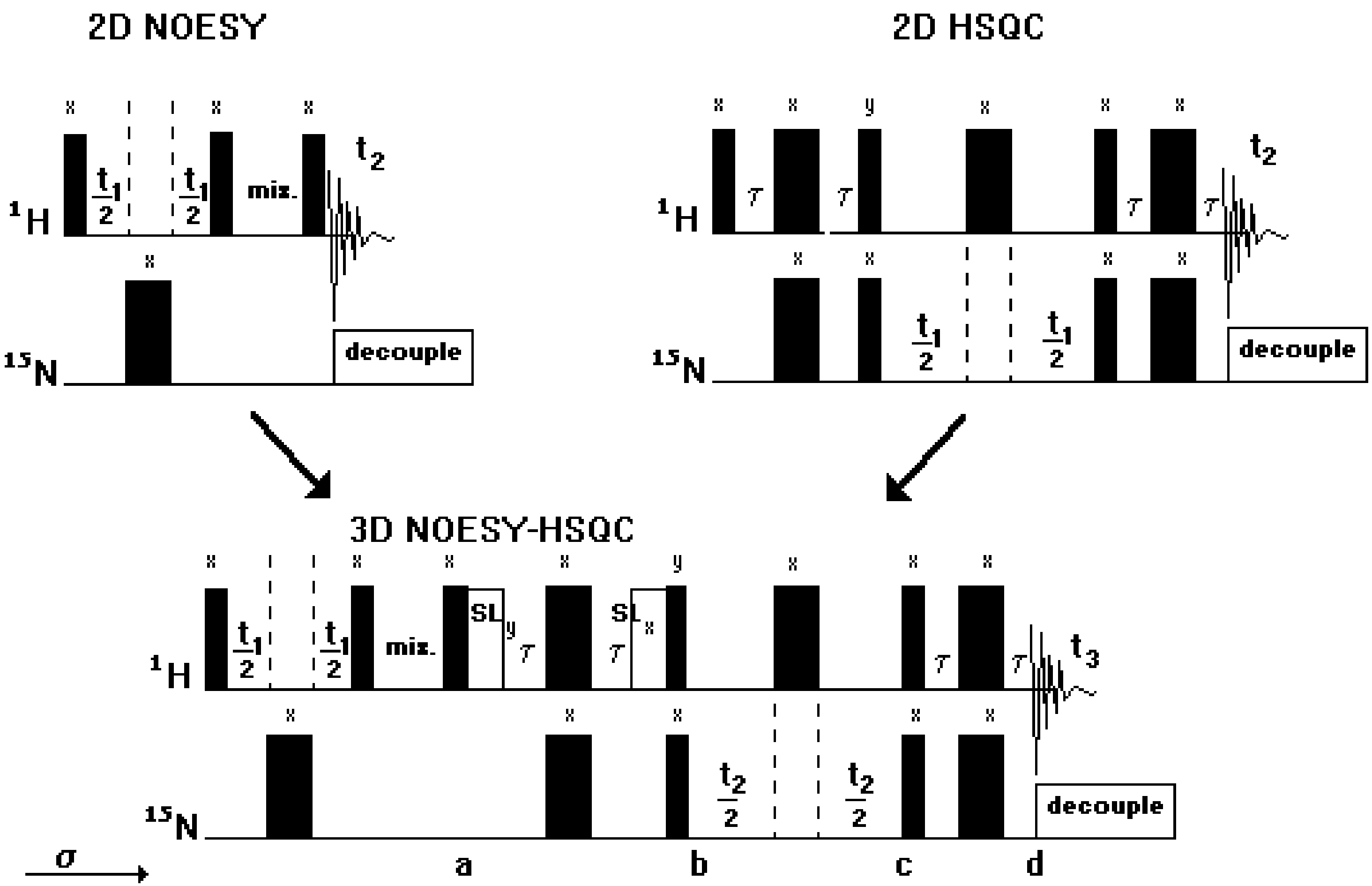
3D NOESY-HSQC



Consider: homonuclear spins I, K with chemical shift Ω_I, Ω_K and coupling J_{IK} (e.g. H^{NH} and H^{α})

heteronuclear spin N with chemical shift Ω_N with a coupling J_{IN} (e.g. H^{NH} and N^{NH})

A. module (NOESY with decoupled ^{15}N)

σ[0]"The first step is 90°
and a heteronuclear echo module removes the heteronuclear coupling during t_1"

