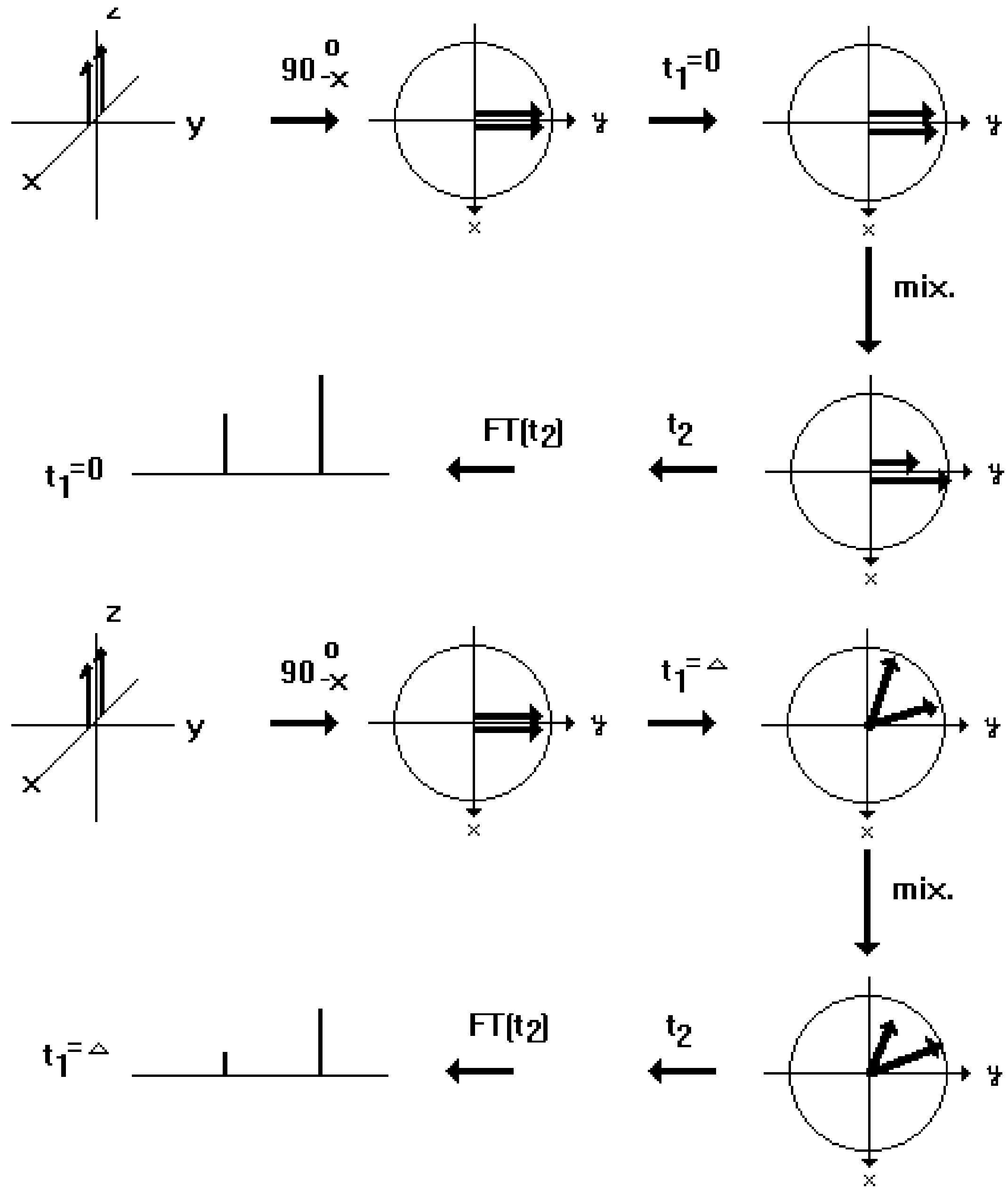
2D spectroscopy via the vector description

