SENSITIVITY OF AN EXPERIMENT AND THE SIZE OF THE MOLECULE

observation : some experiment (*e.g.* COSY, DQF-COSY) don't work for large molecules (M ≅ 10 kDalton) although efficient for smaller ones (M ≅ 1 kDalton). Why is that?

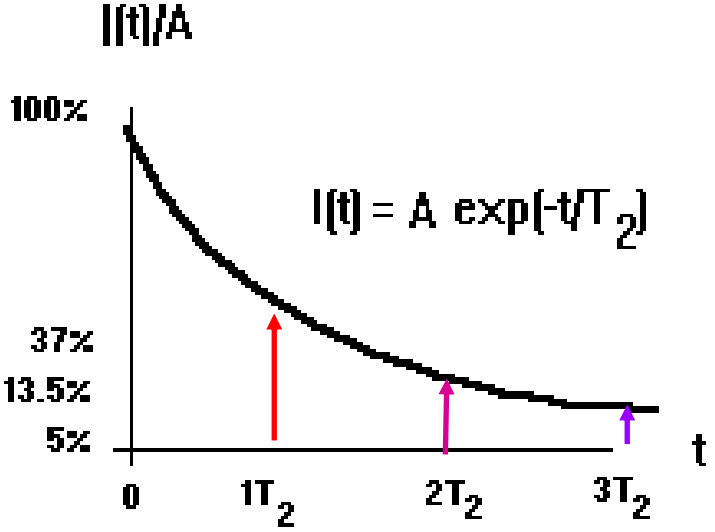
answer: Their T_2 values are different, namely:
- for M ≅ 1 kD the typical T_2 ≅ 1-2 s,
- for M ≅ 10 kD the typical T_2 ≅ 10-20 ms.

explanation: **relaxation*** time (mainly T_2) is to be compared with the time required for the "build up" of the **off-diagonal** signal **intensity**. (A comparison on the absolute time scale.)

1. T_2 relaxation

1.1. ***relative (or T_2) time scale***

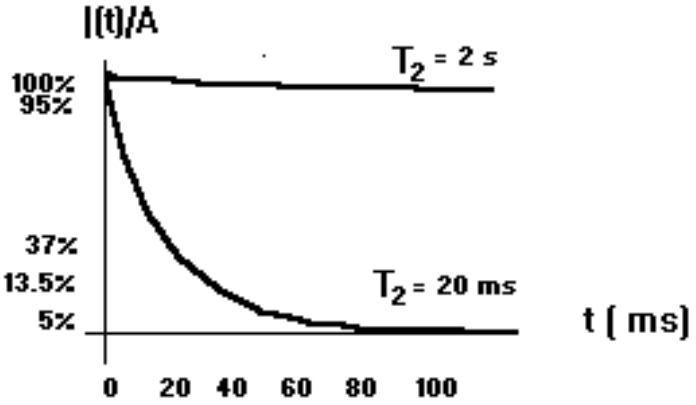
Due to relaxation the signal intensity decays according to $I(t)=A*\exp(-t/T_2)$



: t = $1T_2$ ⇒ intensity of the signal is A*exp(-1) ≅ 36.8%
: t = $2T_2$ ⇒ intensity of the signal is A*exp(-2) ≅ 13.5%
: t = $3T_2$ ⇒ intensity of the signal is A*exp(-3) ≅ 5.0%

relaxation* ~ $R_2 + R_{inhomogenity} + R_{excahnge}$

1.2. ***absolute time scale***



considering two molecules with two different T_2 values:
M ≅ 1 kDalton typical $T_2 \cong$ 1-2 s
M ≅ 10 kDalton typical $T_2 \cong$ 10-20 ms

Since their spin-spin relaxation is different the decay of the signal intensities is different.

For a small molecule with large T_2 {2 s} there is practically no decay due T_2 relaxation **during the first 100 ms** {A*exp(-0.1/2) ≅ 95.1%}
For a large molecule with small T_2 {20 ms} there is practically no signal after the first 100 ms.