σ[eq.] $\quad$ $\mathbf{I}_z$ **and** $\mathbf{K}_z$

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

σ[0] $\quad$ $-\mathbf{I}_y$ **and** $-\mathbf{K}_y$

$\hat{H} = \hat{I}_z(\Omega_I t_1) + K_z(\Omega_K t_1)$ $\quad$ $\downarrow t_1$

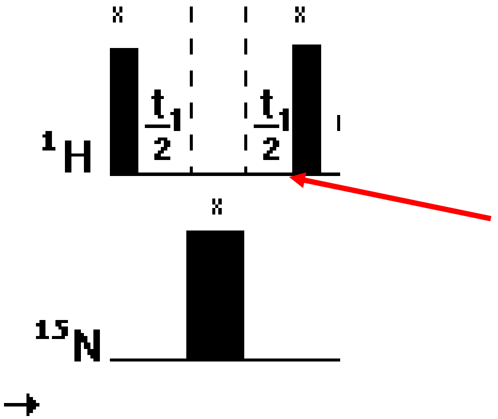
$-\mathbf{I}_y \cos(\Omega_I t_1)$

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

$-\mathbf{K}_y \cos(\Omega_K t_1)$

$+\mathbf{K}_x \sin(\Omega_K t_1)$

$\hat{H} = 2\hat{I}_z K_z(J_{IK}\pi t_1)$



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

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

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

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

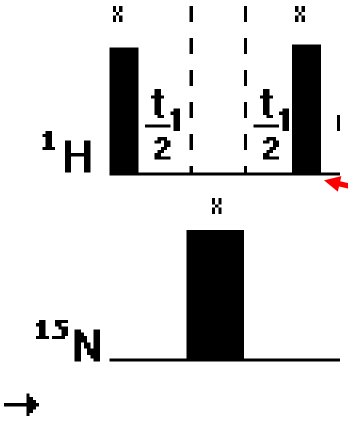
$-\mathbf{K}_y \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)$

$+2\mathbf{K}_x\mathbf{I}_z \cos(\Omega_K t_1)\sin(\pi J_{IK} t_1)$

$+\mathbf{K}_x \sin(\Omega_K t_1)\cos(\pi J_{IK} t_1)$

$+2_y\mathbf{I}_z \sin(\Omega_K t_1)\sin(\pi J_{IK} t_1)$

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



$\downarrow 90°_x$ *memo.= cos(π/2) = 0, sin(π/2) = 1*

$-\mathbf{I}_z \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)$

$-2\mathbf{I}_x\mathbf{K}_y \cos(\Omega_I t_1)\sin(\pi J_{IK} t_1)$

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

$-2\mathbf{I}_z\mathbf{K}_y \sin(\Omega_I t_1)\sin(\pi J_{IK} t_1)$

$-\mathbf{K}_z \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)$

$-2\mathbf{K}_x\mathbf{I}_y \cos(\Omega_K t_1)\sin(\pi J_{IK} t_1)$

$+\mathbf{K}_x \sin(\Omega_K t_1)\cos(\pi J_{IK} t_1)$

$-2\mathbf{K}_z\mathbf{I}_y \sin(\Omega_K t_1)\sin(\pi J_{IK} t_1)$

memo.1: selecting only z magnetization ($-\mathbf{I}_z$ *and* $-\mathbf{K}_z$)

$\sigma[t_1,0]$

$-\mathbf{I}_z \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)$

$-\mathbf{K}_z \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)$

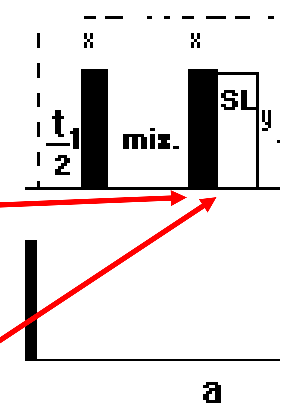
since during the mixing time (τ_m) only the populations ($-\mathbf{I}_z\, a_{II}$, $-\mathbf{I}_z\, a_{IK}$, $-\mathbf{I}_z\, a_{KI}$ and $-\mathbf{I}_z\, a_{KK}$) interact, where the interaction or mixing coefficients are :$a_{I\,I}$, a_{IK}, a_{KI}, a_{KK})

after the mixing the populations are:

$-\mathbf{I}_z\, a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)$ $-\mathbf{I}_z\, a_{IK} \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)$

$-\mathbf{K}_z\, a_{KK} \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)$ $-\mathbf{K}_z\, a_{KI} \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)$

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

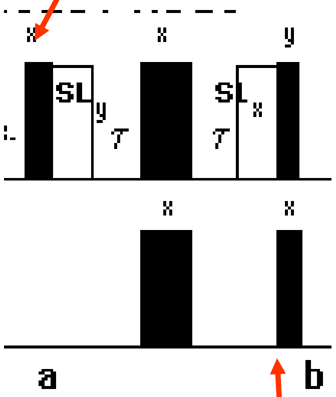


$+\mathbf{I}_y$ $\{a_{II} \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)$ $+ a_{IK} \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)\}$

$+\mathbf{K}_y$ $\{a_{KK} \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)$ $+ a_{KI} \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)\}$

$$\sigma[a] \quad +\mathbf{I}_y \quad \{a_{II}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1) + a_{IK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)\}$$
$$+\mathbf{K}_y \quad \{a_{KK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1) + a_{KI}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)\}$$

B. generating anti-phase magnetization on ^{15}N



memo.2: *The x* and y spin-locks are present for water suppression.
(e.g. on a D_2O sample it would be not needed.)

