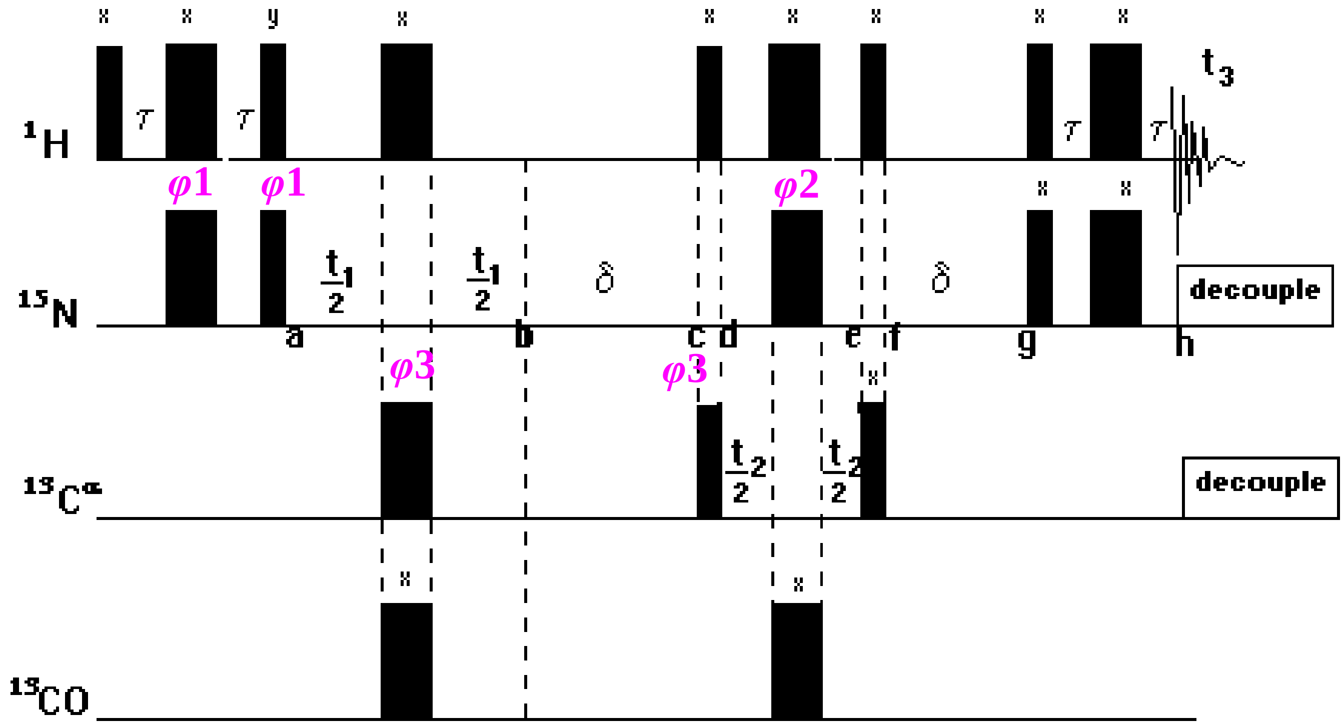
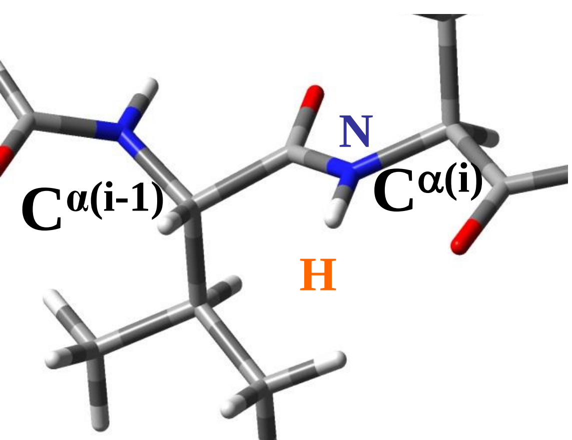
See also Cavanagh et al. 618

The **3D-HNCA** on a ^{15}N,-^{13}C labeled sample:





$\varphi 1$ = x, –x

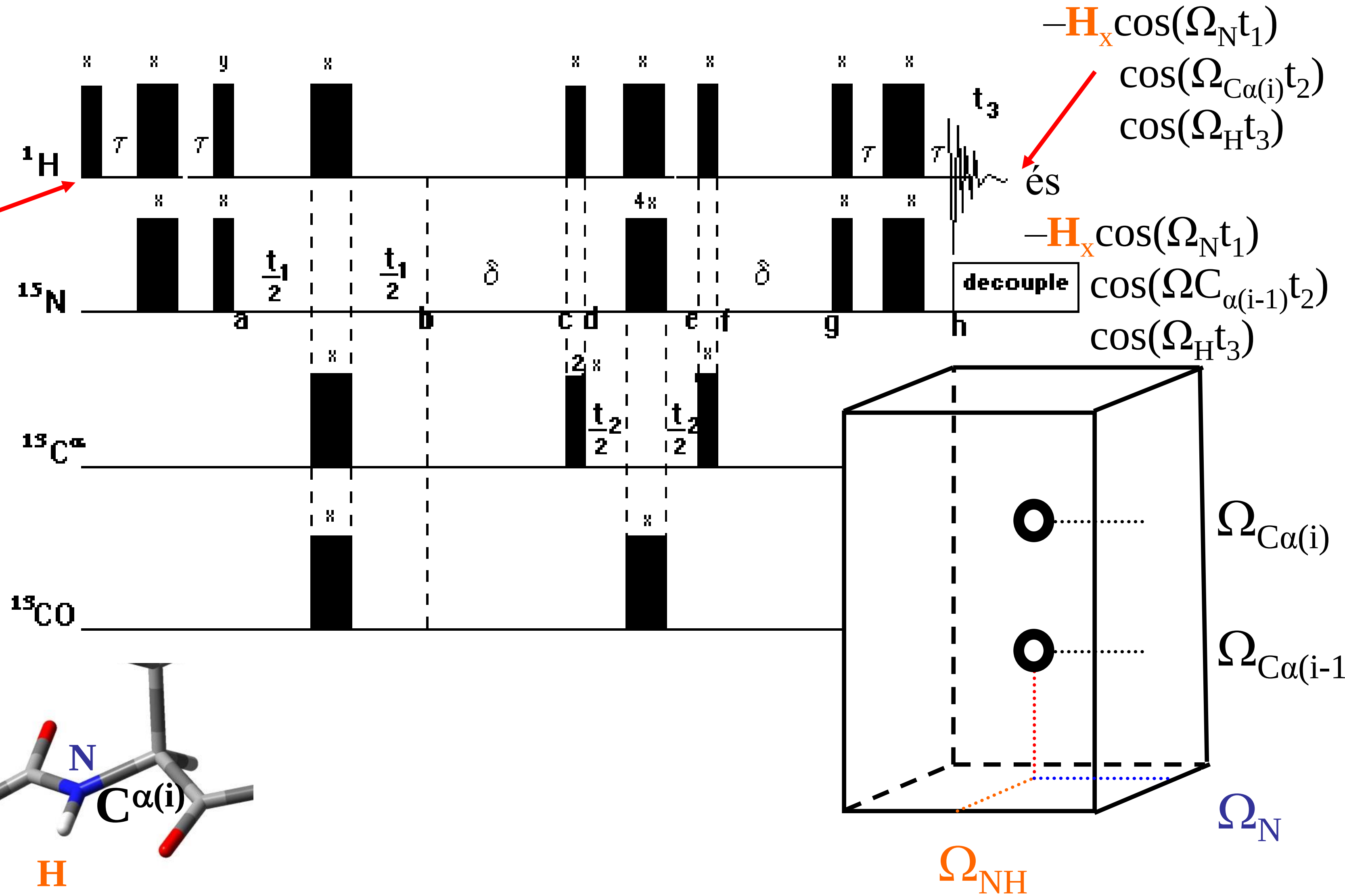
$\varphi 2$ = 4(x), 4(y), 4(–x), 4(–y)

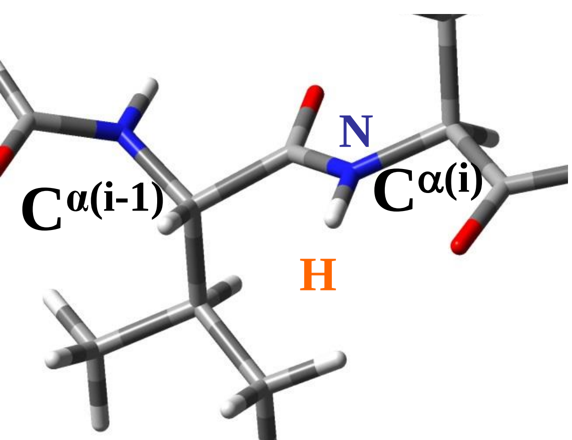
$\varphi 3$ = 2(x), 2(–x)

receiver x = x, –x, –x, x, –x, x, x, –x

The **3D-HNCA** on a ^{15}N,-^{13}C labeled sample:

What do we get at the end?





