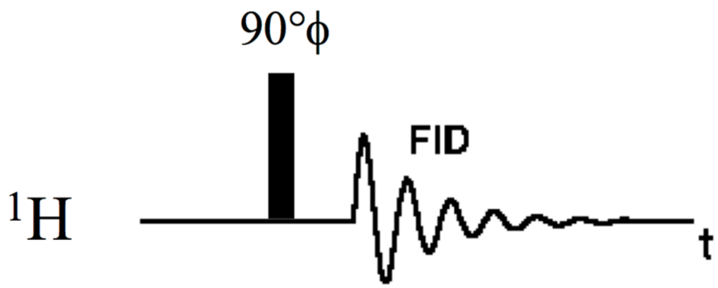
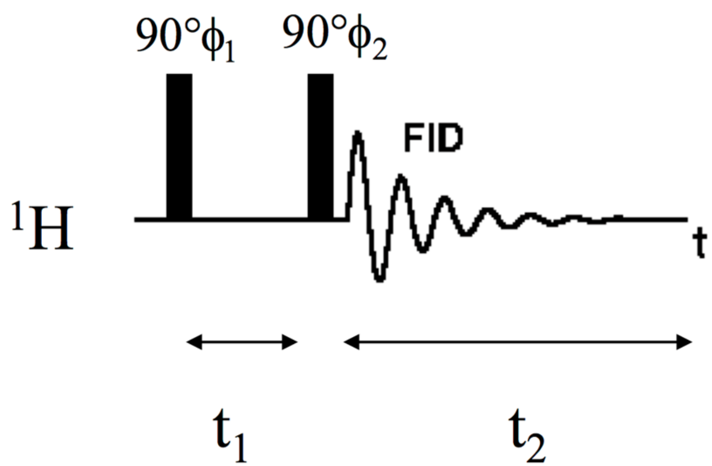
THE 2D NMR EXPERIMENT



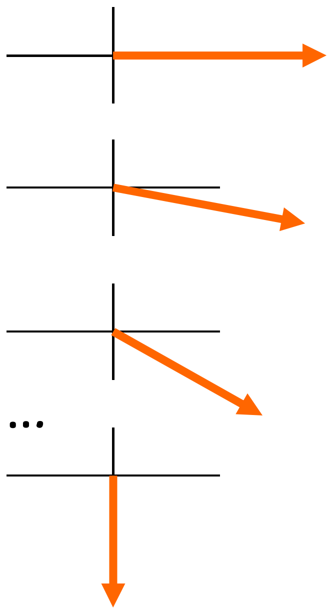
Pulse and collect sequence

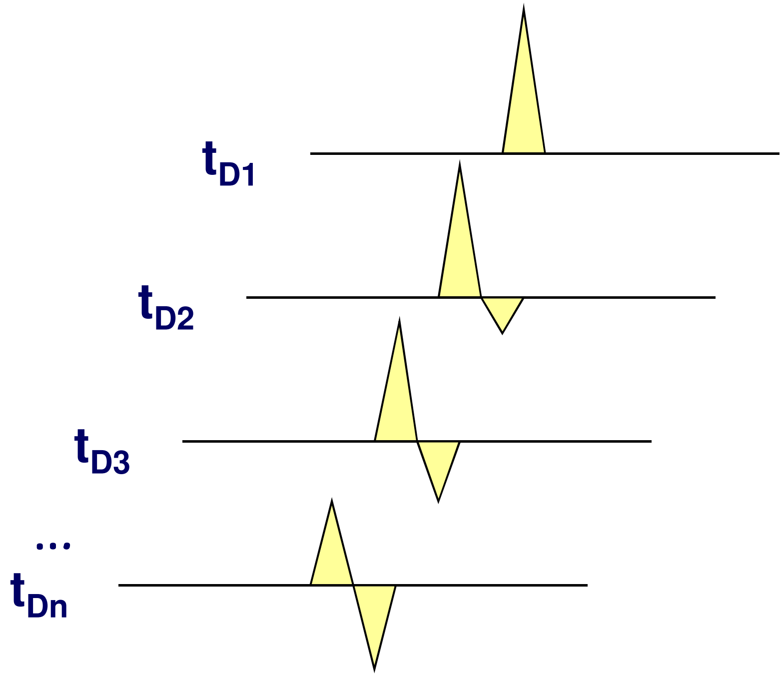


COSY(**CO**rrelated **S**pectroscop**Y**) sequence

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

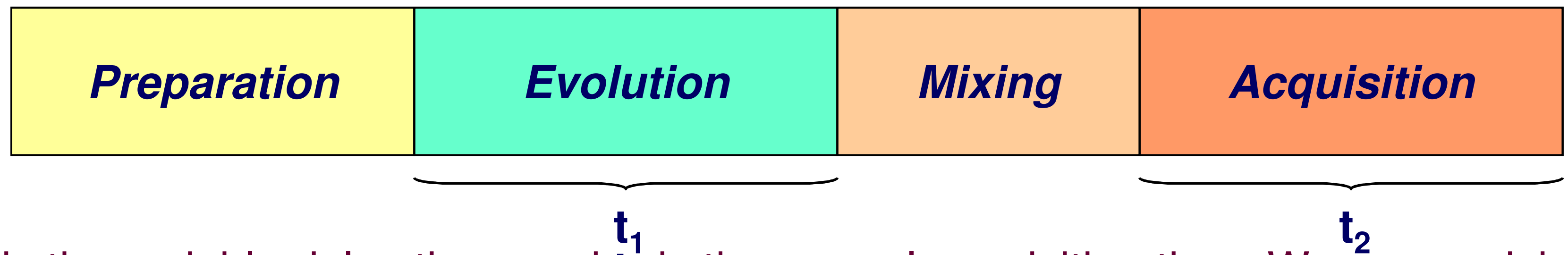
- The first perturbation of the system (pulse) will now be called the ***preparation*** of the spin system.

- The variable $\mathbf{t_D}$ is renamed the ***evolution time***, $\mathbf{t_1}$.

- 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*** ($\mathbf{t_2}$) as with all 1D experiments.

• Schematically, we can draw it like this:

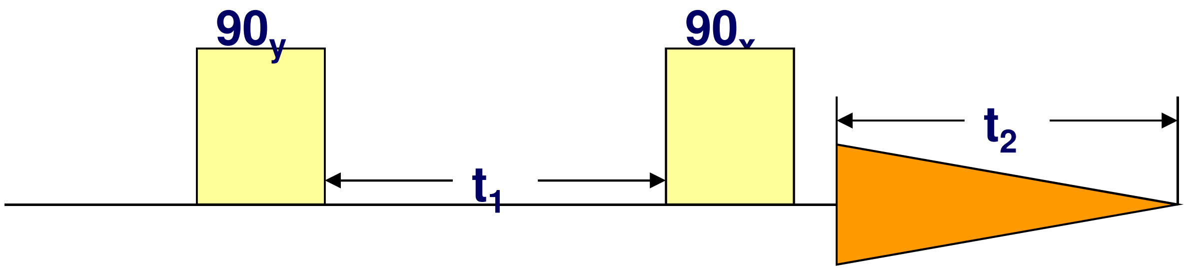


• $\mathbf{t_1}$ is the variable delay time, and $\mathbf{t_2}$ is the normal acquisition time. We can envision having $\mathbf{f_1}$ and $\mathbf{f_2}$, for both frequencies…

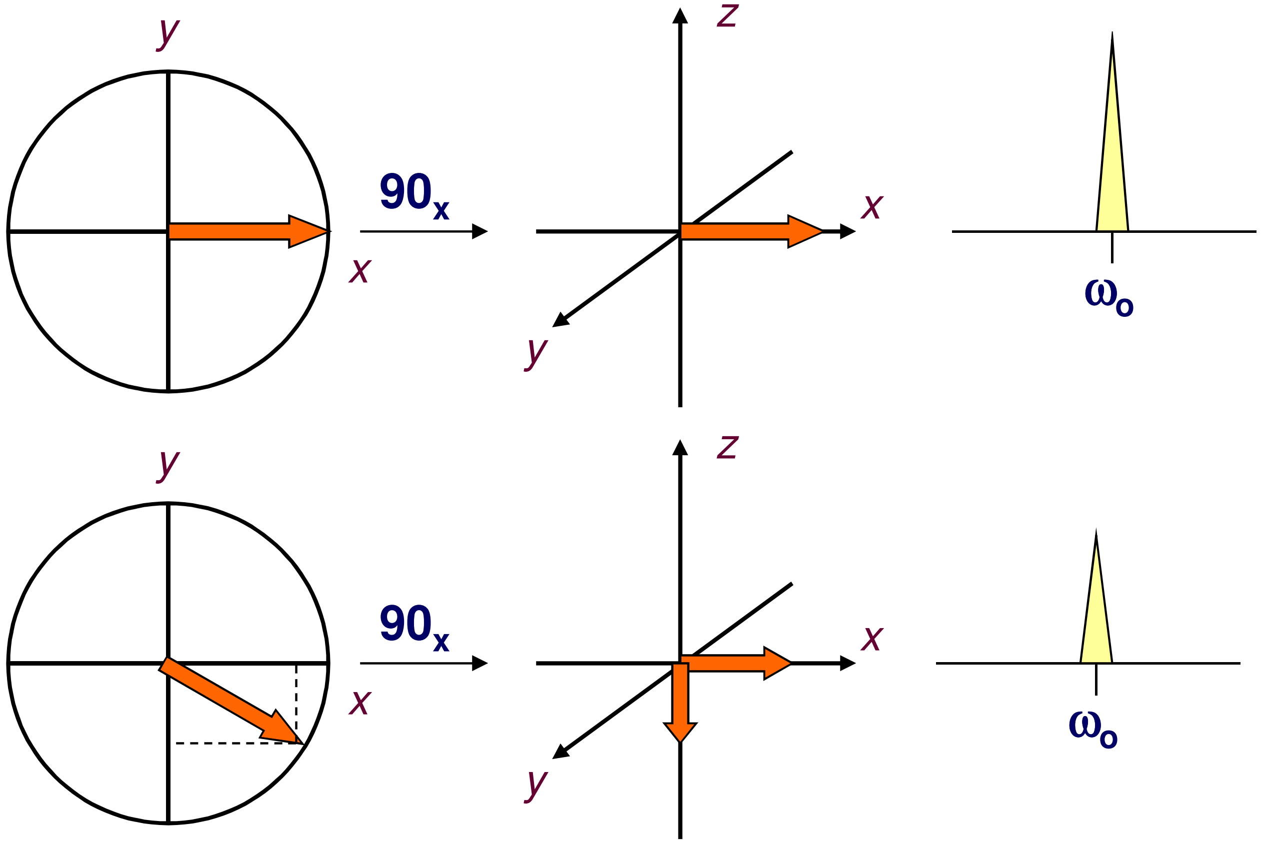
• We'll see that this format is basically the same for all 2D pulse sequences and experiments.

