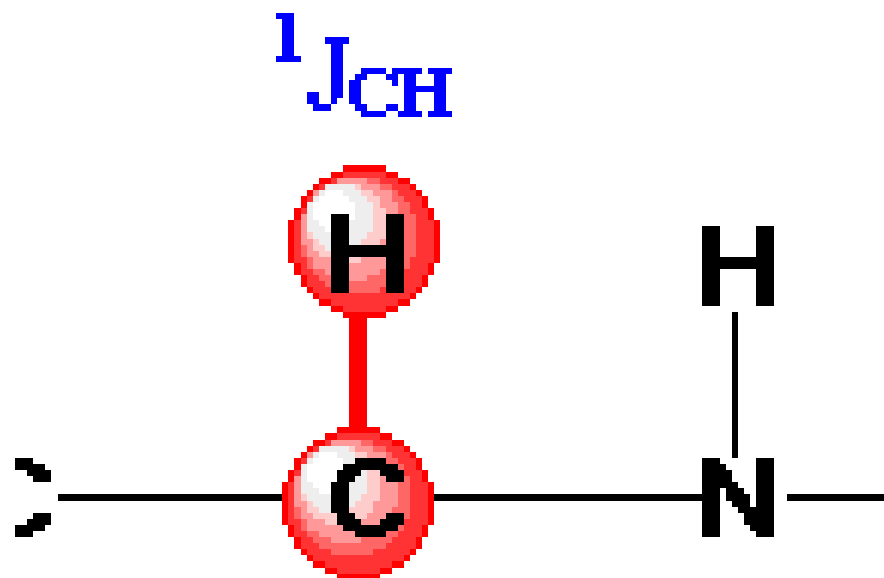
Acquisisco (t1) ^1H –

Perturbo (Trasferisco la magnetizzazione da ^1H a ^{13}C
utilizzando l'accoppiamento scalare $^1J_{\text{HC}}$

