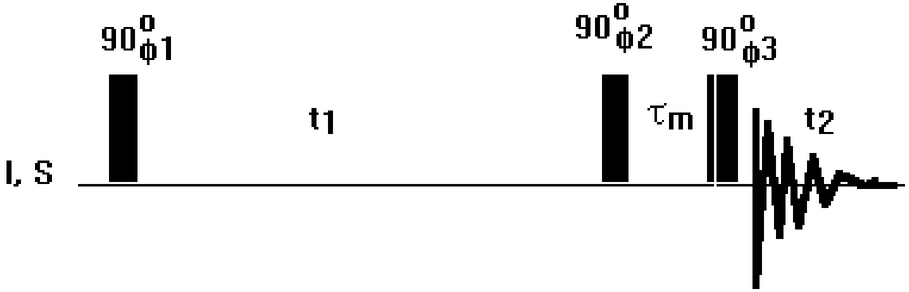
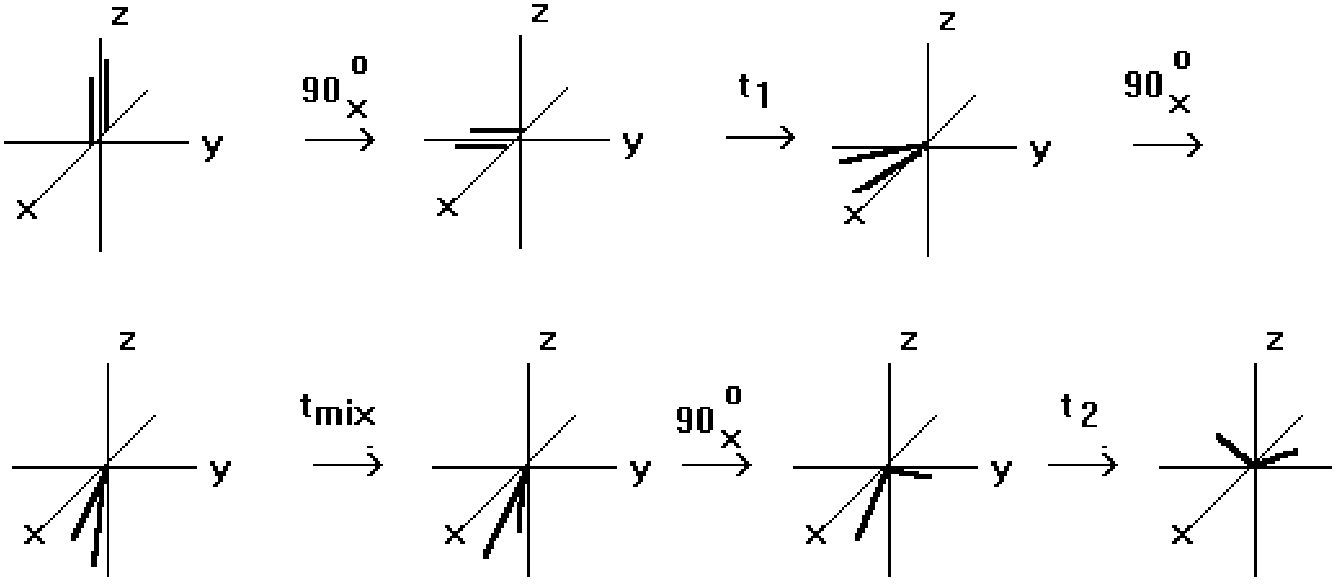
NOESY = Nuclear Overhauser Effect SpectroscopY

The pulse sequence: $90°_x$ - t_1 - $90°_x$ - $t_{mix.}$ - $90°_x$ - t_2



Memo 1: in a NOE type experiment both **magnetization** (I_z and S_z) and **the zero quantum coherence** terms are important.

