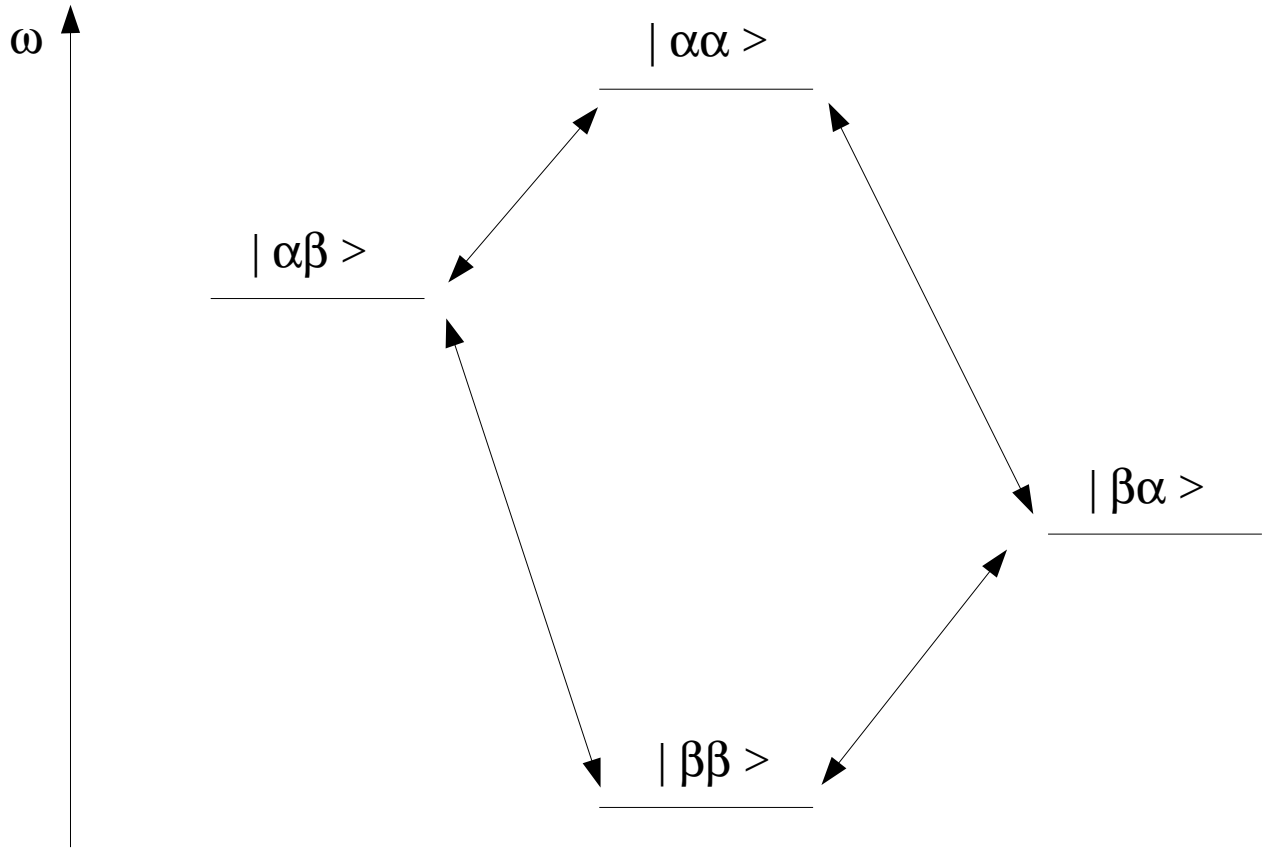
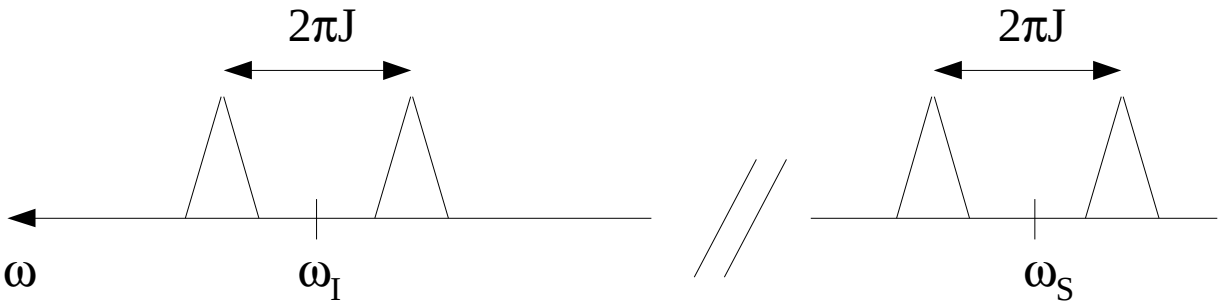
Energy levels of
a two-spin system I,S

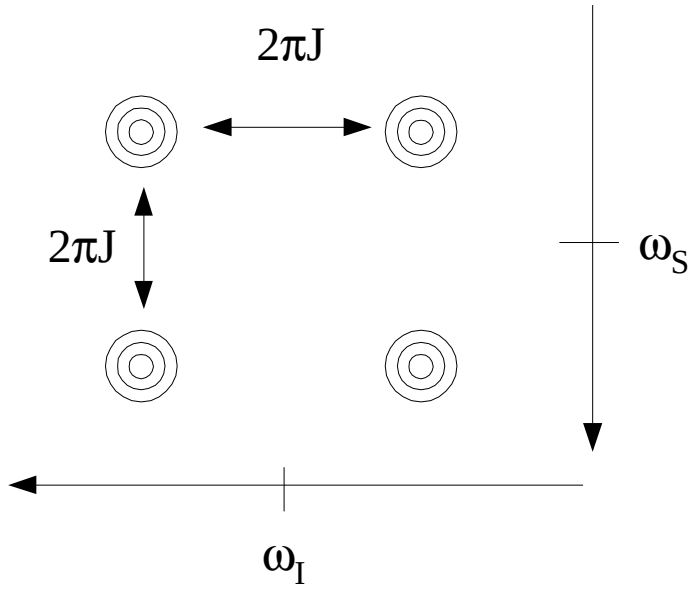


resulting 1D spectrum



classical I,S correlation (HSQC) without decoupling
described with cartesian product operators

$$\boldsymbol{I}_z \longrightarrow 2\,\boldsymbol{I}_z\boldsymbol{S}_y \longrightarrow 2\,\boldsymbol{I}_z\boldsymbol{S}_y \cos(\omega_S t_1)\cos(\pi J_{IS} t_1) \longrightarrow \boldsymbol{I}_x \cos(\omega_S t_1)\cos(\pi J_{IS} t_1)\exp\{i\,\omega_I t_2\}\cos(\pi J_{IS} t_2)$$