The pulse sequence is:
$90°_x$ - t_1 - mixing - t_2
Consider: Ω_I, Ω_S and $J_{IS} = 0$
At $t_1=0$

receiver on y

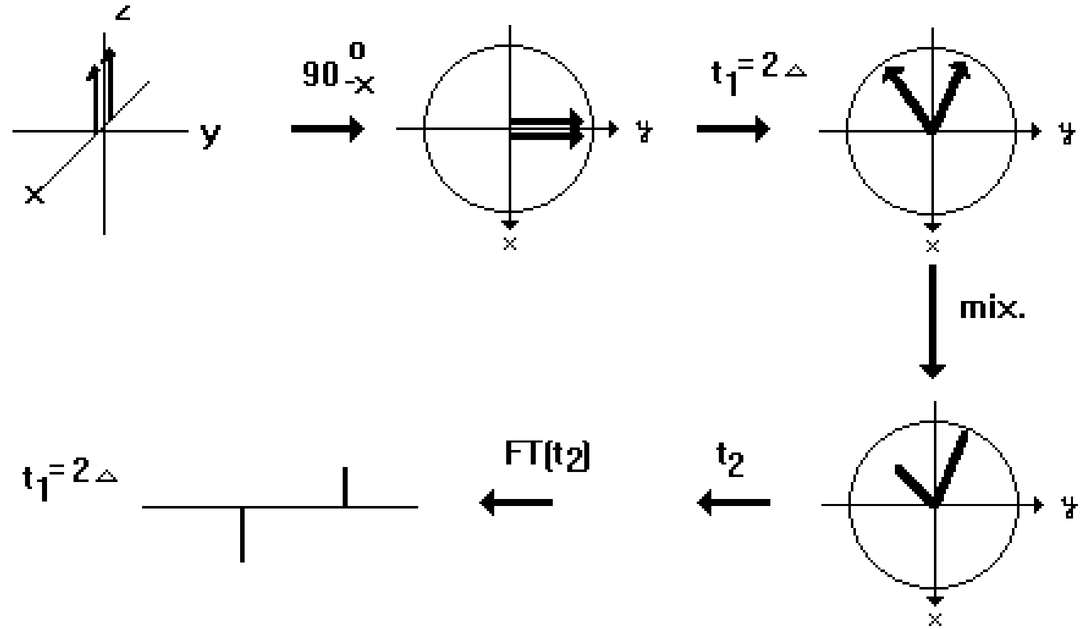
At a new $t_1 = \Delta$

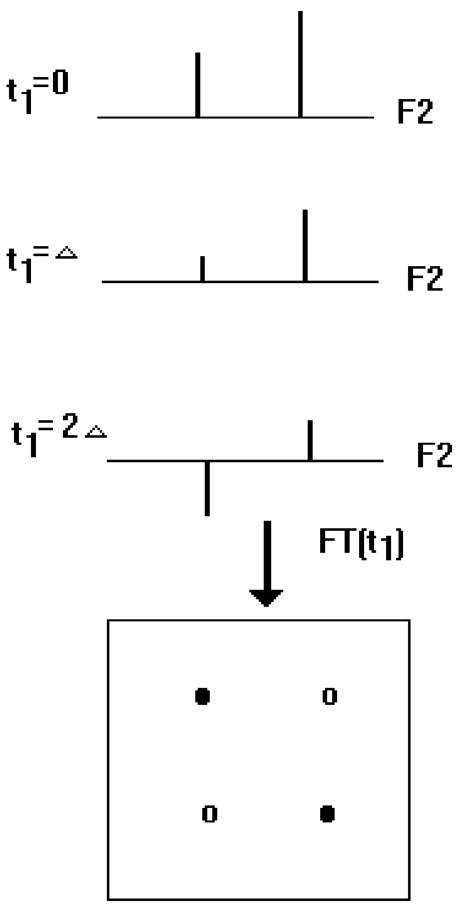
receiver on y



At a new $t_1 = 2\Delta$

receiver on y

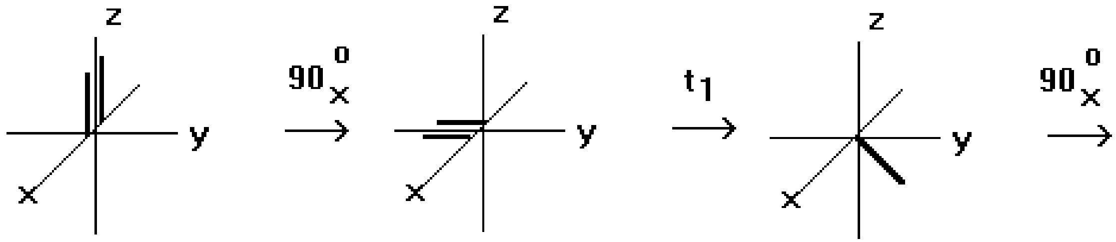


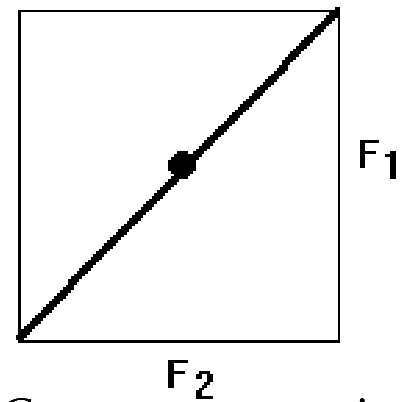


t_1 is further incremented. The obtained data sets (1D spectra) are the Fourier transform of t_2 (acquisition dimension). This set of 1D spectra can also be *Fourier*-transformed according to t_1..

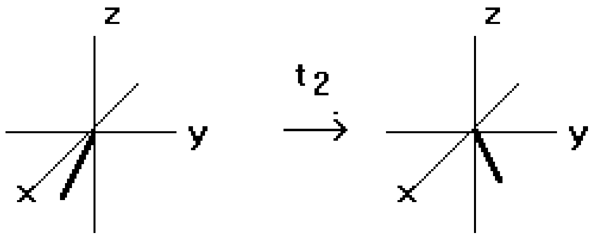
Two Dimensional NMR spectroscopy

A: Consider: $\Omega_I = \Omega_S = \mathbf{\Omega}$ (therefore there is no **J** [or J is infinitely large])





Comment: precession is at the same frequency during both t_1 *and* t_2*, therefore signal is observed only at* $F_1 = F_2$.