Consider: Ω_I, Ω_S and J_{IS}



σ[eq.] $\quad$ $\mathbf{I_z}$ **and** $\mathbf{S_z}$

$\hat{H} = \pi/2 \ (\hat{I}_x + \check{S}_x)$ $\quad$ $\downarrow 90°_x$

σ[0] $\quad$ $\mathbf{-I_y}$ **and** $\mathbf{-S_y}$

$\hat{H} = \hat{I}_z(\Omega_I t_1) + \check{S}_z(\Omega_S t_1)$ $\quad$ $\downarrow \ t_1$

$-\mathbf{I}_y \cos(\Omega_I t_1)$

$+\mathbf{I}_x \sin(\Omega_I t_1)$

$-\mathbf{S}_y \cos(\Omega_S t_1)$

$+\mathbf{S}_x \sin(\Omega_S t_1)$

$\hat{H} = 2\hat{I}_z\check{S}_z(J_{IS}\pi t_1)$ $\quad$ $\downarrow$

$-\mathbf{I}_y \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$

$+2\mathbf{I}_x\mathbf{S}_z \cos(\Omega_I t_1)\sin(\pi J_{IS} t_1)$

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

$+2\mathbf{I}_y\mathbf{S}_z \sin(\Omega_I t_1)\sin(\pi J_{IS} t_1)$

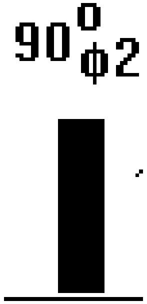
$-\mathbf{S}_y \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$

$+2\mathbf{S}_x\mathbf{I}_z \cos(\Omega_S t_1)\sin(\pi J_{IS} t_1)$

$+\mathbf{S}_x \sin(\Omega_S t_1)\cos(\pi J_{IS} t_1)$

$\sigma[t_1]$ $\quad$ $+2\mathbf{S}_y\mathbf{I}_z \sin(\Omega_S t_1)\sin(\pi J_{IS} t_1)$

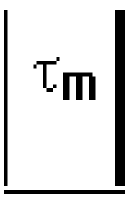
$\hat{H} = \pi/2 \ (\hat{I}_x + \check{S}_x)$ $\quad\downarrow 90°_x$ *memo.= cos(π/2) = 0, sin(π/2) = 1*



$$-\mathbf{I}_z \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$$
$$-2\mathbf{I}_x\mathbf{S}_y \cos(\Omega_I t_1)\sin(\pi J_{IS} t_1)$$
$$+\mathbf{I}_x \sin(\Omega_I t_1)\cos(\pi J_{IS} t_1)$$
$$-2\mathbf{I}_z\mathbf{S}_y \sin(\Omega_I t_1)\sin(\pi J_{IS} t_1)$$
$$-\mathbf{S}_z \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$$
$$-2\mathbf{S}_x\mathbf{I}_y \cos(\Omega_S t_1)\sin(\pi J_{IS} t_1)$$
$$+\mathbf{S}_x \sin(\Omega_S t_1)\cos(\pi J_{IS} t_1)$$
$$-2\mathbf{S}_z\mathbf{I}_y \sin(\Omega_S t_1)\sin(\pi J_{IS} t_1)$$

$\sigma[t_1,0]$

Memo 2. $\mathbf{I}_z$ *and* $\mathbf{S}_z$ are z magnetizations,
$\mathbf{I}_x$, $\mathbf{S}_x$ as well as $\mathbf{I}_z\mathbf{S}_y$ *and* $\mathbf{S}_z\mathbf{I}_y$ are single-quantum coherences,
$\mathbf{I}_x\mathbf{S}_y$ *and* $\mathbf{S}_x\mathbf{I}_y$ are double-quantum coherences.



Memo 3: Both **Z magnetizations** ($\mathbf{I}_z$ and $\mathbf{S}_z$) as well as the **Zero Quantum parts** (**ZQ**) of the Double Quantum terms (DQ) are relevant for NOE.