Description in mixed spin operators

$$I_z = \tfrac{1}{2}(I^\alpha - I^\beta) \qquad E = \tfrac{1}{2}(I^\alpha + I^\beta)$$

$$2I_zS_x = \tfrac{1}{2}(I^\alpha - I^\beta)S_x \longrightarrow I_x = \tfrac{1}{2}(S^\alpha + S^\beta)I_x$$

$$I^\alpha S_x \xrightarrow{(\Omega_S S_z + \pi J 2 I_z S_z)t} I^\alpha S_x \cos((\Omega_S+\pi J)t)$$

$$I^\alpha S_x \cos((\Omega_S+\pi J)t_1) \xrightarrow{\text{rev. INEPT}} I_x S^\alpha \cos((\Omega_S+\pi J)t_1)\cos((\Omega_I+\pi J)t_2) + I_x S^\beta \cos((\Omega_S+\pi J)t_1)\cos((\Omega_I-\pi J)t_2)$$

$$-I^\beta S_x \cos((\Omega_S-\pi J)t_1) \xrightarrow{\text{rev. INEPT}} I_x S^\alpha \cos((\Omega_S-\pi J)t_1)\cos((\Omega_I+\pi J)t_2) + I_x S^\beta \cos((\Omega_S-\pi J)t_1)\cos((\Omega_I-\pi J)t_2)$$

Refocusing of scalar coupling J_{IS} by a 180° pulse

$$I^{\alpha}S^{+} \xrightarrow{(\Omega_S S_z + \pi J 2 I_z S_z)t} I^{\alpha}S^{+} \exp\{-i(\Omega_S+\pi J)t\}$$

$$I^{\beta}S^{+} \xrightarrow{(\Omega_S S_z + \pi J 2 I_z S_z)t} I^{\beta}S^{+} \exp\{-i(\Omega_S-\pi J)t\}$$

$$I^{\alpha}S^{+} \xrightarrow{½\ t} I^{\alpha}S^{+} \exp\{-i(\Omega_S+\pi J)\ ½\ t\} \xrightarrow{180°\ I_x} I^{\beta}S^{+} \exp\{-i(\Omega_S+\pi J)\ ½\ t\} \xrightarrow{½\ t} I^{\beta}S^{+}\ [\exp\{-i(\Omega_S+\pi J)\ ½\ t\} * \exp\{-i(\Omega_S-\pi J)\ ½\ t\}]$$

$$= I^{\beta}S^{+} \exp\{-i\Omega_S\ t\}$$

Decoupling in a HSQC experiment

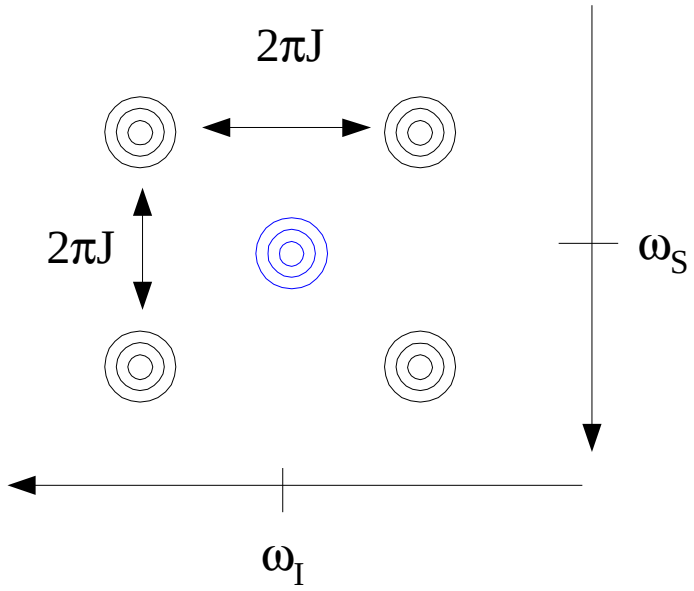
180° (I) pulse in the center of the S-evolution

composite pulse decoupling (cpd) of the S spin during detection of spin I

→ collapsing of doublet fine structure

minimizing number of resonance lines

increasing intensity



Effect of different transverse relaxation rates of individual coherences

$$I^{\alpha}S^{+} \xrightarrow{\text{precession + trans. relaxation}} I^{\alpha}S^{+}\exp\{-i(\Omega_S+\pi J)t - R_{2a}t\}$$

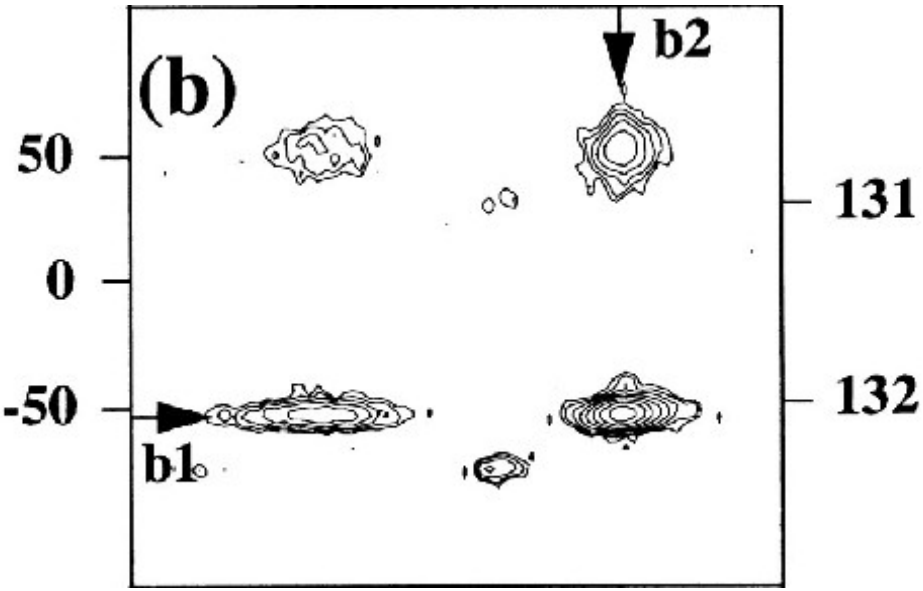
$$I^{\beta}S^{+} \xrightarrow{\text{precession + trans. relaxation}} I^{\beta}S^{+}\exp\{-i(\Omega_S-\pi J)t - R_{2b}t\}$$