$\sigma[a]$ "The first INEPT module"

$$+\mathbf{I}_y$$

$\hat{H}$ = echo (homo) $\downarrow$

$$\{-\mathbf{I}_y\cos(J_{HN}\pi 2\tau) + 2\mathbf{I}_x\mathbf{N}_z\sin(J_{HN}\pi 2\tau) \text{ with } 2\tau = 1/\,2J_{IN}\}$$

$$+2\mathbf{I}_x\mathbf{N}_z$$

$\hat{H} = I_y(\pi/2)$ $\downarrow$

$$-2\mathbf{I}_z\mathbf{N}_z$$

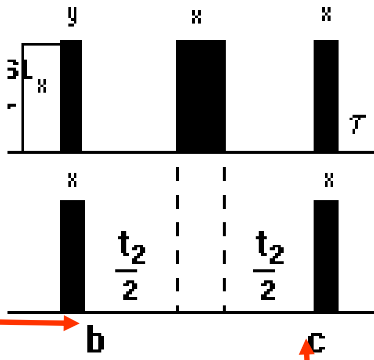
$\hat{H} = N_x(\pi/2)$ $\downarrow$

$$+2\mathbf{I}_z\mathbf{N}_y$$

$\sigma[b]$ "at the end of the INEPT module"

$$+2\mathbf{I}_z\mathbf{N}_y \quad \{a_{II}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1) + a_{IK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)\}$$
$$+2\mathbf{K}_y\mathbf{N}_y \quad \{a_{KK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1) + a_{KI}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)\}$$

C. module (frequency labelling by N)



σ[a] -> σ[b] "frequency labelling by ^{15}N"

memo.3: echo decouples from N all protons

σ[b] "at the beginning of t_2"

$+2\mathbf{I}_z\mathbf{N}_y$

$\hat{H}$ = echo (hetero) $\downarrow$

σ[c] "at the end of t_2" $-2\mathbf{I}_z\mathbf{N}_y\cos(\Omega_N t_2) + 2\mathbf{I}_z\mathbf{N}_x\sin(\Omega_N t_2)$

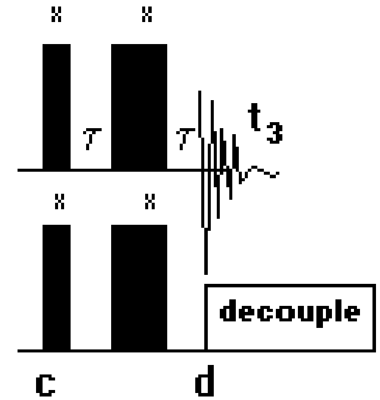
memo.4 : $-2\mathbf{I}_z\mathbf{N}_x\sin(\Omega_N t_2)$ is phase cycled out (in a gradient enhanced version it is kept.)

σ[c] "at the end of the frequency labelling module"

$$-2\mathbf{I}_z\mathbf{N}_y \quad *\cos(\Omega_N t_2)*\{a_{II}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1) \quad + a_{IK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)\}$$

$$-2\mathbf{K}_y\mathbf{N}_y \quad *\cos(\Omega_N t_2)*\{a_{KK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1) \quad + a_{KI}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)\}$$

D. module (finishing with a reverse INEPT)



σ[g] "The reverse INEPT module" $-2\mathbf{I}_z\mathbf{N}_y$

$\hat{H} = I_x(\pi/2)$ ↓

$+2\mathbf{I}_y\mathbf{N}_y$

$\hat{H} = N_x(\pi/2)$ ↓

$+2\mathbf{I}_y\mathbf{N}_z$

$\hat{H}$ = echo (homo) ↓

$\{-\mathbf{I}_x \sin(J_{HN}\pi 2\tau) + 2\mathbf{I}_y\mathbf{N}_z \cos(J_{IN}\pi 2\tau)$ with $2\tau = 1/2J_{IN}\}$

$-\mathbf{I}_x$

σ[h]" at the end": $-\mathbf{I}_x$ $*\cos(\Omega_N t_2)*\{a_{II}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1) + a_{IK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)\}$

$-\mathbf{K}_x$ $*\cos(\Omega_N t_2)*\{a_{KK}\cos(\Omega_K t_1)\cos(\pi J_{IK} t_1) + a_{KI}\cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)\}$