A rudimentary 2D experiment

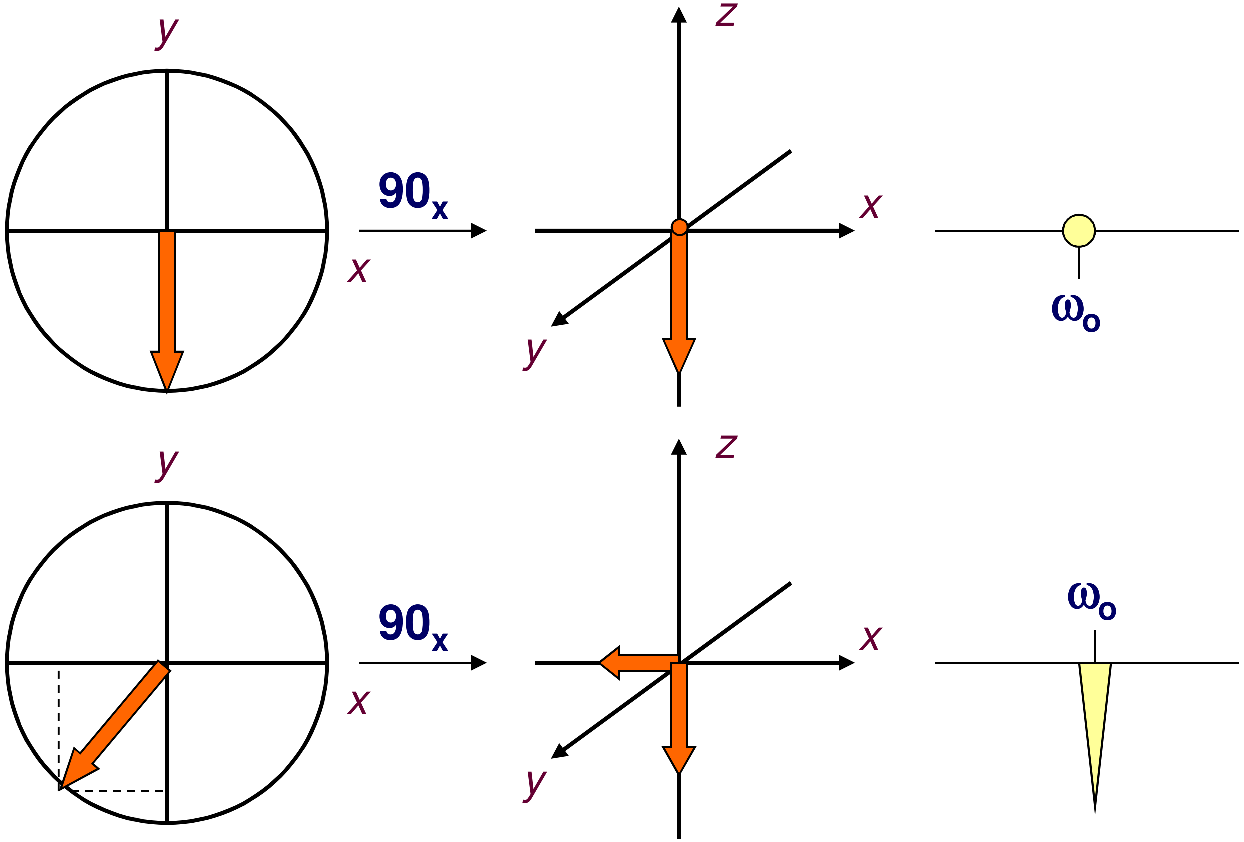
• We'll see how it works with the backbone of what will become the ***COSY*** pulse sequence. Think of theses pulses, were $\mathbf{t_1}$ is the preparation time:



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



The rudimentary 2D (continued)



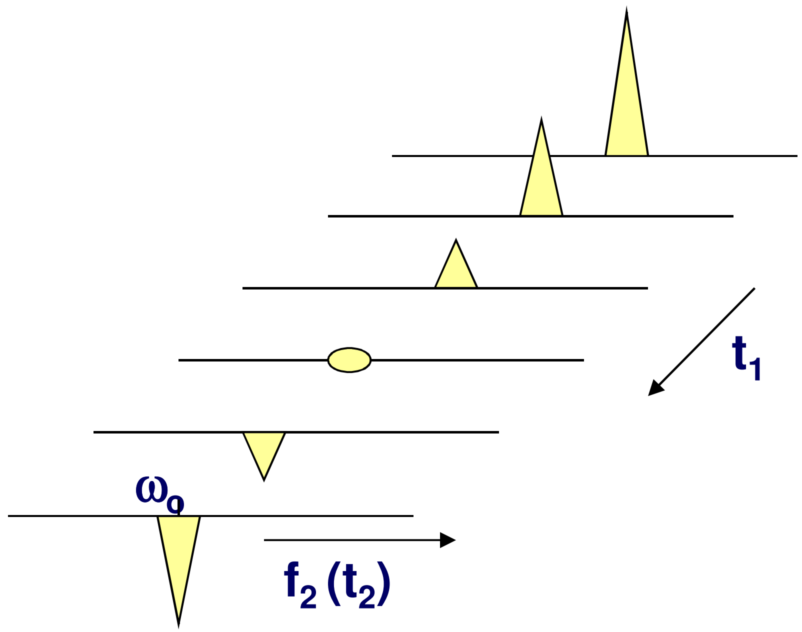
- The second π / **2** pulse acts only on the **y** axis component of the magnetization of the **<xy>** plane.

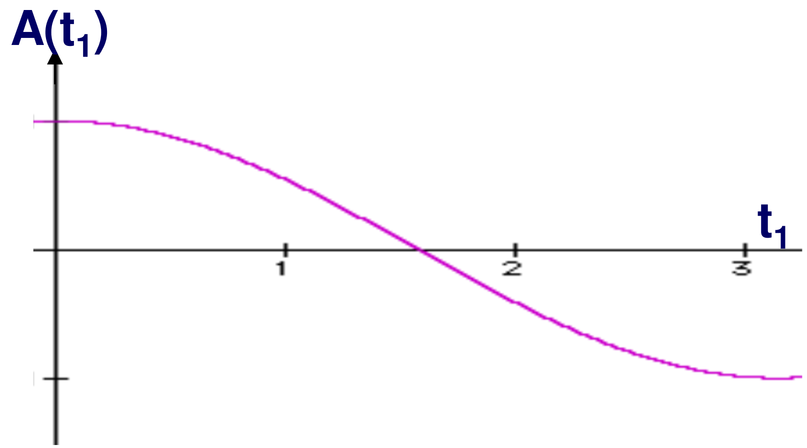
- The **x** axis component is not affected, but its amplitude will depend on the frequency of the line.

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

The rudimentary 2D (…)

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





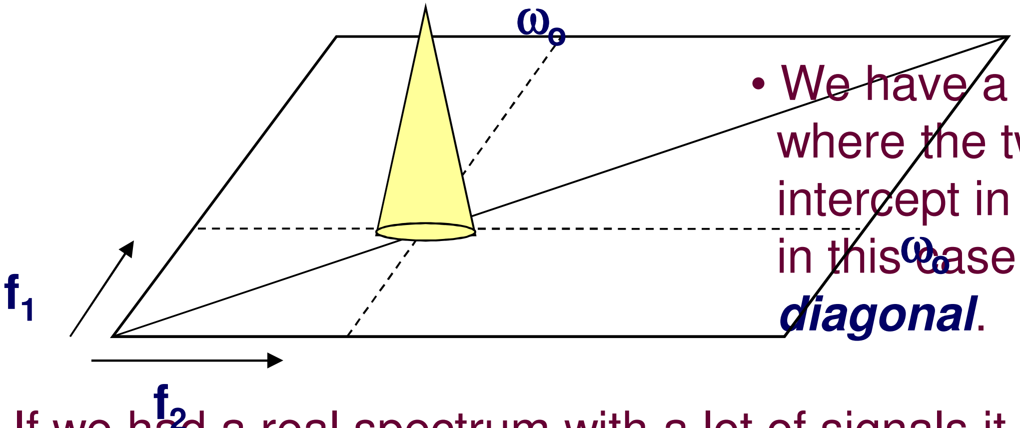
• Now, we have frequency data in one axis (**f_2**, which came from **t_2**), and time domain data in the other (**t_1**).

• 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.

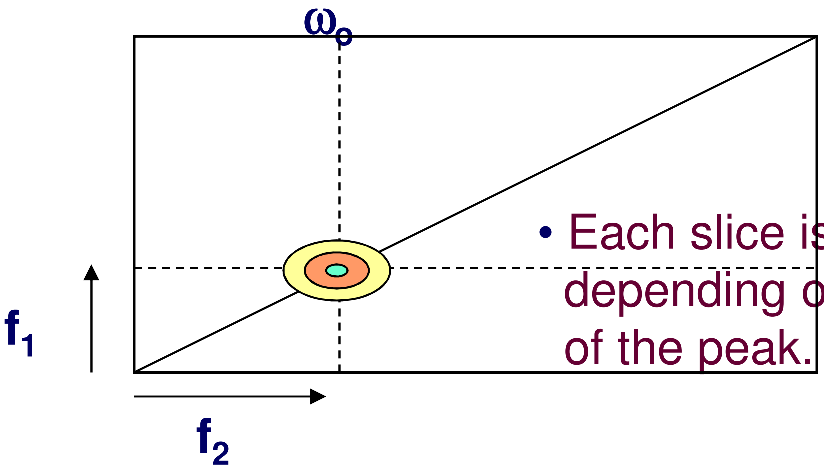
The rudimentary 2D (…)

• 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***.

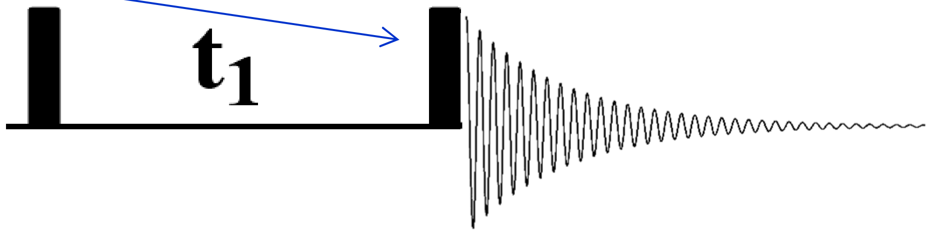
• If we had a real spectrum with a lot of signals it would be a royal mess. We look it from above, and draw it as a ***contour plot*** - we chop all the peaks with planes at different heights.

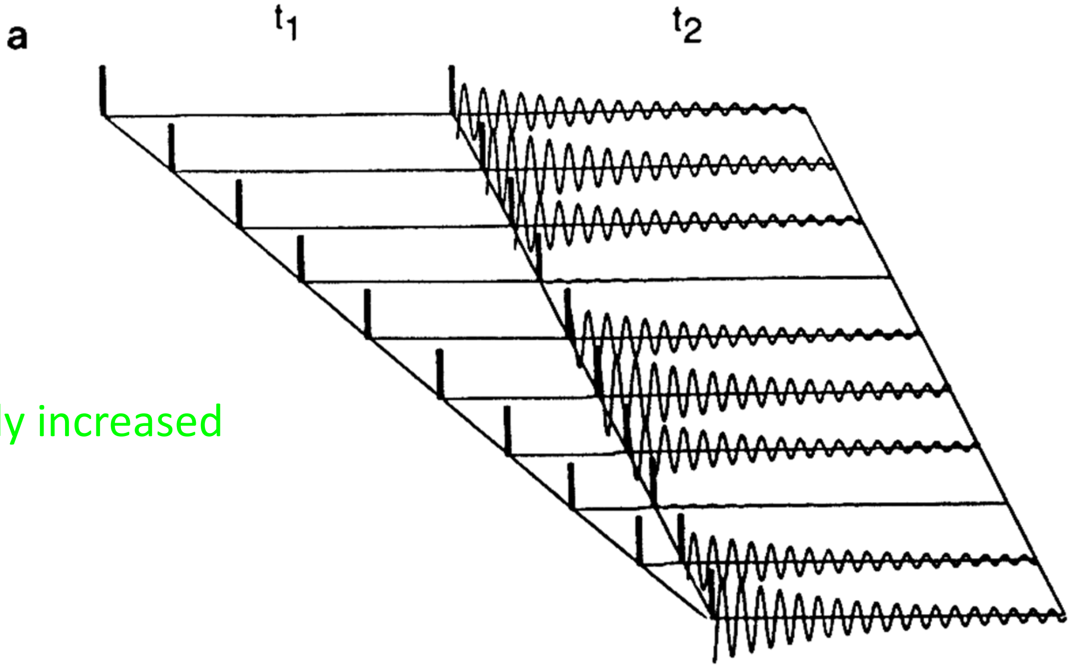


• Each slice is color-coded depending on the height of the peak.