- Note that ZQ terms can't be removed from the spectrum with phase-cycling and thus bellow we will work out only the ZQ part of the DQ term.

memo : $I^{+} = I_x + iI_y$

$I^{-} = I_x - iI_y$

$S^{+} = S_x + iS_y$

$S^{-} = S_x - iS_y$

consequently

$1/2\ [I^{+} + I^{-}] = I_x$ and $1/(2i)[I^{+} - I^{-}] = I_y$

$1/2[S^{+} + S^{-}] = S_x$ and $1/(2i)[S^{+} - S^{-}] = S_y$

therefore

$-2\mathbf{I}_x\mathbf{S}_y = -2\{1/2\ [I^{+} + I^{-}]1/(2i)[S^{+} - S^{-}]\} = -1/(2i)\ \{I^{+}S^{+} - \mathbf{I^{+}S^{-}} + \mathbf{I^{-}S^{+}} - I^{-}S^{-}\}$

only $I^{+}S^{+}$ and $I^{-}S^{-}$ are double quant. coh. (or coherence order 2),

$I^{+}S^{-}$ and $I^{-}S^{+}$ are zero quant. coh. (or coherence order 0)

through phase cycling the double quantum coherences are removed,

while $-1/(2i)\ \{-I^{+}S^{-} + I^{-}S^{+}\}$ terms remain.

finally: $-1/(2i)\ [-1\{(I_x + iI_y)(S_x - iS_y)\} + \{(I_x - iI_y)(S_x + iS_y)\}] =$

$-1/(2i)\ [-I_xS_x - iI_yS_x + iI_xS_y - I_yS_y + I_xS_x - iI_yS_x + iI_xS_y + I_yS_y]$

$-1/(2i)\ [-i2I_yS_x + i2I_xS_y]$

$$\underbrace{[+\mathbf{I_yS_x} - \mathbf{I_xS_y}]}$$

In summary : $-2\mathbf{I}_x\mathbf{S}_y\cos(\Omega_I t_1)\sin(\pi J_{IS} t_1) \Rightarrow [+I_yS_x - I_xS_y]\cos(\Omega_I t_1)\sin(\pi J_{IS} t_1)$

for the same reason from the $-2\mathbf{I}_y\mathbf{S}_x$ term, the $+[I_xS_y - I_yS_x]\cos(\Omega_S t_1)\sin(\pi J_{IS} t_1)$ terms have zero quantum coherence.

$$[+I_yS_x - I_xS_y]\cos(\Omega_I t_1)\sin(\pi J_{IS} t_1)$$
$$[+I_xS_y - I_yS_x]\cos(\Omega_S t_1)\sin(\pi J_{IS} t_1)$$
$$\Downarrow$$

term A: $[+I_yS_x - I_xS_y]\cos(\Omega_I t_1)\sin(\pi J_{IS} t_1)$
term B: $[+I_xS_y - I_yS_x]\cos(\Omega_S t_1)\sin(\pi J_{IS} t_1)$

The result of the addition of term A plus B results in the following 4 terms:

$$A + B \text{ term} = +I_yS_x \cos(\Omega_I t_1)\sin(\pi J_{IS} t_1) - I_xS_y \cos(\Omega_I t_1)\sin(\pi J_{IS} t_1)$$
$$+I_xS_y\cos(\Omega_S t_1)\sin(\pi J_{IS} t_1) - I_yS_x\cos(\Omega_S t_1)\sin(\pi J_{IS} t_1)$$

Written in a condensed form: $[I_yS_x - I_xS_y]\,[\cos(\Omega_I t_1) - \cos(\Omega_S t_1)]\sin(\pi J_{IS} t_1)$

Before the mixing starts we have the following terms with quant. number 0.

$$-\mathbf{I}_z \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$$
$$-\mathbf{S}_z \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$$

$\sigma[t_1,0]$ $[I_yS_x - I_xS_y]\,[\cos(\Omega_I t_1) - \cos(\Omega_S t_1)]\sin(\pi J_{IS} t_1)$

with mix. coeff. :$a_{I\,I}$,$a_{I\,S}$,$a_{S\,I}$,a_{SS}
during the mixing time (τ_m) the populations ($-\mathbf{I}_z\, a_{II}$, $-\mathbf{I}_z\, a_{IS}$, $-\mathbf{S}_z\, a_{SI}$ and $-\mathbf{S}_z\, a_{SS}$) interact, while the zero-quantum term ($[I_yS_x - I_xS_y]$) coherence continues to precess.