2. the build up of the signal intensity according to t_1 (t_2 during ACQ is unimportant)

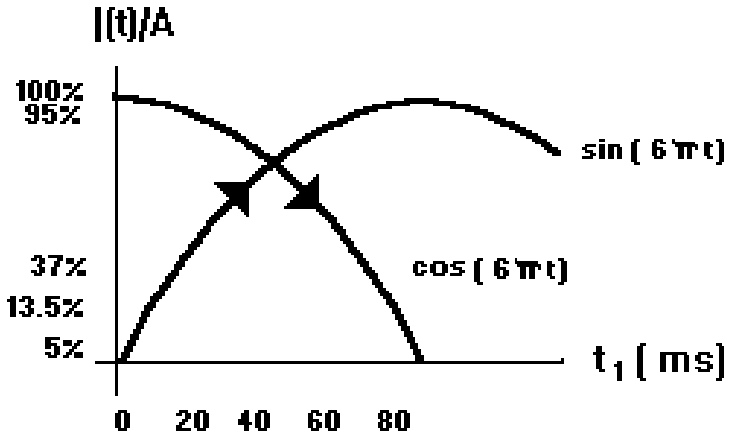
In homonuclear experiments the $^{1,3}J$ type-couplings have
-high conformational dependence,
-an average value of 6 Hz,
-and builds up with *sin* or *cos* modulation.

e.g. in a 2D-COSY
diagonal peak: $+\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$
off-diagonal peak: $+\mathbf{I}_x \sin(\Omega_S t_1)\sin(\pi J_{IS} t_1)\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$

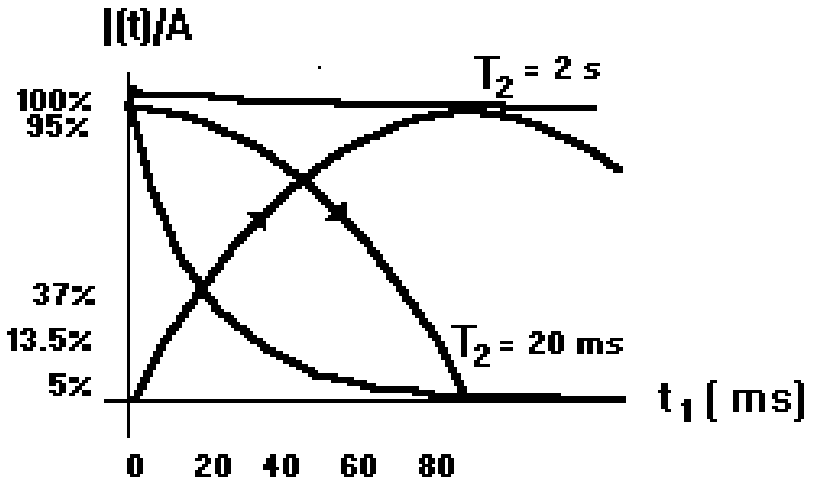
J_{IS} is cos modulated {$\cos(\pi J_{IS} t_1)$} in the diagonal peak and
J_{IS} is sin modulated {$\sin(\pi J_{IS} t_1)$} in the off-diagonal peak

At t=0 the cos is at its maximum and at $\pi/2$ it is 0, so the intensity of the diagonal decays.
At t=0 the sin is 0 and its maximum is at $\pi/2$ so the off-diagonal signal is building up.