(1D spectrum along the diagonal.)



$\mathbf{I}_z$

$\downarrow 90°_x$

$-\mathbf{I}_y$

$\downarrow\ t_1$

$\hat{H} = \hat{I}_z(\Omega t_1)$

$-\mathbf{I}_y(\cos\Omega t_1)$ $+\mathbf{I}_x(\sin\Omega t_1)$

$\downarrow 90°_x$

$\hat{H} = \pi/2\ (\hat{I}_x)$

$-\mathbf{I}_z(\cos\Omega t_1)$ $+\mathbf{I}_x(\sin\Omega t_1)$

$\downarrow\ t_2$

$\hat{H} = \hat{I}_z(\Omega t_2)$

$-\mathbf{I}_z(\cos\Omega t_1)$ $+\mathbf{I}_x(\sin\Omega t_1)(\cos\Omega t_2)$ $+\mathbf{I}_y(\sin\Omega t_1)(\sin\Omega t_2)$

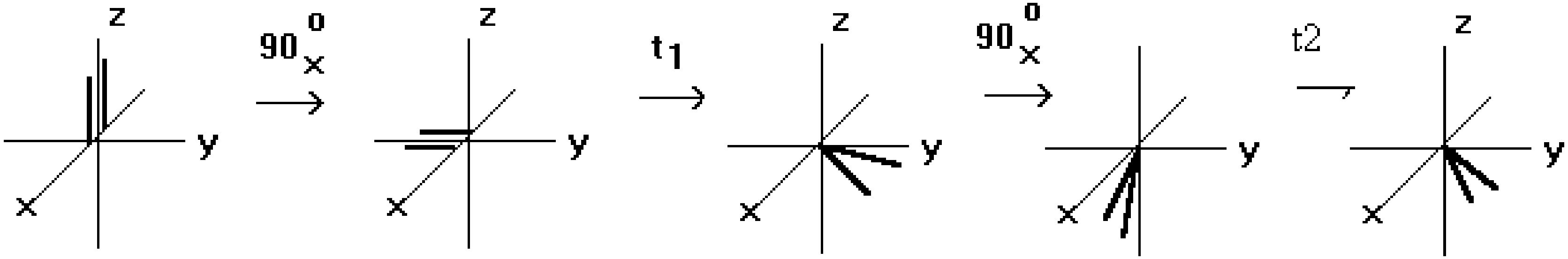
memo: $\exp(-ix) = \cos(x) - i.\sin(x)$

the complex signal is : $(\sin\Omega t_1)\exp(-i\Omega t_2)$

signal =0 if $\Omega t_1 = k\pi$
signal =max. if $\Omega t_1 = k\pi/2$
signal =invert. if $\Omega t_1 = 3k\pi/2$