after the mixing the populations are:

$$-\mathbf{I}_z\, a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$$
$$-\mathbf{I}_z\, a_{IS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$$
$$-\mathbf{S}_z\, a_{SS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$$
$$-\mathbf{S}_z\, a_{SI} \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$$

and the zero-quantum coh. term is

$$[I_yS_x - I_xS_y]\,[\cos(\Omega_I t_1) - \cos(\Omega_S t_1)]\sin(\pi J_{IS} t_1)$$

$\hat{H} = \pi/2 \ (\hat{I}_x + \check{S}_x)$ $\downarrow 90°_x$ *memo. = cos(π/2) = 0, sin(π/2) = 1*

$\mathbf{I}_y\, a_{II}\ \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$
$\mathbf{I}_y\, a_{IS}\ \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$
$\mathbf{S}_y\, a_{SS}\ \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$
$\mathbf{S}_y\, a_{SI}\ \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$
$[I_z S_x - I_x S_z]\ [(\cos(\Omega_I t_1) - \cos(\Omega_S t_1)]\sin(\pi J_{IS} t_1)$

The unwanted zero-quantum diagonal and cross-peaks ($I_z S_x - I_x S_z$) (both anti-phased and dispersive) can removed at this point.
a. in small molecule several spec. are recorded with diff. mixing time and coadded.
b. in large molecule they can be ignored because –> zero-quant. relaxation is fast
–> large linewidth with antiphased disp. line shape cancel each other.

$\mathbf{I}_y\, a_{I\,I}\ \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$
$\mathbf{I}_y\, a_{IS}\ \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$
$\mathbf{S}_y\, a_{SS}\ \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)$
$\mathbf{S}_y\, a_{SI}\ \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)$

the diagonal $\mathbf{I}_y$ term during ACQ $\Rightarrow$

$$+\mathbf{I}_y\, a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{I}_x\mathbf{S}_z\, a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$$
$$-\mathbf{I}_x\, a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{I}_y\mathbf{S}_z\, a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\sin(\pi J_{IS} t_2)$$

the diagonal $\mathbf{S}_y$ term during ACQ $\Rightarrow$

$$+\mathbf{S}_y\, a_{SS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_S t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{S}_x\mathbf{I}_z\, a_{SS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_S t_2)\sin(\pi J_{IS} t_2)$$
$$-\mathbf{S}_x\, a_{SS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_S t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{S}_y\mathbf{I}_z\, a_{SS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_S t_2)\sin(\pi J_{IS} t_2)$$

off-diagonal $\mathbf{I}_y$ term during ACQ $\Rightarrow$

$$+\mathbf{I}_y\, a_{IS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{I}_x\mathbf{S}_z\, a_{IS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$$
$$-\mathbf{I}_x\, a_{IS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{I}_y\mathbf{S}_z a_{IS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\sin(\pi J_{IS} t_2)$$

off-diagonal $\mathbf{S}_y$ term during ACQ $\Rightarrow$

$$+\mathbf{S}_y\, a_{SI} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{S}_x\mathbf{I}_z\, a_{SI} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\cos(\Omega_I t_2)\sin(\pi J_{IS} t_2)$$
$$-\mathbf{S}_x\, a_{SI} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-2\mathbf{S}_y\mathbf{I}_z\, a_{SI} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\sin(\pi J_{IS} t_2)$$

memo 1: put the receiver on x

therefore only the four x term remain

$$-\mathbf{I}_x\, a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-\mathbf{S}_x\, a_{SS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_S t_2)\cos(\pi J_{IS} t_2)$$
$$-\mathbf{I}_x\, a_{IS} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$
$$-\mathbf{S}_x\, a_{SI} \cos(\Omega_S t_1)\cos(\pi J_{IS} t_1)\sin(\Omega_I t_2)\cos(\pi J_{IS} t_2)$$