The scalar coupling induced splitting is removed

The **3D-HNCA**

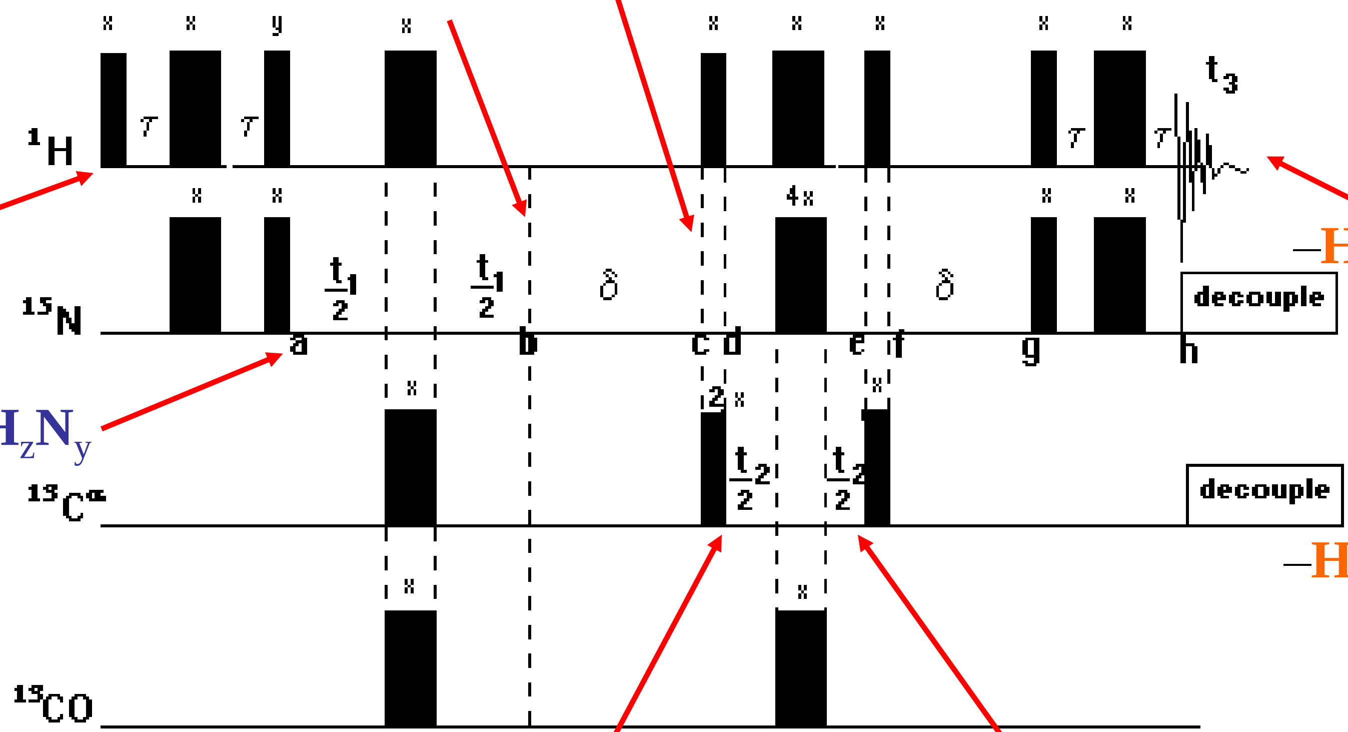
How do we get it?

$-2\mathbf{H}_z\mathbf{N}_x C_z^{\alpha(i-1)} \cos(\Omega_N t_1)$
és
$-2\mathbf{H}_z\mathbf{N}_x C_z^{\alpha(i)} \cos(\Omega_N t_1)$

$-2\mathbf{H}_z\mathbf{N}_y \cos(\Omega_N t_1)$

$\mathbf{H}_z$

$-2\mathbf{H}_z\mathbf{N}_y$

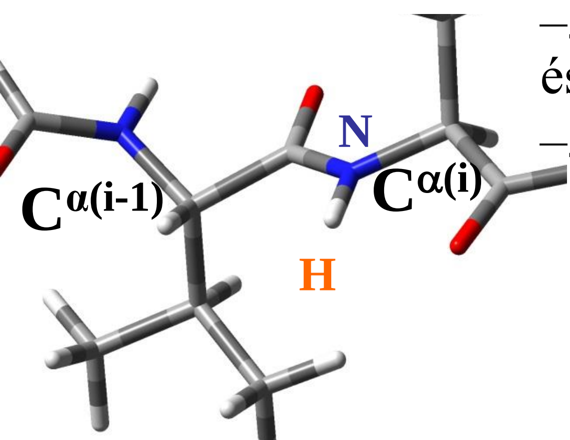


$-\mathbf{H}_x \cos(\Omega_N t_1) \cos(\Omega_{C\alpha(i)} t_2) \cos(\Omega_H t_3)$

$-\mathbf{H}_x \cos(\Omega_N t_1) \cos(\Omega C_{\alpha(i-1)} t_2) \cos(\Omega_H t_3)$

$-2\mathbf{H}_y\mathbf{N}_x C_y^{\alpha(i-1)} \cos(\Omega_N t_1)$
és
$-2\mathbf{H}_y\mathbf{N}_x C_y^{\alpha(i)} \cos(\Omega_N t_1)$

$-2\mathbf{H}_y\mathbf{N}_x C_y^{\alpha(i-1)} \cos(\Omega_N t_1) \cos(\Omega_{C\alpha(i)} t_2)$
és
$-2\mathbf{H}_y\mathbf{N}_x C_y^{\alpha(i)} \cos(\Omega_N t_1) \cos(\Omega C_{\alpha(i-1)} t_2)$



The **3D-HNCA**

How do we get it?

freq. labeling by Ω_N during t_1

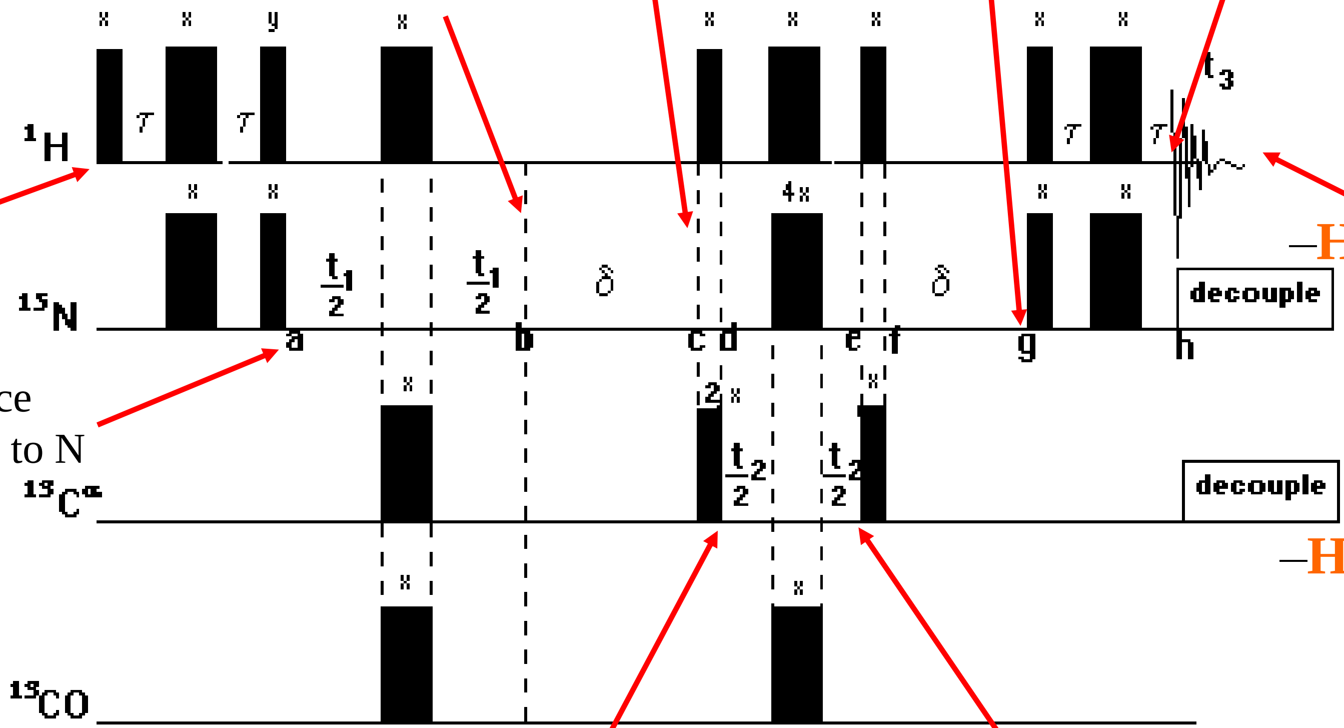
N-coherence is now transferred to Cα

C-coherence is back transferred now to N

N-coherence is back transferred now to H

H_z

H-coherence transferred to N

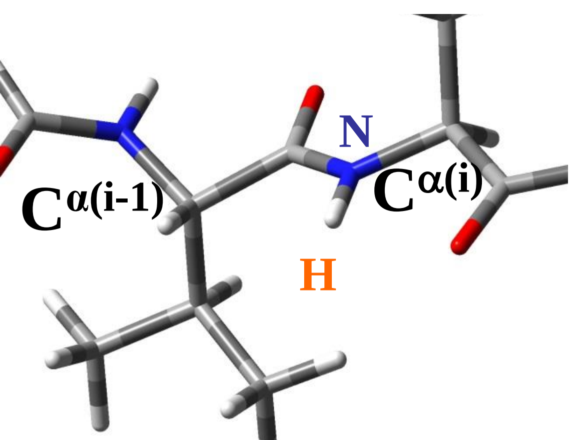


$-H_x \cos(\Omega_N t_1)$
$\cos(\Omega_{C\alpha(i)} t_2)$
$\cos(\Omega_H t_3)$

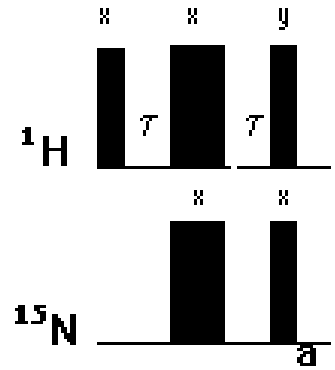
$-H_x \cos(\Omega_N t_1)$
$\cos(\Omega C_{\alpha(i-1)} t_2)$
$\cos(\Omega_H t_3)$

generating multiple-quantum coherences

freq. labeling by $\Omega_{C\alpha}$ during t_2



A. module (getting started)



σ[0] "The first INEPT module" $+\mathbf{H_z}$

$\hat{H} = H_x(\pi/2)$ ↓

$-\mathbf{H_y}$

$\hat{H}$ = echo (homo) ↓

$\{+\mathbf{H_y}\cos(J_{HN}\pi 2\tau) - 2\mathbf{H_x N_z}\sin(J_{HN}\pi 2\tau)$ with $2\tau = 1/2J_{HN}\}$