Summary: a correlation is established between the two dimension

B: Consider: $\Omega_I \neq \Omega_S$ and J



$\hat{H} = \pi/2 \ (\hat{I}_x)$

$$\mathbf{I}_z \xrightarrow{90^\circ_x} -\mathbf{I}_y \xrightarrow{t_1}$$

$-\mathbf{I}_y \cos(\Omega_I t_1)\cos(\pi J t_1)$ $+2\mathbf{I}_x\mathbf{S}_z \cos(\Omega_I t_1)\sin(\pi J t_1)$ only I type magnetization;

$+\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J t_1)$ $+2\mathbf{I}_y\mathbf{S}_z \sin(\Omega_I t_1)\sin(\pi J t_1)$ (in- and anti-phase)

$\hat{H} = \pi/2(\hat{I}_x)$ and $\pi/2(\hat{S}_x)$ $\downarrow 90^\circ_x$ *mixing pulse*

$-\mathbf{I}_z \cos(\Omega_I t_1)\cos(\pi J t_1)$ $-2\mathbf{I}_x\mathbf{S}_y \cos(\Omega_I t_1)\sin(\pi J t_1)$ both I and S type magnetization;

$+\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J t_1)$ $-2\mathbf{I}_z\mathbf{S}_y \sin(\Omega_I t_1)\sin(\pi J t_1)$ (in-phase on I and anti-phase on S)

$\downarrow t_2$

Comment: I_z (z magnetization) and I_xS_y (double quant. coherence) will not evolve during Acq to observables, thus we can eliminate both of it

Acquisition starts here

$\downarrow$ t_2

$$+\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J t_1) \rightarrow \begin{cases} +\mathbf{I}_y \sin(\Omega_I t_1)\cos(\pi J t_1)\sin(\Omega_I t_2)\cos(\pi J t_2) \\ +\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J t_1)\cos(\Omega_I t_2)\cos(\pi J t_2) \end{cases}$$

and

$$-2\mathbf{I}_z\mathbf{S}_y \sin(\Omega_I t_1)\sin(\pi J t_1) \rightarrow \begin{cases} +2\mathbf{I}_z\mathbf{S}_y \sin(\Omega_I t_1)\sin(\pi J t_1)\cos(\Omega_S t_2)\cos(\pi J t_2) \\ -2\mathbf{I}_z\mathbf{S}_x \sin(\Omega_I t_1)\sin(\pi J t_1)\sin(\Omega_S t_2)\cos(\pi J t_2) \end{cases}$$

$$\left.\begin{array}{l} +\mathbf{I}_y \sin(\Omega_I t_1)\cos(\pi J t_1)\ \sin(\Omega_I t_2)\ \cos(\pi J t_2) \\ +\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J t_1)\ \cos(\Omega_I t_2)\ \cos(\pi J t_2) \end{array}\right\} \rightarrow \underbrace{\sin(\Omega_I t_1)\cos(\pi J t_1)}_{t_1}\ \underbrace{\exp(-i\Omega_I t_2)\cos(\pi J t_2)}_{t_2}$$

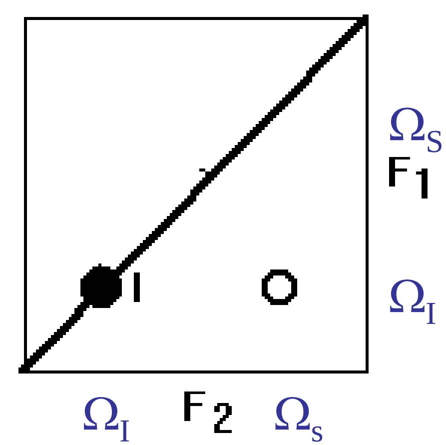
and

$$\left.\begin{array}{l} 2\mathbf{I}_z\mathbf{S}_y \sin(\Omega_I t_1)\sin(\pi J t_1)\ \cos(\Omega_S t_2)\ \cos(\pi J t_2) \\ -2\mathbf{I}_z\mathbf{S}_x \sin(\Omega_I t_1)\sin(\pi J t_1)\ \sin(\Omega_S t_2)\ \cos(\pi J t_2) \end{array}\right\} \rightarrow \underbrace{\sin(\Omega_I t_1)\sin(\pi J t_1)}_{t_1}\ \underbrace{\exp(-i\Omega_S t_2)\cos(\pi J t_2)}_{t_2}$$