$J_{IS} \approx 6$Hz $I(t) = \sin(\pi 6 t_1)$ has its maximum at $t_1 = 1/(6*2) = 83$ ms
$J_{IS} \approx 6$Hz $I(t) = \cos(\pi 6 t_1)$ reaches zero at $t_1 = 1/(6*2) = 83$ ms



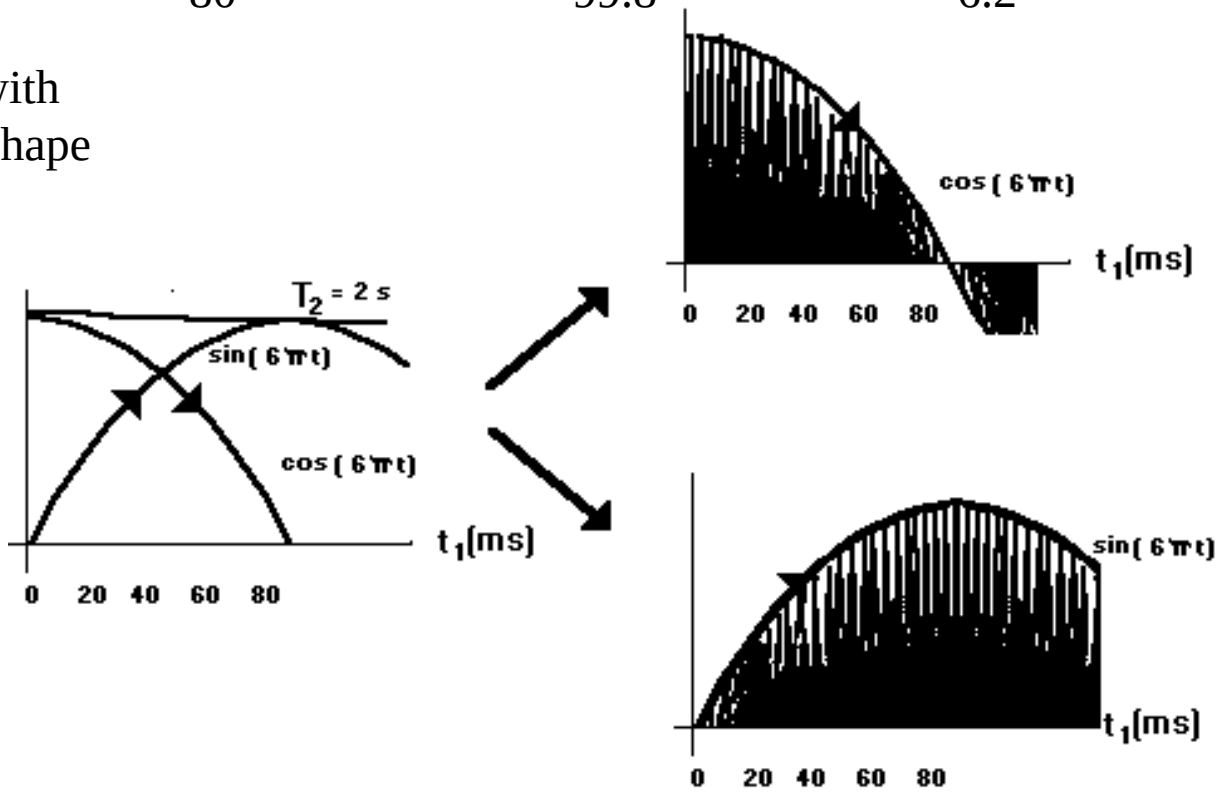
3. the build up or decay of the signal with relaxation



For a small molecule with large T_2 relaxation:

t	relat. signal intensity (%) (decay due to T2 relax.)	time (ms) (T2 = 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 large T_2 relaxation the shape of the FID