2D Spectroscopy

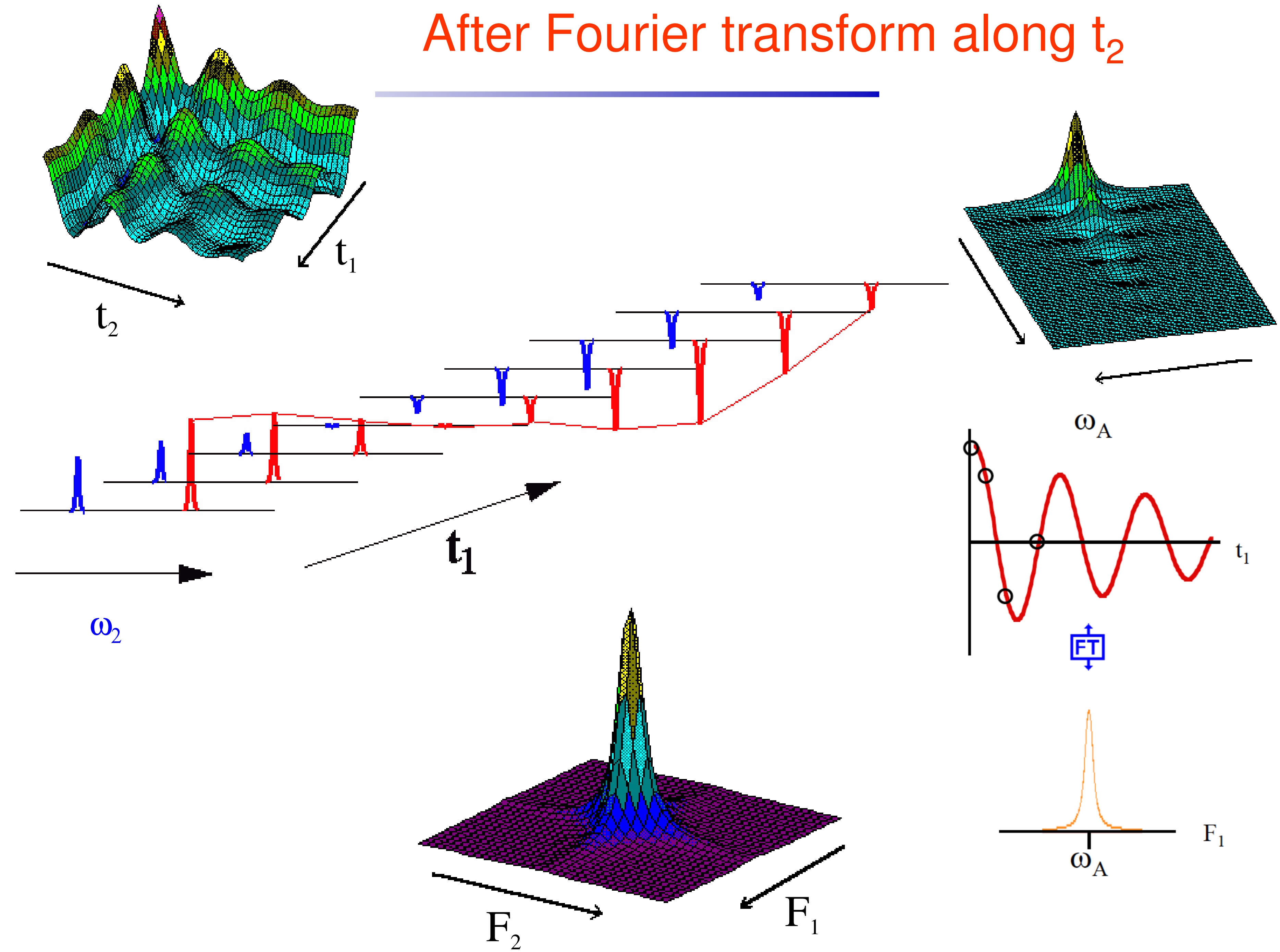
- Introduction of a second pulse and of a variable delay t_1