ACQ: $\{\hat{H} = I_z(\Omega_I[t_3])$ and $K_z(\Omega_K[t_3])\}$

memo.5: heteronuclear coupling is not affective during acquisition but homonuclear coupling J_{IK} is present.

memo.6: The four terms of σ[h] evolves during ACQ into 16 terms (see NOESY). Putting the receiver on x the above 4 terms remains:

$-\mathbf{I}_x\, a_{II}\ \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)\sin(\Omega_I t_3)\cos(\pi J_{IK} t_3)*\cos(\Omega_N t_2)$

$-\mathbf{K}_x\, a_{KK}\ \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)\sin(\Omega_K t_3)\cos(\pi J_{IK} t_3)*\cos(\Omega_N t_2)$

$-\mathbf{I}_x\, a_{IK}\ \cos(\Omega_K t_1)\cos(\pi J_{IK} t_1)\sin(\Omega_I t_3)\cos(\pi J_{IK} t_3)*\cos(\Omega_N t_2)$

$-\mathbf{K}_x\, a_{KI}\ \cos(\Omega_I t_1)\cos(\pi J_{IK} t_1)\sin(\Omega_K t_3)\cos(\pi J_{IK} t_3)*\cos(\Omega_N t_2)$

memo.7: $\sin(A)\cos(B) = 1/2[\sin(A+B)+\sin(A-B)]$

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

therefore:

$-1/4\mathbf{I}_x\, a_{II}$ $[+\cos\{(\Omega_I+\pi J_{IK})t_1\} + \cos\{(\Omega_I-\pi J_{IK})t_1\}]^*$

$[+\sin\{(\Omega_I+\pi J_{IK})t_3\} + \sin\{(\Omega_I-\pi J_{IK})t_3\}]^*\cos(\Omega_N t_2)$

$-1/4\mathbf{K}_x\, a_{KK}$ $[+\cos\{(\Omega_K+\pi J_{IK})t_1\} + \cos\{(\Omega_K-\pi J_{IK})t_1\}]^*$

$[+\sin\{(\Omega_K+\pi J_{IK})t_3\} + \sin\{(\Omega_K-\pi_{IK})t_3\}]^*\cos(\Omega_N t_2)$

$-1/4\mathbf{I}_x\, a_{IK}$ $[+\cos\{(\Omega_K+\pi J_{IK})t_1\} + \cos\{(\Omega_K-\pi J_{IK})t_1\}]^*$

$[+\sin\{(\Omega_I+\pi J_{IK})t_3\} + \sin\{(\Omega_I-\pi J_{IK})t_3\}]^*\cos(\Omega_N t_2)$

$-1/4\mathbf{K}_x\, s_{KI}$ $[+\cos\{(\Omega_I+\pi J_{IK})t_1\} + \cos\{(\Omega_I-\pi J_{IK})t_1\}]^*$

$[+\sin\{(\Omega_K+\pi J_{IK})t_3\} + \sin\{(\Omega_K-\pi_{IK})t_3\}]^*\cos(\Omega_N t_2)$

the following terms can be found

- $\mathbf{I}_x\, a_{II}$ [+ .. + .. + .. + ..] at Ω_I, Ω_I
- $\mathbf{I}_x\, a_{IK}$ [+ .. + .. + .. + ..] at Ω_K, Ω_I
- $\mathbf{K}_x\, a_{KK}$ [+ .. + .. + .. + ..] at Ω_K, Ω_K
- $\mathbf{K}_x\, a_{KI}$ [+ .. + .. + .. + ..] at Ω_I, Ω_K