For a large molecule with serious T2 relaxation the shape of the interferrogram:

t	relat. signal intensity (%) (decay due to T2 relax.)	time (ms) (T2 = 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
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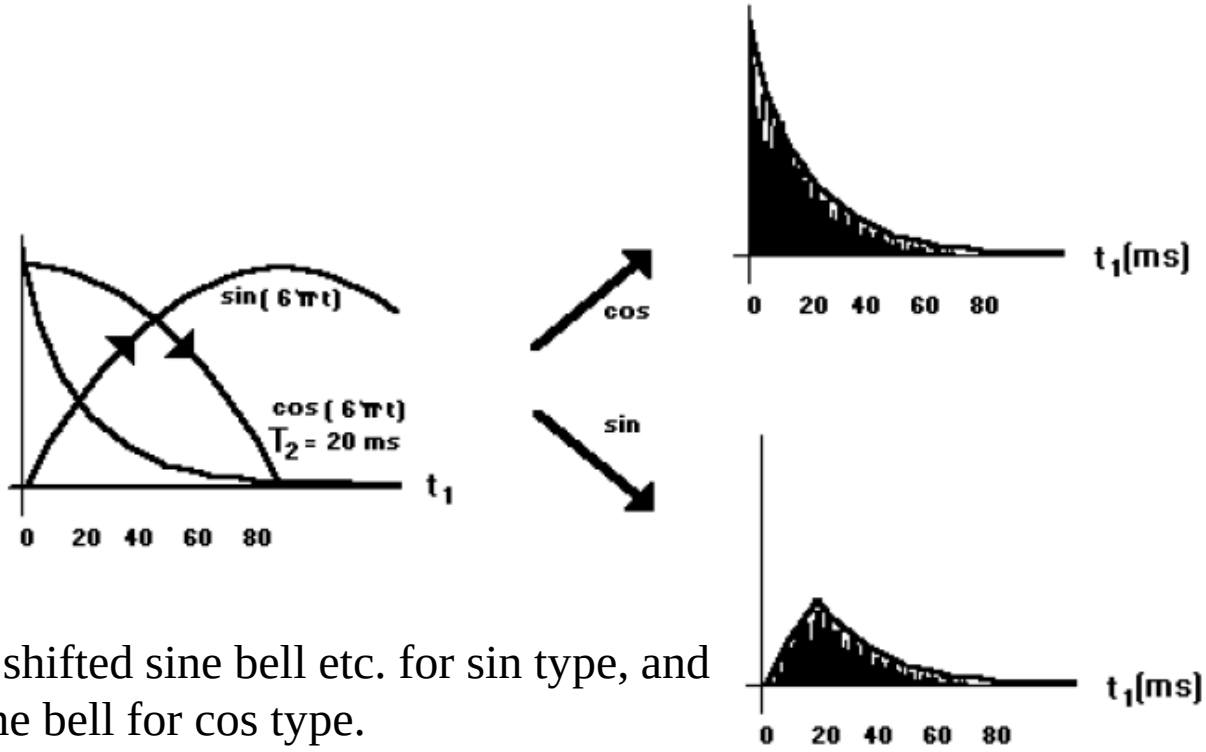


memo: when data is apodized
- use sine bell, squared sine bell, shifted sine bell etc. for sin type, and
- use exponential, gaussian, cosine bell for cos type.

final explanation:

In a **COSY-type experiments** we observe the following
memo: (receiver on x)

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

comment: so for a mol. with a fast $\mathbf{T_2}$ relaxation ($\mathbf{T_2}$ small, broad linewidth [*e.g.* protein]) the decay of the signal 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.

The RELAY is similar to COSY since:

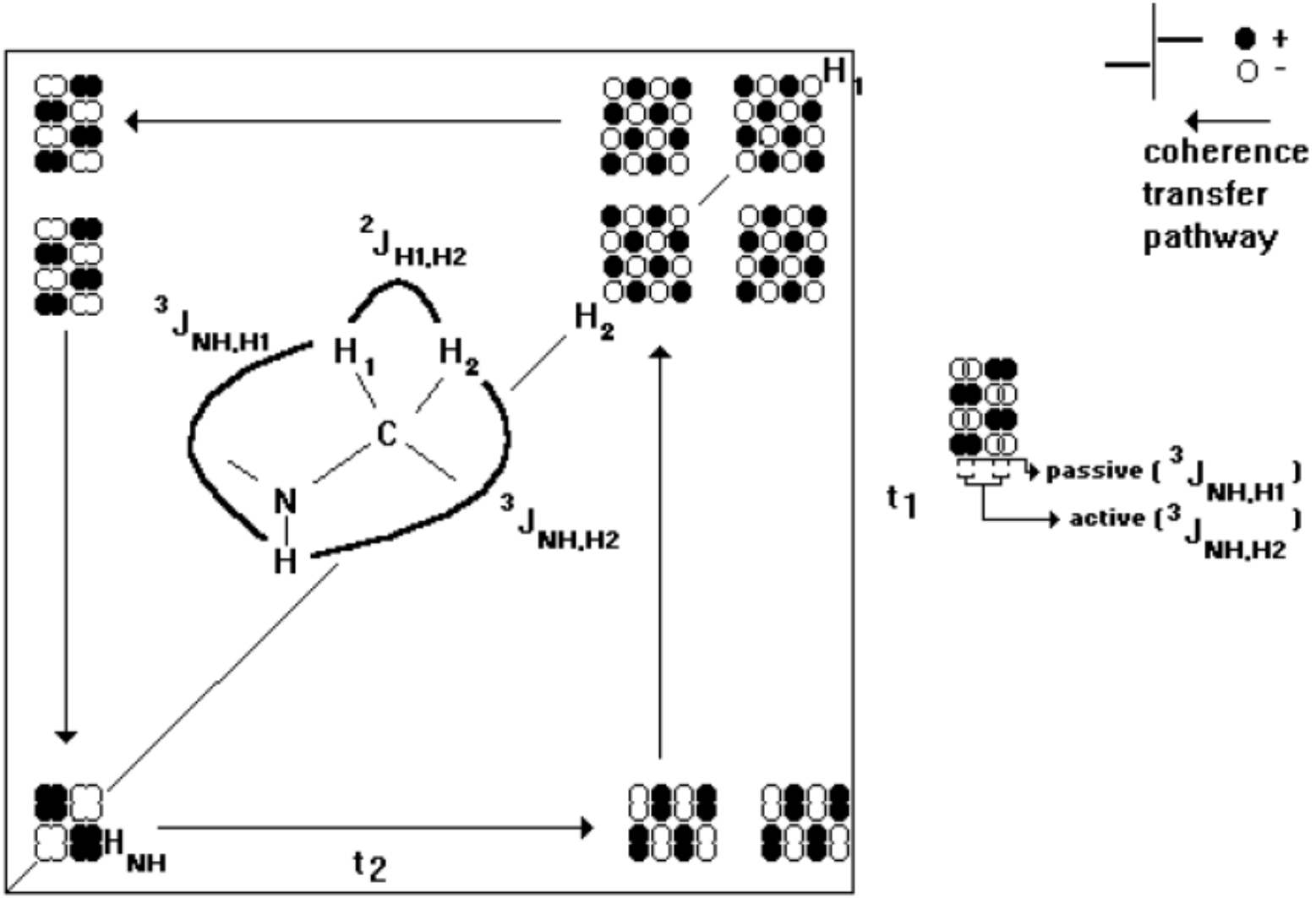
diagonal term $+\mathbf{I}_x \sin(\Omega_I t_1)\mathbf{\cos(\pi J_{IS} t_1)}\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$
off-diagonal term $+\mathbf{I}_x \alpha \sin(\Omega_M t_1)\mathbf{\sin(\pi J_{SM} t_1)}\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$

The DQF-COSY experiment: both the diagonal and the off-diagonal could vanish.

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

Resolution, self-cancellation, fine structure of 2D-experiments

e.g. 2D-DQF-COSY of a Gly residue



- For a molecule with large line width (protein) the fine structure could be lost.
(*e.g.* the passive coupling $^{3}J_{H1,H2}$ is not observed)

- If the number of increments are too small in t1 (*e.g.* t1,max.= 128*t1dw instead of t1,max=512*t1dw) then self-cancellation can occur: