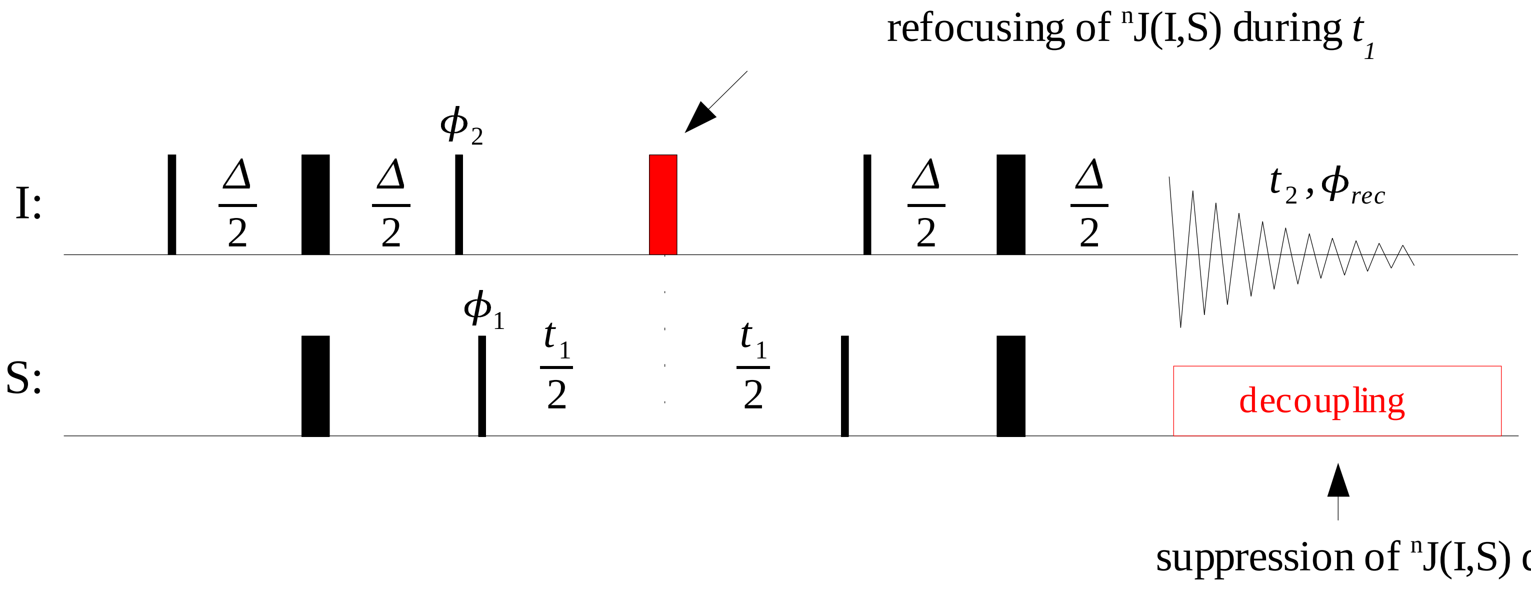
Determination of $^1J$ coupling constants in HSQC experiments
→ omit of the decoupling (in F1 or F2)

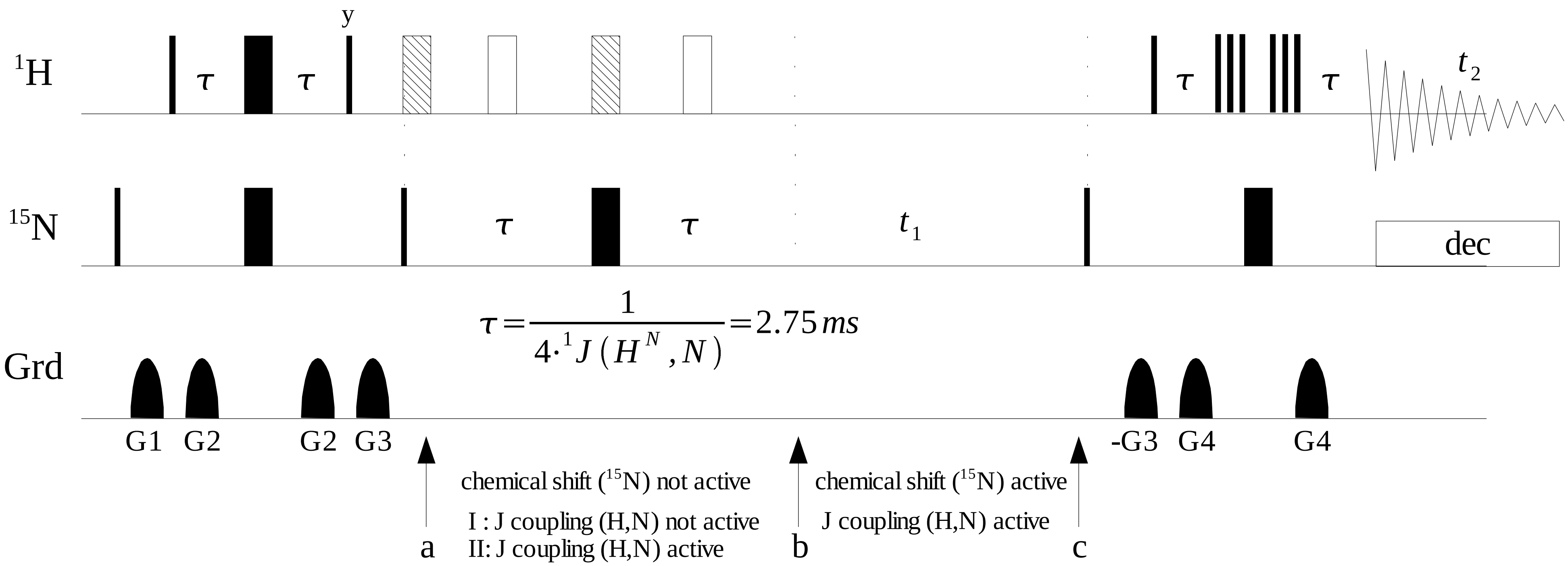



$$\Delta \approx \frac{1}{2 J_{IS}}$$
$$\phi_1 = \{x, -x\} + SHR$$
$$\phi_2 = \{y, y, -y, -y\}$$
$$\phi_{rec} = \{x, -x, -x, x\}$$

| | |
|---|---|
| $^{15}N$, $^1H$ HSQC | $^{15}N$ relaxes slower than $^1H$, therefore determination along F1<br>additional: more passive couplings along F2 ($^3J(H^N, H^\alpha)$) |
| $^{13}C$, $^1H$ | constant time evolution suppresses $^{13}C$,$^{13}C$ couplings<br>along F2 several passive $^1H$, $^1H$ couplings |

→ general problem: signal splitting due to $^1J$ couplings increases number of resonances
probability of signal overlap increases.