$-2\mathbf{H_x N_z}$

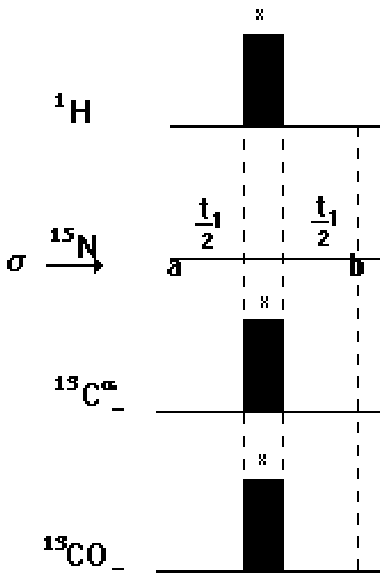
$\hat{H} = H_y(\pi/2)$ ↓

$+2\mathbf{H_z N_z}$

$\hat{H} = N_x(\pi/2)$ ↓

σ[a] "end of this INEPT" $\mathbf{-2H_z N_y}$

B. module (frequency labeling by N)

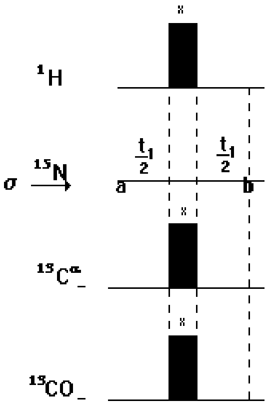


σ[a] → σ[b] "frequency labeling" by N^{15}
The echo module decouples N from all the other nuclei (*e.g.* H, C^{α} ,C'). In other words, all scalar couplings between ^{15}N and $^1H^{NH}$, ^{13}Ca and ^{13}CO spins are removed by the 180^o refocusing pulses (echo) positioned at the middle of t_1.

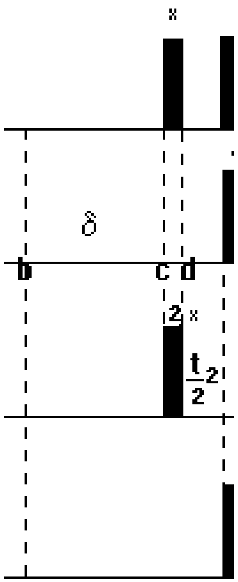
σ[a] "at the beginning of t_1" $-2\mathbf{H}_z\mathbf{N}_y$

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

σ[b] "at the end of t_1" $+2\mathbf{H}_z\mathbf{N}_y \cos(\Omega_N t_1)$ $-2\mathbf{H}_z\mathbf{N}_x \sin(\Omega_N t_1)$

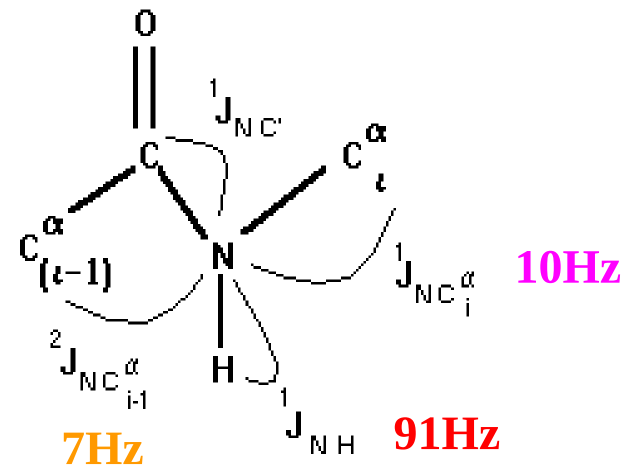


memo : $-2\mathbf{H}_z\mathbf{N}_x \sin(\Omega_N t_1)$ is phase cycled out, as it would evolve into a $+4\mathbf{H}_z\mathbf{N}_y\mathrm{C}_z$ type anti-phase „tri-spin" magnetization.



C. module (transferring coherence from N to Cα(s))

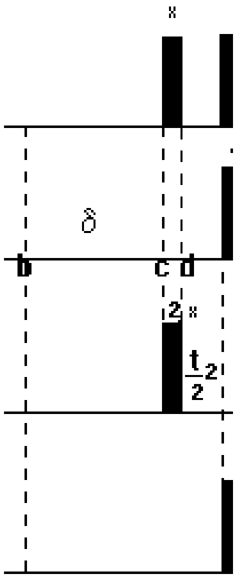
σ[b] from the beginning of delay δ term $+2\mathbf{H}_z\mathbf{N}_y \cos(\Omega_N t_1)$ is to be considered only *memo:* during the delay-time δ "all" couplings ($^1J_{HN}$, $^1J_{NC'}$ $^1J_{NC^\alpha}$ $^2J_{NC^\alpha}$) are active.



$+2\mathbf{H_zN_y}\cos(\Omega_N t_1)$

$\downarrow$

$\hat{H} = 2H_zN_z({}^1J_{NH}\,\pi\delta)$



$+2\mathbf{H_zN_y}\cos(\Omega_N t_1)\cos({}^1J_{NH}\,\pi\delta)\ +2\mathbf{H_zN_x}\cos(\Omega_N t_1)\sin({}^1J_{NH}\,\pi\delta)$

memo 3: If δ is set to a value $k/{}^1J_{HN}$ (where k = 1, 2,...) [1/91, 2/91, *etc*.] then ${}^1J_{NH}\,\pi\delta \rightarrow k^*\pi$ [1π, 2π, *etc*.]. As $\sin(k^*\pi) = 0$, the $+2\mathbf{H_zN_x}\cos(\Omega_N t_1)\sin({}^1J_{NH}\pi\delta)$ term must vanishes.

Thus, $+2\mathbf{H_zN_y}\cos(\Omega_N t_1)\cos({}^1J_{NH}\,\pi\delta)$ is to be considered only:

$+2\mathbf{H_zN_y}$

$\downarrow$

$\hat{H} = 2N_zC_z({}^1J_{NC\alpha^{(i)}}\,\pi\delta)$

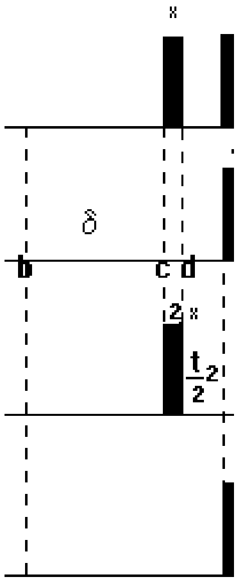
$+2\mathbf{H_zN_y}$ $\downarrow$ $\qquad -4\mathbf{H_zN_x}C\alpha^{(i)}_z$ $\downarrow$

$\hat{H} = 2N_zC_z({}^2J_{NC\alpha^{(i-1)}}\,\pi\delta)$

$+2\mathbf{H_zN_y}\qquad -4\mathbf{H_zN_x}C\alpha^{(i-1)}_z\qquad -4\mathbf{H_zN_x}C\alpha^{(i)}_z\qquad -8\mathbf{H_zN_y}\mathbf{C}\alpha^{(i)}_z\mathbf{C}\alpha^{(i-1)}_z$

$+2\mathbf{H_zN_y}$	$^*\cos({}^1J_{NC\alpha^{(i)}}\,\pi\delta)$	$^*\cos({}^2J_{NC\alpha^{(i-1)}}\,\pi\delta)$	a 2-spin term
$-4\mathbf{H_zN_x}\mathbf{C}\alpha^{(i-1)}_z$	$^*\cos({}^1J_{NC\alpha^{(i)}}\,\pi\delta)$	$^*\sin({}^2J_{NC\alpha^{(i-1)}}\,\pi\delta)$	a 3-spin term
$-4\mathbf{H_zN_x}\mathbf{C}\alpha^{(i)}_z$	$^*\sin({}^1J_{NC\alpha^{(i)}}\,\pi\delta)$	$^*\cos({}^2J_{NC\alpha^{(i-1)}}\,\pi\delta)$	a 3-spin term
$-8\mathbf{H_zN_y}\mathbf{C}\alpha^{(i)}_z\mathbf{C}\alpha^{(i-1)}_z$	$^*\sin({}^1J_{NC\alpha^{(i)}}\,\pi\delta)$	$^*\sin({}^2J_{NC\alpha^{(i-1)}}\,\pi\delta)$	a 4-spin term

memo: recall that ${}^1J_{NC\alpha^{(i)}} \neq {}^2J_{NC\alpha^{(i-1)}}$ **as** $10 \neq 7$Hz

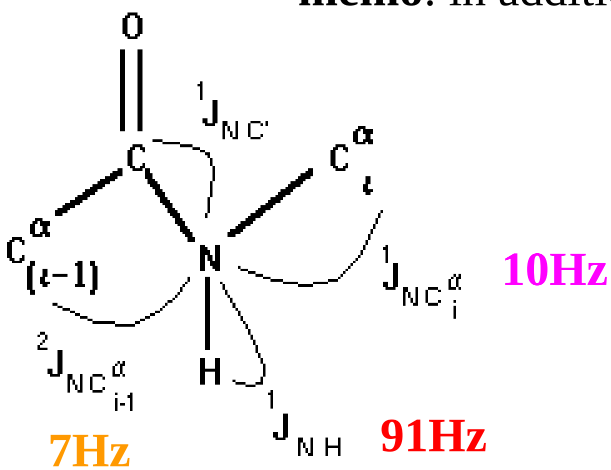


question: how to set optimally delay time δ?

answer: the $\cos(^1J_{NC\alpha^{(i)}}\pi\delta)$ *$\sin(^2J_{NC\alpha^{(i-1)}}\pi\delta)$ and the $\sin(^1J_{NC\alpha^{(i)}}\pi\delta)$ *$\cos(^2J_{NC\alpha^{(i-1)}}\pi\delta)$ terms are called as the "transfer” functions; terms to be maximized.