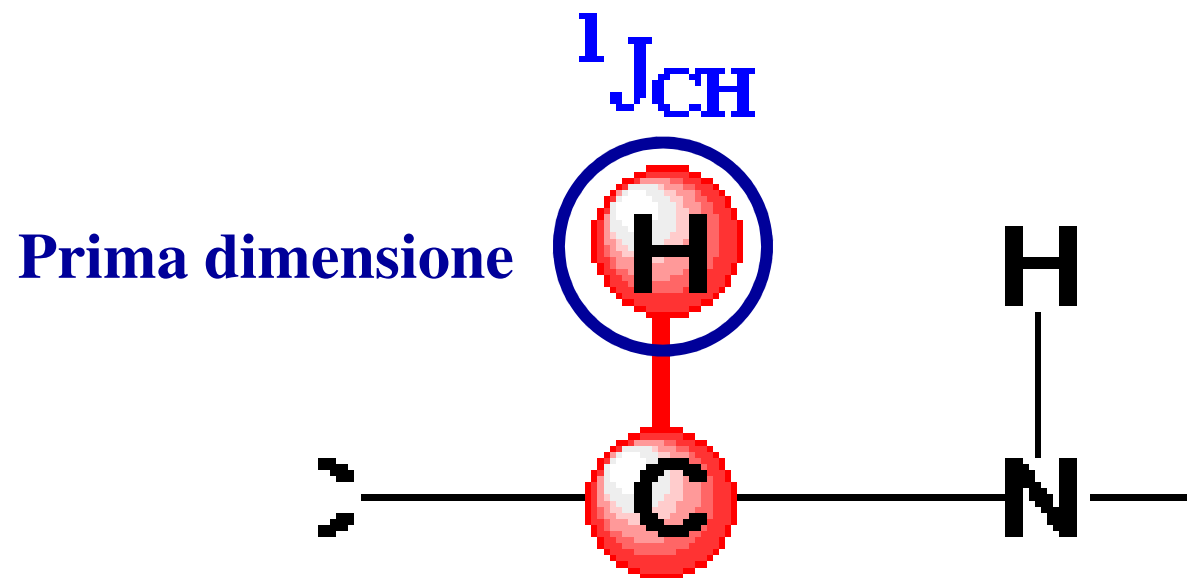
Acquisisco (t2) ^{13}C



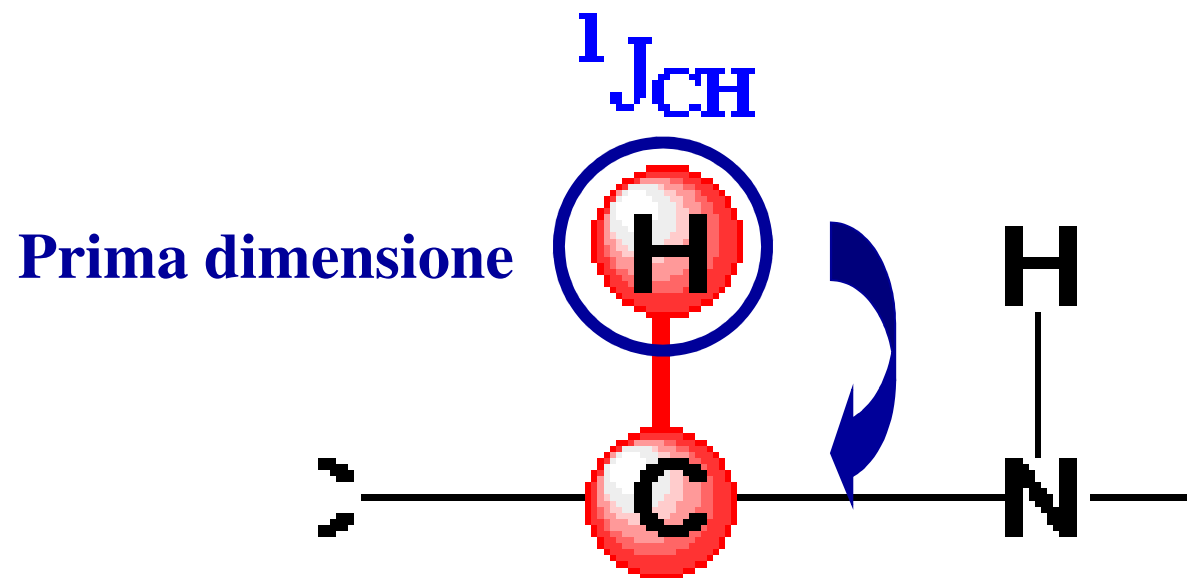
2D HETCOR Experiment



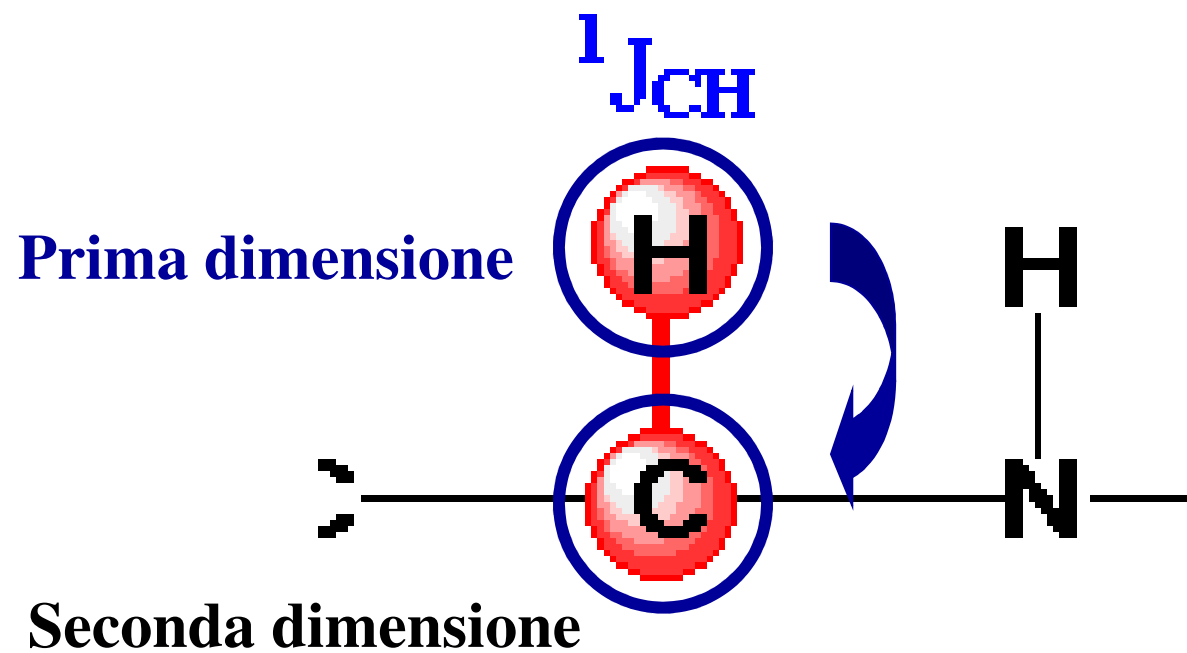
2D HETCOR Experiment



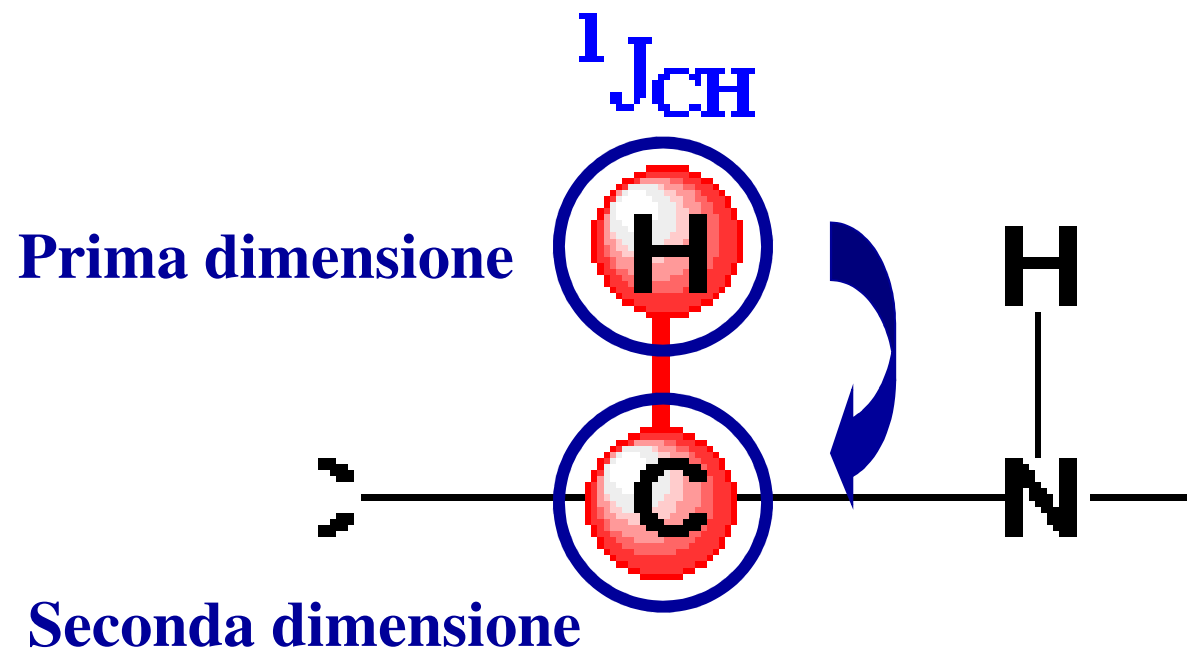
2D HETCOR Experiment



2D HETCOR Experiment

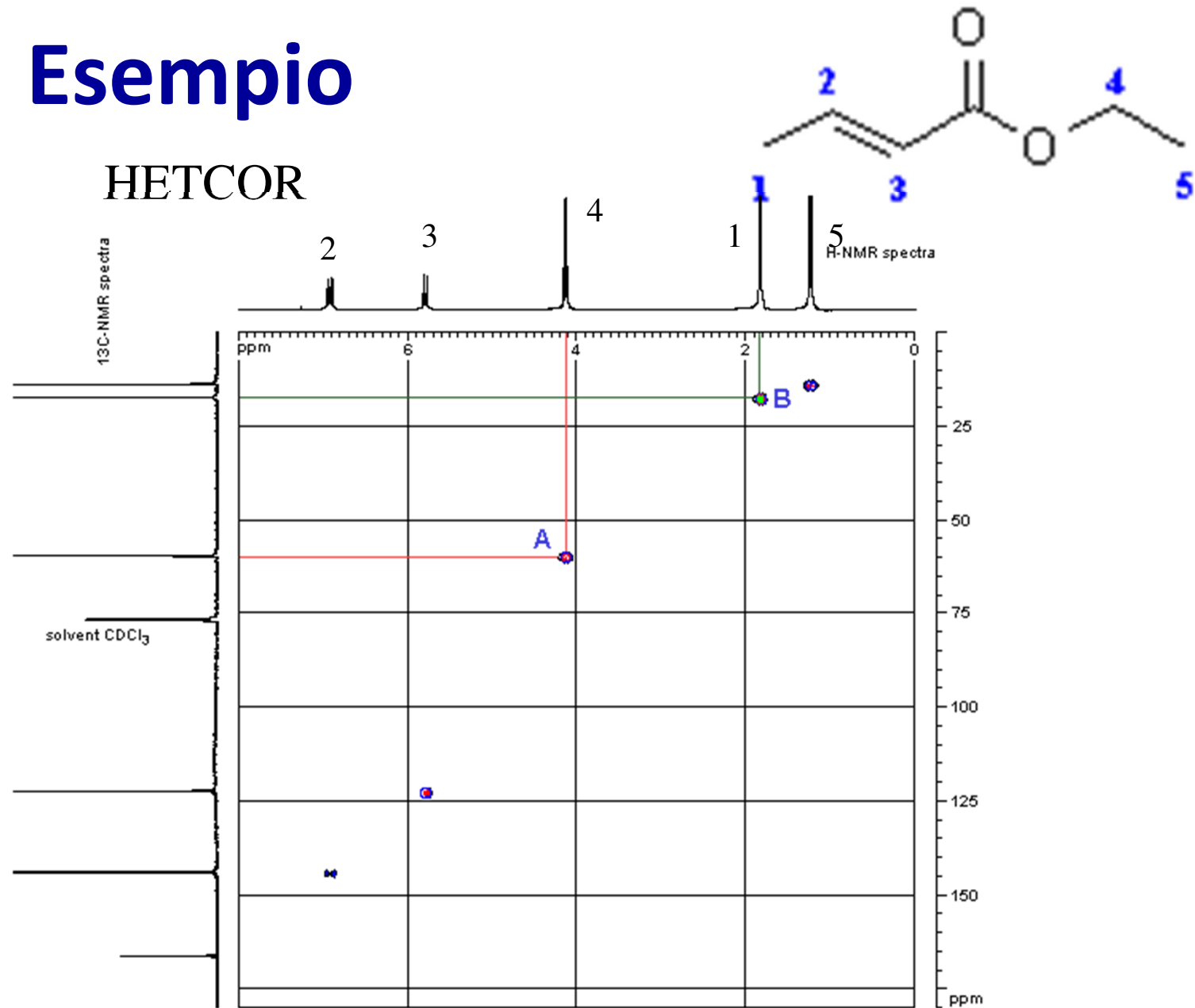


2D HETCOR Experiment



Esempio

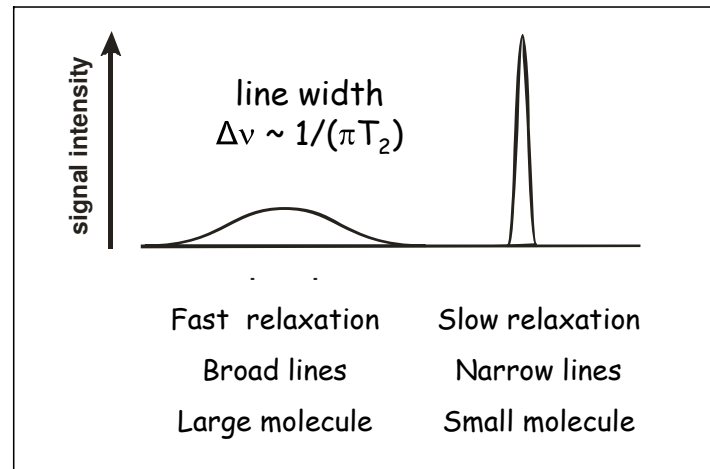
HETCOR



Sensitivity of NMR spectroscopy

$$S/N \sim N \gamma_{\text{exc}} \gamma_{\text{det}}^{3/2} B_0^{3/2} NS T_2^{1/2}$$