Pulse sequence for the $^1$H, $^{15}$N IPAP experiment (IPAP = Inphase-Antiphase)



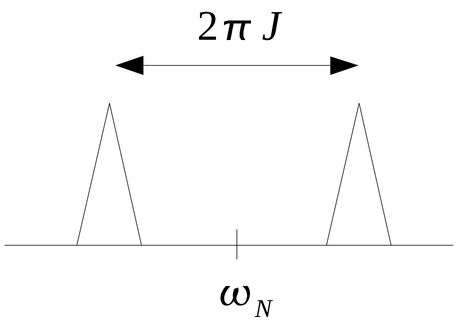

I: $\boldsymbol{H_z} \longrightarrow \boldsymbol{2H_zN_y} \longrightarrow \boldsymbol{2H_zN_y} \longrightarrow \boldsymbol{2H_zN_y} \longrightarrow \boldsymbol{H_x}$

II: $\boldsymbol{H_z} \longrightarrow \boldsymbol{2H_zN_y} \longrightarrow \boldsymbol{N_x} \longrightarrow \boldsymbol{2H_zN_y} \longrightarrow \boldsymbol{H_x}$

I. experiment (white 180° pulses) $\quad S(t_1, t_2) = \cos(\pi J_{NH} t_1) \cos(\omega_N t_1) \exp\{i \omega_H t_2\}$

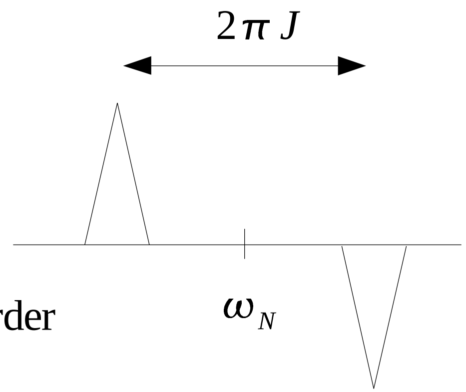
II. experiment (hatched 180° pulses) $\quad S(t_1, t_2) = \sin(\pi J_{NH} t_1) \cos(\omega_N t_1) \exp\{i \omega_H t_2\}$

$$\cos(\pi J t)\cos(\omega_N t) = \cos((\omega_N + \pi J)t) + \cos((\omega_N - \pi J)t) \xrightarrow{\text{F.T}}$$




InPhase

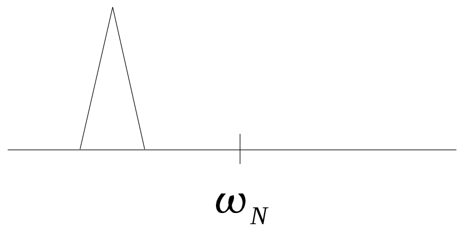
$$\sin(\pi J t)\cos(\omega_N t) = \sin((\omega_N + \pi J)t) - \sin((\omega_N - \pi J)t) \xrightarrow{\text{F.T.}}$$



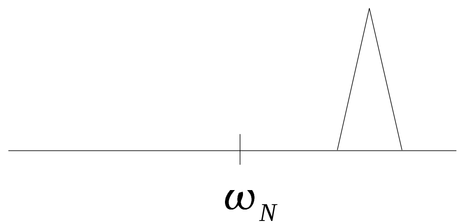

AntiPhase

90° phase correction 0. order

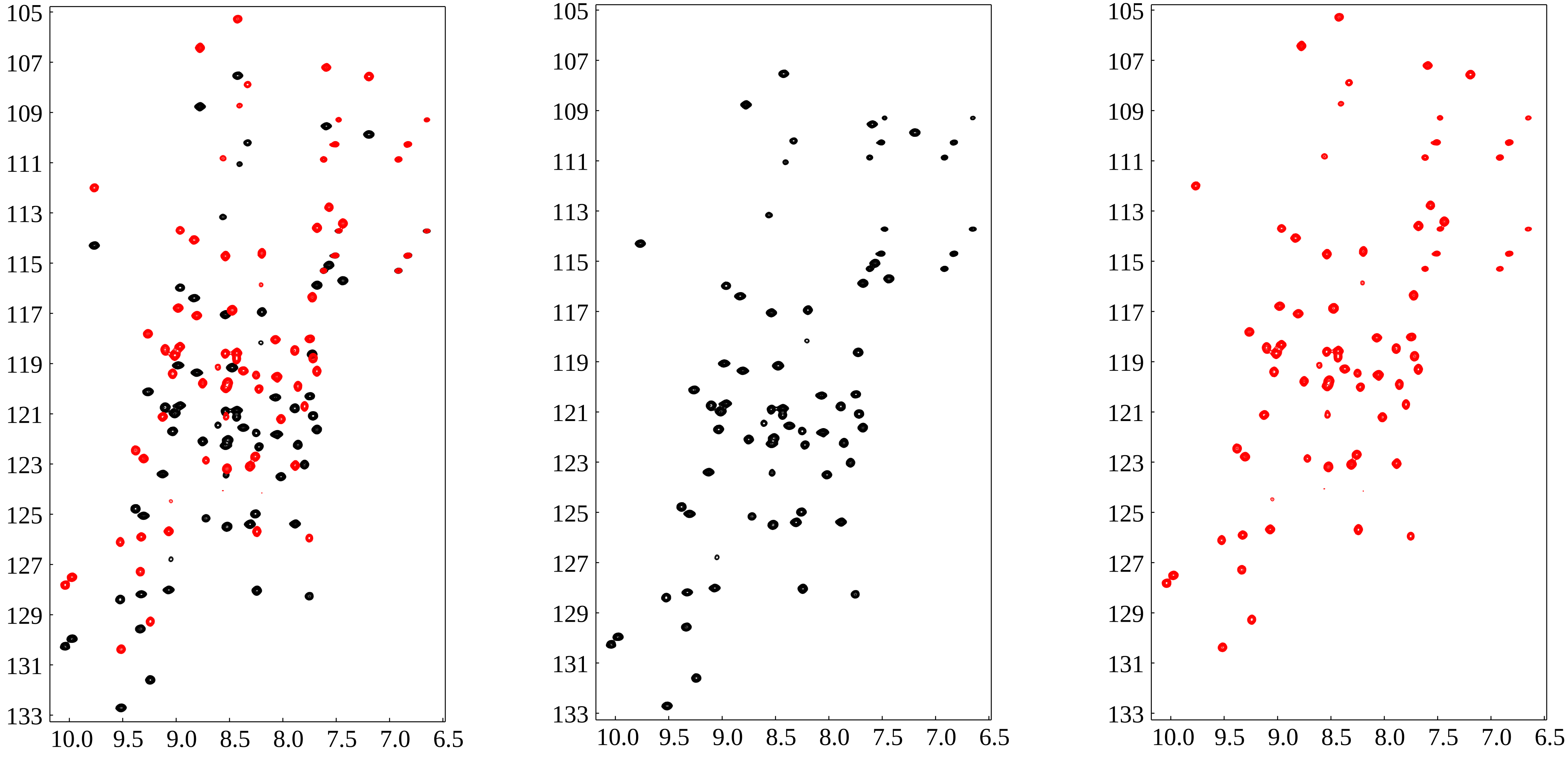
InPhase + AntiPhase




InPhase - AntiPhase

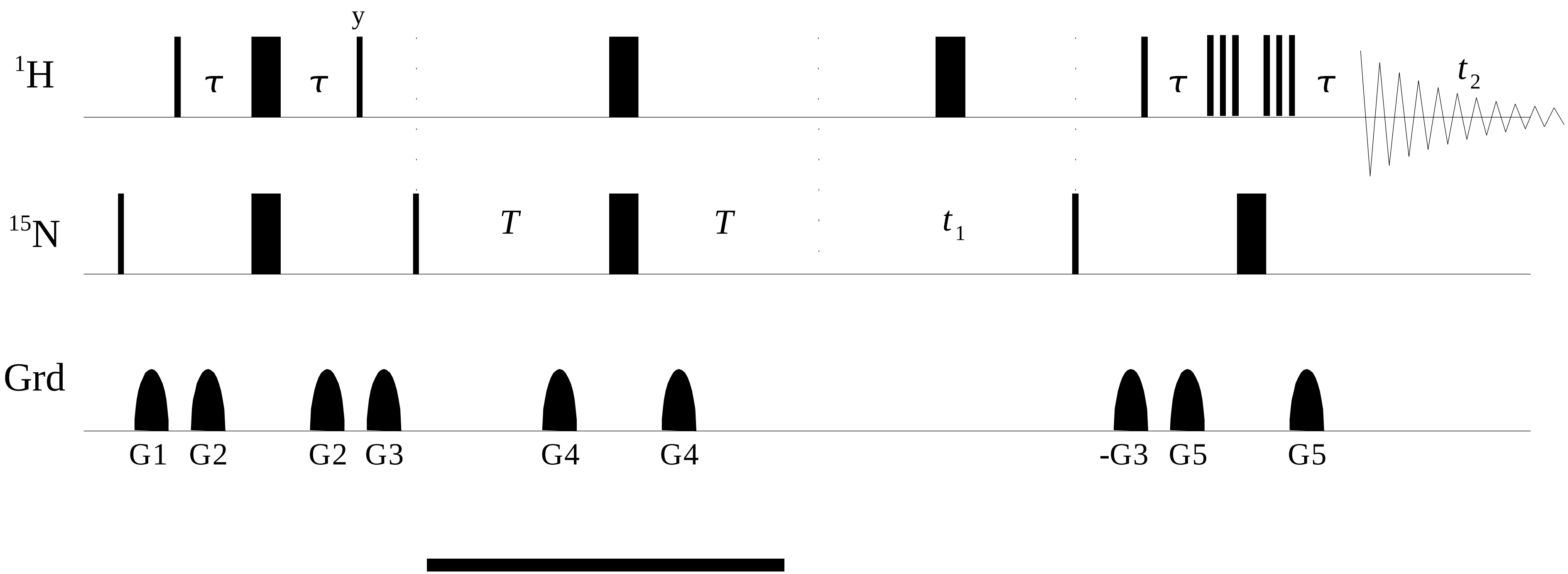


# $^{1}H^{N}$, $^{15}N$ IPAP HSQC spectrum of a small protein



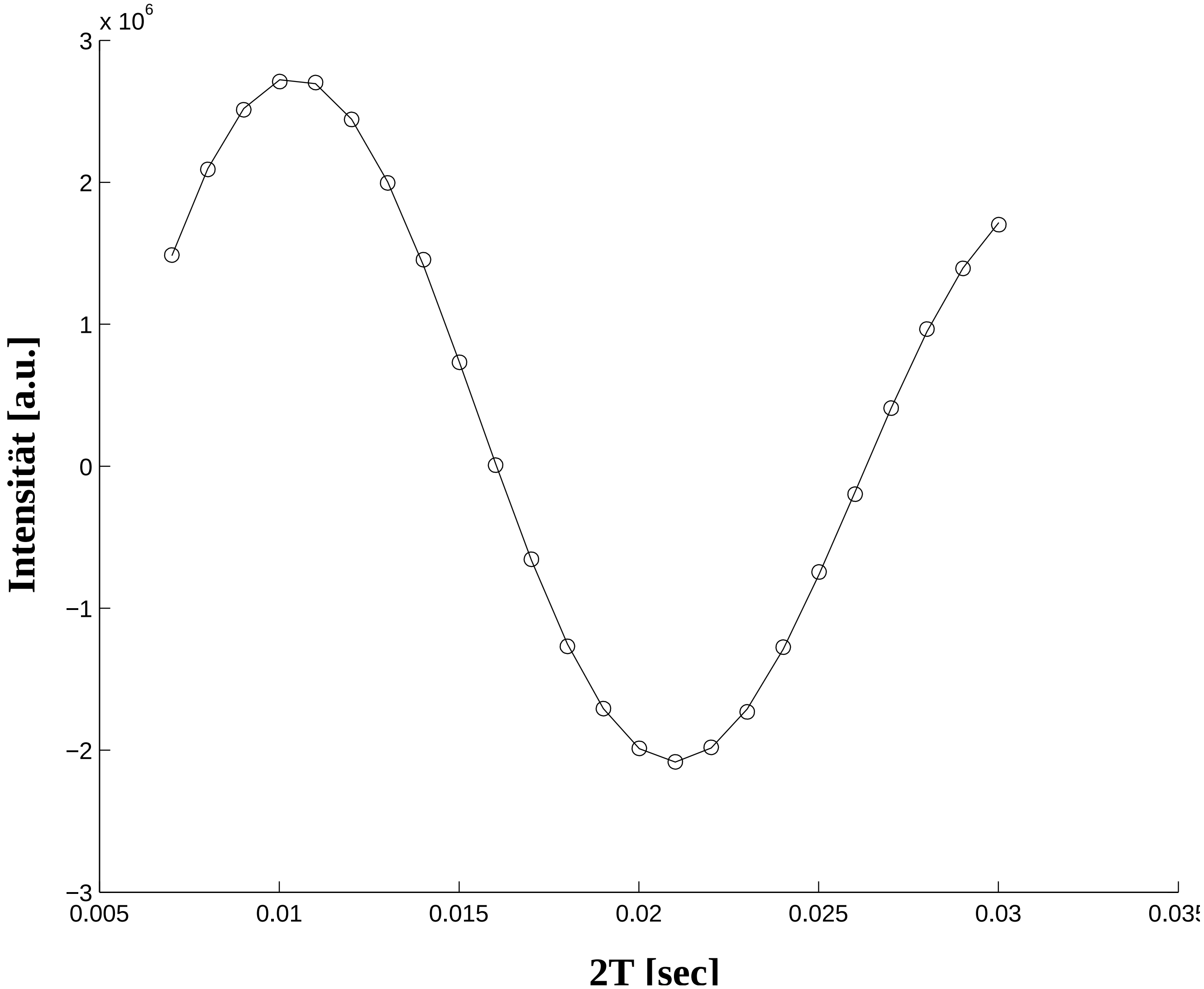

**Überlagerung ADD, SUB** **ADD−Subspektrum** **SUB−Subspektrum**

pulse sequence for the J-modulated $^{1}$H, $^{15}$N HSQC experiment

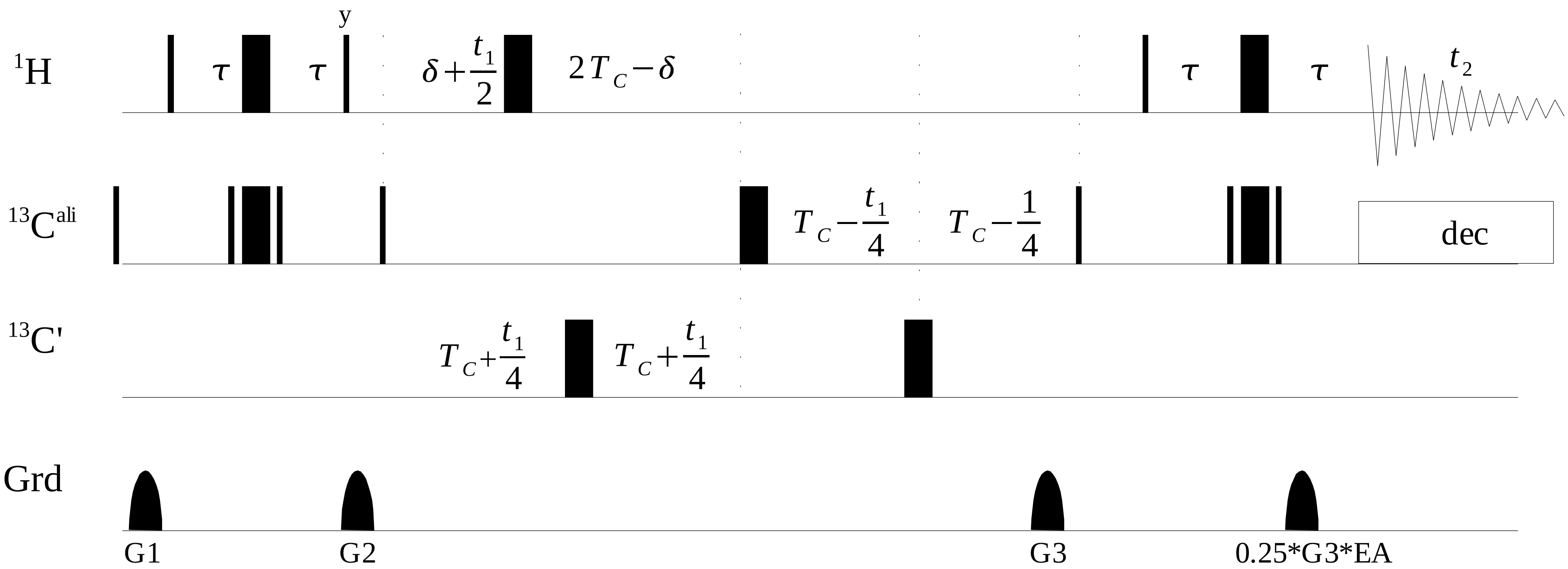



$$J(^{15}N, H^{N})\,active \rightarrow S(T) \propto \cos(2\pi J T)$$

The signal intensity is modulated by the coupling constant in a series with different values of T example for the amplitude modulation in a J-modulated $^1H$, $^{15}N$ HSQC experiment



pulse sequence for the J-modulated $^{1}$H, $^{13}$C constant-time HSQC experiment

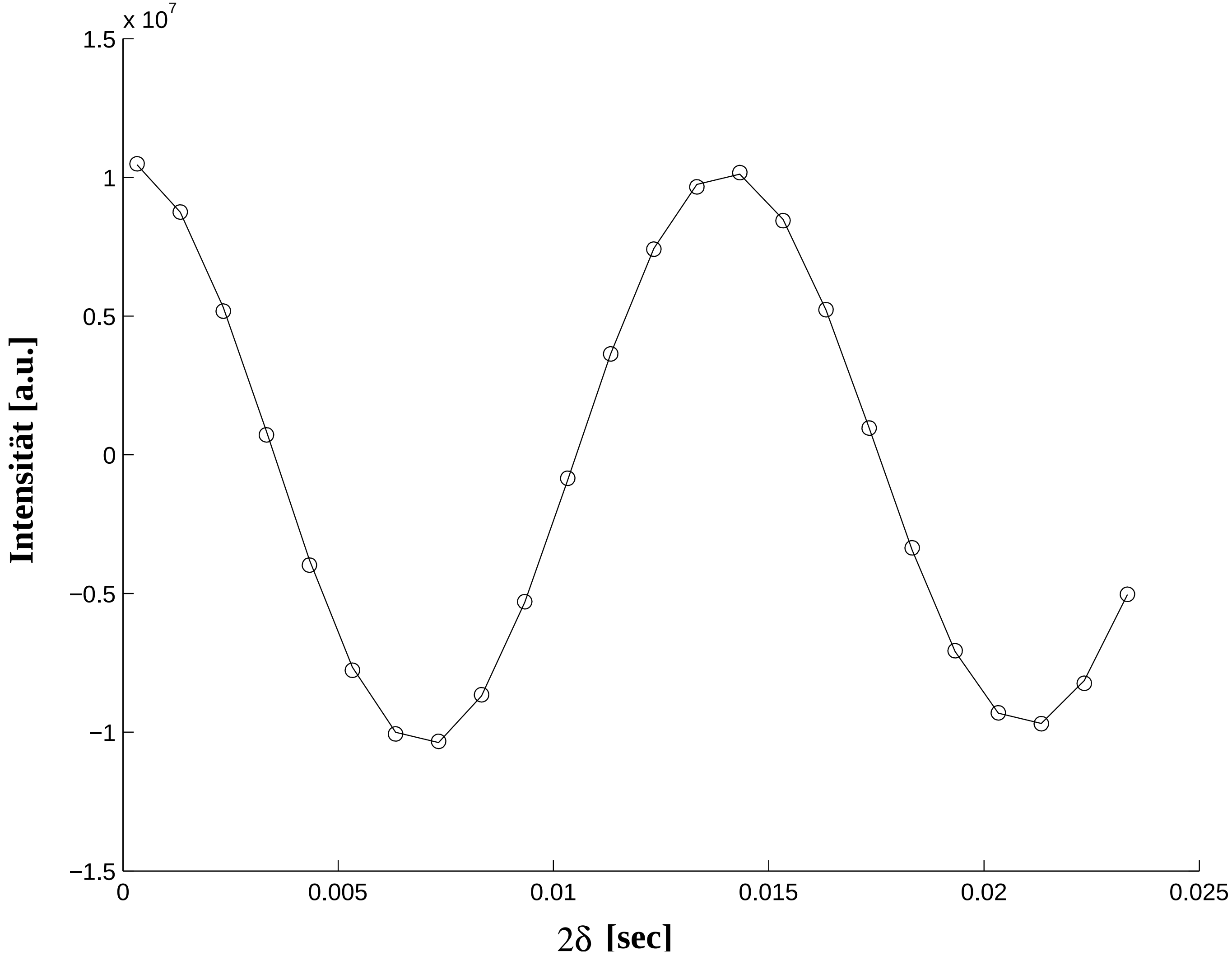



$T_C$ = 6.5 ms, τ = 1.7 ms, δ = 0 - 12 ms

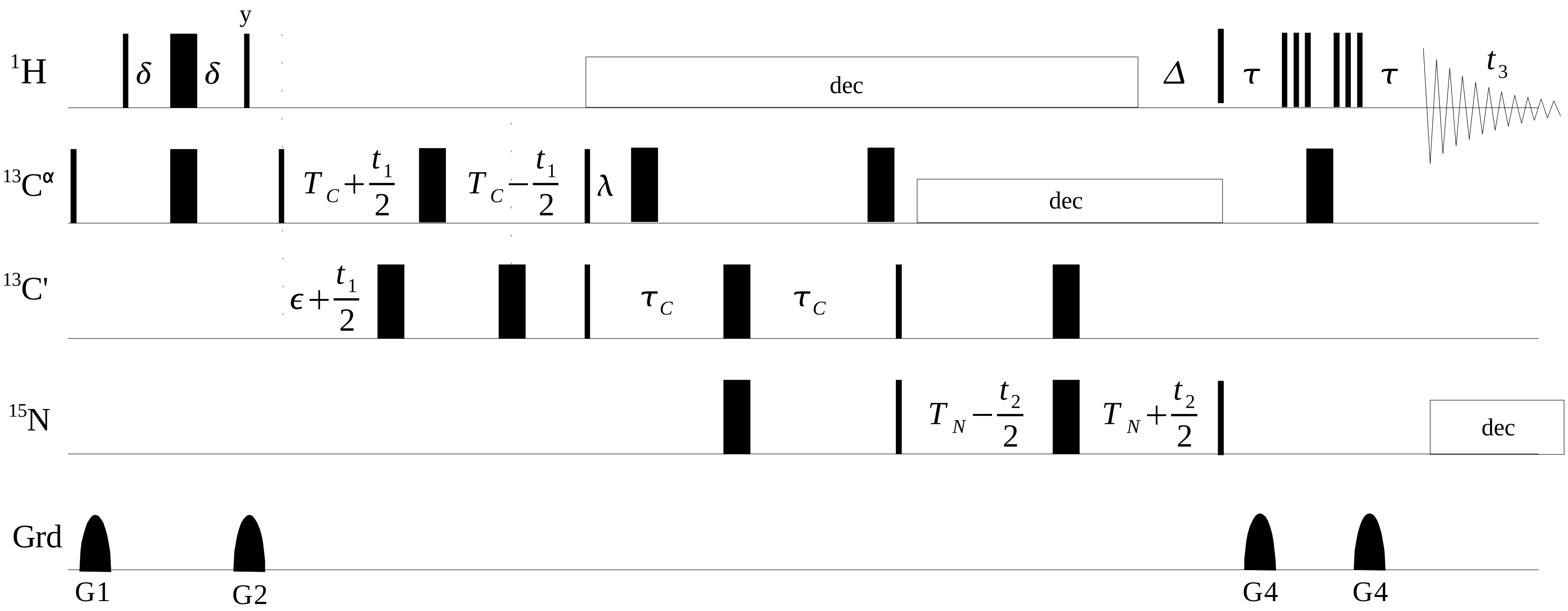
$^{n}J(^{13}C, H)$ active during 2 δ $\rightarrow S(T) \propto \cos(2\pi J \delta)$

example of the modulation in the J-modulated $^{1}H$, $^{13}C$ ct-HSQC

'decay' of the modulation : small $^{n}J(^{1}H, ^{13}C)$ couplings , not transverse relaxation !



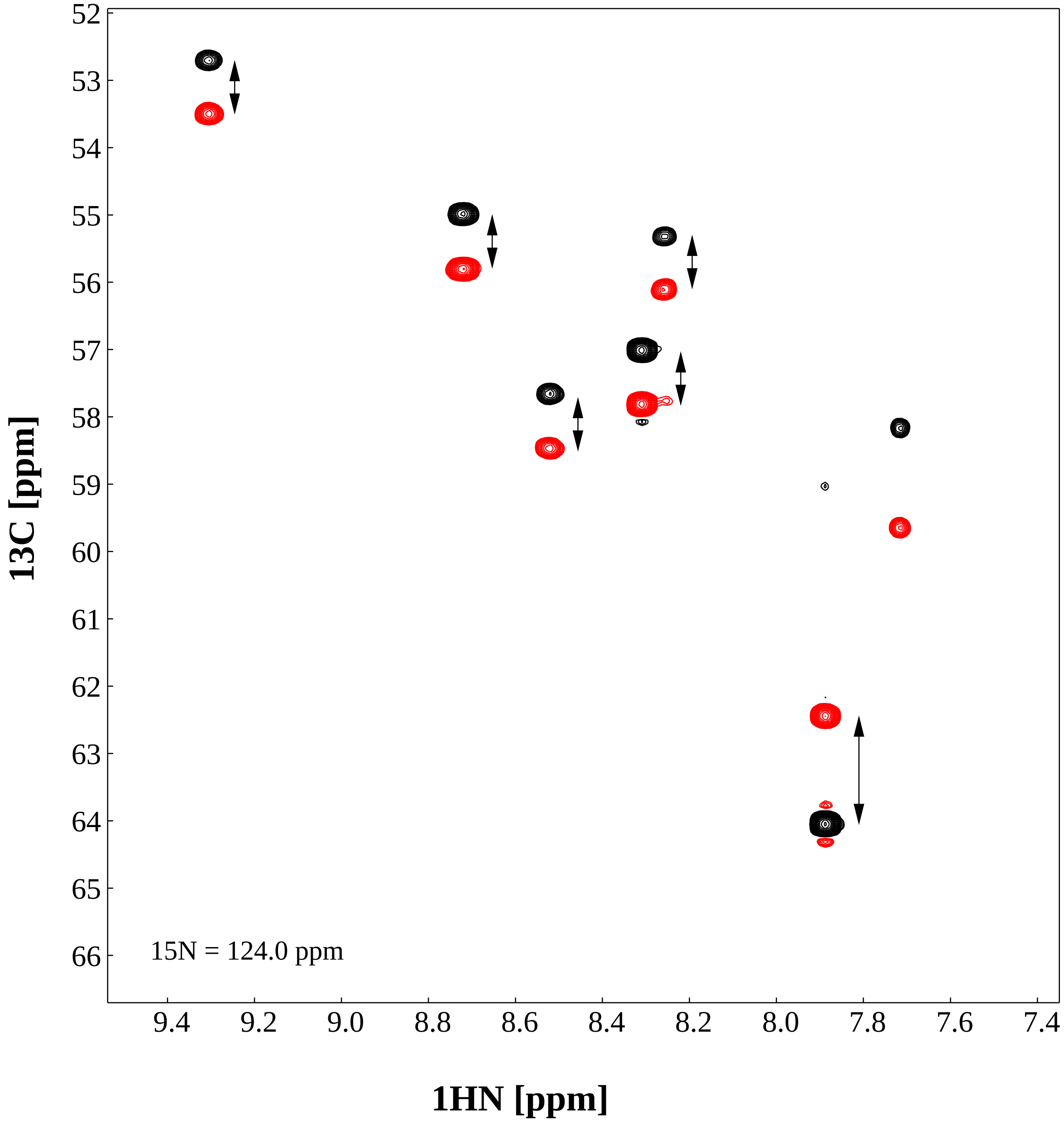
pulse sequence for the 3D CA(CO)NH-J experiment



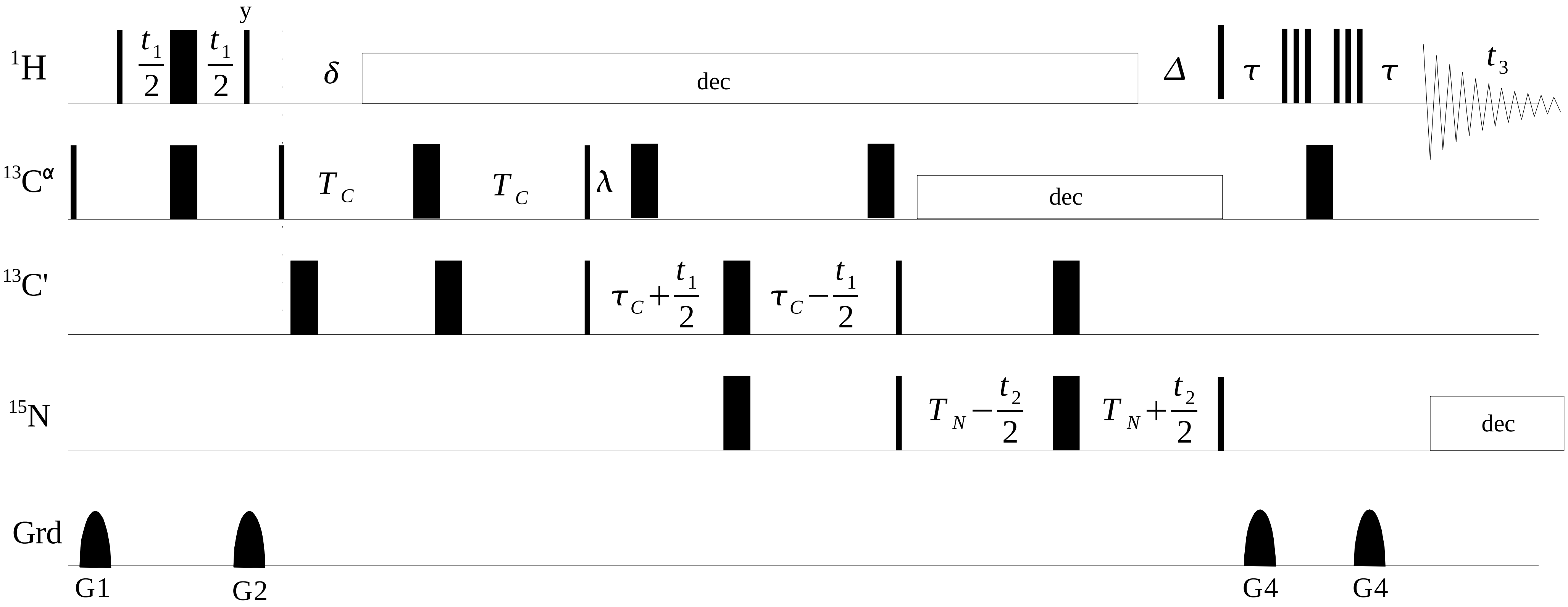

$\delta$ = 1.7 ms, $T_C$ = 13 ms, $T_N = \tau_C$ = 12 ms, $\Delta$ = 5.5 ms, $\tau$ = 2.5 ms

$$\boldsymbol{H_z^\alpha} \longrightarrow \underbrace{\boldsymbol{2H_z^\alpha C_y^\alpha}}_{\sin(\pi J_{H\alpha C\alpha} t_1)\cos(\omega_{C\alpha} t_1)} \longrightarrow \boldsymbol{2C_y^\alpha C'_z} \longrightarrow \boldsymbol{2C_z^\alpha C'_y} \longrightarrow \boldsymbol{2N_z C'_y} \longrightarrow \underbrace{\boldsymbol{2N_y C'_z}}_{\cos(\omega_N t_2)} \longrightarrow \boldsymbol{2H_z^N N_y} \longrightarrow \boldsymbol{H_x^N}$$