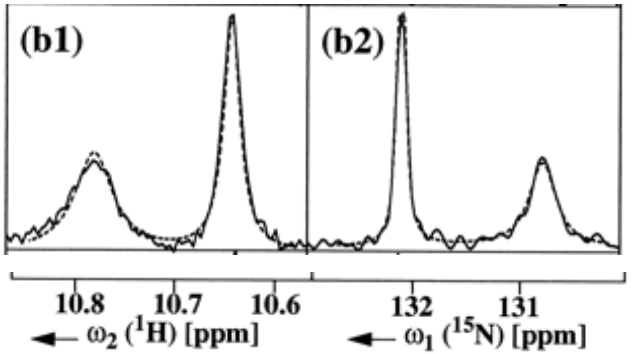
$$I^{\alpha}S^{+} \xrightarrow{\frac{1}{2}t} \xrightarrow{180^\circ I_x} \xrightarrow{\frac{1}{2}t} I^{\beta}S^{+}\exp\{-i\Omega_S t - \tfrac{1}{2}(R_{2a}+R_{2b})t\}$$

$\longrightarrow$ decoupling averages individual relaxation rates

Are the relaxation rates of different doublet components equal ?

HSQC without decoupling

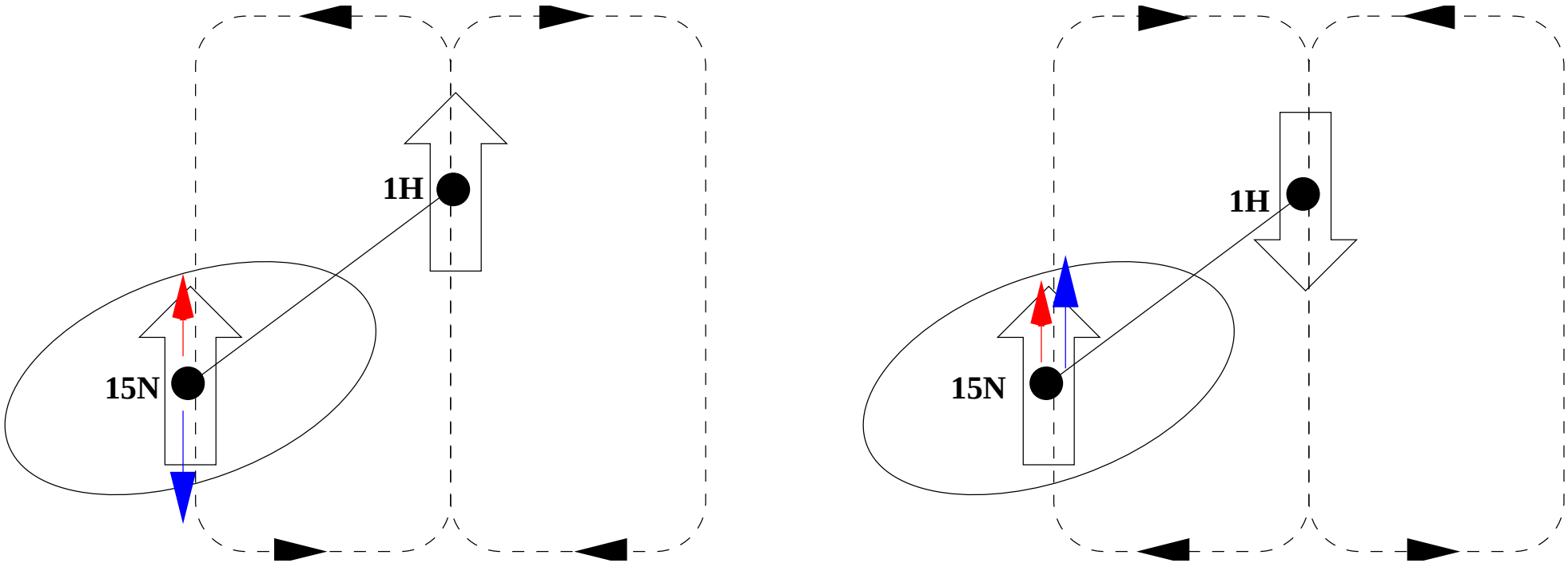




Tryptophan-Indole resonance of a
protein-DNA complex at 4°C, 750 MHz
τ_c ca. 20 ns
(comparable to a 40 kDa protein at 35 °C)

Explanation of different relaxation rates of individual multiplet components

→ Cross correlation, interference of two different relaxation mechanisms
(M.J. Goldman (1984), J. Magn. Res. 60, 437-452)



important relaxation mechanisms:

a) dipole-dipole interaction
b) anisotropy of chemical shift (depends on B_0 !)