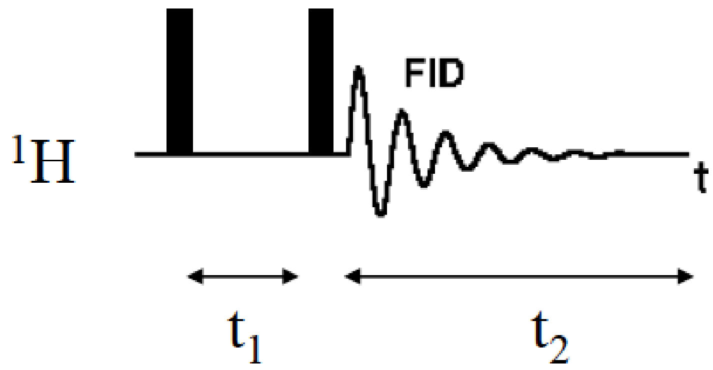




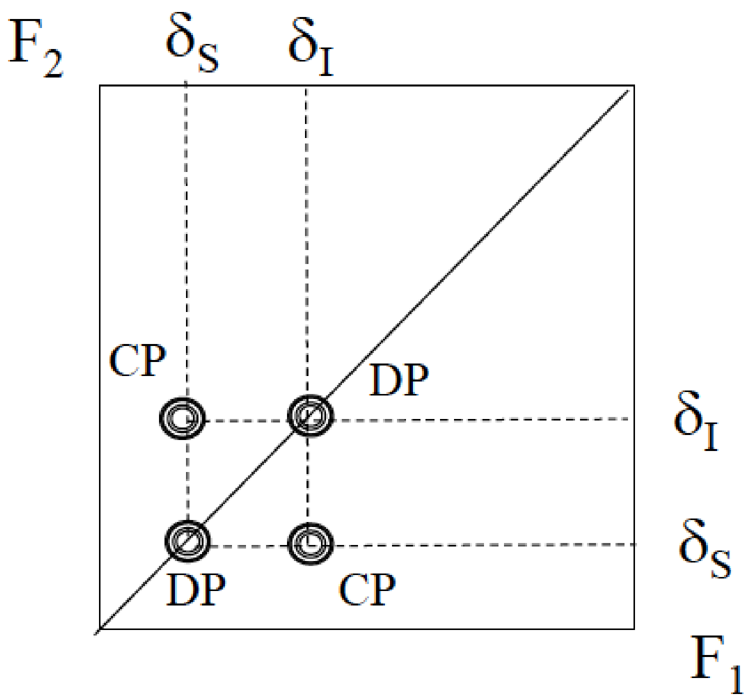
t_1 is systematically increased

After Fourier transform along t_2

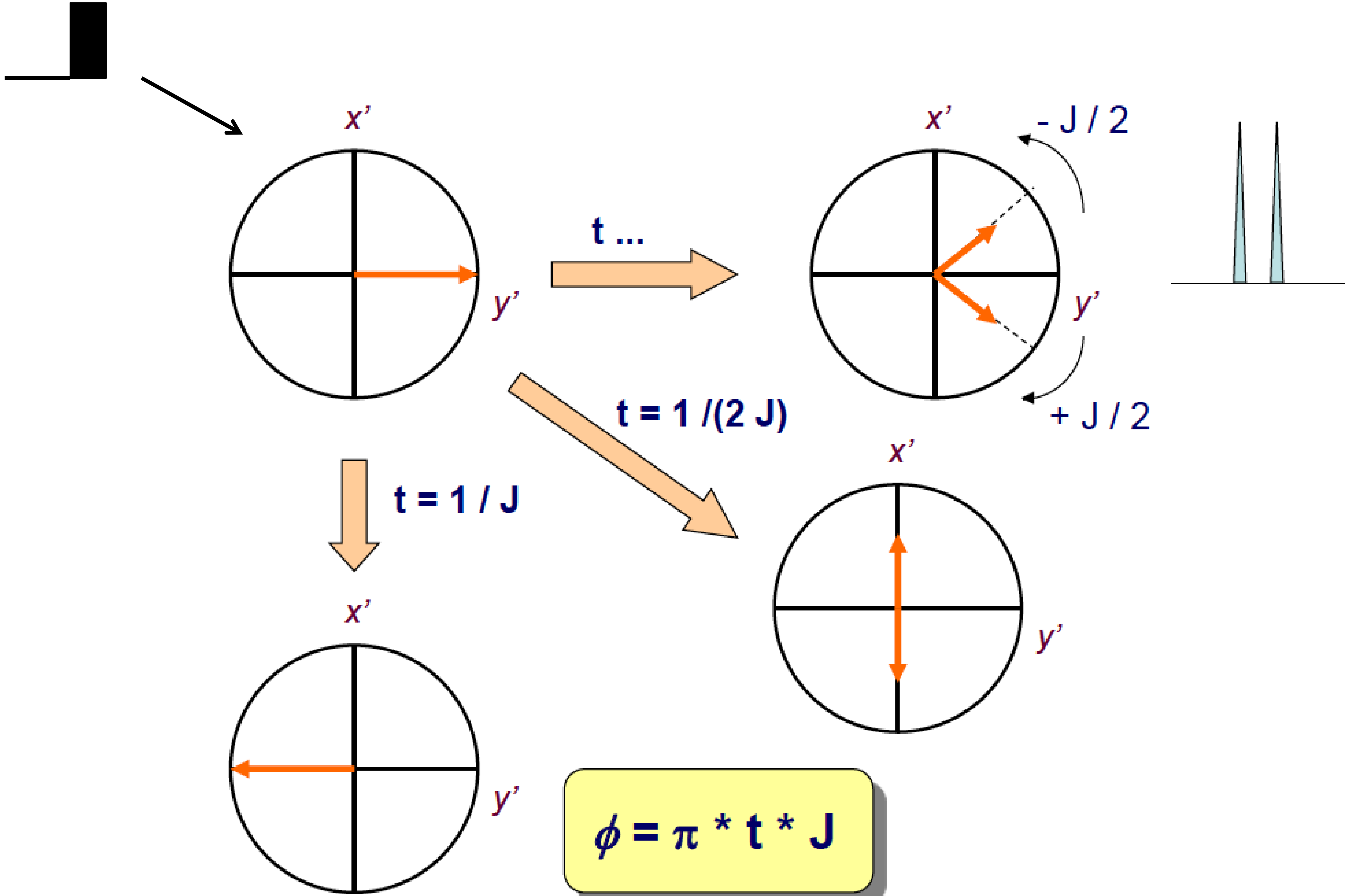




I and S are coupled

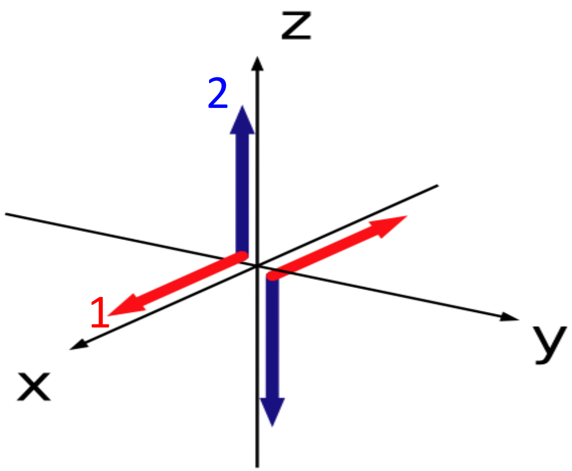


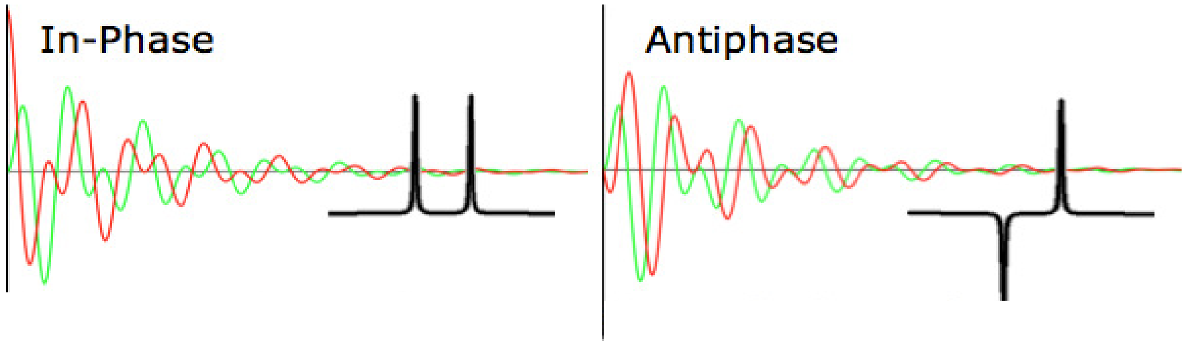
COUPLING CONSTANTS IN THE ROTATING FRAME



$$\phi = \pi * t * J$$

Antiphase magnetisation represents the coherence of one spin (red vectors) linked to the population of the coupling partner (blue vectors) and is denoted as the product of a transverse magnetisation with a z-magnetisation ($2I_{1x}I_{2z}$)



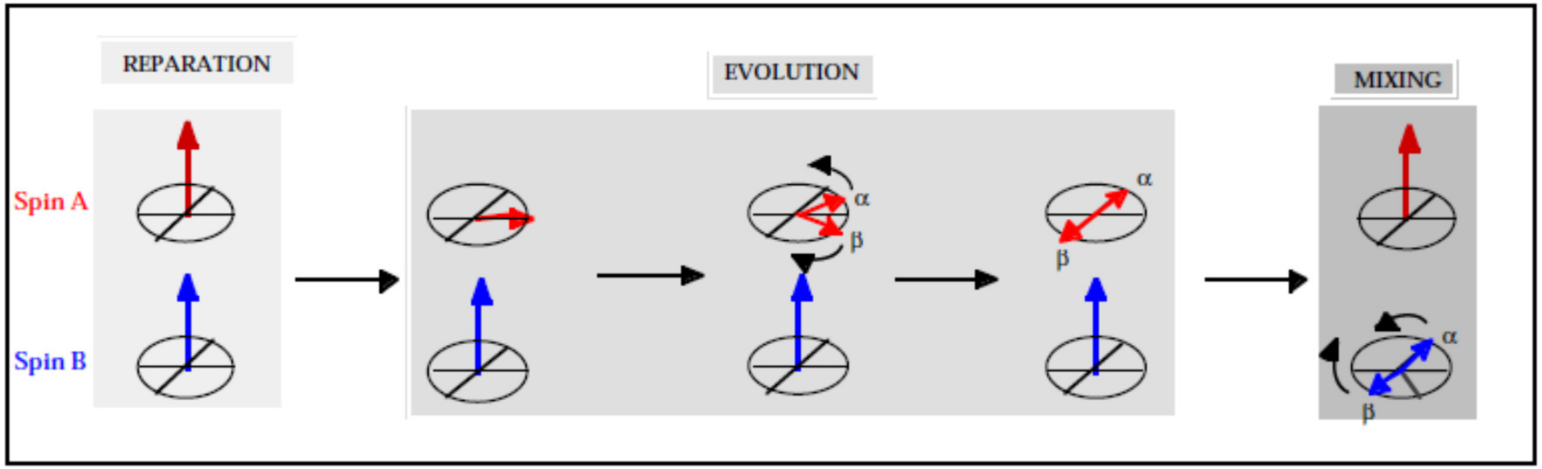


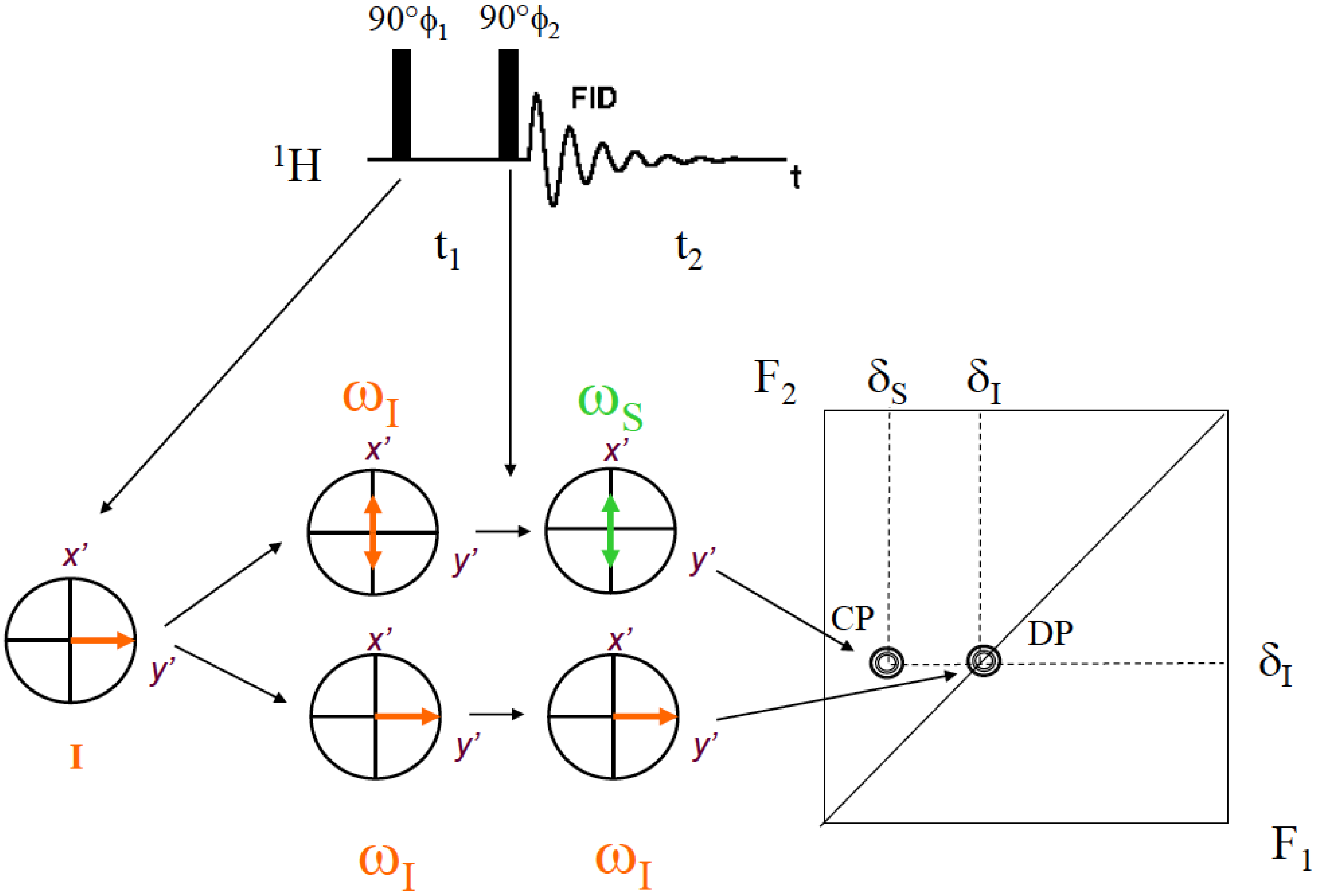
One vector results from spin 1 bound to spin 2 in α-state, while the second vector corresponds to spin 1 bound to spin 2 in the β-state

Coherence refers to the presence of phase relationship between excited spins

in-phase, coherence **-I1y,** causes a normal doublet, where both peaks have the same sign and phase

antiphase magnetisation, coherence **2I1yI2z** results in a characteristic doublet with the two peaks having opposite signs.

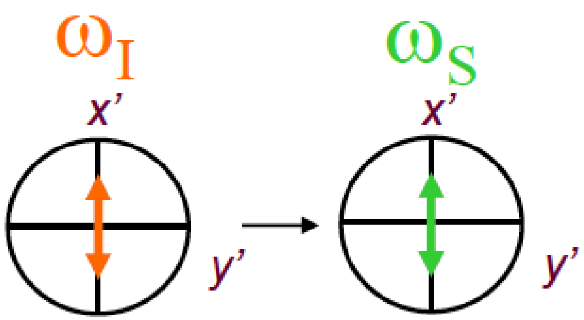

REPARATION
EVOLUTION
MIXING
Spin A
Spin B
α
β
α
β
α
β



90°φ1
90°φ2
FIG
1H
t
t1
t2
ωI
ωS
x'
y'
I
ωI
ωI
F2
δS
δI
CP
DP
δI
F1

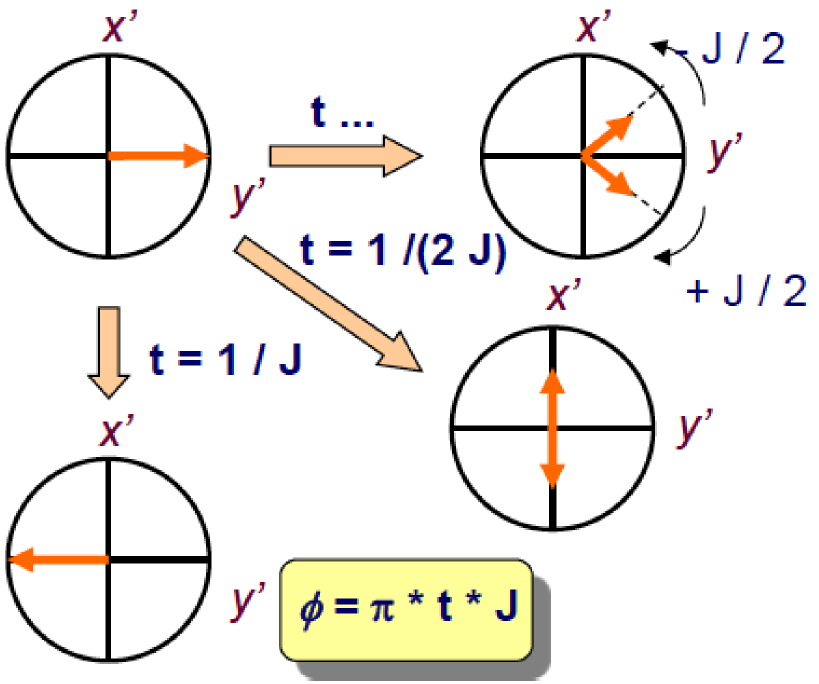
Summarizing the important aspects of the COSY experiment

- Diagonal peaks represent no coherence transfer, whereas cross peaks represent the transfer of coherence from spin 1 to spin 2. Recall that "coherence" refers to the presence of a defined phase relationship between excited spins; coherence transfer means that this phase relationship has been transferred to a "non-excited" spin through a scalar coupling mechanism.