memo 2: $\sin(A)\cos(B) = 1/2[\sin(A+B)+\sin(A-B)]$

$\cos(A)\cos(B) = 1/2[\cos(A+B)+\cos(A-B)]$

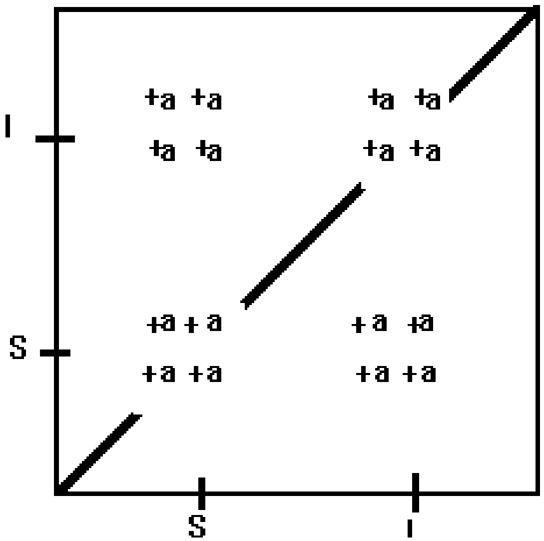
therefore

$$-1/4\mathbf{I}_x\, a_{II}\, [+\cos\{(\Omega_I+\pi J_{IS})t_1\} +\cos\{(\Omega_I-\pi J_{IS})t_1\}][+\sin\{(\Omega_I+\pi J_{IS})t_2\}+ \sin\{(\Omega_I-\pi J_{IS})t_2\}]$$
$$-1/4\mathbf{S}_x\, a_{II}\, [+\cos\{(\Omega_S+\pi J_{IS})t_1\}+\cos\{(\Omega_S-\pi J_{IS})t_1\}][+\sin\{(\Omega_S+\pi J_{IS})t_2\}+ \sin\{(\Omega_S-\pi J_{IS})t_2\}]$$
$$-1/4\mathbf{I}_x\, a_{IS}\, [+\cos\{(\Omega_S+\pi J_{IS})t_1\}+\cos\{(\Omega_S-\pi J_{IS})t_1\}][+\sin\{(\Omega_I+\pi J_{IS})t_2\}+ \sin\{(\Omega_I-\pi J_{IS})t_2\}]$$
$$-1/4\mathbf{S}_x\, a_{SI}\, [+\cos\{(\Omega_I+\pi J_{IS})t_1\} +\cos\{(\Omega_I-\pi J_{IS})t_1\}][+\sin\{(\Omega_S+\pi J_{IS})t_2\}+ \sin\{(\Omega_S-\pi J_{IS})t_2\}]$$

the following terms can be found

- $\mathbf{I}_x\, a_{II}$ [+ .. + .. + .. + ..] at Ω_I , Ω_I
- $\mathbf{I}_x\, a_{IS}$ [+ .. + .. + .. + ..] at Ω_S , Ω_I
- $\mathbf{S}_x a_{SS}$ [+ .. + .. + .. + ..] at Ω_S , Ω_S
- $\mathbf{S}_x a_{SI}$ [+ .. + .. + .. + ..] at Ω_I , Ω_S

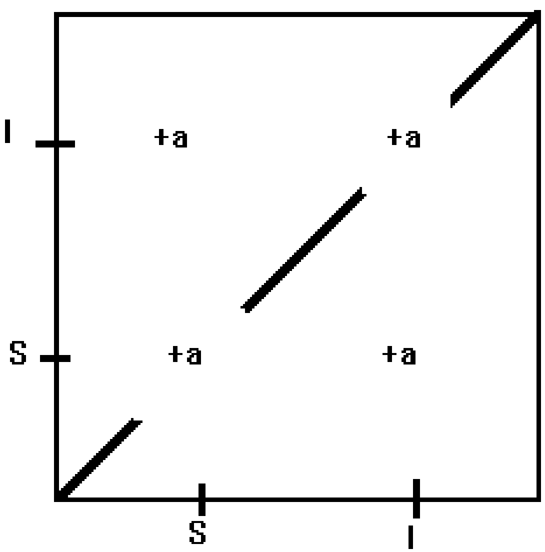
if one sets the phase that *cos is absorptive (a) in t_1*
sin is absorptive (a) in t_2