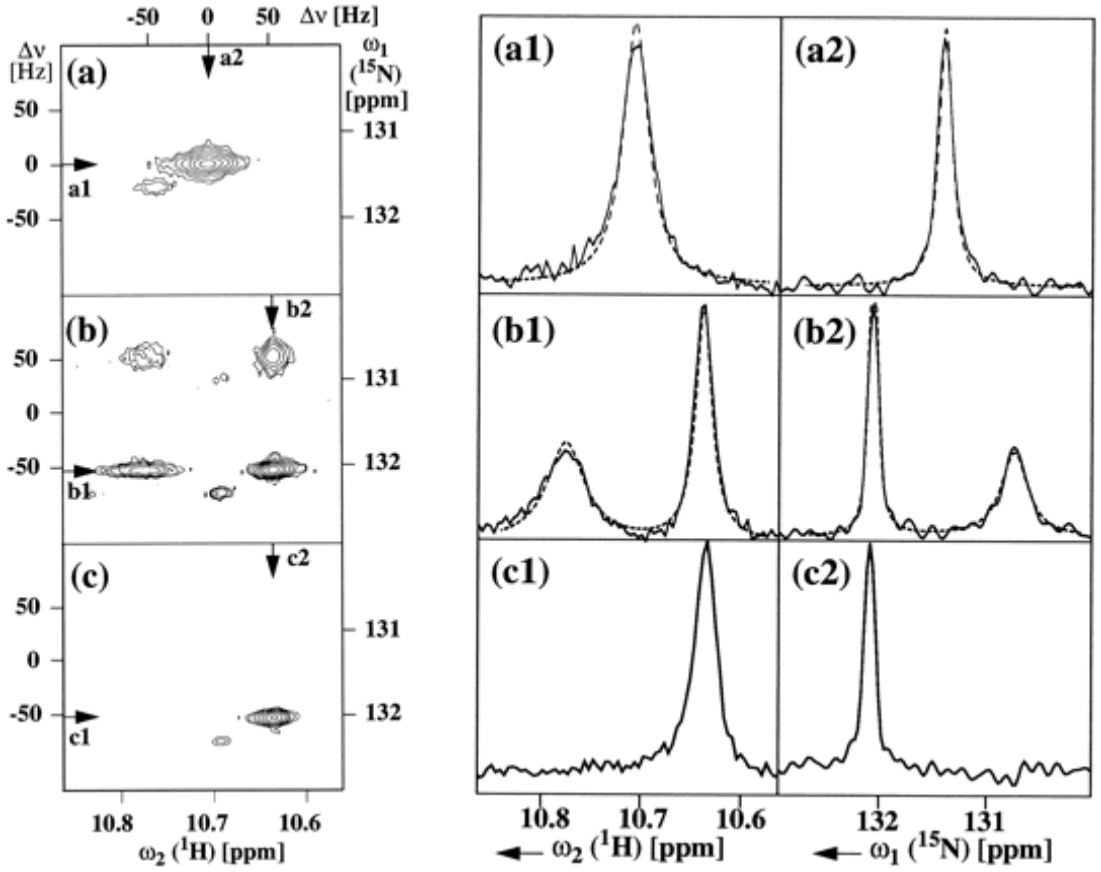
TROSY = transverse relaxation optimized spectroscopy

→ Selection of the slowest relaxing multiplet component

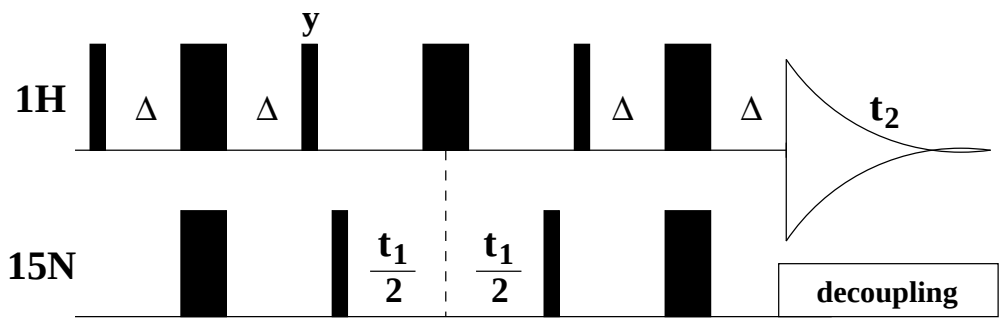
HSQC with decoupling in F1 and F2

HSQC without decoupling in F1 and F2

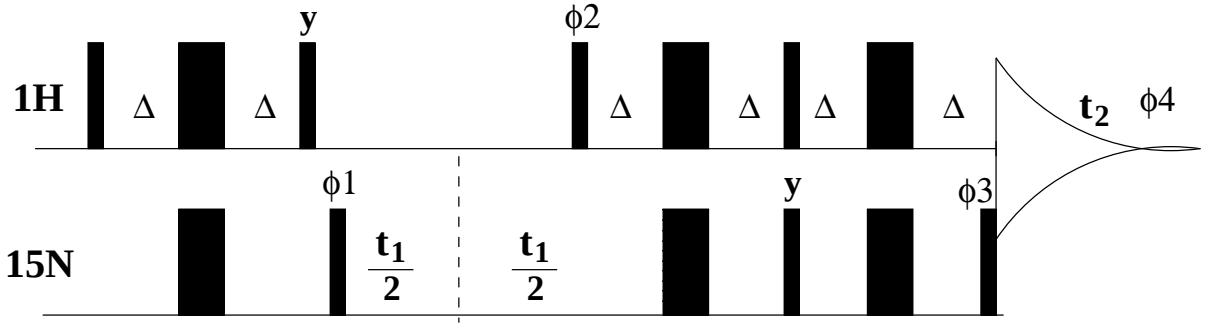
TROSY $^{1}H^{N}$, ^{15}N correlation



basic HSQC pulse sequence (Bodenhausen, G. & Ruben, D.J.,1980)



basic TROSY pulse sequence (Pevushin, K. Riek, R., Wider, G. & Wüthrich, K., 1997)



$\phi1 = \{y, -y, -x, x, y, -y, -x, x\}$

$\phi2 = \{y, y, y, y, -y, -y, -y, -y\}$

$\phi3 = \{x, x, x, x, -x, -x, -x, -x\}$

$\phi4 = \{x, -x, -y, y, x, -x, y, -y\}$

phase cycle selects the desired multiplet component

TROSY transfer in cartesian product operators

$H^{\alpha}N_x = ½ (N_x + 2H_zN_x)$

$H^{\beta}N_x = ½ (N_x - 2H_zN_x)$

The transfers resulting in the four possible multiplet components

$½ (N_x + 2H_zN_x) \longrightarrow ½ (H_x + 2H_xN_z)$

$½ (N_x + 2H_zN_x) \longrightarrow ½ (H_x - 2H_xN_z)$

$½ (N_x - 2H_zN_x) \longrightarrow ½ (H_x + 2H_xN_z)$

$½ (N_x - 2H_zN_x) \longrightarrow ½ (H_x - 2H_xN_z)$

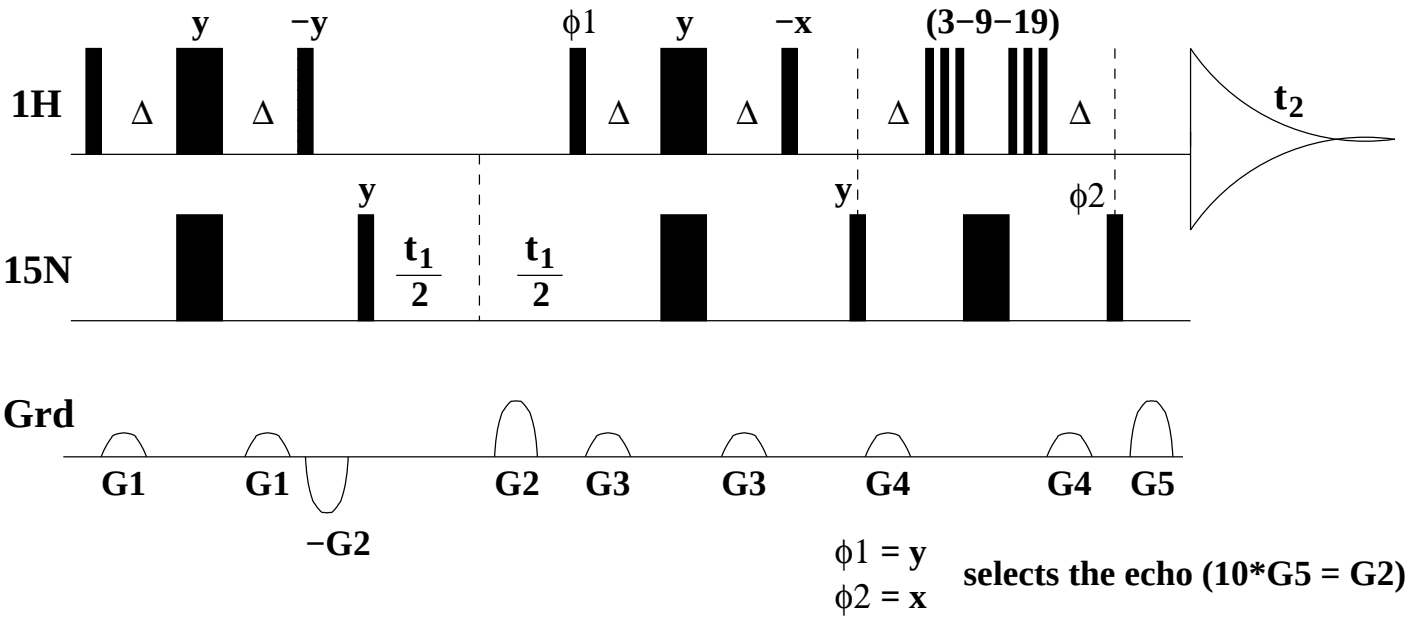
Optimization of TROSY pulse sequence

coherence order selctive transfer (’sensitivity enhancement, with / without gradients)
water−flipback (minimizing water saturation)

(M.Czisch & R. Boelens, 1998; M. Rance, J.P. Loria & A.G. Palmer III, 1999)

(J. Weigelt, 1998; Pervushin, K., Wider, G., & Wüthrich, K. 1998)

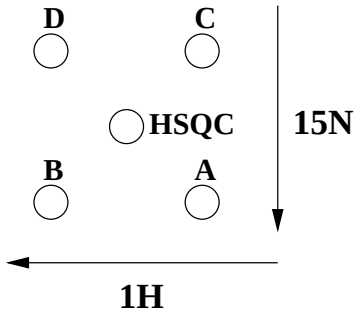
improved TROSY pulse sequence



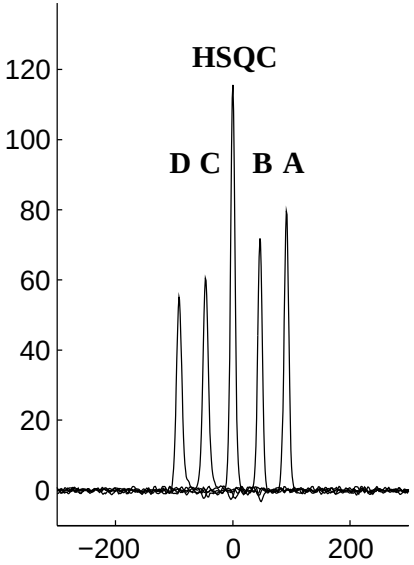
ϕ1 = y
ϕ2 = x **selects the echo (10*G5 = G2)**

Inverting of G2 , ϕ1, ϕ2 **selects the antiecho**

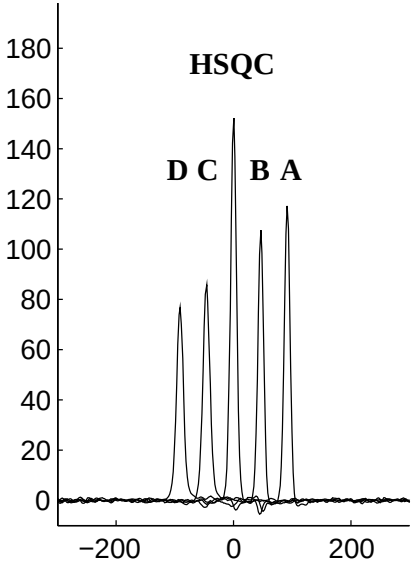
TROSY selection in a single scan
(incl. sensitivity enhancement, water−flipback and minimal number of pulses)



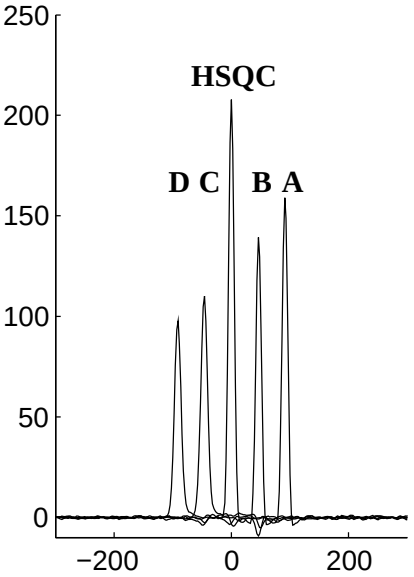
400 MHz



700 MHz



800 MHz

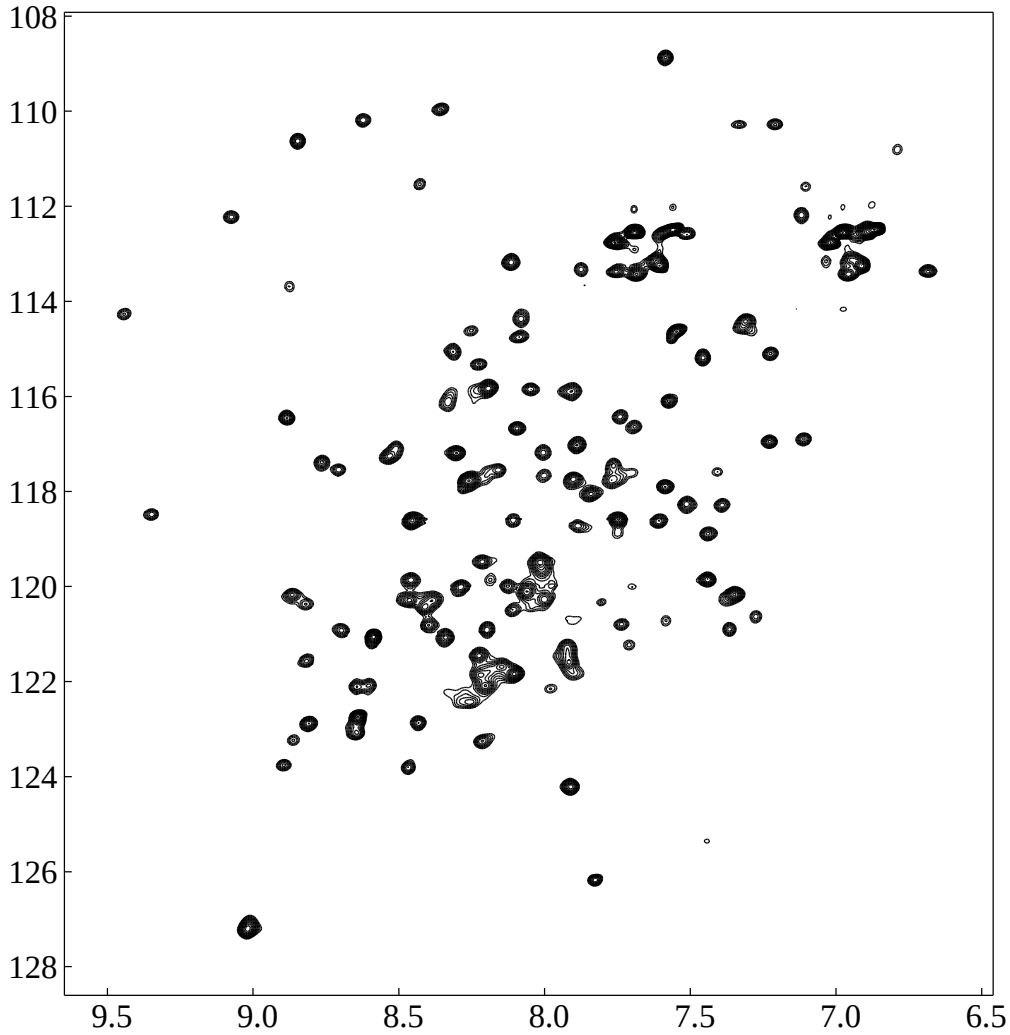


av. relative intensities (50 peaks, structured)

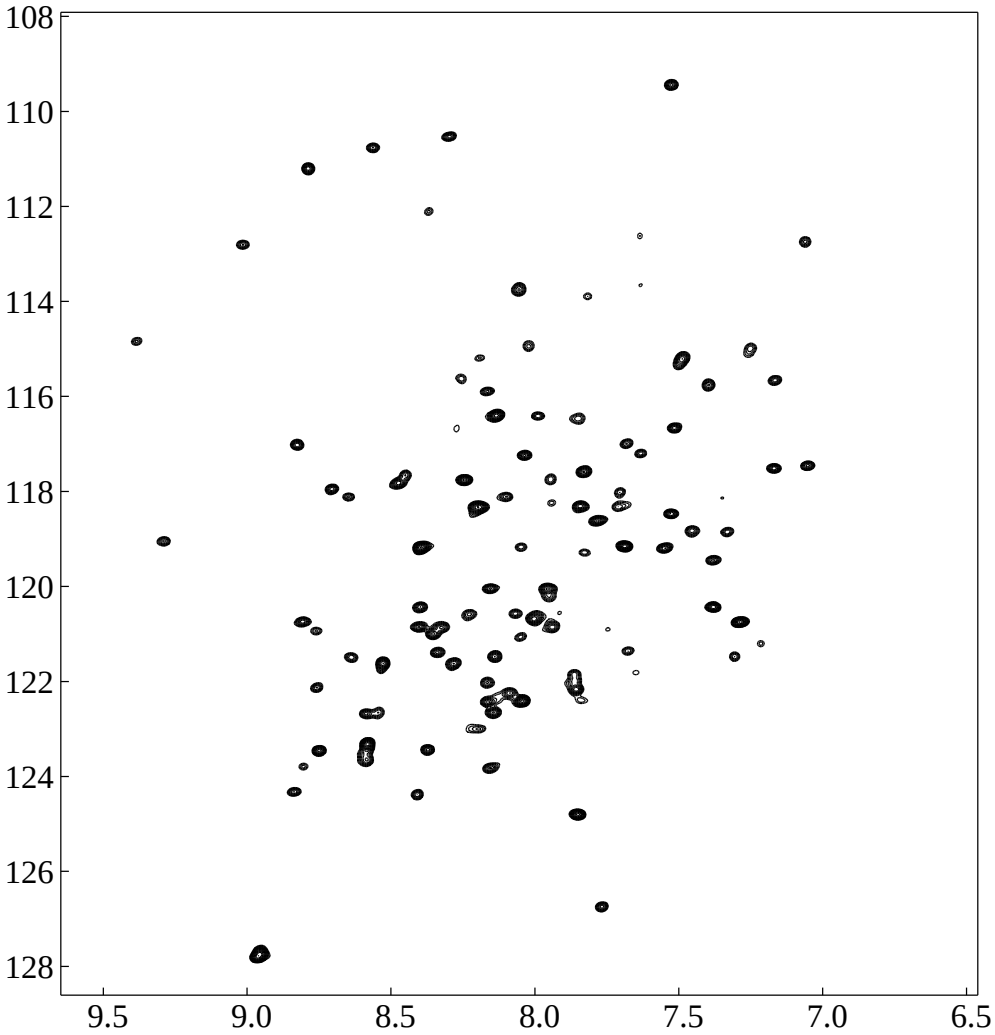
HSQC	**1.53 (0.19)**	**HSQC**	**1.37 (0.17)**	**HSQC**	**1.38 (0.18)**
A	**1.0**	**A**	**1.0**	**A**	**1.0**
B	**0.93 (0.02)**	**B**	**0.94 (0.02)**	**B**	**0.89 (0.02)**
C	**0.77 (0.04)**	**C**	**0.73 (0.04)**	**C**	**0.69 (0.04)**
D	**0.70 (0.04)**	**D**	**0.67 (0.04)**	**D**	**0.63 (0.04)**