setting the phase such as *cos is absorptive (a) in* t_1

sin is absorptive (d) in t_2

cos is absorptive (a) in t_3

$\mathbf{I}_x\, a_{II}$ [+a .. +a .. +a .. +a ..] at Ω_I, Ω_I and [+a] in Ω_N

$\mathbf{I}_x\, a_{IK}$ [+a .. +a .. +a ..+a ..] at Ω_K, Ω_I and [+a] in Ω_N

$\mathbf{K}_x\, a_{KK}$ [+a .. +a .. +a .. +a ..] at Ω_K, Ω_K and [+a] in Ω_N

$\mathbf{K}_x\, a_{KI}$ [+a .. +a .. +a .. +a ..] at Ω_I, Ω_K and [+a] in Ω_N

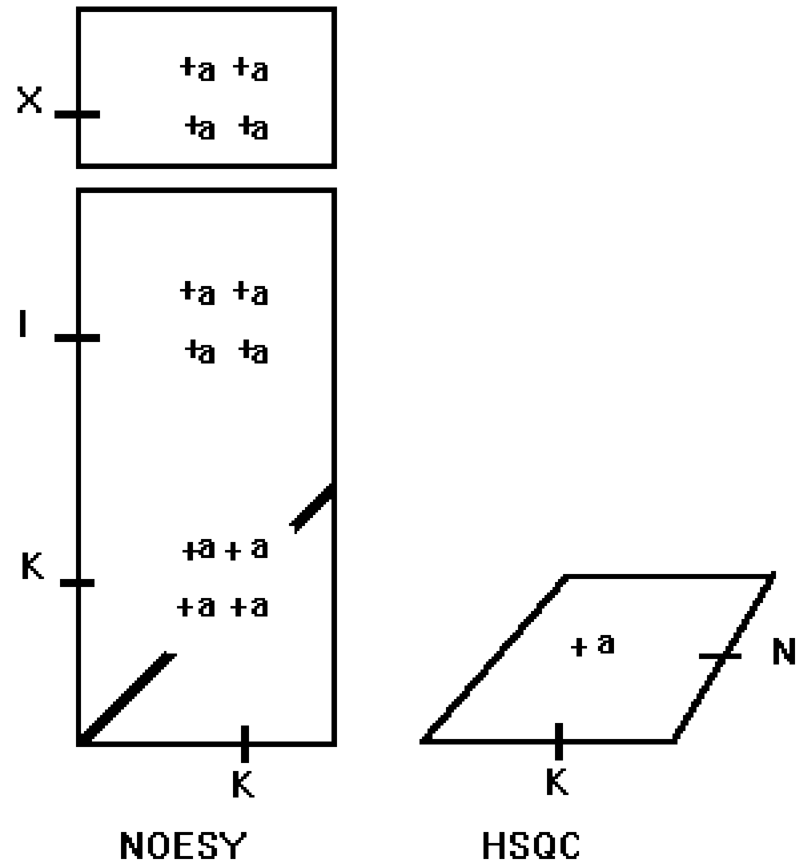
Conclusions (e.g. {^{1}H-^{15}N} 3D NOESY-HSQC):

memo.8: homonuclear spins I, K with chemical shift Ω_I, Ω_K and coupling J_{IK} (e.g. H^{NH} and H^{α}) were involved in the analysis.

Because of the HSQC module of the experiment the NH region [H_{NH} {≈7.2 ± 2.5 ppm} and N{≈117 ± 20 ppm}] is detected therefore only the "left" region of the NOESY is only present.

memo.9: heteronuclear spin N with chemical shift Ω_N with a coupling J_{IN} (e.g. H^{NH} and N^{NH}) provides a "normal" HSQC.

memo10:{^{1}H-^{13}C} 3D NOESY-HSQC only the aliphatic carbons [C^{α},C^{β},..,C^{ε}] {≈40 ± 30 ppm} and protons [H^{α},H^{β},..,H^{ε}]{≈3 ± 3 ppm} are recorded.



The NOESY and the HSQC planes.

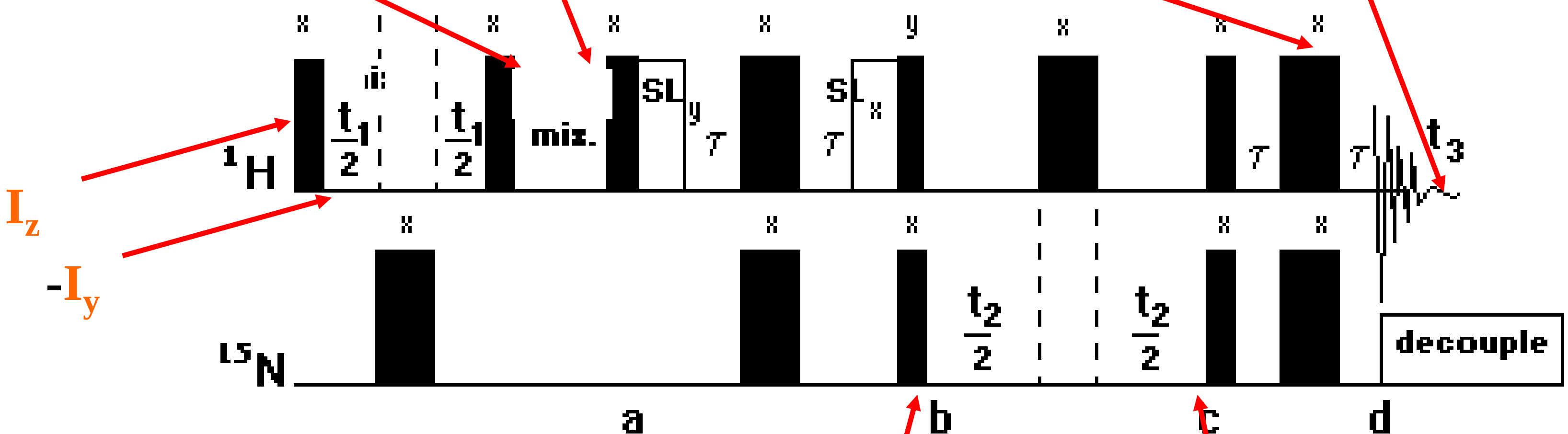
Összefoglalás: a ^{15}N-**szerkesztett 3D-NOESY-HSQC**

$-2\mathbf{I}_z\cos(\Omega_I t_1)$

$-2\mathbf{I}_z a_{IK}\cos(\Omega_K t_1)$

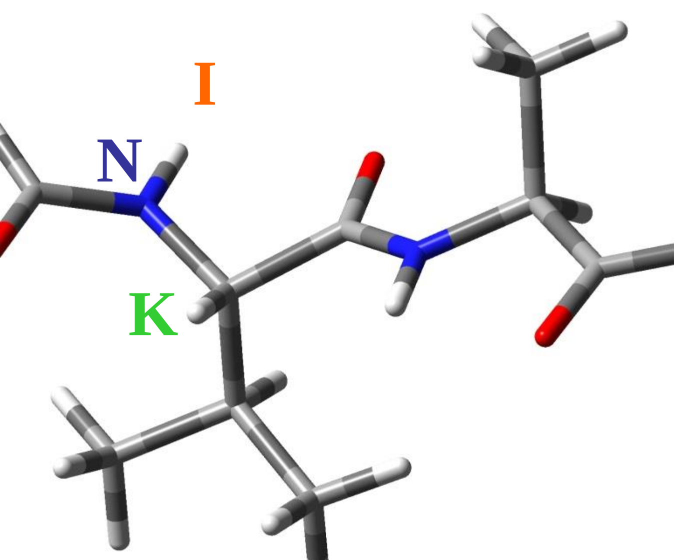
$-\mathbf{I}_x a_{IK}\cos(\Omega_K t_1)\cos(\Omega_N t_2)$

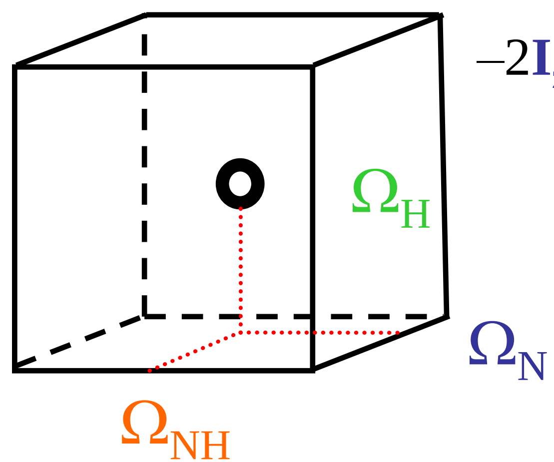
$-\mathbf{I}_x a_{IK}\sin(\Omega_I t_3)\cos(\Omega_N t_2)\cos(\Omega_K t_1)$



$-2\mathbf{I}_z\mathbf{N}_y\,\beta_{od}\cos(\Omega_K t_1)$

$-2\mathbf{I}_z\mathbf{N}_y\,a_{IK}\cos(\Omega_K t_1)\cos(\Omega_N t_2)$

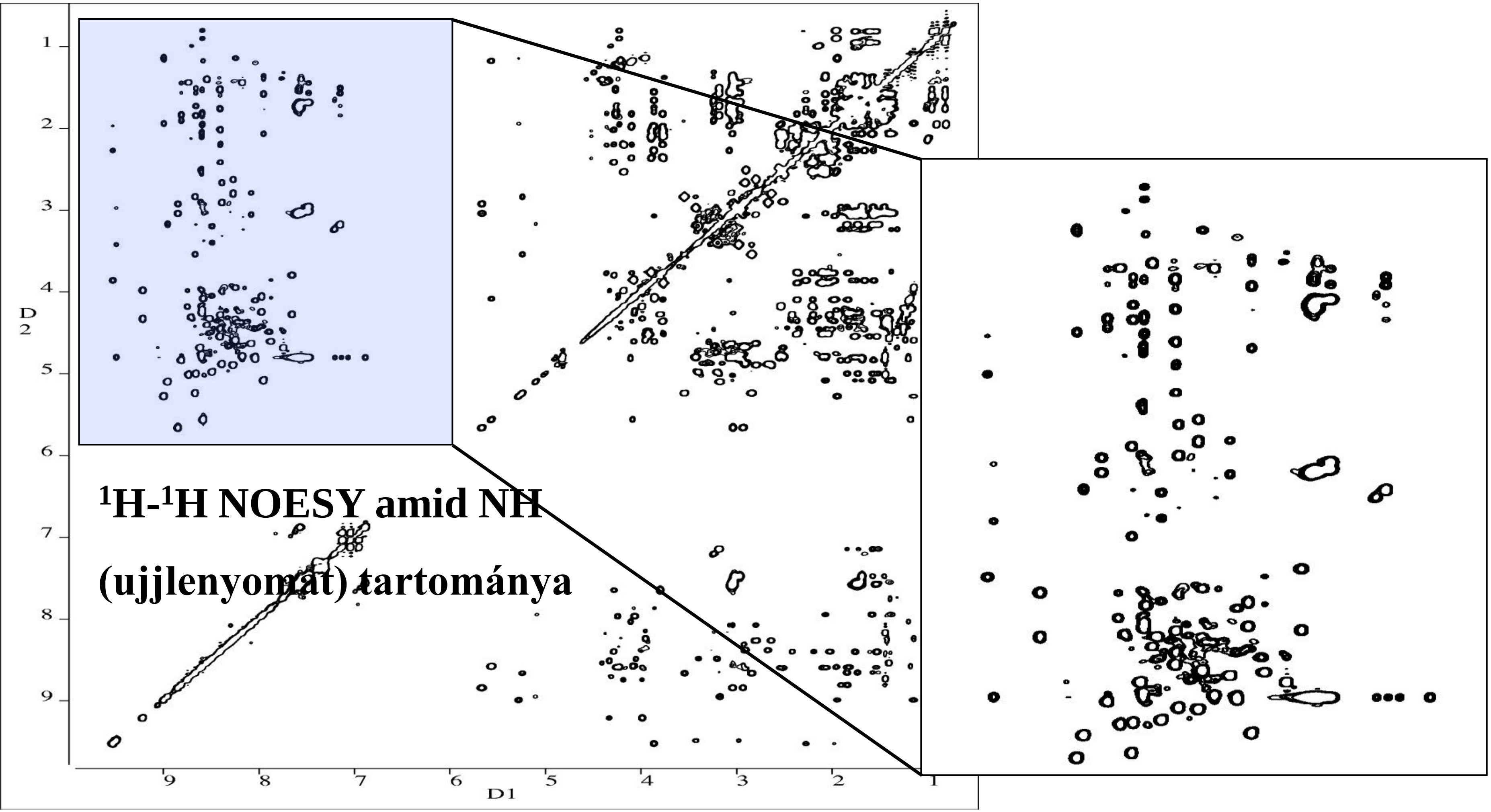




a_{IK} = off diagonális intenzitás

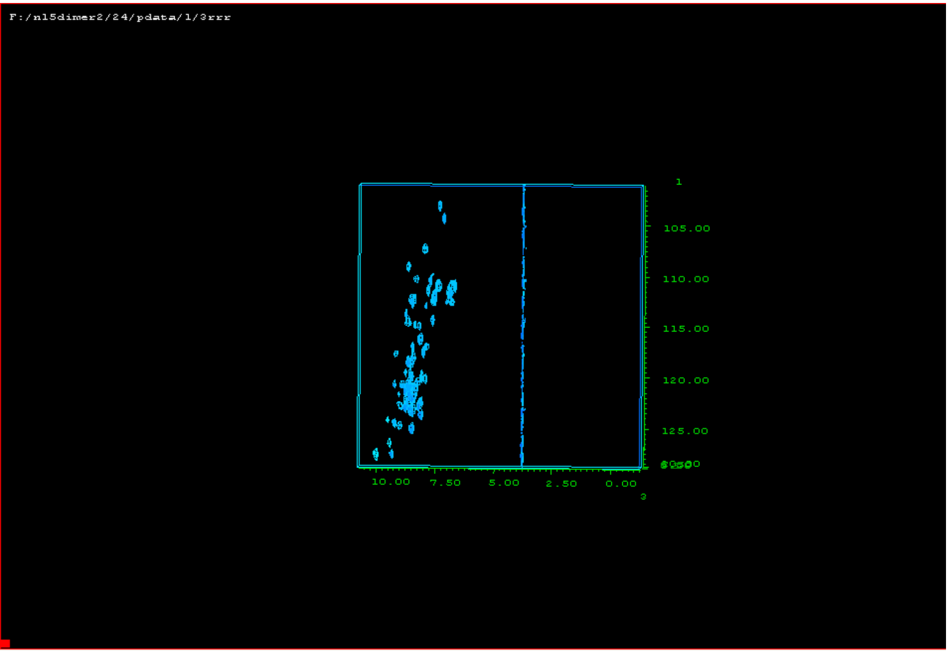
A J_{IK} okozta modulációtól eltekintünk

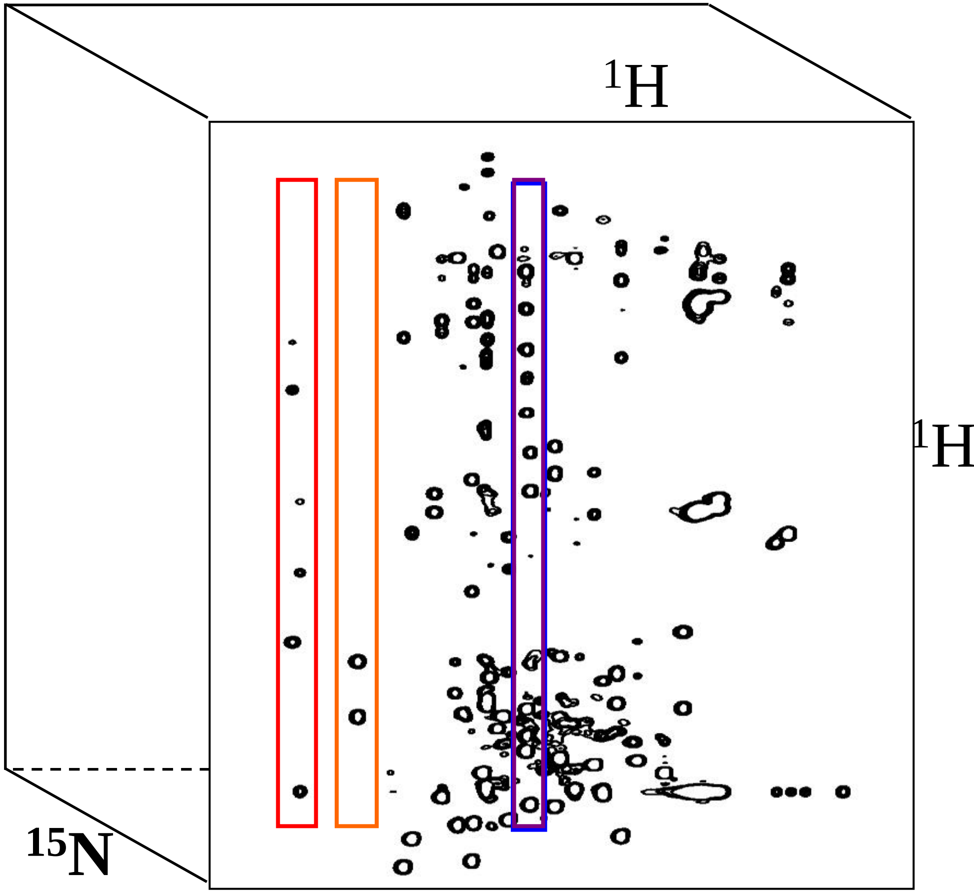
^{15}N**-szerkesztett NOESY spektrum**



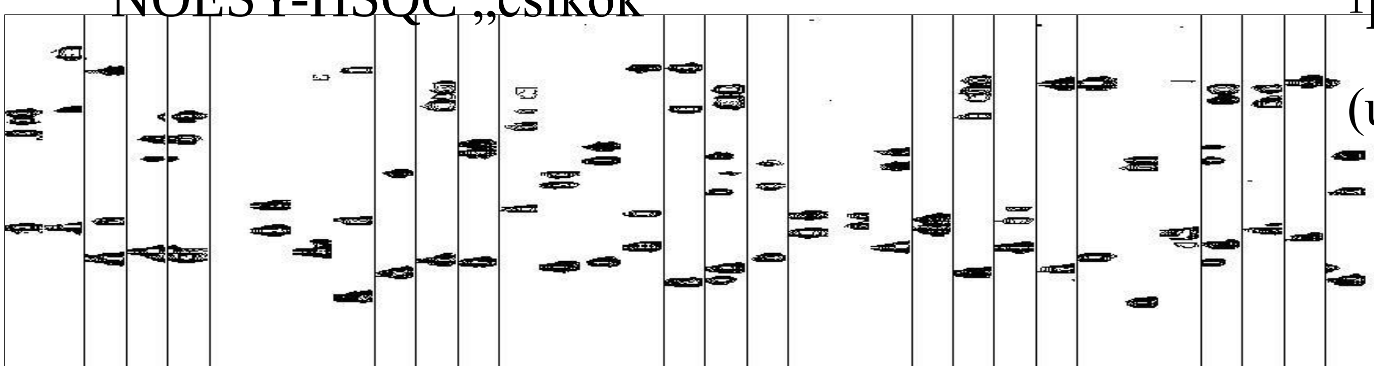
Homonukleáris 2D NOESY

^{15}N-szerkesztett 2D NOESY





NOESY-HSQC „csíkok”



EVTCEPGTTFKDKCNTCRCGSDGKSAACTLKACPQ

^{1}H-^{1}H TOCSYamid NH (ujjlenyomat) tartománya