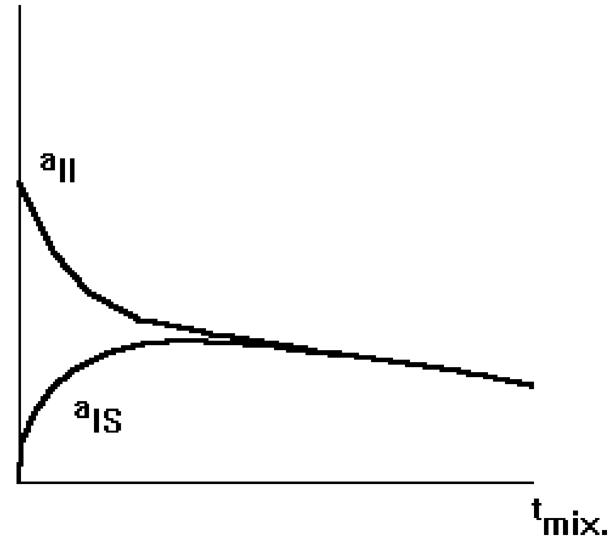


I_x a_{II} [+a .. +a .. +a .. +a ..] at Ω_I , Ω_I
I_x a_{IS} [+a .. +a .. +a ..+a ..] at Ω_S , Ω_I
S_x a_{SS} [+a .. +a .. +a .. +a ..] at Ω_S , Ω_S
S_x a_{SI} [+a .. +a .. +a .. +a ..] at Ω_I , Ω_S

If $J_{IS} = 0$ (through bond coupling ignored) then

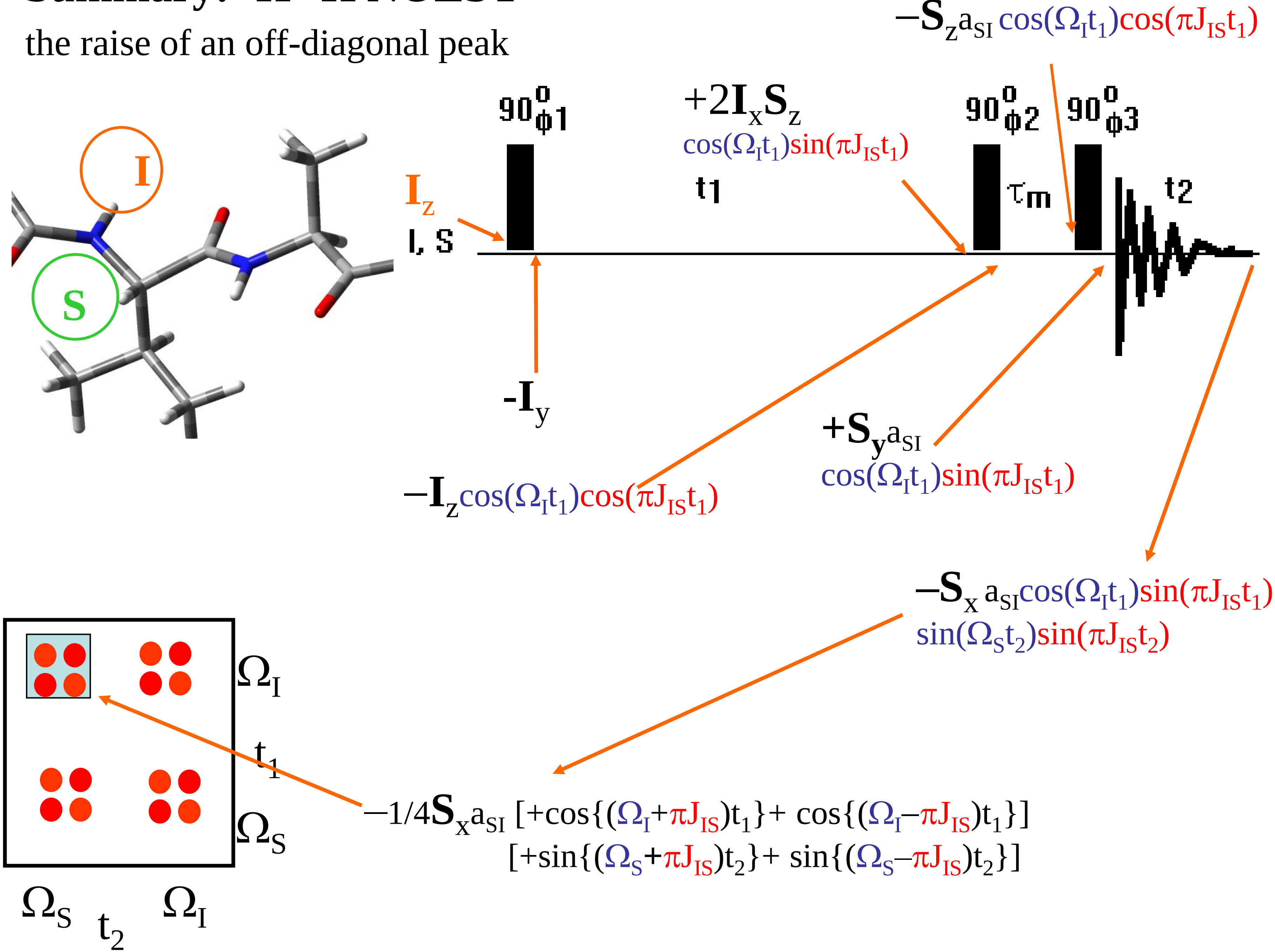
The sign of the peaks is determined
by the mix. coeff. :a_{II}, a_{IS}, a_{SI}, a_{SS}