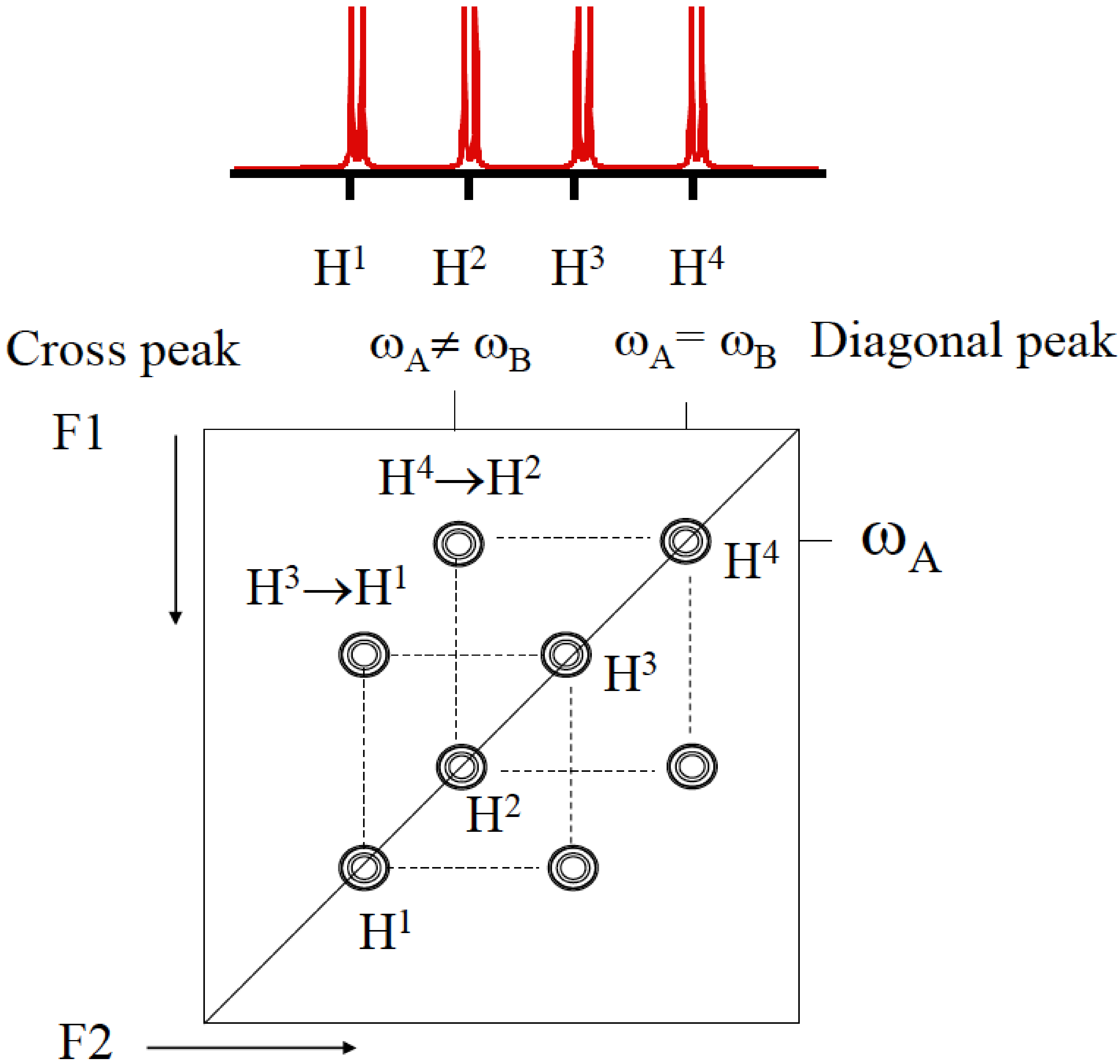


Summarizing the important aspects of the COSY experiment

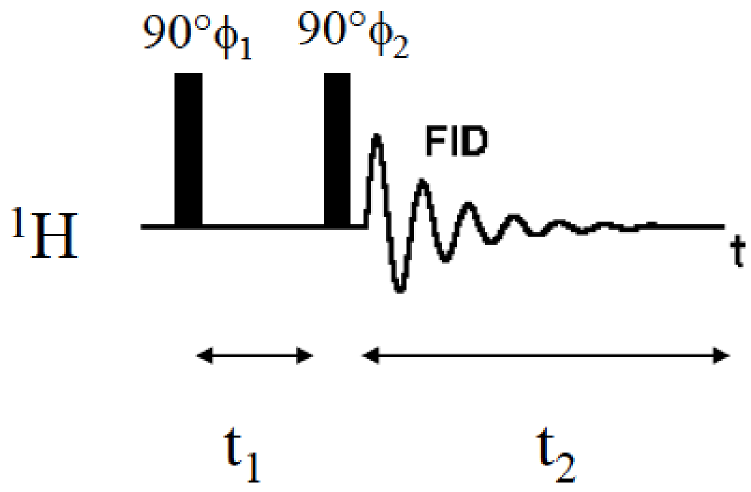
- In the COSY experiment, intensity variation in t_2 is dependent upon the amount of anti-phase state that builds up during the t_1 period ($\sin\pi Jt_1$), not just on the chemical shift of the nucleus during t_1. For a fixed t_1 value, the amount of anti-phase character will be less for two spins coupled by a small J (weakly coupled) than for two spins that are more strongly coupled. Recall that the scalar coupling constant decreases with increasing number of bonds between the two nuclei. In other words, the COSY experiment gives correlations between spins that are strongly coupled.



$$\text{Int } \alpha \sin(\pi^* t_1^* J)$$

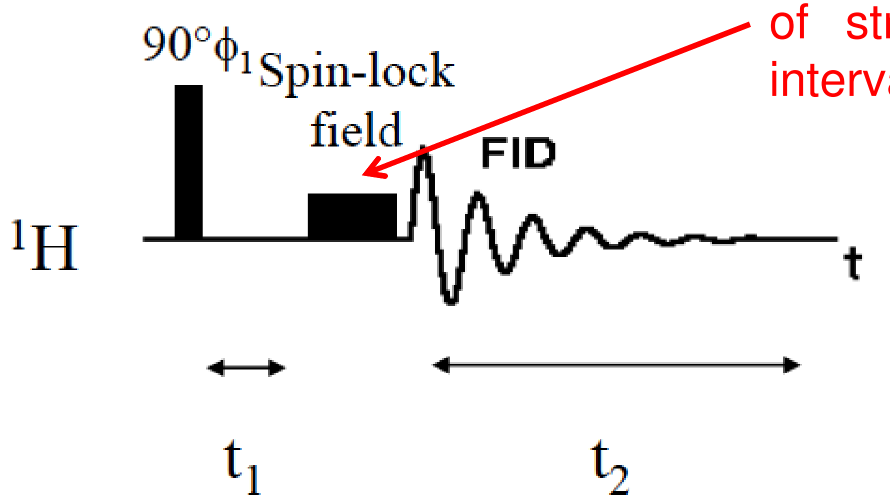

H1 H2 H3 H4
Cross peak
ωA≠ ωB
ωA= ωB
Diagonal peak
F1
H4→H2
H3→H1
H4
H3
H2
H1
ωA
F2

Total correlation spectroscopy



COSY(**CO**rrelated **S**pectroscop**Y**) sequence

The second $\pi/2$ pulse is replaced by a train of strong π pulses spanning the mixing interval τ_m



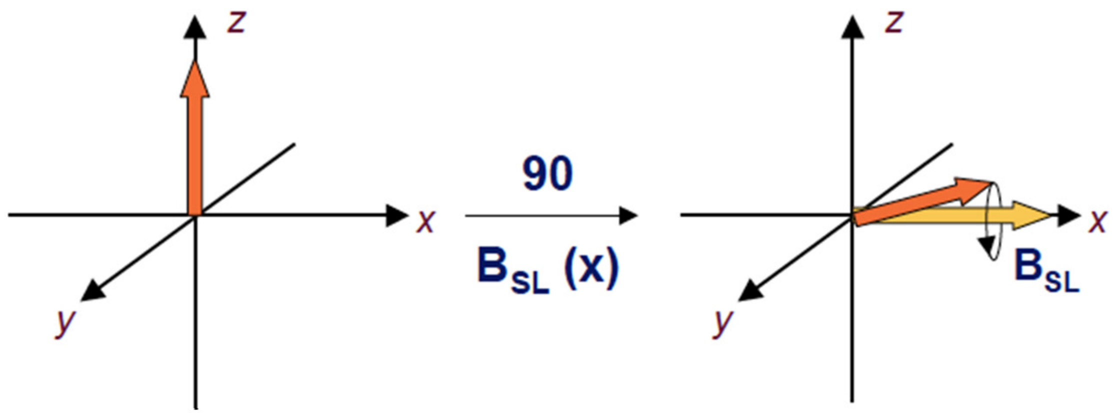
TOCSY(**TO**tal **C**orrelation **S**pectroscop**Y**) sequence

At $B_0\,\Delta\delta(\text{Hz}) >> J(\text{Hz})$ the effects on the energy of the system arising from couplings are much smaller than those due to chemical shifts

$$\mathbf{H} = \mathbf{H}_\delta + \mathbf{H}_J + \ldots \quad \text{with } \mathbf{H}_\delta >> \mathbf{H}_J + \ldots$$

H_δ is called the *Halmitonian* and represents the energy of the system

If the magnetisation is spin-locked in the xy plane by a composite pulse



$$\text{before SL: } \omega_o = \gamma B_o \qquad \text{after SL: } \omega_{SL} = \gamma B_{SL}$$

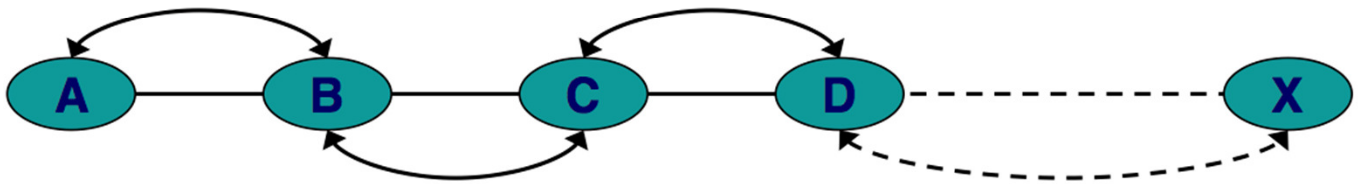
The frequencies of all the transitions of the system are proportional to B_{SL}

$$\mathbf{H}_J >> \mathbf{H}_\delta$$

coherence transfer occurs due to scalar coupling
Strong coupling condition

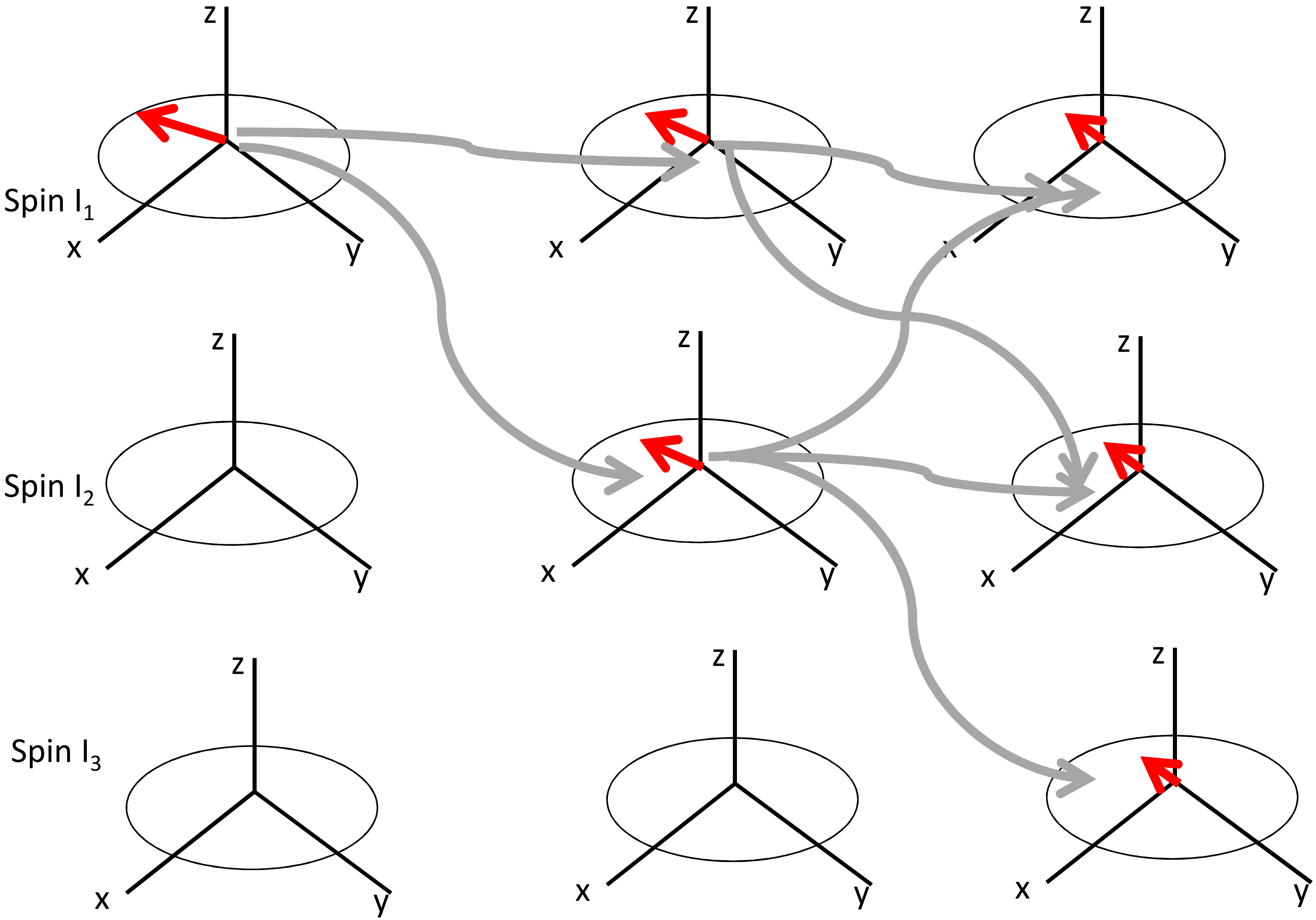
- Now the coupling term dominates the energy of the system, and coherence transfer occurs due to scalar coupling.

- 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:

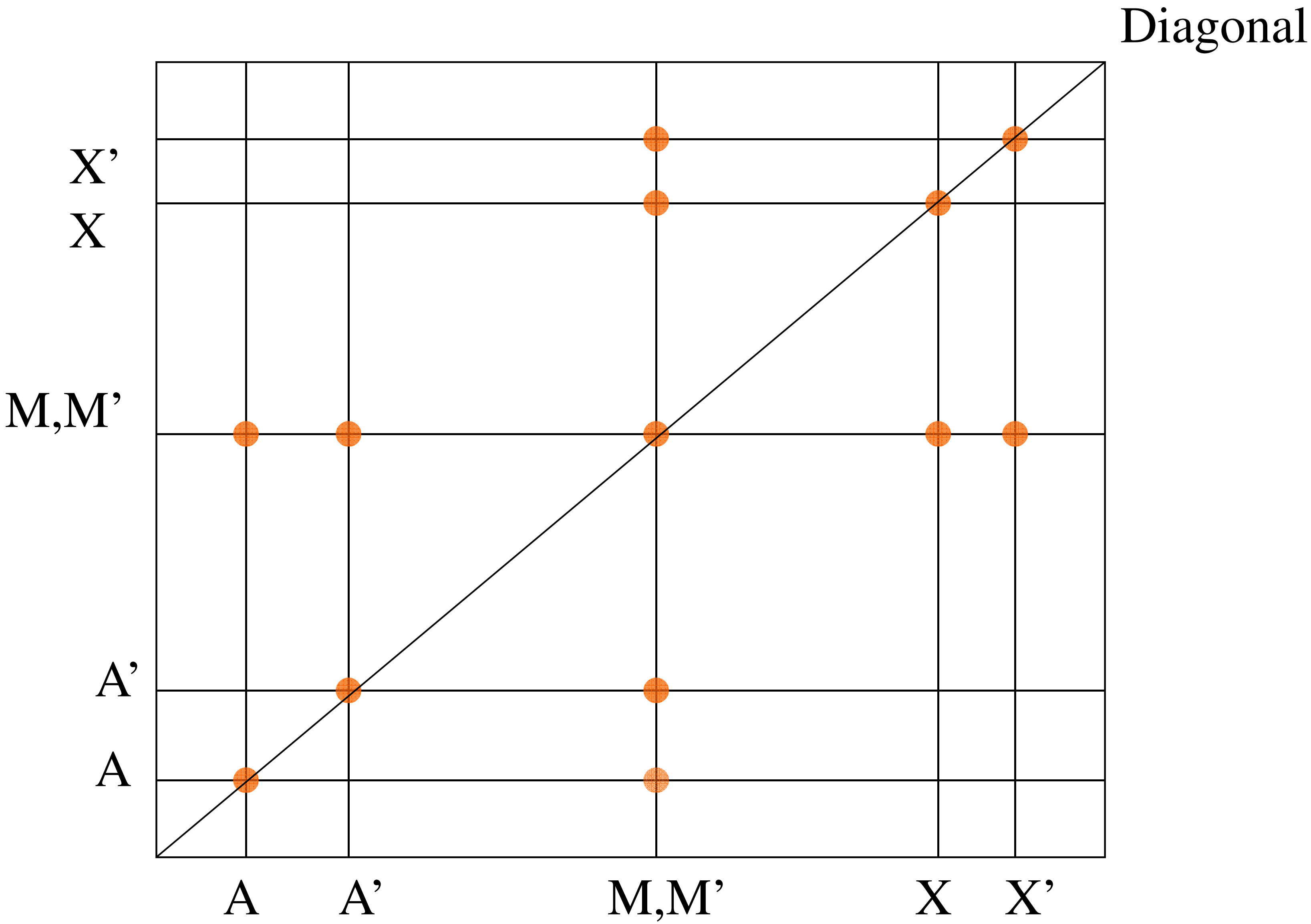


- The maximum transfer between two spins with a coupling of **J** Hz is optimal when $\mathbf{t_m} = \mathbf{1 / 2J}$. Longer $\mathbf{t_m}$s allow transfer to weakly coupled spins: We go deeper in the spin system.

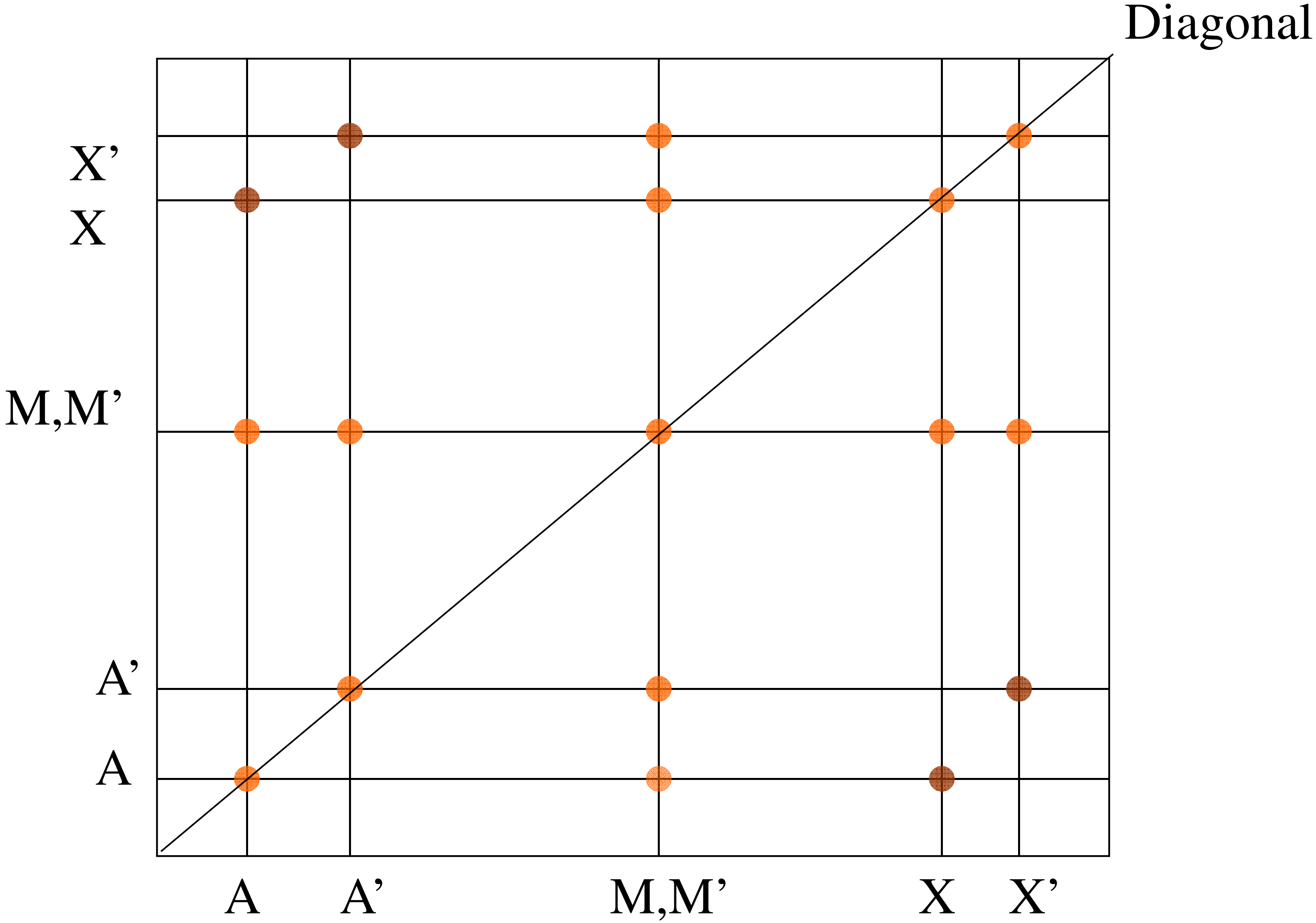
In-phase transfer magnetisation under strong coupling in TOCSY

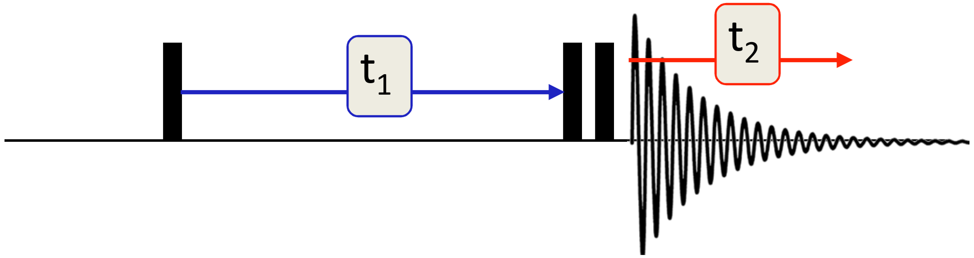


Ambiguity in a COSY spectrum



If we only could detect spin-spin couplings bejond ^{3}J… TOCSY spectra!

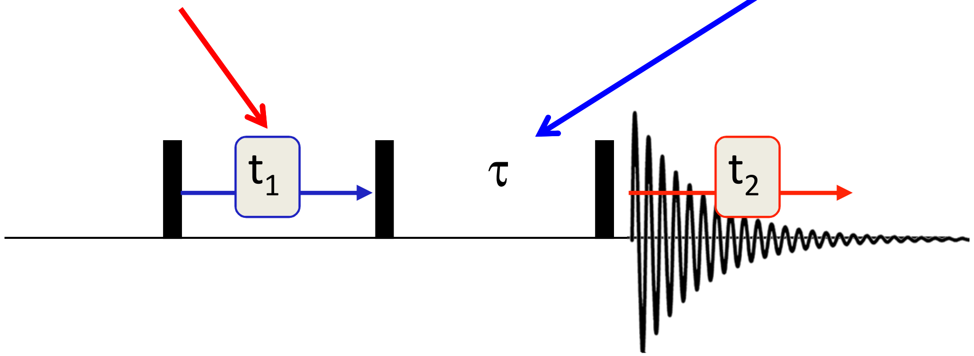




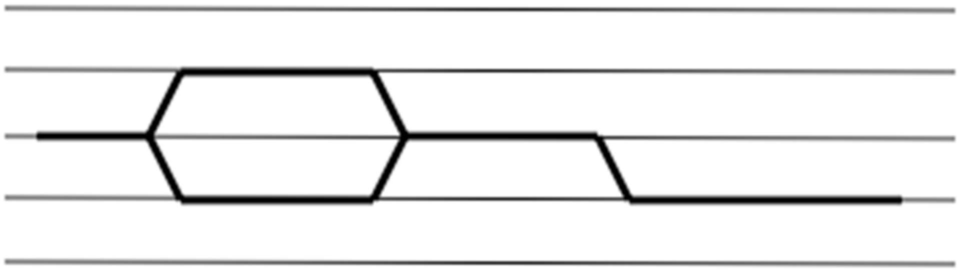
DQF-COSY

t_1: frequency labelling

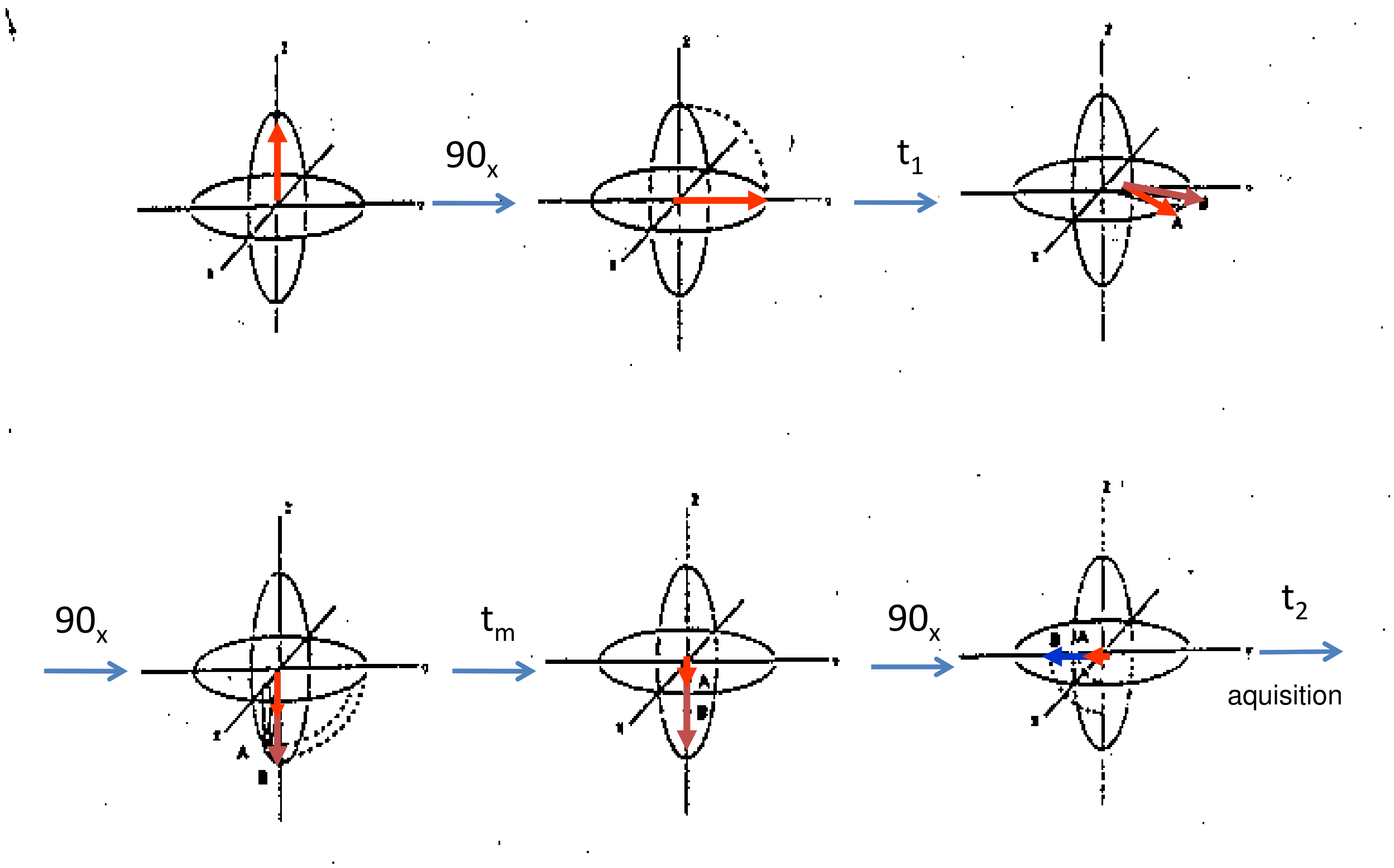
τ: cross-relaxation between close nuclei can take place