$^{1}H^{N}$, $^{13}C^{\alpha}$ plane of a 3D CA(CO)NH-J spectrum



pulse sequence for the 3D HACONH-J experiment

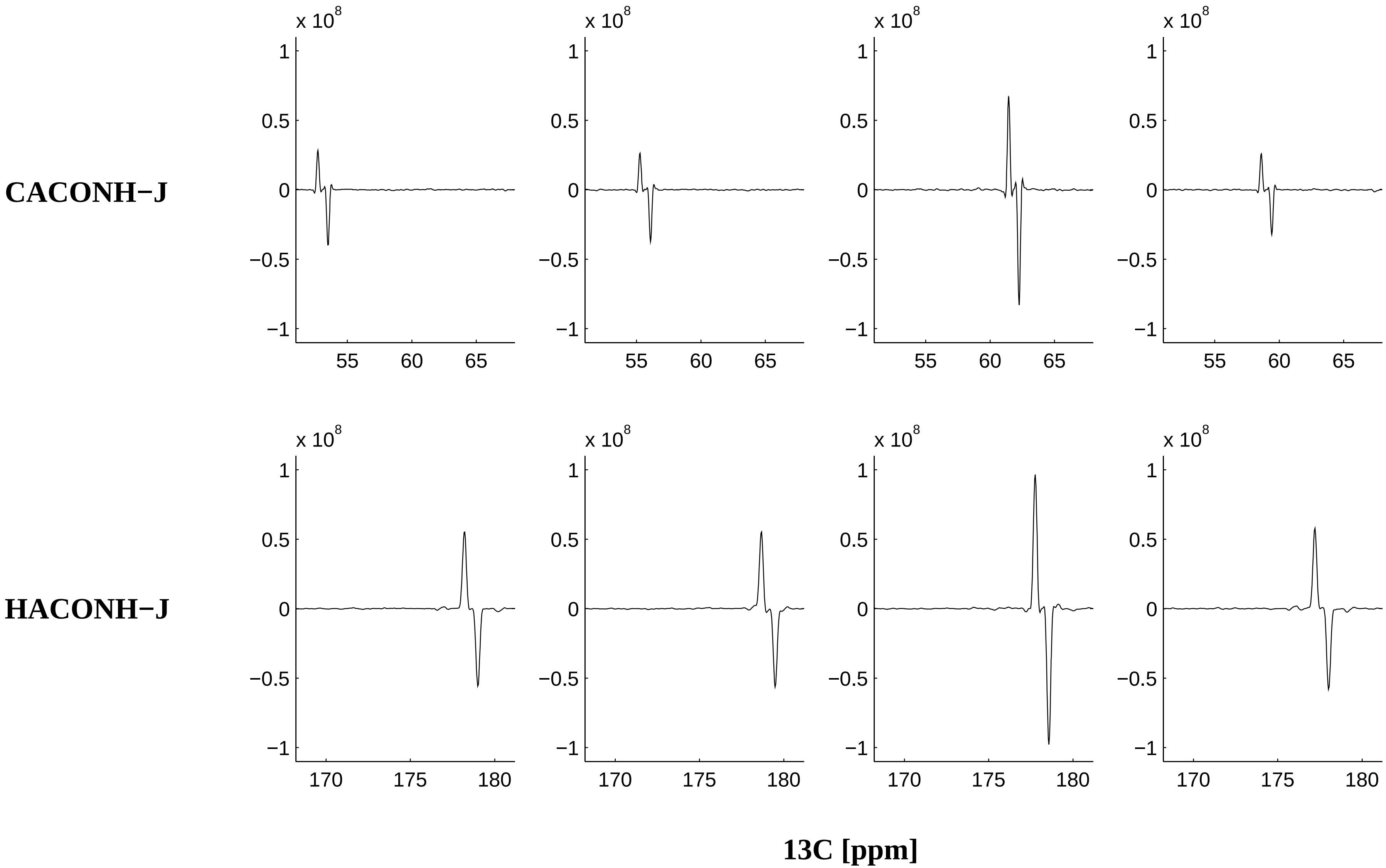



$\delta = 1.7$ ms, $T_C = 13$ ms, $T_N = \tau_C = 12$ ms, $\Delta = 5.5$ ms, $\tau = 2.5$ ms

$$\boldsymbol{H^{\alpha}_{y}} \longrightarrow \boldsymbol{2H^{\alpha}_{z}C^{\alpha}_{y}} \longrightarrow \boldsymbol{2C^{\alpha}_{y}C'_{z}} \longrightarrow \boldsymbol{2C^{\alpha}_{z}C'_{y}} \longrightarrow \boldsymbol{2N_{z}C'_{y}} \longrightarrow \boldsymbol{2N_{y}C'_{z}} \longrightarrow \boldsymbol{2H^{N}_{z}N_{y}} \longrightarrow \boldsymbol{H^{N}_{x}}$$

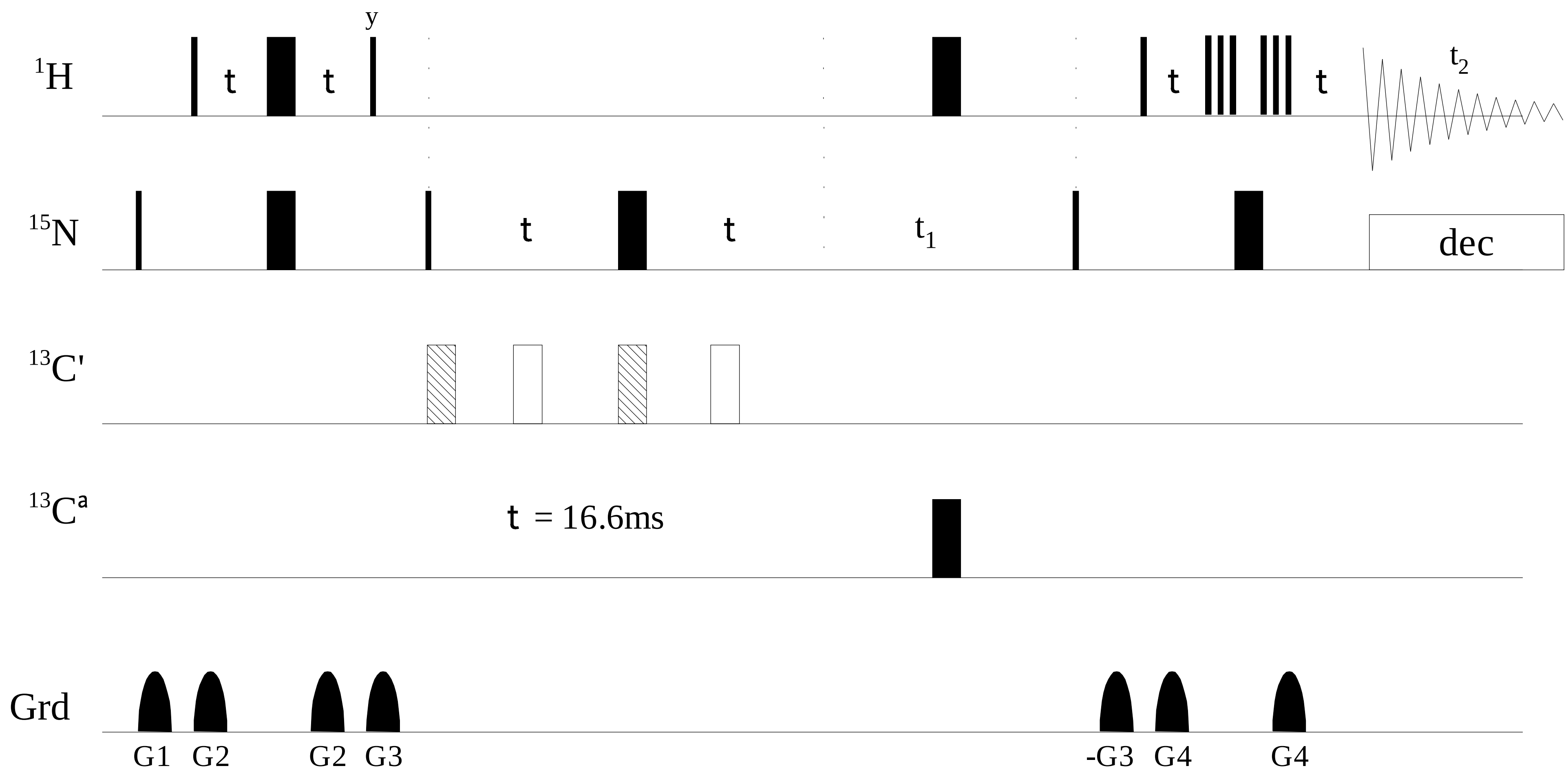
$\sin(\pi J_{H\alpha C\alpha} t_1)$ $\qquad$ $\cos(\omega_{C'} t_1)$ $\qquad$ $\cos(\omega_N t_2)$

# Comparison of the 3D CA(CO)NH-J with the 3D HACONH-J




HACONH-J : approx. sensitivity gain of 40 % (SH3 domain, 700 MHz)

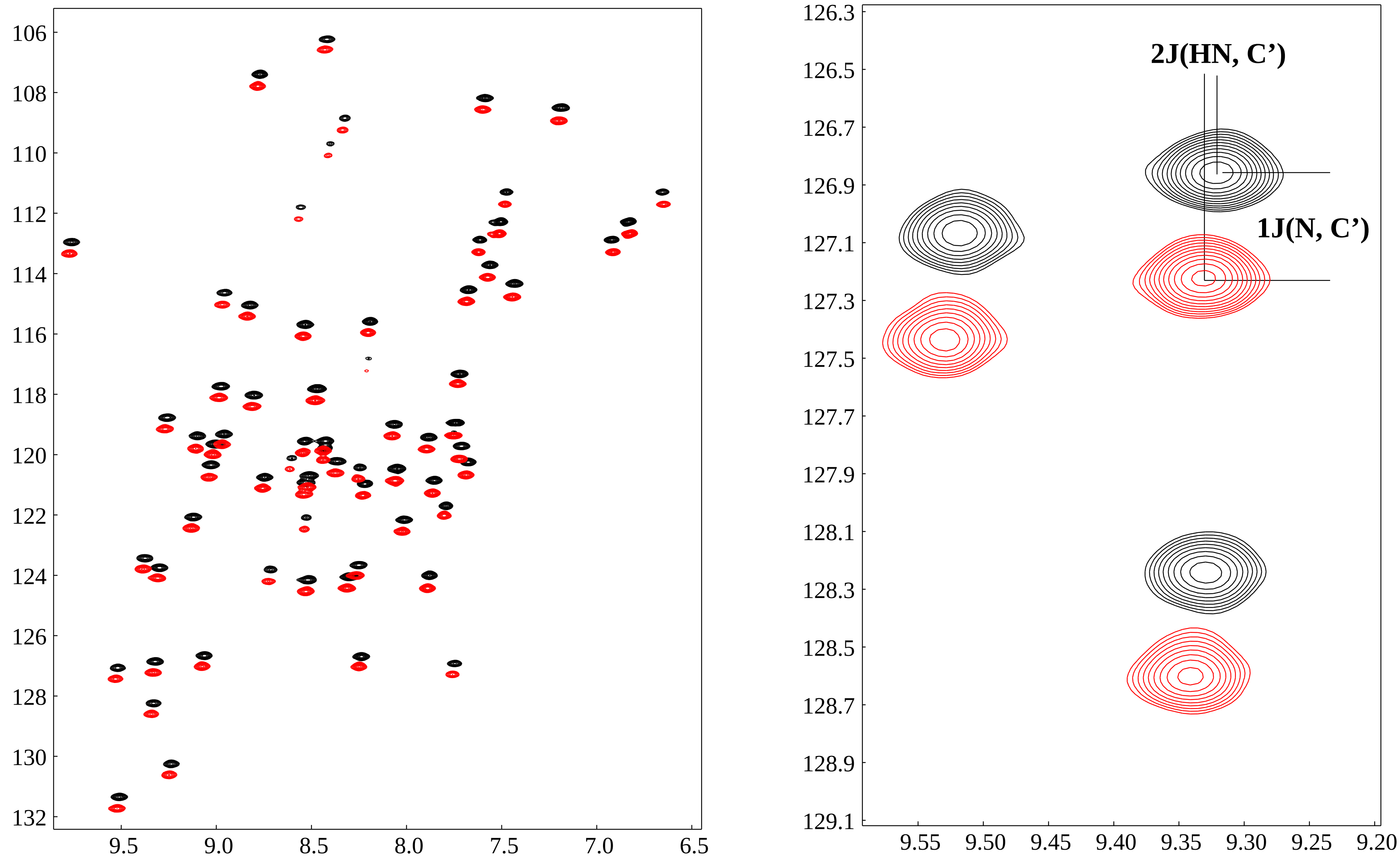
# Pulse sequence for the NCO-IPAP experiment




I. experiment (white 180° pulses) $S(t_{1,}\, t_2) \propto \cos(\pi J_{NC'} t_1) \cos(\omega_N) \exp\{i \omega_{HN} t_2\}$

II. experiment (hatched 180° pulses) $S(t_{1,}\, t_2) \propto \sin(\pi J_{NC'} t_1) \cos(\omega_N) \exp\{i \omega_{HN} t_2\}$

# NCO-IPAP spectra of a small protein



# Distribution of ${}^{1}J(H^{N}, N)$ and ${}^{1}J(H^{\alpha}, C^{\alpha})$ couplings in a small protein