Summary: ignoring multiplet structure
ignoring peak shape

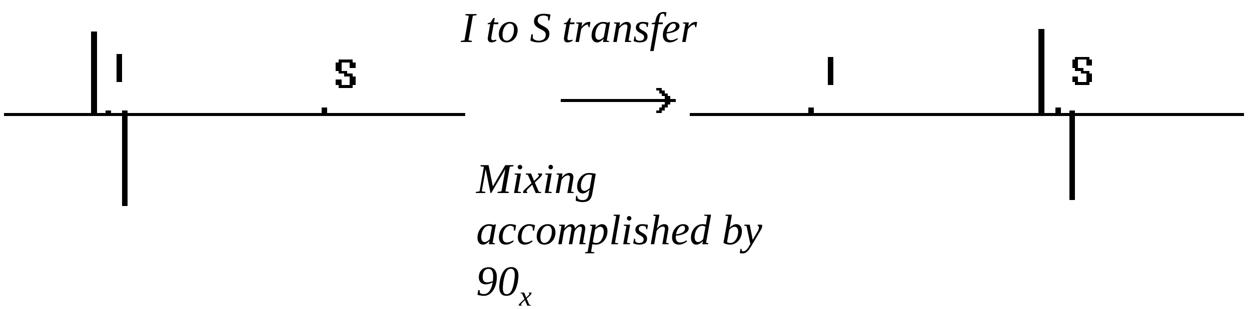
Comment 1: precession is at different frequency during t_1 and t_2,
therefore signals are observed at Ω_I,Ω_I and Ω_I,Ω_S

Conclusion: there is the 1D spectrum along the diagonal (Ω_I,Ω_I).
There is the off-diagonal element (Ω_I,Ω_S).

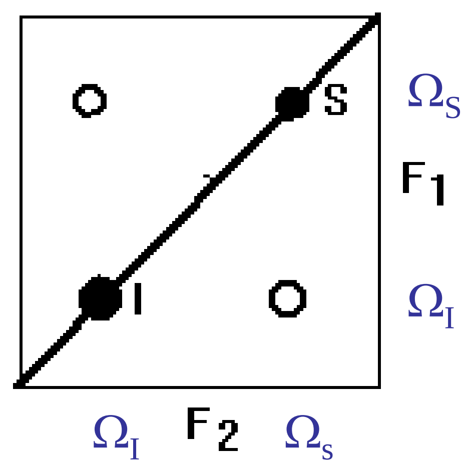


Comment 2: Coherence transfer pathway:
magnetization originated on spin I ends on spin I as well as on spin S.
The transfer is performed during the mixing period.

Here this is the second 90° pulse, where
the $2\mathbf{I}_y\mathbf{S}_z$ (anti-phased magnetization on spin **I**) is transferred to
$2\mathbf{I}_z\mathbf{S}_y$ (anti phased magnetization on spin **S**).



There is an alternative coherence transfer pathway when magnetization originated on spin S ends on spin S as well as on spin I.



Two Dimensional experiments in general

The density operator (σ) is transformed from its initial value (σ[eq.]) to its final stage ($\sigma[t_1,t_2]$).

σ[eq.] ----> $\sigma[0]$ *preparation:* from equilibrium to non-equilibrium state.
in the simplest situation the transverse magnetization is gener.

$\sigma[0]$ ----> $\sigma[t_1]$ *evolution:* the off-diagonal component of the density operator prepared in step 1 is evolving under the Hamiltonian. The Hamiltonian can be free-precession *etc*. The coherence evolves during t_1 resulting in signals detected at frequencies in the F_1 dimension. This is called **F1 frequency labelling** of the coherence.