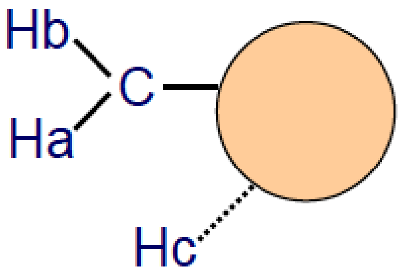
NOESY

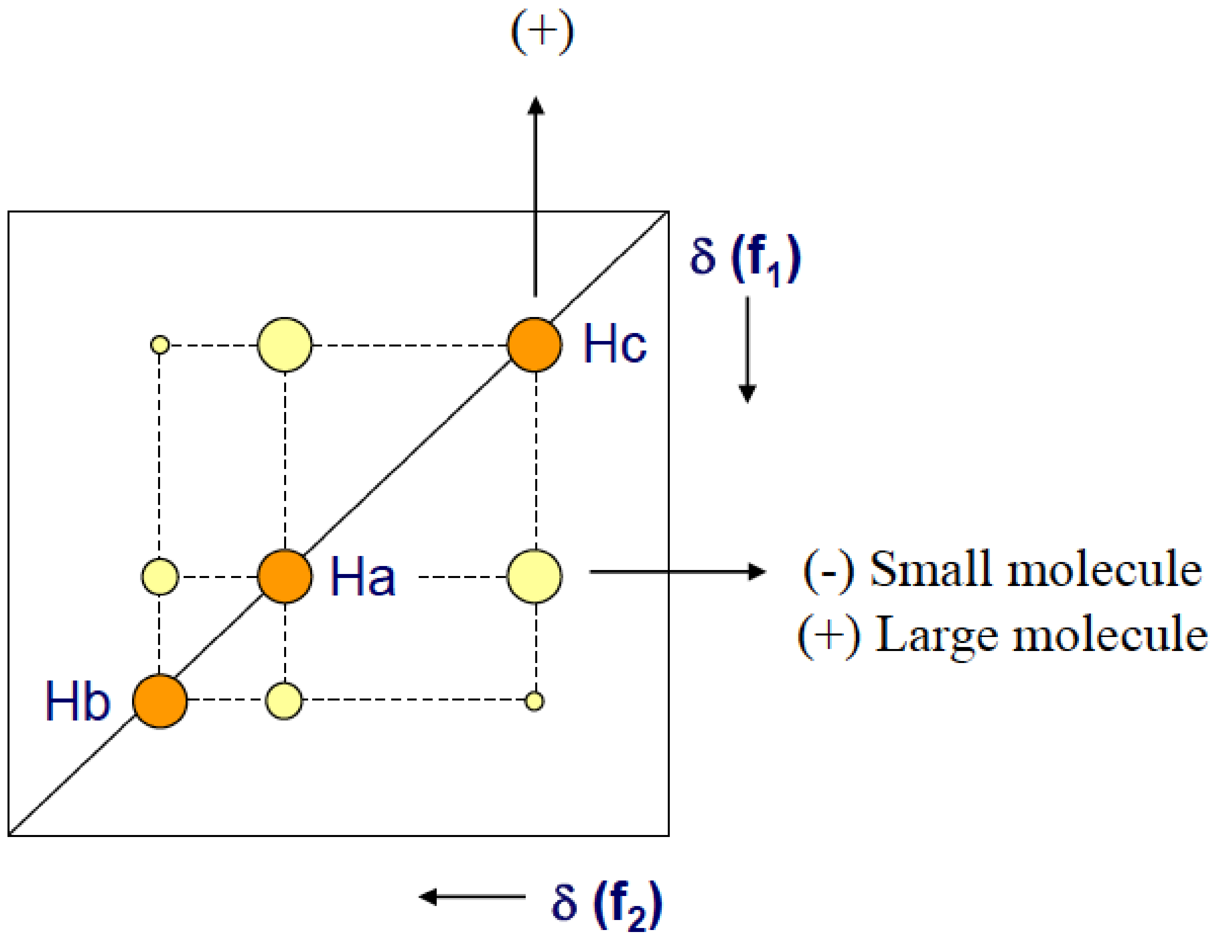


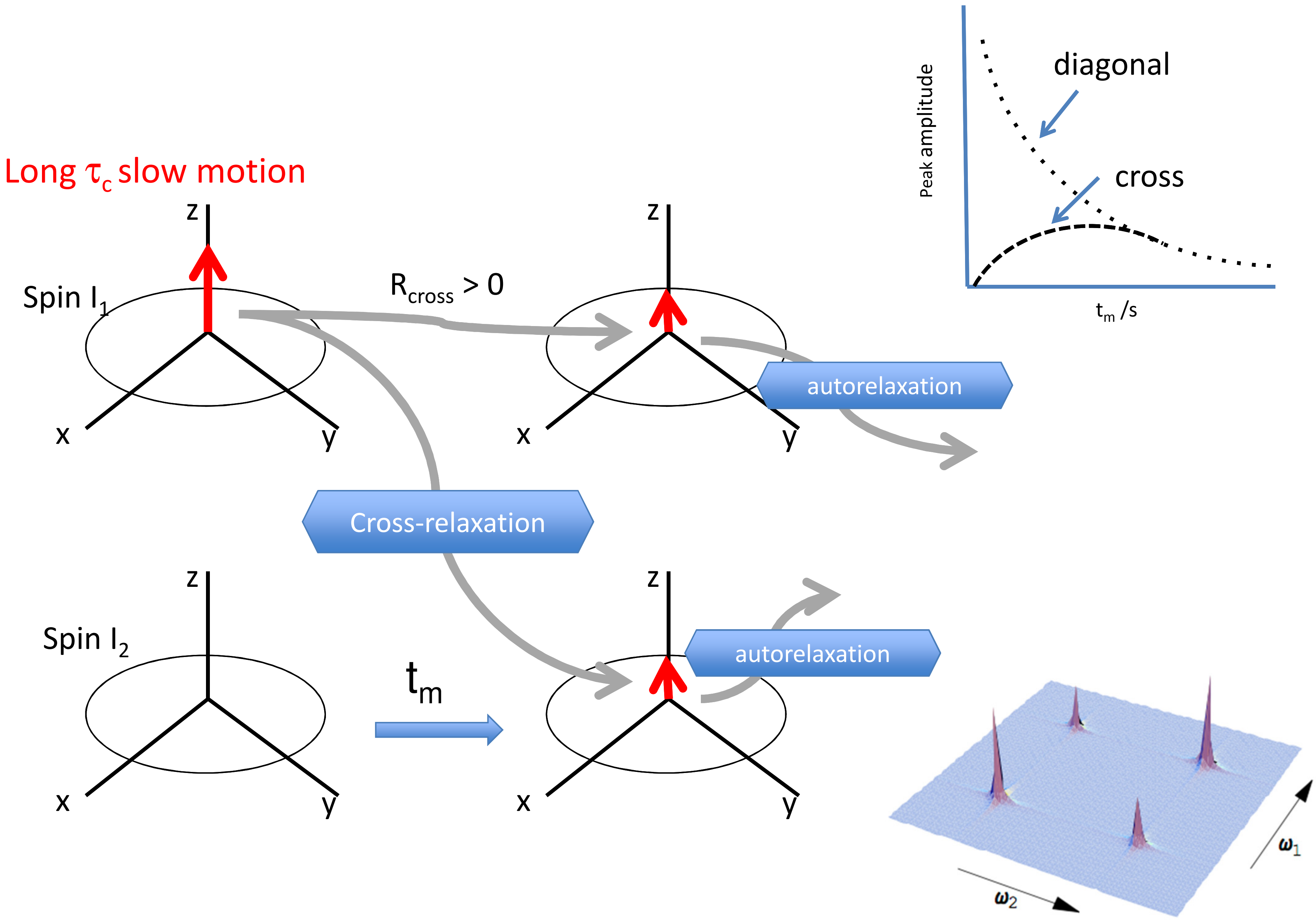
NOESY

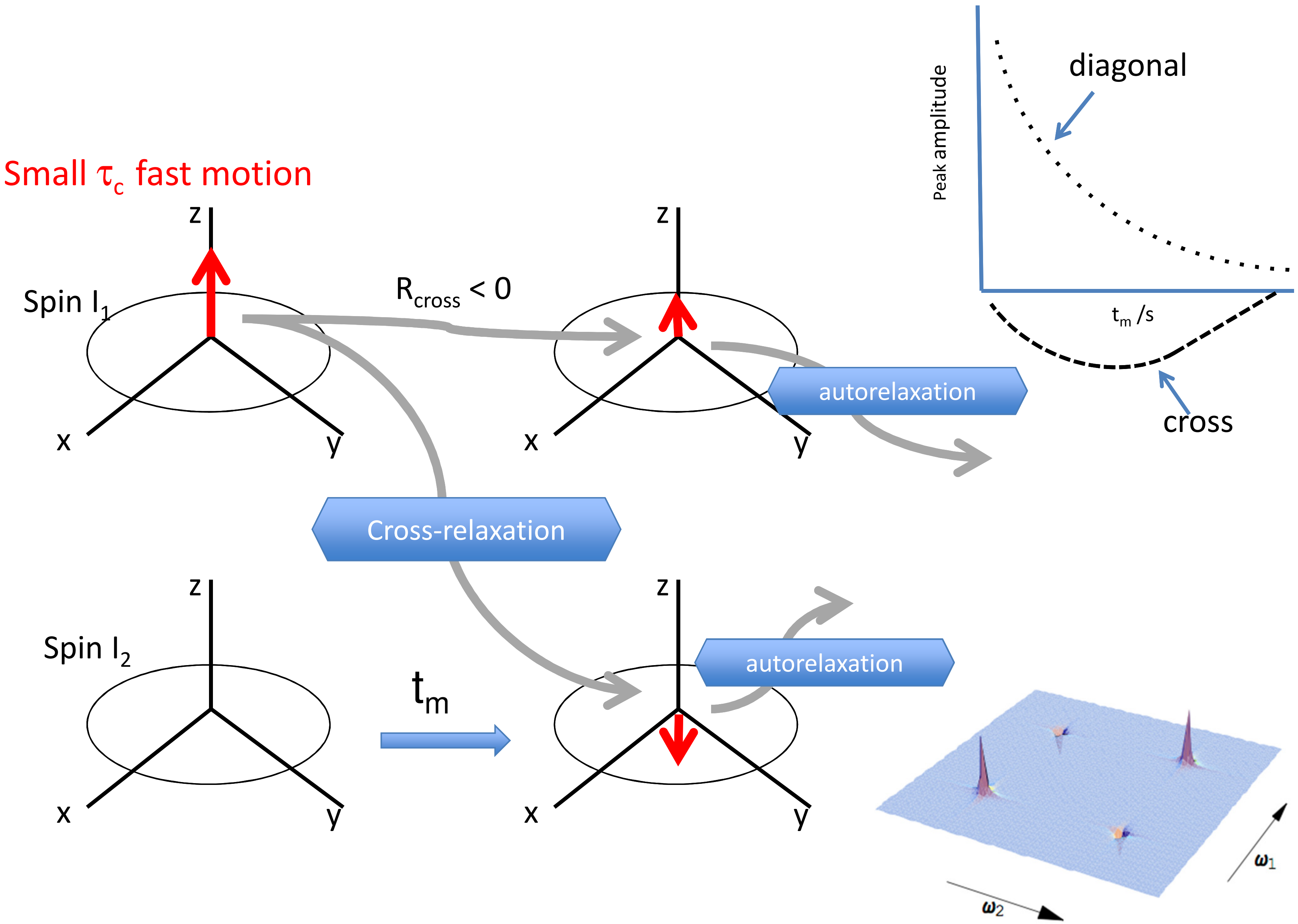


NOESY (NOE SpectroscopY)






Long τ_c slow motion
Spin I_1
Spin I_2
$R_{cross} > 0$
Cross-relaxation
autorelaxation
autorelaxation
t_m
z
x
y
Peak amplitude
diagonal
cross
t_m /s
ω_1
ω_2



Small τc fast motion
Spin I1
Spin I2
Rcross < 0
z
x
y
tm
Cross-relaxation
autorelaxation
autorelaxation
Peak amplitude
diagonal
cross
tm /s
ω1
ω2

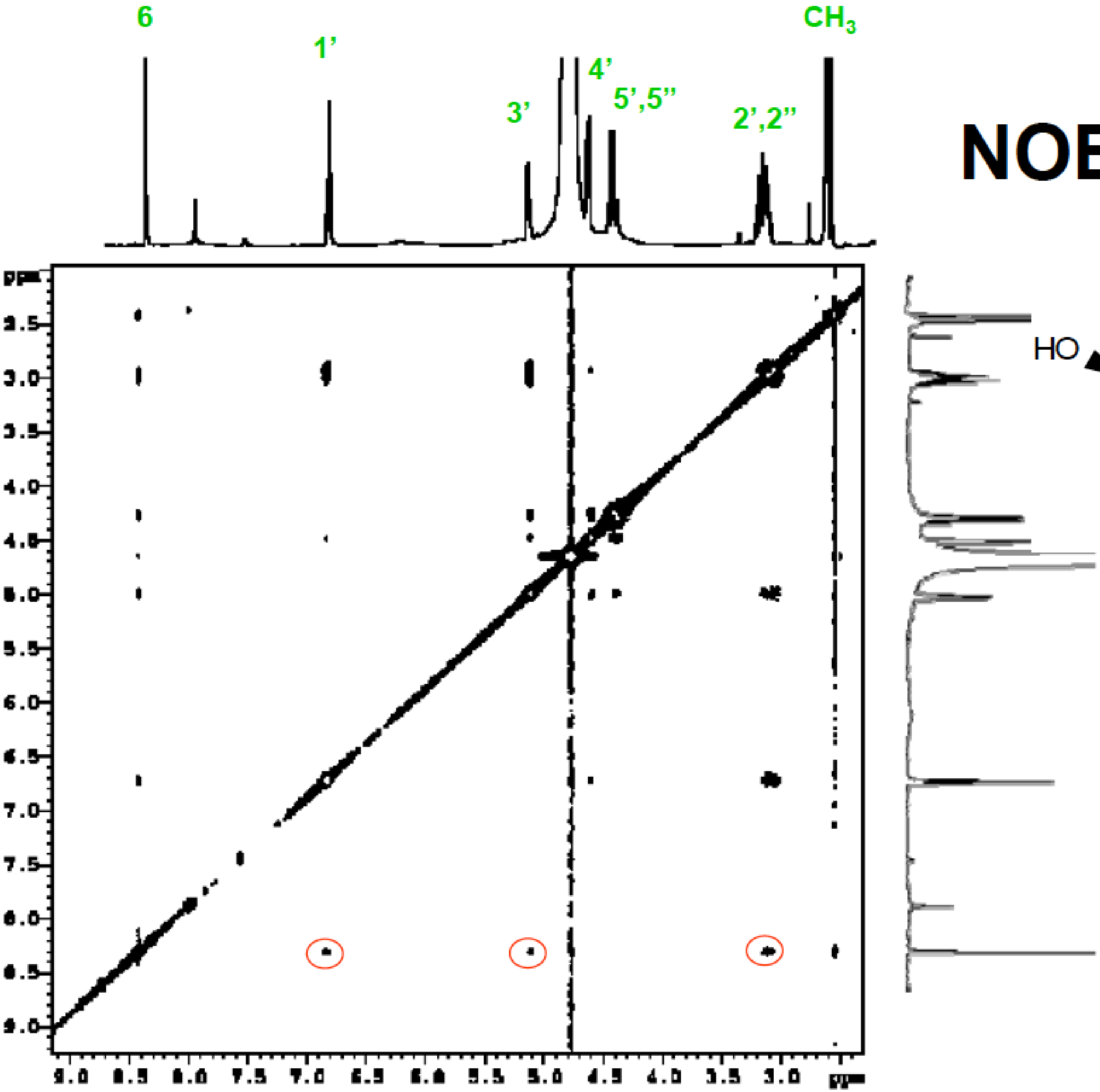
The power of the NOESY experiment is that the intensity of an NOE peak will be related to the nuclear separation.

Strong NOE crosspeaks - 2.5 Å
Weak NOE crosspeaks - 2.5-3.5 Å

Extending the mixing time will permit the observation of nuclei separated by 5Å – Not all spin systems will give a detectable peak though. The absence of a peak does not preclude close approach. Similarly a weaker crosspeak does not always prove a larger internuclear distance.

Therefore tend to be cautious and define distance ranges.
Strong (1.8-2.5Å), medium (1.8-3.5Å), weak (1.8-5.0Å).

Since this works through space we can use the NOE to connect spin systems that we assigned with the COSY and TOCSY spectra.



NOESY