Summary: ^{1}H-^{1}H NOESY

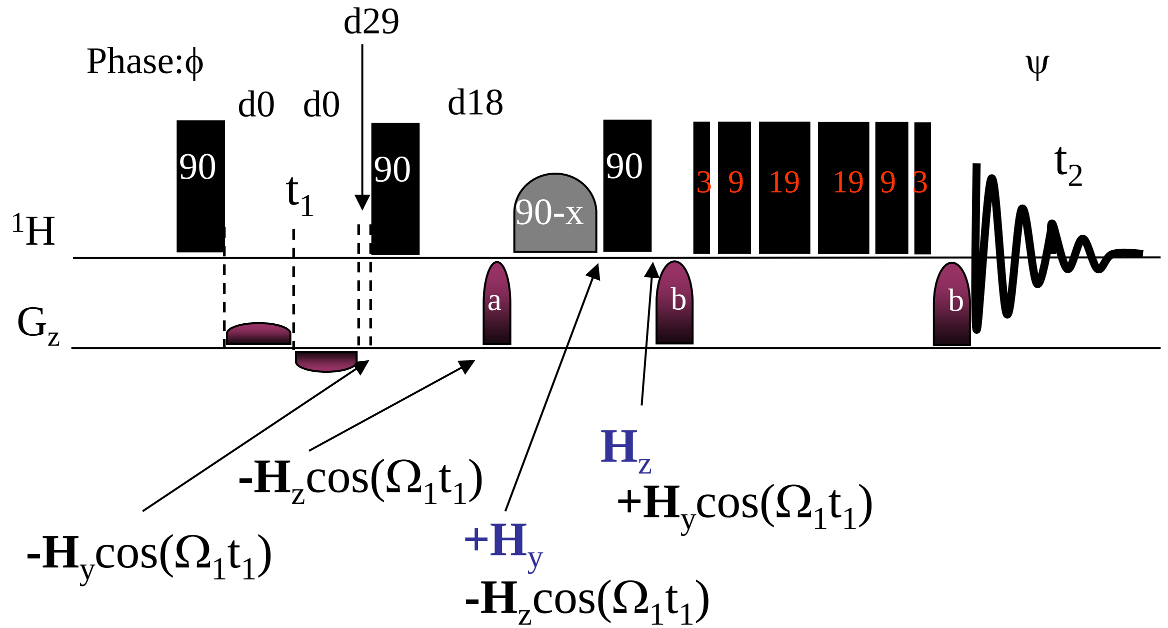
the raise of an off-diagonal peak



NOESY-with bipolar gradient, water flip-back pulse and -3-9-19:

↑ Net magnetization form H_2O (on-resonance)

↑ Net magnetization form protein (off-resonance)



bipolar gradient taking care of water during evolution:

a pair of gradients (typically of low power *e.g.* 0.5%) of increasing length covering the overall time of evolution (t1). Radiation dumping is minimized since the otherwise bulk water, as well as any other signals, are dephased and rephased in a symmetric manner during evolution. (No uniform (big) water, no radiation dumping occurs.)