Considering that $^1J_{NC\alpha^{(i)}}$ and $^2J_{NC\alpha^{(i-1)}}$ are 10 and 7 Hz, respectively (with $^1J_{NH} = 91$ Hz) both transfer functions have a maximum at $\delta \sim$ 22 ms.

So one can now **set** δ **at a sensible value (**~ 22 ms) for globular proteins.
memo: in addition, if δ~22 ms, then $\cos(^1J_{NH}\pi\delta) \sim \cos(2{,}002\pi) \sim 1$ **.**

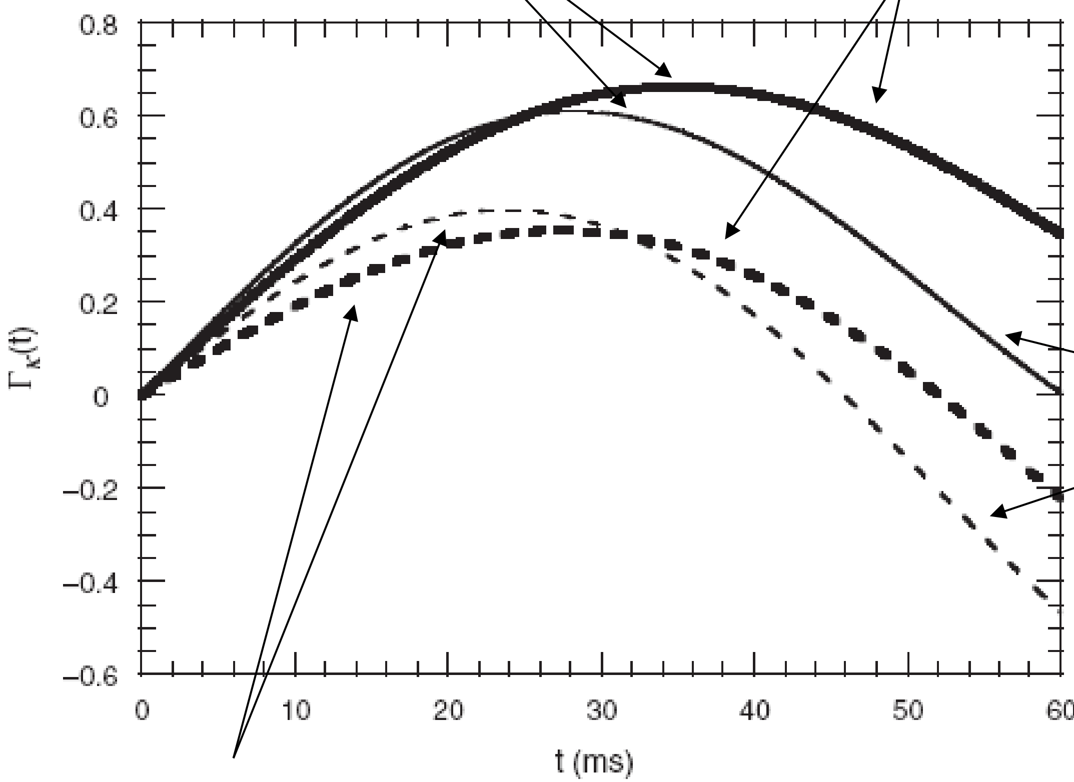


$^1J_{NC'}$ refocused with the 180^o suite centered in the middle of t_2 (or more typically it is decoupled).

The two "transfer" functions, f_1 and f_2, as function of the delay-time (δ)

$f_1(\delta) = \cos({}^1J_{NC}\alpha^{(i)}\,\pi\delta)\,\sin({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta)$

${}^1J_{NC}\alpha$ and ${}^2J_{NC}\alpha$ set for a β-sheet backbone structure



For α-helix ${}^1J_{NC}\alpha$ = 10.9Hz and ${}^2J_{NC}\alpha$ = 8.3Hz

${}^1J_{NC}\alpha$ and ${}^2J_{NC}\alpha$ set for an α-helical backbone structure

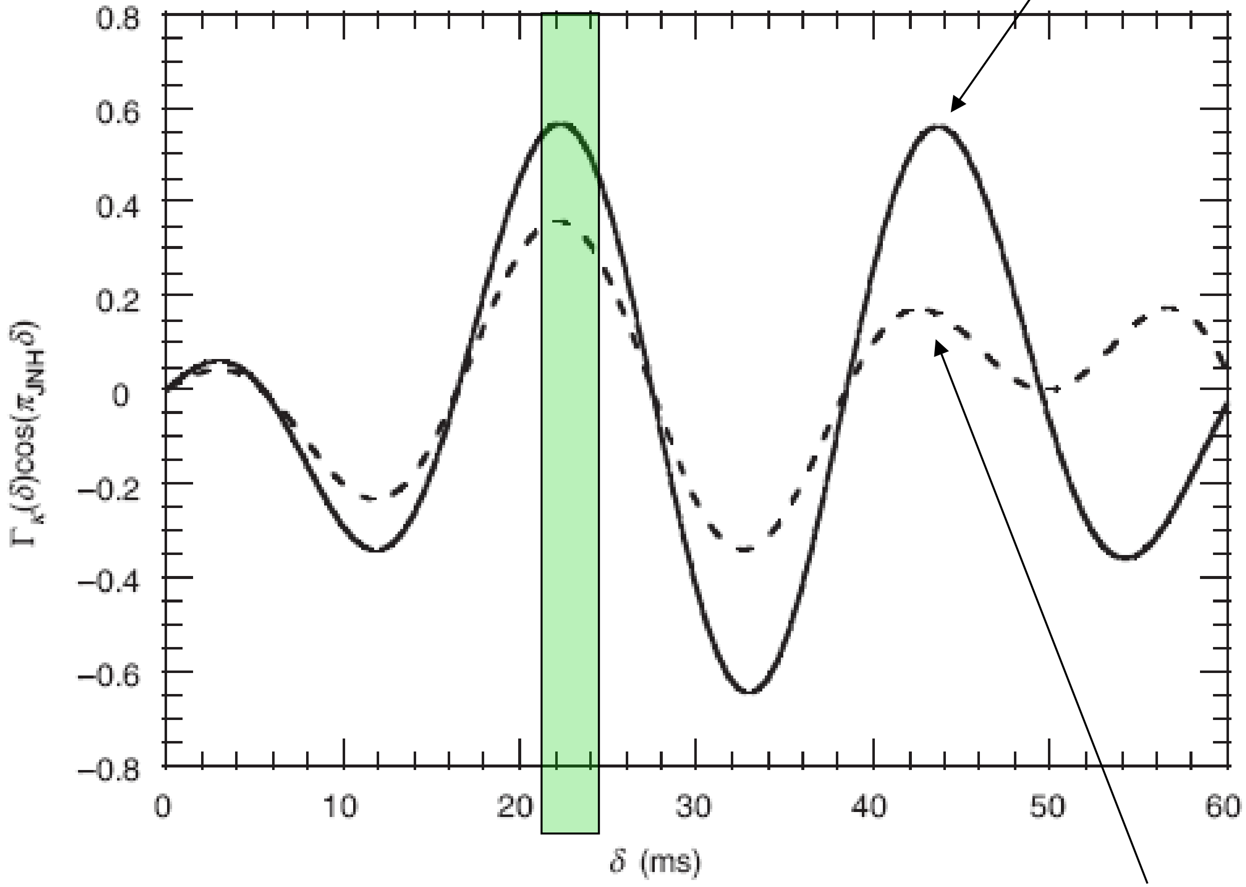
For α-helix ${}^1J_{NC}\alpha$ = 9.6Hz and ${}^2J_{NC}\alpha$ = 6.4Hz

$f_2(\delta) = \sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta)\,\cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta)$

See also Cavanagh et al. 622

The two "transfer" functions, $f_1 \cos(^1J_{NH}\pi\delta)$ and $f_2 \cos(^1J_{NH}\pi\delta)$ as function of the delay-time (δ)

$f_1(\delta) = \cos(^1J_{NC\alpha^{(i)}}\,\pi\delta)\ \sin(^2J_{NC\alpha^{(i-1)}}\,\pi\delta)\ \cos(^1J_{NH}\pi\delta)$



$f_2(\delta) = \sin(^1J_{NC\alpha^{(i)}}\,\pi\delta)\ \cos(^2J_{NC\alpha^{(i-1)}}\,\pi\delta)\ \cos(^1J_{NH}\pi\delta)$

One can **set δ at a sensible value (**~ 22 ms) for globular proteins**.**

See also Cavanagh et al. 623

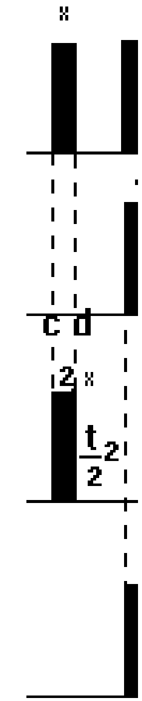
memo 4: - since ${}^{1}J_{NC\alpha^{(i)}}$ and ${}^{2}J_{NC\alpha^{(i-1)}}$ couplings are different, they do not cancel out,

- the anti-phase two spin ($+2\mathbf{H}_z\mathbf{N}_y$) and four-spin ($-8\mathbf{H}_z\mathbf{N}_y C\alpha(i)_z C\alpha(i-1)_z$) terms are removed by phase cycling during the next 90° on C (of phase $\varphi 3$).