$\sigma[t_1]$ ----> $\sigma[t_1,0]$ *mixing:* coherence is transferred from one spine to another. One can established here the desired type of correlation between the two dimensions.

$\sigma[t_1,0]$ ----> $\sigma[t_1,t_2]$ *detection (or acquisition):* The FID is recorded. If more than one coherence transfer path is possible, then phase cycle or field gradient pulse are used to discriminate.

A: through-bond coherence transfer
migration of coherence between scalar coupled nuclei ---> COSY, RELAY, TOCSY
B: through-space coherence transfer
dipolar coupled spins change their intensities via nuclear Overhauser effect (NOE)
---> NOESY, ROESY

SENSITIVITY OF AN EXPERIMENT AND THE SIZE OF THE MOLECULE

At a magnetic field (e.g. 500 MHz), for a typical concentration of (*e.g.* 1 mM) certain experiments (e.g. COSY) don't work for large molecules (M ≅ 10 kDalton) but are efficient for small ones (M ≅ 1 kDalton). The reason for this is because their T_2 values are different:

M ≅ 1 kDalton typical $\mathbf{T_2} \cong$ 1-2 s,
M ≅ 10 kDalton typical $\mathbf{T_2} \cong$ 10-20 ms.

The signal intensity (I) goes down according to $I(t) = A*\exp(-t/\mathbf{T_2})$, therefore their spin-spin relaxation is different.

so at t = $1T_2 \Rightarrow$ intensity of the signal is A*exp(-1) ≅ 36.8%
so at t = $2T_2 \Rightarrow$ intensity of the signal is A*exp(-2) ≅ 13.5%
so at t = $3T_2 \Rightarrow$ intensity of the signal is A*exp(-3) ≅ 5.0%

In homonuclear experiments the $^{1,3}J$ type-couplings have

a) high **conformational** dependence,
b) an average value of ~**6 Hz**,
c) and **builds up** with *sin* or *cos* modulation.

e.g. in COSY the J is cos modulated $\{\cos(\pi J_{IS} t)\}$ in the diagonal peak and
J is sin modulated $\{\sin(\pi J_{IS} t)\}$ in the off-diagonal peak.
With J = 6Hz, the $I(t) = \sin(\pi 6t)$ has its maximum at t = 1/(6*2) = 83 ms.

t	relat. signal intensity (%) (decay due to T_2 relax.)	time (ms) (T_2 = 20)	build up (%) sin modulated J (6Hz)	decay (%) cos modulated J (6Hz)
$1T_2$	36.8	20	36.8	93.0
$2T_2$	13.5	40	68.5	72.9
$3T_2$	5.0	60	90.5	42.6
$4T_2$	2.0	80	99.8	6.2

For a small molecule with **T_2 = 2 (s) there is practically no decay due T_2 relaxation in the first 100 ms** ($A*\exp(-0.1/2) \approx 95.1\%$)

THEREFORE in a **COSY-type experiments** we observe the following

memo : $M_x(t_2) = \langle I_x \rangle = Tr\{I_x\sigma\}$

diagonal term $+\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$

off-diagonal term $+\mathbf{I}_x \sin(\Omega_S t_1)\sin(\pi J_{IS} t_1)\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$

comment: so for a mol. with a fast $\mathbf{T_2}$ relaxation ($\mathbf{T_2}$ small, broad linewidth [e.g. protein]) the signal decay is fast while the build up of the sin modulated coupling is slow. In conclusion, the off-diagonal peak **can't be detected**. For the same reason the diagonal is there.

p.s.1. The RELAY is similar to COSY since :

diagonal term $+\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$

off-diagonal term $+\mathbf{I}_x\alpha \sin(\Omega_M t_1)\sin(\pi J_{SM} t_1)\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$

p.s.2. In the DQF-COSY experiment both the diagonal and the off-diagonal could vanish.

diagonal term $-1/2\mathbf{I}_x\cos(\Omega_I t_1)\sin(\pi J_{IS} t_1)\sin(\Omega_I t_2)\sin(\pi J_{IS} t_2)$

off-diagonal term $-1/2\mathbf{I}_x\cos(\Omega_S t_1)\sin(\pi J_{IS} t_1)\sin(\Omega_I t_2)\sin(\pi J_{IS} t_2)$