Gradient "a": dephases all coherences of the x,y plane (general clean up before bringing magnetization back into the x,y palne)

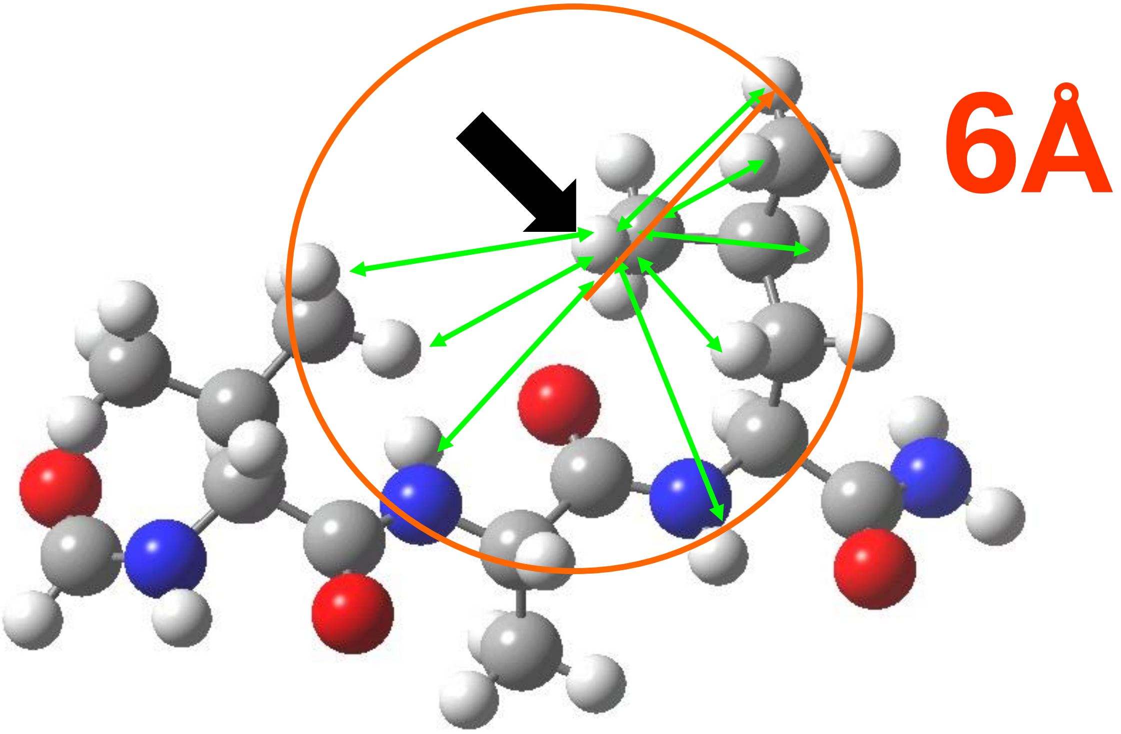
Gradient "b" is that of the "standard" watergate.

The water flip back pulse The on-resonance magnetization of H_2O, is first selectively rotated into the transverse plane (along y) by the shaped low power 90-x, and subsequently returned along z

by the non-selective hard 90x. All magnetization related to off-resonance signals are "simply" rotated to –y axis.

The 3-9-19 watergate or binominal water suppression is to remove water before acquisition

A szekvenciális hozzárendelés és a szerkezetszámolás alapja a nukláris *Overhauser*- effektus (NOE)



(NOE)

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Távolság jellegű adatok

Fehérje modul ^{1}H-^{1}H NOESY spektruma