Thus, the two 3-spin terms of interest are as follows:

$$-4\mathbf{H}_z\mathbf{N}_x C\alpha^{(i-1)}_z \quad *\cos({}^{1}J_{NC\alpha^{(i)}}\,\pi\delta) \quad *\sin({}^{2}J_{NC\alpha^{(i-1)}}\,\pi\delta) \quad *\cos({}^{1}J_{NH}\,\pi\delta) * \cos(\Omega_N t_1)$$

$$-4\mathbf{H}_z\mathbf{N}_x C\alpha^{(i)}_z \quad *\sin({}^{1}J_{NC\alpha^{(i)}}\,\pi\delta) \quad *\cos({}^{2}J_{NC\alpha^{(i-1)}}\,\pi\delta) \quad *\cos({}^{1}J_{NH}\,\pi\delta) * \cos(\Omega_N t_1)$$



D. module (generating multiple-quantum coherences)

σ[c]

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

$\hat{H} = C\alpha_x(\pi/2)$

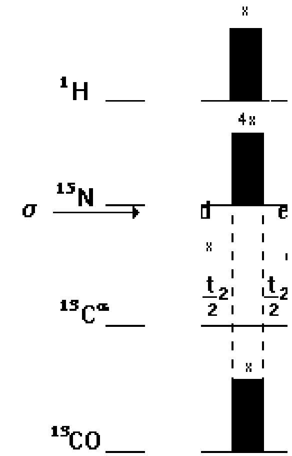
$$-4\mathbf{H}_z\mathbf{N}_x C_z \;\downarrow\; +4\mathbf{H}_y\mathbf{N}_x C_z \;\downarrow\; -4\mathbf{H}_y\mathbf{N}_x C_y$$

σ[d]

$$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i-1)}_y *\cos({}^{1}J_{NC\alpha^{(i)}}\,\pi\delta) *\sin({}^{2}J_{NC\alpha^{(i-1)}}\,\pi\delta) *\cos({}^{1}J_{NH}\,\pi\delta) *\cos(\Omega_N t_1)$$

$$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i)}_y *\sin({}^{1}J_{NC\alpha^{(i)}}\,\pi\delta) *\cos({}^{2}J_{NC\alpha^{(i-1)}}\,\pi\delta) *\cos({}^{1}J_{NH}\,\pi\delta) *\cos(\Omega_N t_1)$$

E. module (frequency labeling by Cα(s))



σ[d] "at the beginning of t_2"

$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i-1)}{}_y * \cos(^1J_{NC}\alpha^{(i)}\,\pi\delta) * \sin(^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos(^1J_{NH}\,\pi\delta) * \cos(\Omega_N t_1)$

$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i)}{}_y * \sin(^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos(^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos(^1J_{NH}\,\pi\delta) * \cos(\Omega_N t_1)$

$\hat{H}$ = echo (hetero) $\hat{H} = C_z(\Omega_{C\alpha}[t_2])$ ↓

at the end of t_2

$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i-1)}{}_y * \cos(^1J_{NC}\alpha^{(i)}\,\pi\delta) * \sin(^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos(^1J_{NH}\,\pi\delta) * \cos(\Omega_N t_1) * \mathbf{\cos(\Omega_C\alpha^{(i-1)}t_2)}$

$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i)}{}_y * \sin(^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos(^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos(^1J_{NH}\,\pi\delta) * \cos(\Omega_N t_1) * \mathbf{\cos(\Omega_C\alpha^{(i)}t_2)}$

memo: Both of the two 3-spin terms contain the $\mathbf{\sin(\Omega_C\alpha^{(i-1)}t_2)}$ modulation (not shown above) were phase cycled out (φ2).

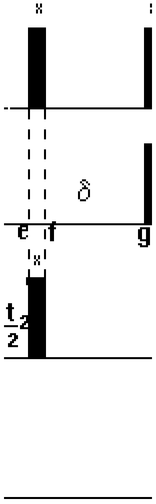
memo: 180^o applied on both ^{1}H and ^{15}N refocuses their chemical shifts (echo), and thus the evolution of $\mathbf{H}_y\mathbf{N}_x C\alpha_y$ term depends only on the chemical shift of Cα, therefore the effective $\hat{H} = C_z(\Omega_{C\alpha}[t_2])$.

Note however, that because the $^1J_{C\alpha C\beta}$ copling is active during t_2, the $\cos(^1J_{C\alpha C\beta}\,\pi t_2)$ modulation is effective, at the end of t_2. Thus, at **σ[e]** is :

$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i-1)}{}_y * \cos(^1J_{NC}\alpha^{(i)}\,\pi\delta) * \sin(^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos(^1J_{NH}\,\pi\delta) * \cos(\Omega_N t_1) * \cos(\Omega_C\alpha^{(i-1)}t_2)$
$* \cos(^1J_C\alpha_C\beta\,\pi t_2)$

$-4\mathbf{H}_y\mathbf{N}_x C\alpha^{(i)}{}_y * \sin(^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos(^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos(^1J_{NH}\,\pi\delta) * \cos(\Omega_N t_1) * \cos(\Omega_C\alpha^{(i)}t_2)$
$* \cos(^1J_C\alpha_C\beta\,\pi t_2)$

F. module (back transforming the multiple-quantum coherences to an anti-phase coherence on N)



σ[e]

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

$\hat{H} = C\alpha_x(\pi/2)$

$$-4\mathbf{H}_y\mathbf{N}_x C_y \downarrow +4\mathbf{H}_z\mathbf{N}_x C_y \downarrow -4\mathbf{H}_z\mathbf{N}_x C_z$$

σ[f]

$-4\mathbf{H_z N_x C}\alpha^{\mathbf{(i\text{-}1)}}{}_{\mathbf{z}}$ *$\cos({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ *$\sin({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta)$ *$\cos({}^1J_{NH}\,\pi\delta)$ *$\cos(\Omega_N t_1)$ *$\cos(\Omega_C\alpha^{(i-1)}t_2)$ *$\cos({}^1J_{C\alpha C\beta}\,\pi t_2)$

$-4\mathbf{H_z N_x C}\alpha^{\mathbf{(i)}}{}_{\mathbf{z}}$ *$\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ *$\cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta)$ *$\cos({}^1J_{NH}\,\pi\delta)$ *$\cos(\Omega_N t_1)$ *$\cos(\Omega_C\alpha^{(i)}t_2)$ *$\cos({}^1J_{C\alpha C\beta}\,\pi t_2)$

G. module (back transferring coherence from Cα(s) to N during the second delay-time δ)

σ[f]

$\hat{H} = 2N_zC_z({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$

$\hat{H} = 2N_zC_z({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta)$

$$-4\mathbf{H_z N_x C_z} \downarrow$$

$$-4\mathbf{H_z N_x C}\alpha^{(i)}{}_{\mathbf{z}} \downarrow \quad -2\mathbf{H}_z\mathbf{N}_y \downarrow$$

$$-4\mathbf{H_z N_x C}\alpha^{(i)}{}_{\mathbf{z}} \quad -8\mathbf{H_z N_y C}\alpha^{(i)}{}_{\mathbf{z}}\mathbf{C}\alpha^{(i-1)}{}_{\mathbf{z}} \quad -2\mathbf{H}_z\mathbf{N}_y \quad +4\mathbf{H_z N_x C}\alpha^{(i-1)}{}_{\mathbf{z}}$$

memo : The reverse INEPT pulses (as it is the terminating module of this pulse sequence) has to result in observable magnetization, thus among the 4 terms:

$-4\mathbf{H_zN_xC}\alpha^{(i)}{}_{\mathbf{z}} \rightarrow -4\mathbf{H_yN_xC}\alpha^{(i)}{}_{\mathbf{z}}$ is a multiple-quantum coh. → **not** observable

$+4\mathbf{H_zN_xC}\alpha^{(i\text{-}i)}{}_{\mathbf{z}} \rightarrow +4\mathbf{H_yN_xC}\alpha^{(i\text{-}1)}{}_{\mathbf{z}}$ is amultiple-quantum coh. → **not** observable

$-8\mathbf{H_zN_yC}\alpha^{(i)}{}_{\mathbf{z}}\mathbf{C}\alpha^{(i\text{-}1)}{}_{\mathbf{z}} \rightarrow -4\mathbf{H_xC}\alpha^{(i)}{}_{\mathbf{z}}\mathbf{C}\alpha^{(i\text{-}1)}{}_{\mathbf{z}}$ is an anti-phase coh.→ observable **but ignored**, as it is modulated by unwanted proton-carbon couplings: ${}^2J_{HC}\alpha^{(i)}$ and ${}^3J_{HC}\alpha^{(i)}$

$\mathbf{-2H_zN_y \rightarrow -H_x}$ **the only important term to be considered**

Therefore from:

σ[f]

$-4\mathbf{H_zN_xC}\alpha^{\mathbf{(i\text{-}1)}}{}_{\mathbf{z}}$ $*\cos({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ $*\sin({}^2J_{NC}\alpha^{(i\text{-}1)}\,\pi\delta)$ $*\cos({}^1J_{NH}\,\pi\delta)$ $*\cos(\Omega_N t_1)$ $*\cos(\Omega_C\alpha^{(i\text{-}1)}t_2)$ $*\cos({}^1J_C\alpha_C\beta\ \pi t_2)$

$-4\mathbf{H_zN_xC}\alpha^{\mathbf{(i)}}{}_{\mathbf{z}}$ $*\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ $*\cos({}^2J_{NC}\alpha^{(i\text{-}1)}\,\pi\delta)$ $*\cos({}^1J_{NH}\,\pi\delta)$ $*\cos(\Omega_N t_1)$ $*\cos(\Omega_C\alpha^{(i)}t_2)$ $*\cos({}^1J_C\alpha_C\beta\ \pi t_2)$

one gets the following two terms:

σ[g]