S/N	signal-to-noise	
N	number of spins	→ sample concentration
γ_{exc}	gyromagnetic ratio of excited spins	→ isotope labeling
γ_{det}	gyromagnetic ratio of detected spins	
B₀	static magnetic field (e.g. 14.1 Tesla or 600 MHz for ¹ H)	→ magnet "size"
NS	number of scans	→ measurement time
T₂	transverse relaxation ~1/(line width)	→ molecular weight



Proton detection

Today, most of the heteronuclear experiments are performed in a ^1H detected version, also called “inverse detection” (in contrast to the classical X nucleus detection described so far). If the proper equipment is available (re-wired spectrometer console; inverse detection probe!), then inverse detection offers such an immense gain in sensitivity that there is (almost) no reason to run any “conventional” heteronuclear correlation experiments anymore.

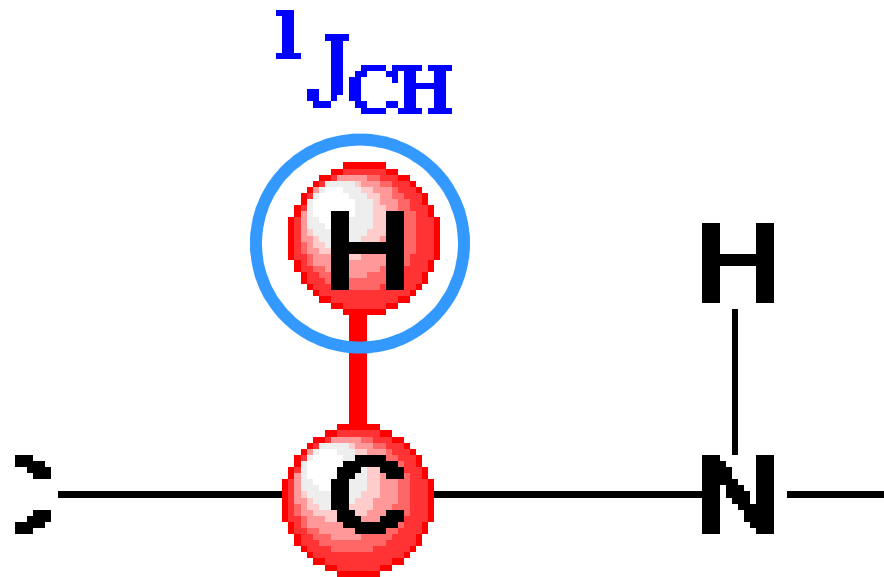
Theoretical relative sensitivities (S/N) for H,X correlation spectra (X= ^{13}C , ^{15}N)*.

Method	$\gamma_{\text{exc.}} \gamma_{\text{det.}}^{3/2}$	^{13}C	^{15}N
<i>direct detection</i>	$\gamma_{\text{X}} \gamma_{\text{X}}^{3/2}$	1.0	1.0
<i>INEPT / DEPT</i>	$\gamma_{\text{H}} \gamma_{\text{X}}^{3/2}$	4.0	9.9
<i>reverse INEPT</i>	$\gamma_{\text{X}} \gamma_{\text{H}}^{3/2}$	7.9	31.0
	(relative to INEPT=1)	2.0	3.1
<i>invers</i>	$\gamma_{\text{H}} \gamma_{\text{H}}$	31.6	306.0
	(relative to INEPT=1)	7.9	31.0

* not taking into account other factors, e.g., T_1 , heteronucl. NOE, linewidths etc.

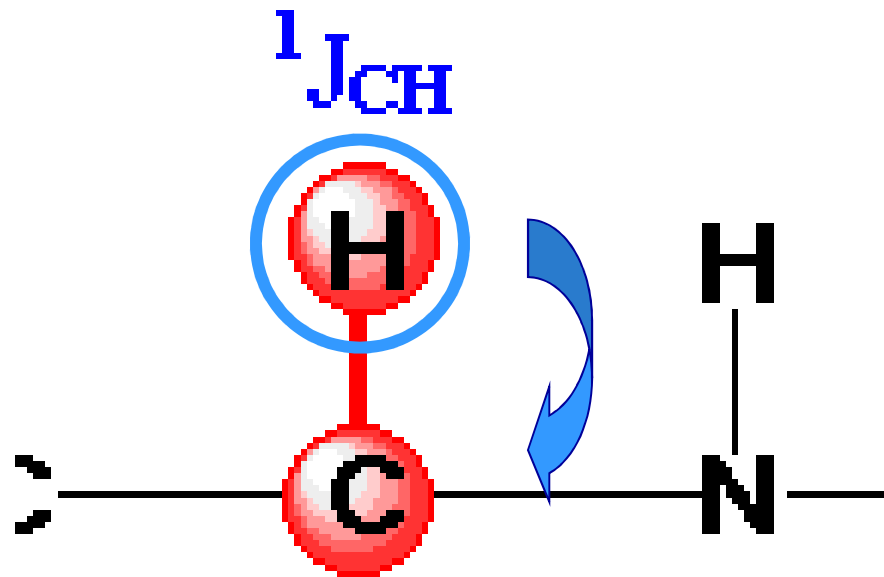
2D HSQC Experiment

Heteronuclear Single Quantum coherence



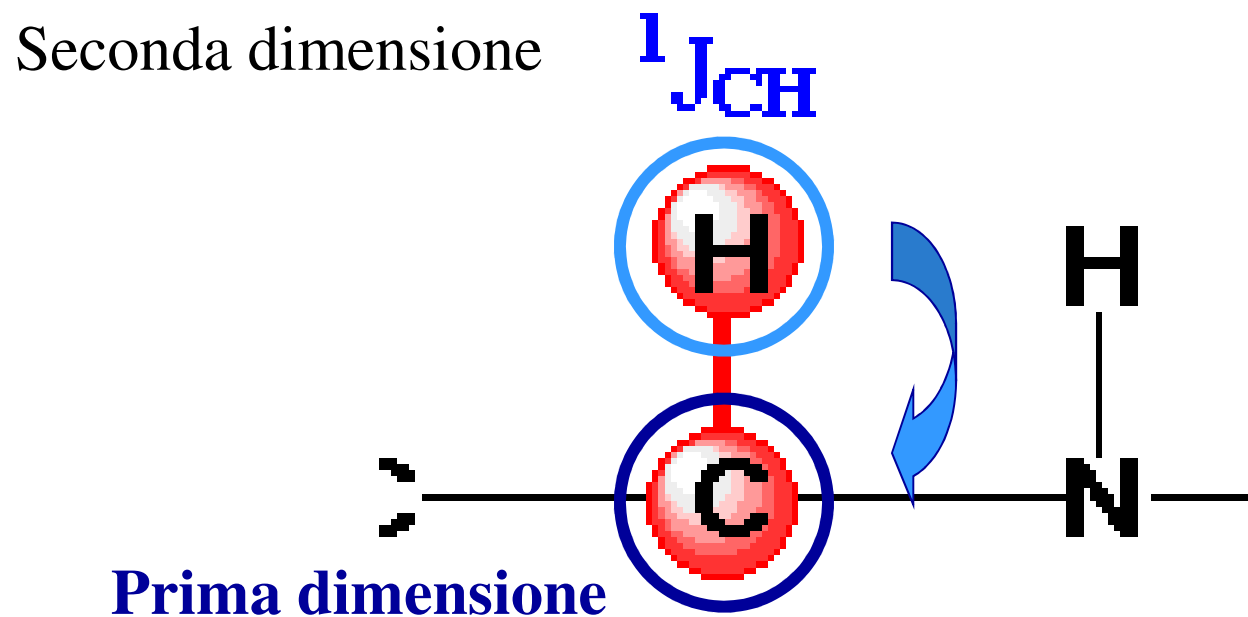
2D HSQC Experiment

Heteronuclear Single Quantum coherence



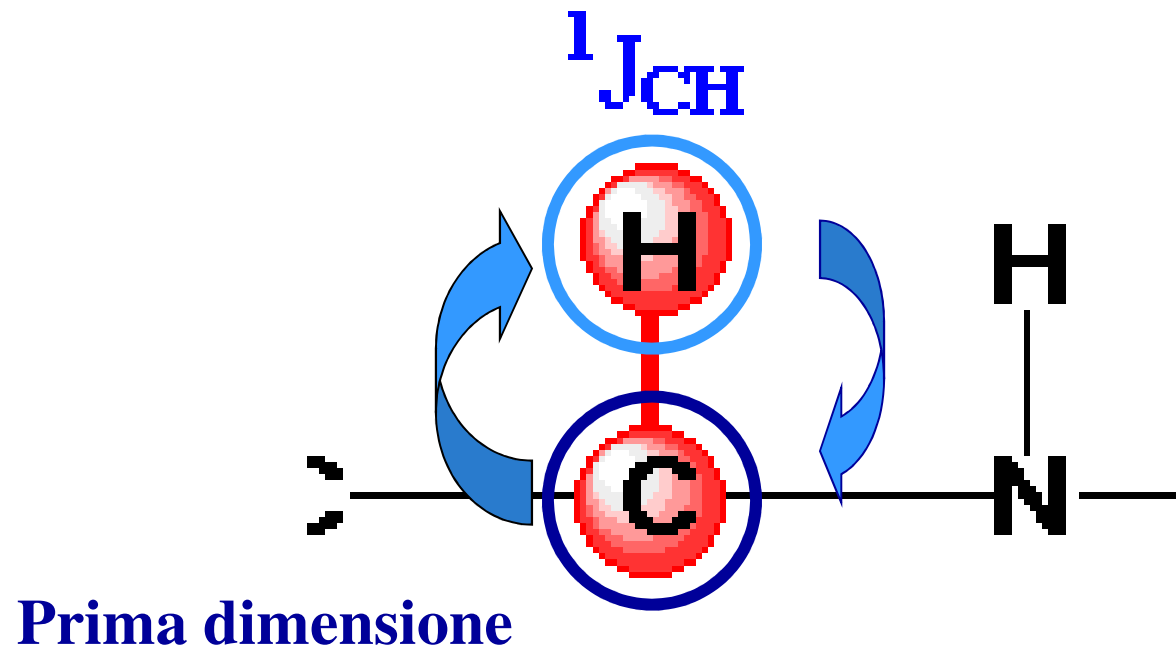
2D HSQC Experiment

Heteronuclear Single Quantum coherence



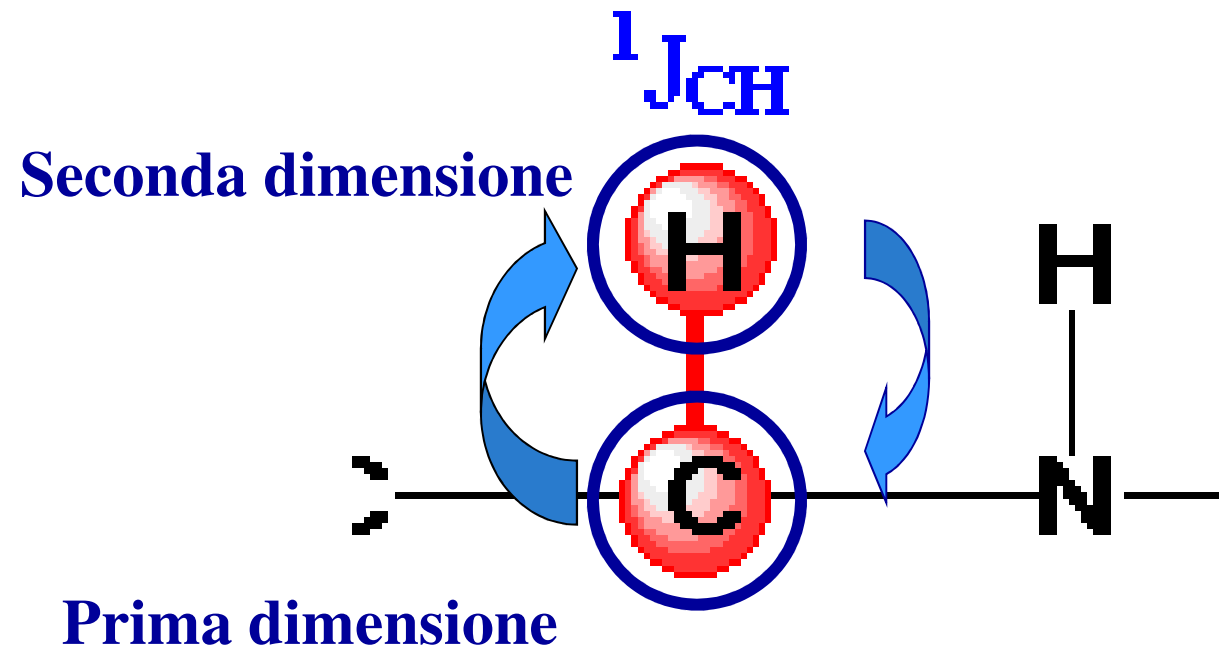
2D HSQC Experiment

Heteronuclear Single Quantum coherence



2D HSQC Experiment

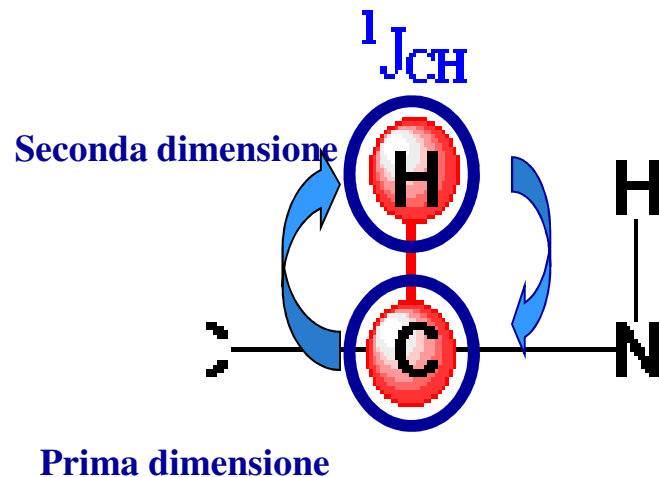
Heteronuclear Single Quantum coherence



2D HSQC Experiment

Heteronuclear Single Quantum coherence

E' possibile progettare esperimenti per trasferire la magnetizzazione da un nucleo all'altro anche indipendentemente dall'acquisizione



In questo esperimento il primo spin che viene eccitato è ^1H , la magnetizzazione viene trasferita da ^1H a ^{13}C PRIMA della acquisizione della prima dimensione, che quindi è ^{13}C .

SOLO i ^{13}C che sono accoppiati ad ^1H possono essere osservati!

Successivamente la magnetizzazione è di nuovo trasferita ^1H utilizzando sempre l'accoppiamento scalare ed alla fine osservo ^1H

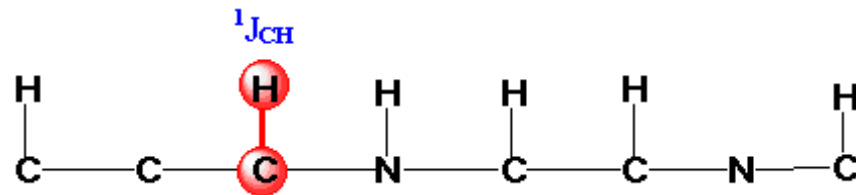
2D HSQC Experiment

Eccito (impulso a 90°) ^1H Trasferisco la magnetizzazione da ^1H a ^{13}C utilizzando l'accoppiamento scalare $^1J_{\text{HC}}$

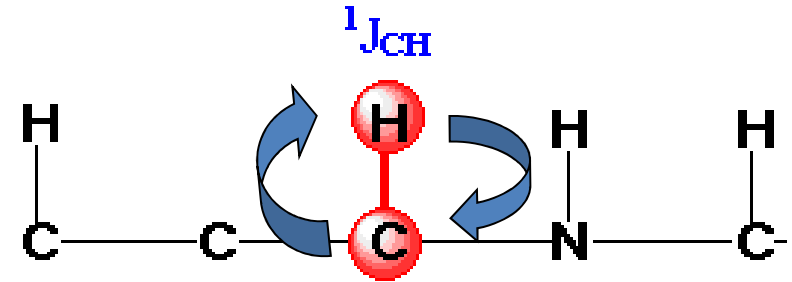
Acquisisco (t1) ^{13}C –

Perturbo -Trasferisco la magnetizzazione da ^{13}C a ^1H utilizzando l'accoppiamento scalare $^1J_{\text{HC}}$

Acquisisco (t2) ^1H



2D HSQC Experiment



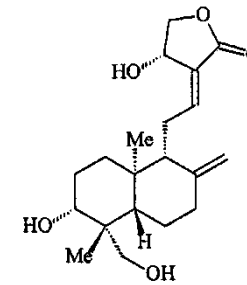
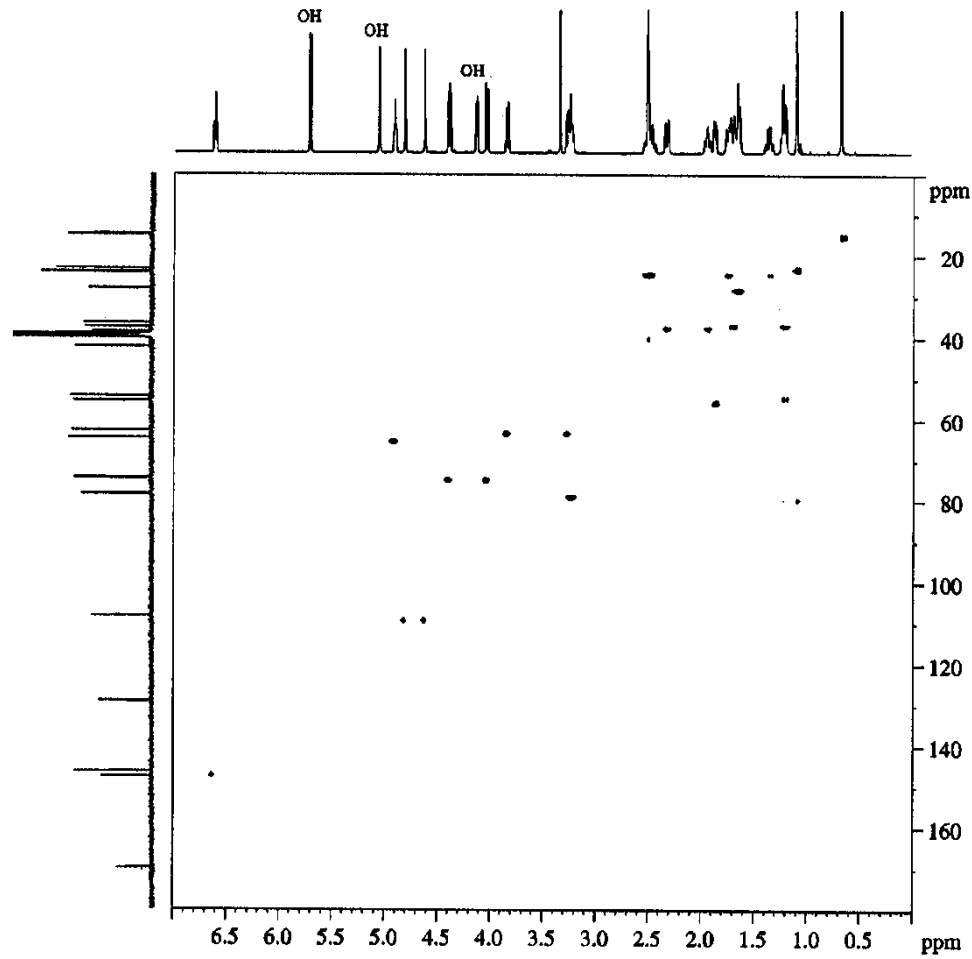
Questo tipo di esperimento si chiama anche
Out and back

Significa che parto da 1H , trasferisco da 1H a ^{13}C (out), acquisisco ^{13}C nella prima dimensione e poi torno (back) sullo stesso nucleo da cui sono partito

Il doppio trasferimento fa sì che l'esperimento sia molto più selettivo

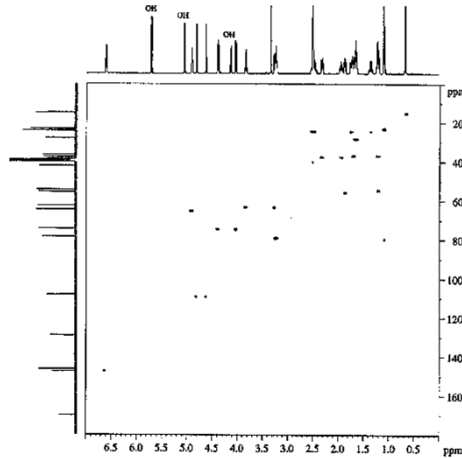
Osservo **solo** 1H e ^{13}C che sono accoppiati tra di se per effetto di 1J

The HSQC experiment



[2]

Caratteristiche dell'esperimento HSQC

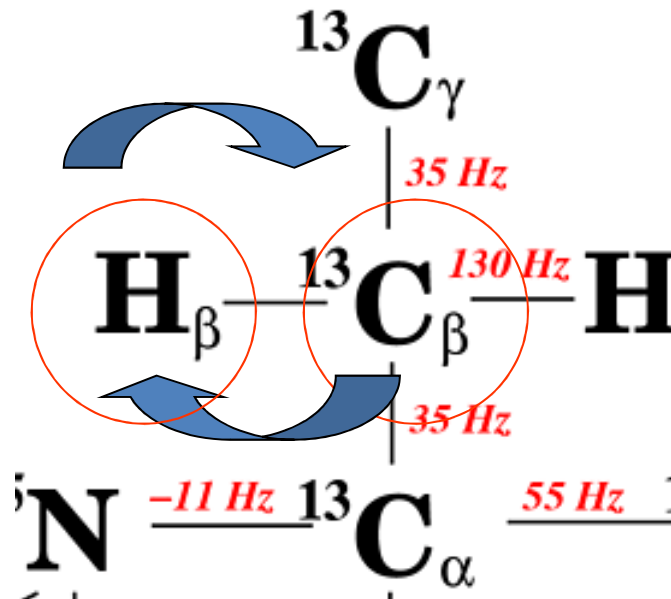


Non esiste la diagonale

La magnetizzazione viene trasferita da ^1H al ^{13}C ad esso accoppiato

Successivamente si acquisisce, nella dimensione indiretta, ^{13}C

Infine si ri-trasferisce su ^1H e si osserva ^1H



Tutti gli ^1H che non sono accoppiati a ^{13}C NON si osservano