$-2\mathbf{H}_z\mathbf{N}_y$ $*\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ $*\cos({}^2J_{NC}\alpha^{(i\text{-}1)}\,\pi\delta)$ $*\cos({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ $*\sin({}^2J_{NC}\alpha^{(i\text{-}1)}\,\pi\delta)$ $*\cos({}^1J_{NH}\,\pi\delta)$ $*\cos(\Omega_N t_1)$ $*\cos(\Omega_C\alpha^{(i\text{-}1)}t_2)$ $*\cos({}^1J_C\alpha_C\beta\ \pi t_2)$

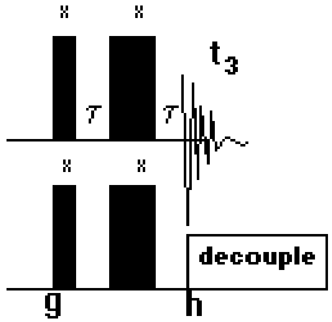
$-2\mathbf{H}_z\mathbf{N}_y$ $*\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ $*\cos({}^2J_{NC}\alpha^{(i\text{-}1)}\,\pi\delta)$ $*\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta)$ $*\cos({}^2J_{NC}\alpha^{(i\text{-}1)}\,\pi\delta)$ $*\cos({}^1J_{NH}\,\pi\delta)$ $*\cos(\Omega_N t_1)$ $*\cos(\Omega_C\alpha^{(i)}t_2)$ $*\cos({}^1J_C\alpha_C\beta\ \pi t_2)$

$\sigma[g]$

$-2\mathbf{H}_z\mathbf{N}_y * \sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \sin({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos^2({}^1J_{NH}\,\pi\delta)$
$* \cos(\Omega_N t_1) * \cos(\Omega_C\alpha^{(i-1)} t_2) * \cos({}^1J_C\alpha_C\beta\,\pi t_2)$

$-2\mathbf{H}_z\mathbf{N}_y * \sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos^2({}^1J_{NH}\,\pi\delta)$
$* \cos(\Omega_N t_1) * \cos(\Omega_C\alpha^{(i)} t_2) * \cos({}^1J_C\alpha_C\beta\,\pi t_2)$

H. module (finishing with a reverse INEPT)



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

$\hat{H} = H_x(\pi/2)$ ↓

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

$\hat{H} = N_x(\pi/2)$ ↓

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

$\hat{H}$ = echo (homo) ↓

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

$\sigma[h]$ „at the end, before acquisition (t_3) starts„ : $-\mathbf{H}_x$

Note that this final observable in-phased $-\mathbf{H}_x$ coherence (or transverse magnetization) is modulated as follows:

$-\mathbf{H}_x * \sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \sin({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos^2({}^1J_{NH}\,\pi\delta)$
$* \cos(\Omega_N t_1) * \cos(\Omega_C\alpha^{(i-1)} t_2) * \cos({}^1J_C\alpha_C\beta\,\pi t_2)$

$-\mathbf{H}_x * \sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) * \cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) * \cos^2({}^1J_{NH}\,\pi\delta)$
$* \cos(\Omega_N t_1) * \cos(\Omega_C\alpha^{(i)} t_2) * \cos({}^1J_C\alpha_C\beta\,\pi t_2)$

ACQ:

As no coupling is effective during acquisition

$$\hat{H} = H_z(\Omega_H[t_3])$$

$$-\mathbf{H}_x$$
$$\downarrow$$
$$-\mathbf{H}_x \cos(\Omega_H t_3) + -\mathbf{H}_y \sin(\Omega_H t_3)$$

memo : put the receiver on -x

therefore only the single x term remains: $+\mathbf{H}_x \cos(\Omega_H t_3)$

the following term can be found $\mathbf{H}_x$ [+] at $\Omega_H, \Omega_N, \Omega_C\alpha$

if one sets the phase that *cos* is absorptive in all three dimensions (t_3, t_2, t_1), then:

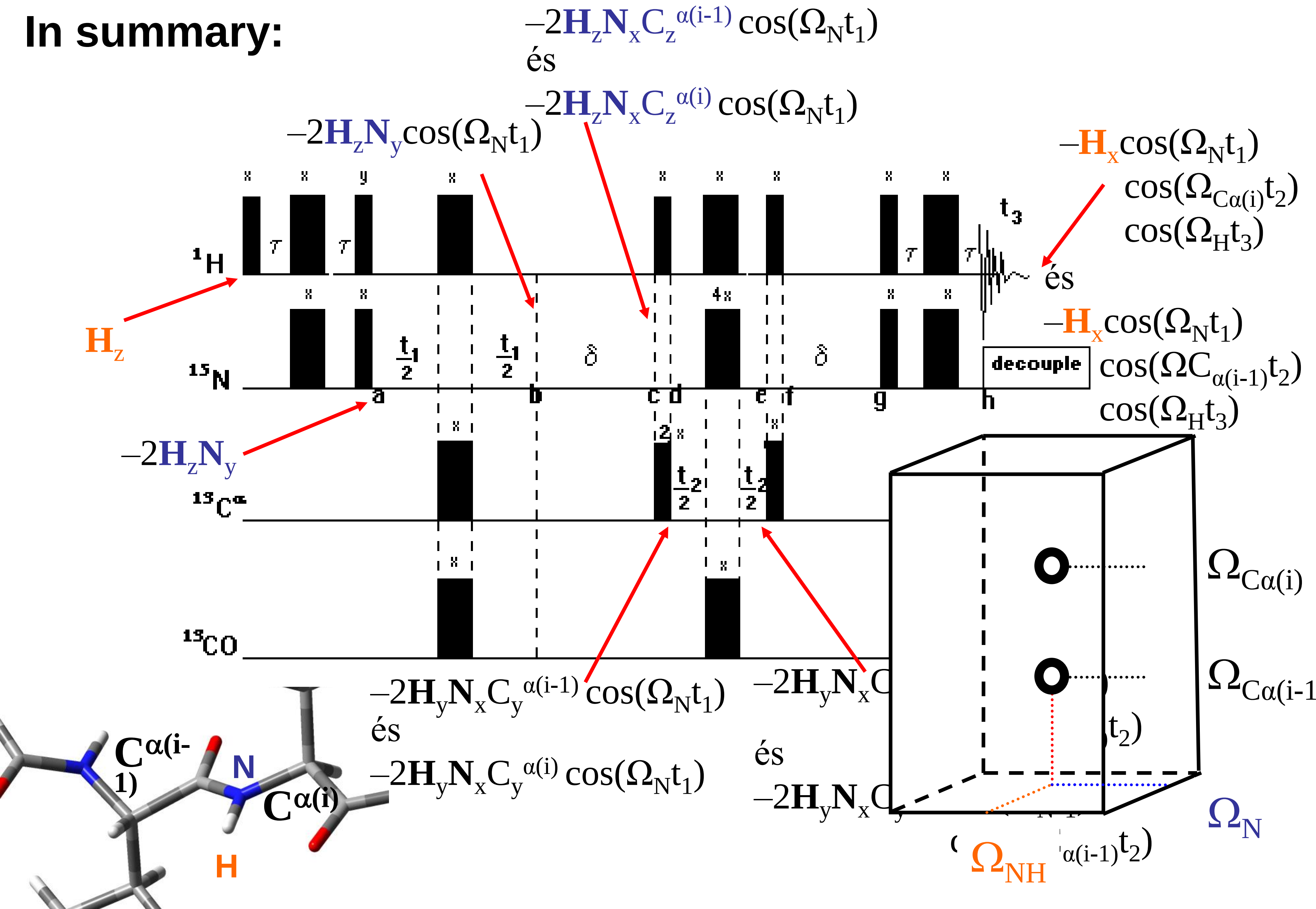
$\mathbf{H}_x$ [+a] at $\Omega_H, \Omega_N, \Omega_C\alpha^{(i-1)}$

$\mathbf{H}_x$ [+a] at $\Omega_H, \Omega_N, \Omega_C\alpha^{(i)}$

$$-\mathbf{H}_x *\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) *\cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) *\cos({}^1J_{NC}\alpha^{(i)}\,\pi\delta) *\sin({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) *\cos^2({}^1J_{NH}\,\pi\delta) *\cos({}^1J_{C\alpha C\beta}\,\pi t_2) *\cos(\Omega_N t_1) *\cos(\Omega_C\alpha^{(i-1)} t_2) *\cos(\Omega_H t_3)$$

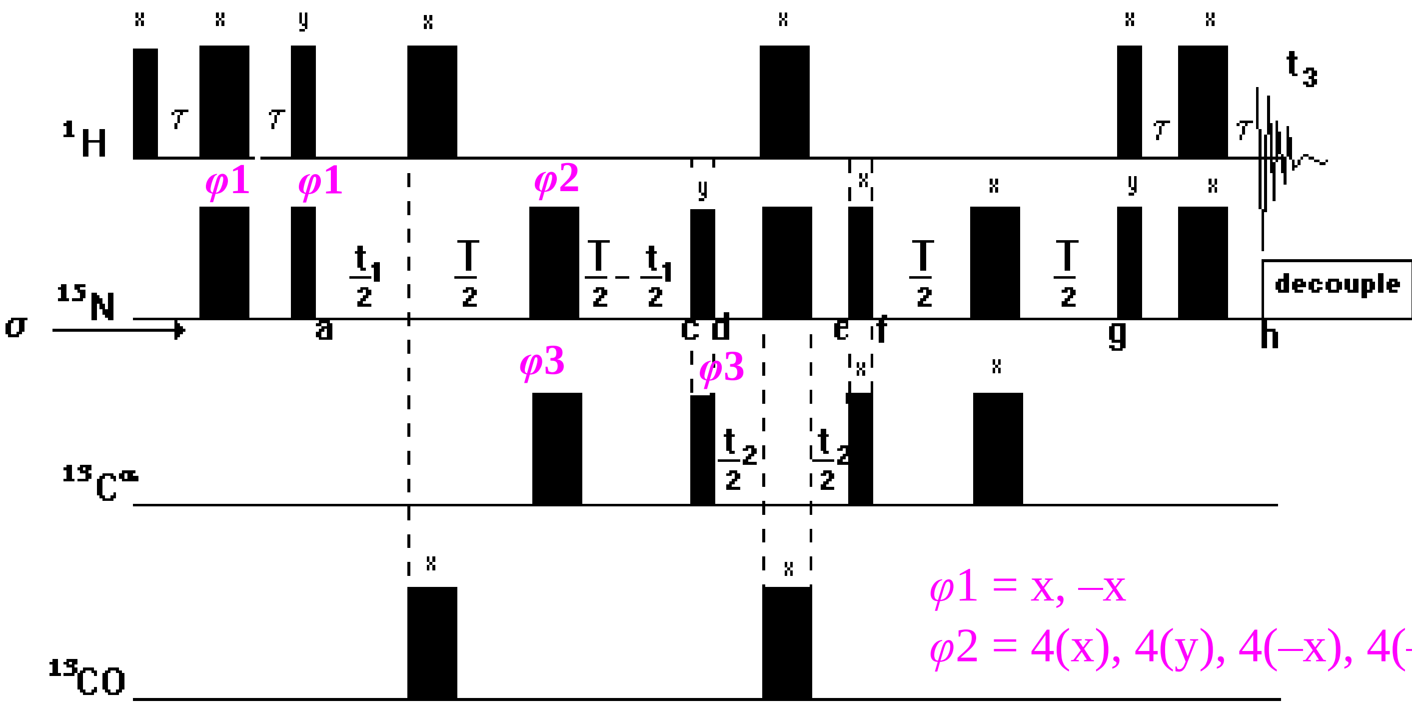
$$-\mathbf{H}_x *\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) *\cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) *\sin({}^1J_{NC}\alpha^{(i)}\,\pi\delta) *\cos({}^2J_{NC}\alpha^{(i-1)}\,\pi\delta) *\cos^2({}^1J_{NH}\,\pi\delta) *\cos({}^1J_{C\alpha C\beta}\,\pi t_2) *\cos(\Omega_N t_1) *\cos(\Omega_C\alpha^{(i)} t_2) *\cos(\Omega_H t_3)$$

In summary:



A skaláris csatolás okozta modulációktól eltekintünk

A constant-time HNCA (CT-HNCA)variant pulse sequence:

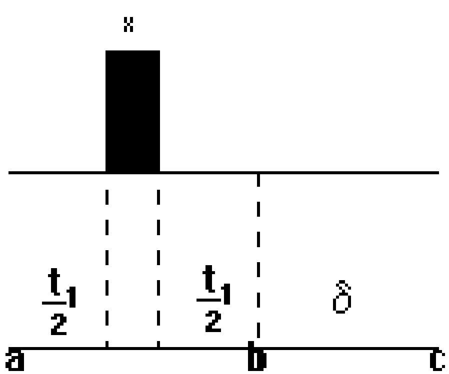


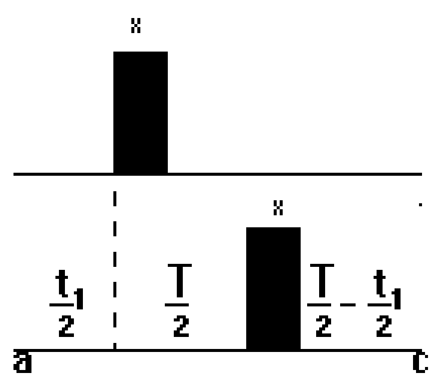
$\varphi1$ = x, –x

$\varphi2$ = 4(x), 4(y), 4(–x), 4(–y)

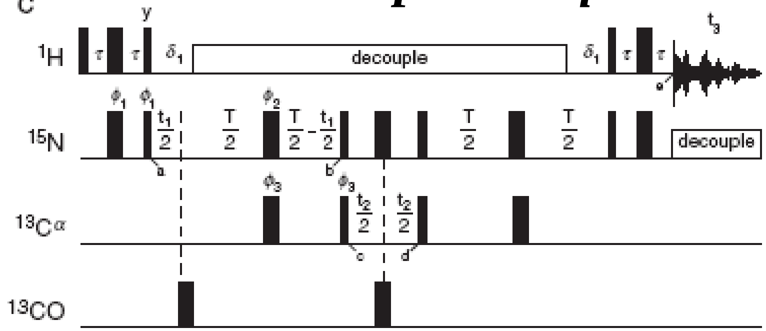
$\varphi3$ = 2(x), 2(–x)

receiver x = x, –x, –x, x, –x, x, x, –x

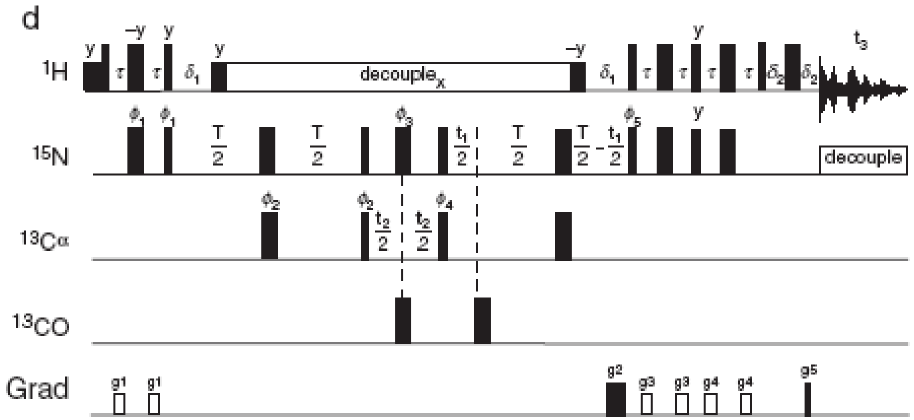




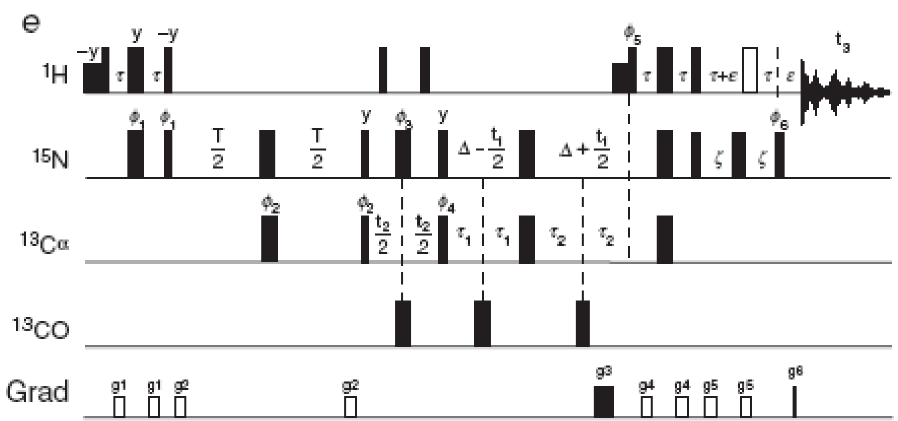
More HNCA variant pulse sequences:



Decoupled-CT-HNCA
synchronous broad band decoupling (*e.g.* WALTZ-16) enhances the sensitivity of a CT-HNCA.



PFG-PEP-HNCA
at the end the reversed-INEPT module is replaced by a PEP-revesed-INEPT.

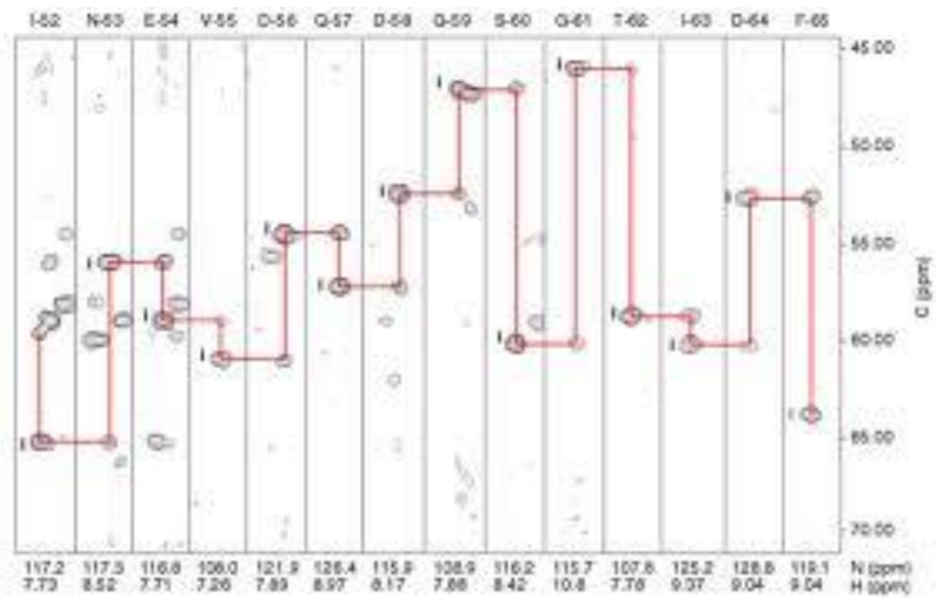


PFG-TROSY-HNCA

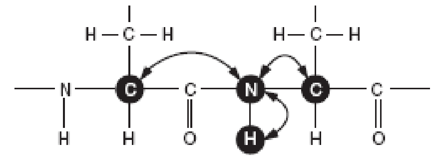
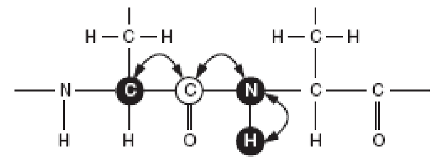
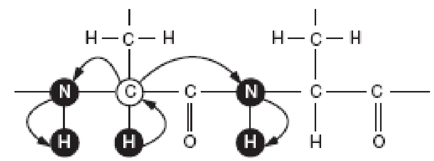
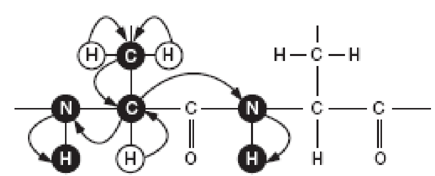
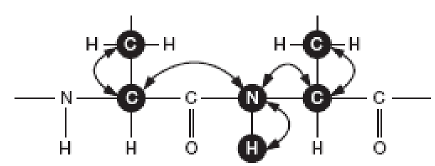
evolution of ^{15}N and
the coherence transfer from N to Cα(s)
are **separated** in time

evolution of ^{15}N and
the coherence transfer from N to Cα(s)
are **simultaneous**

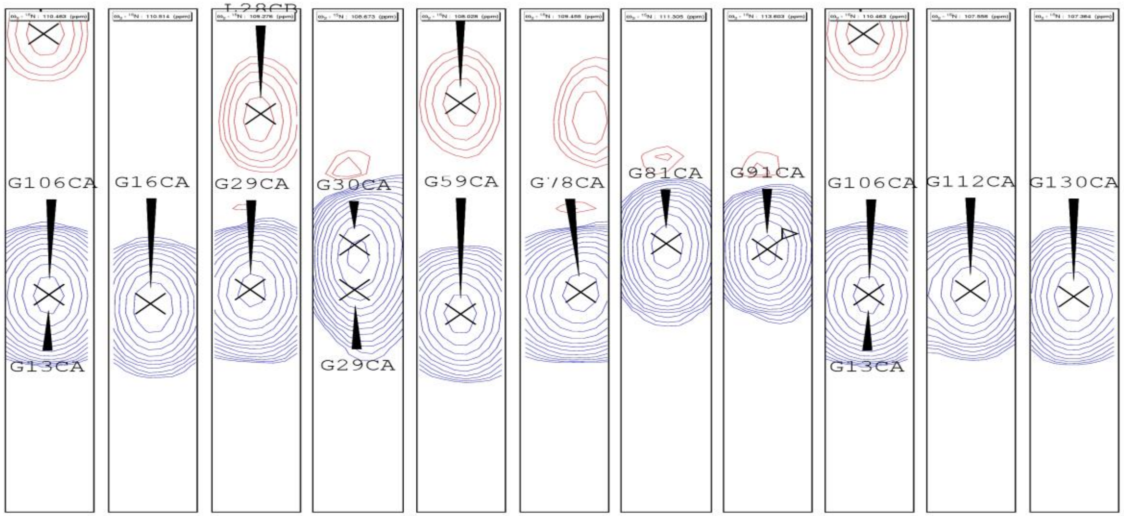
advantage: one saves time with the CT- experiment.
memo: by saving time one may gain in sensitivity as relaxation is reduced.

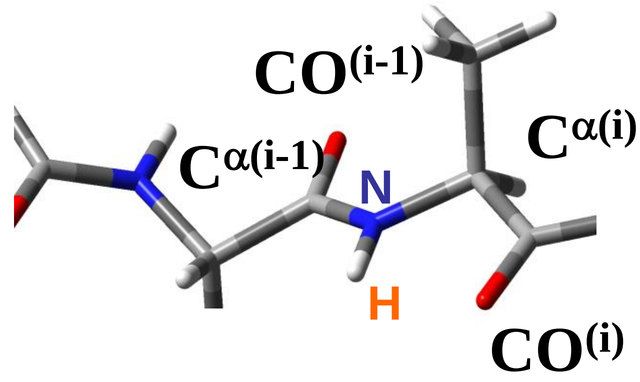


The simplest triple resonance experiments used for backbone resonance assignment

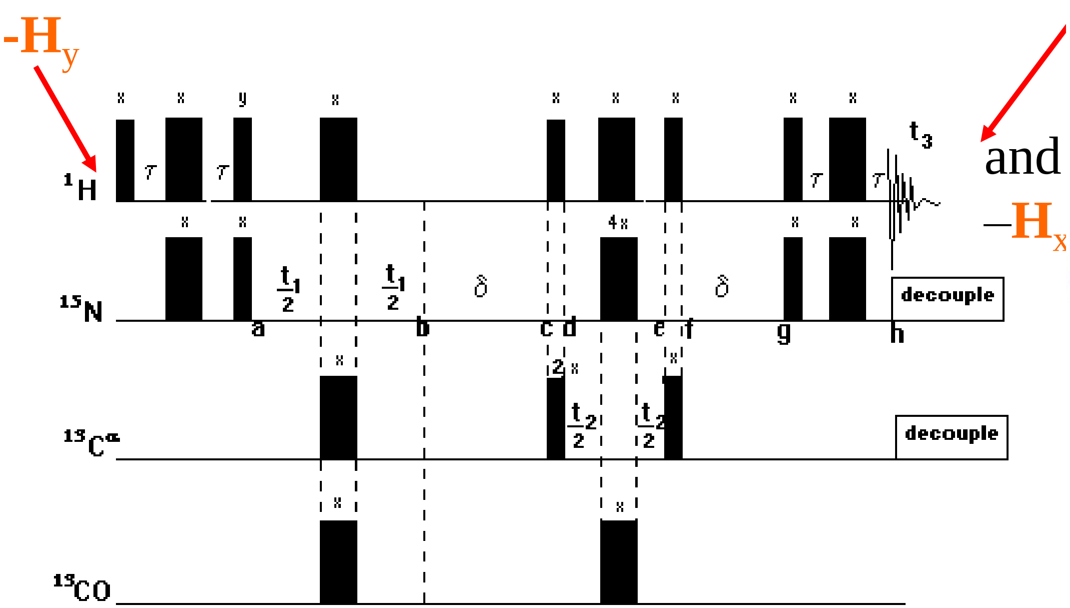
Experiment	Correlations observed	Magnetization transfer	J couplings[b]
HNCA	${}^1H_i^N - {}^{15}N_i - {}^{13}C_i^\alpha$ ${}^1H_i^N - {}^{15}N_i - {}^{13}C_{i-1}^\alpha$		${}^1J_{NH}$ ${}^1J_{NC^\alpha}$ ${}^2J_{NC^\alpha}$
HN(CO)CA	${}^1H_i^N - {}^{15}N_i - {}^{13}C_{i-1}^\alpha$		${}^1J_{NH}$ ${}^1J_{NCO}$ ${}^1J_{C^\alpha CO}$
H(CA)NH	${}^1H_i^\alpha - {}^{15}N_i - {}^1H_i^N$ ${}^1H_i^\alpha - {}^{15}N_{i+1} - {}^1H_{i+1}^N$		${}^1J_{C^\alpha H^\alpha}$ ${}^1J_{NC^\alpha}$ ${}^2J_{NC^\alpha}$ ${}^1J_{NH}$
CBCANH	${}^{13}C_i^\beta/{}^{13}C_i^\alpha - {}^{15}N_i - {}^1H_i^N$ ${}^{13}C_i^\beta/{}^{13}C_i^\alpha - {}^{15}N_{i+1} - {}^1H_{i+1}^N$		${}^1J_{CH}$ ${}^1J_{C^\alpha C^\beta}$ ${}^1J_{NC^\alpha}$ ${}^2J_{NC^\alpha}$ ${}^1J_{NH}$
HNCACB	${}^{13}C_i^\beta/{}^{13}C_i^\alpha - {}^{15}N_i - {}^1H_i^N$ ${}^{13}C_{i-1}^\beta/{}^{13}C_{i-1}^\alpha - {}^{15}N_i - {}^1H_i^N$		${}^1J_{C^\alpha C^\beta}$ ${}^1J_{NC^\alpha}$ ${}^2J_{NC^\alpha}$ ${}^1J_{NH}$

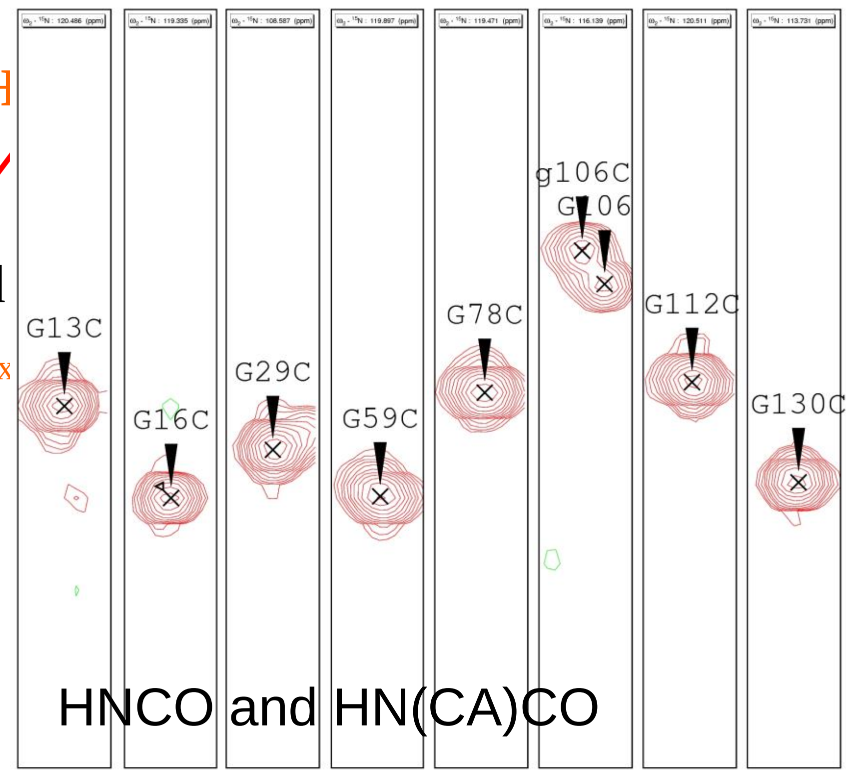
Resonance assignment in ^{15}N-^{13}C-Calpastatin





HNCA and HN(CO)CA





HNCO and HN(CA)CO