1H, 15N Correlations of [2H, 15N]−labeled Tom70−16K at 800 MHz

1H, 15N HSQC

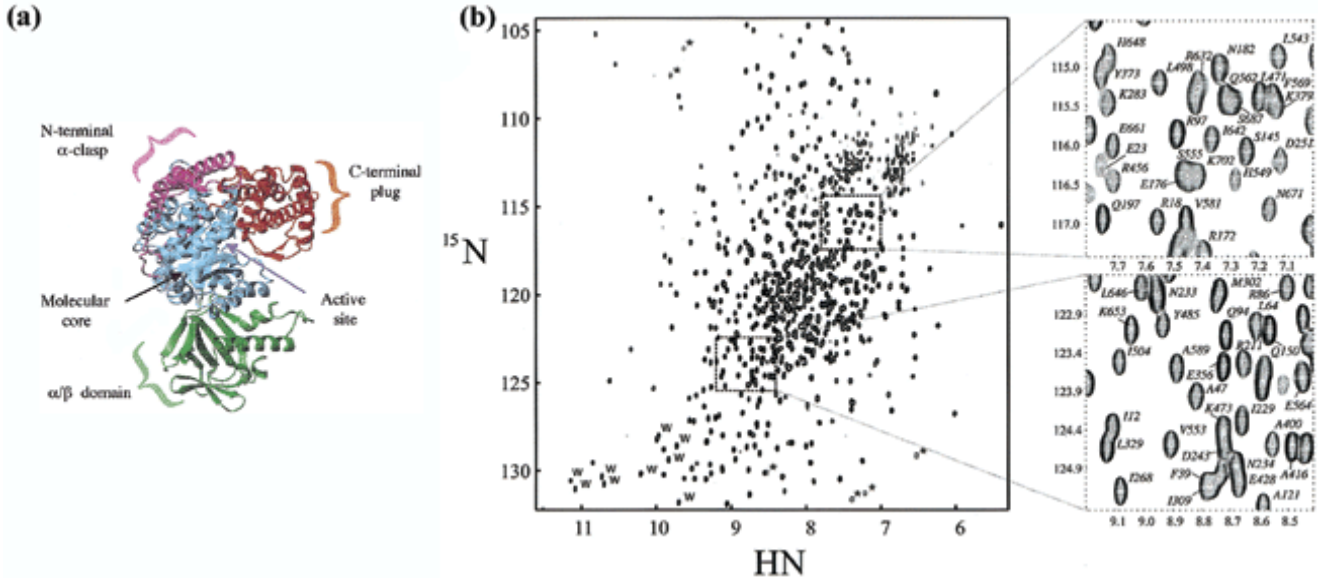


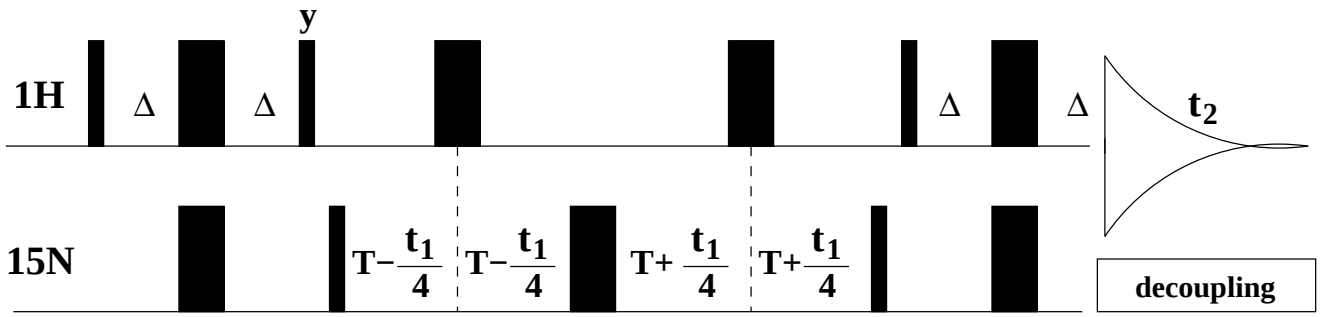
1H, 15N TROSY



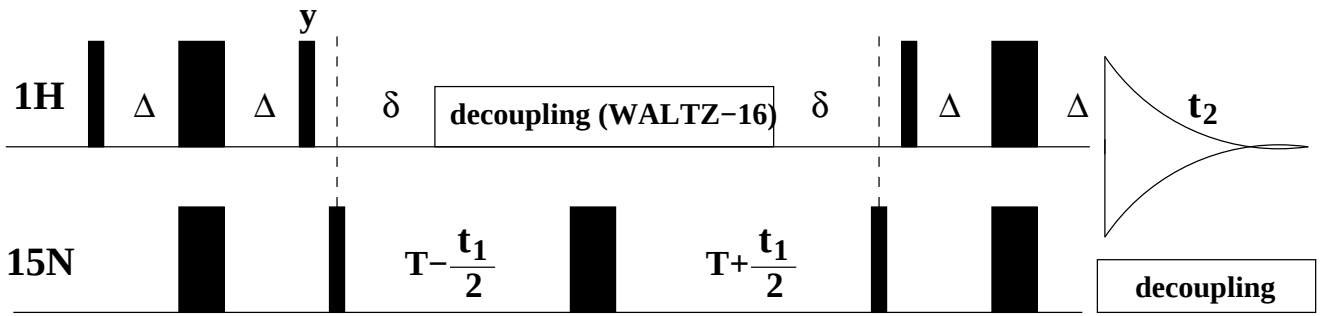
TROSY $^{1}H^{N}$, ^{15}N spectrum of a larger protein

E. coli malate synthase G (723 aa, 81.4 kDa), ^{2}H, ^{15}N, ^{13}C labeled, 0.8 mM, 800 MHz, 37°C

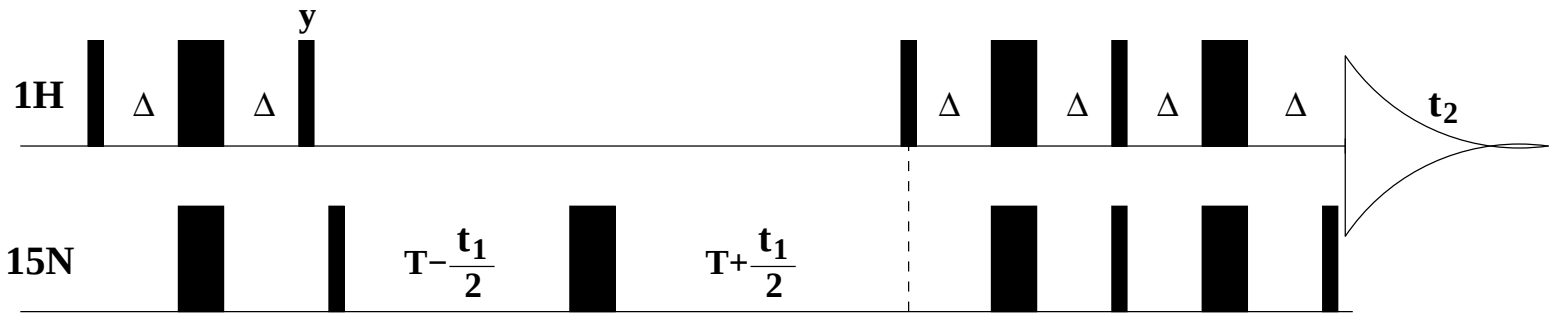




4T = 50 ms

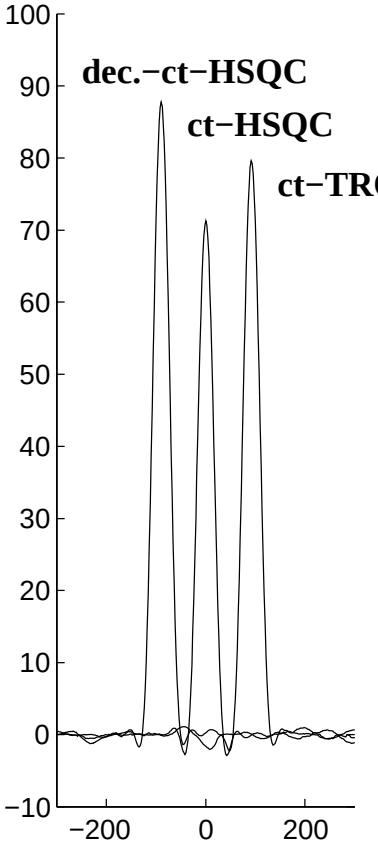


2T = 50 ms



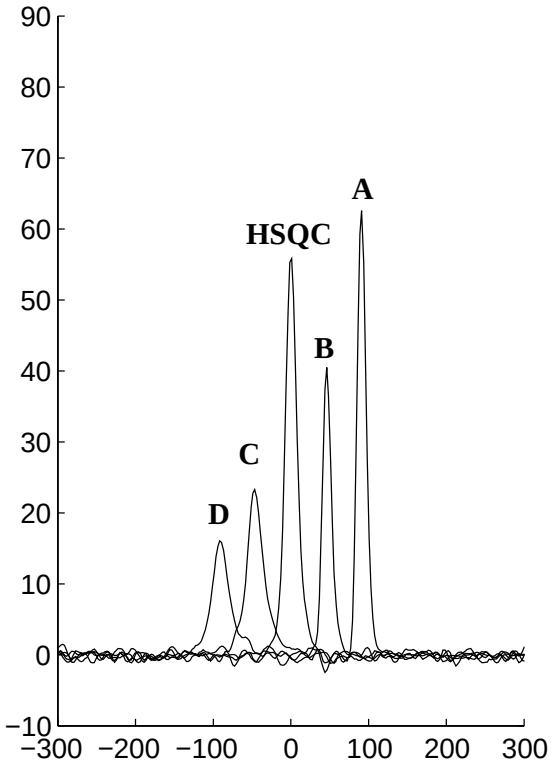
2T = 50 ms

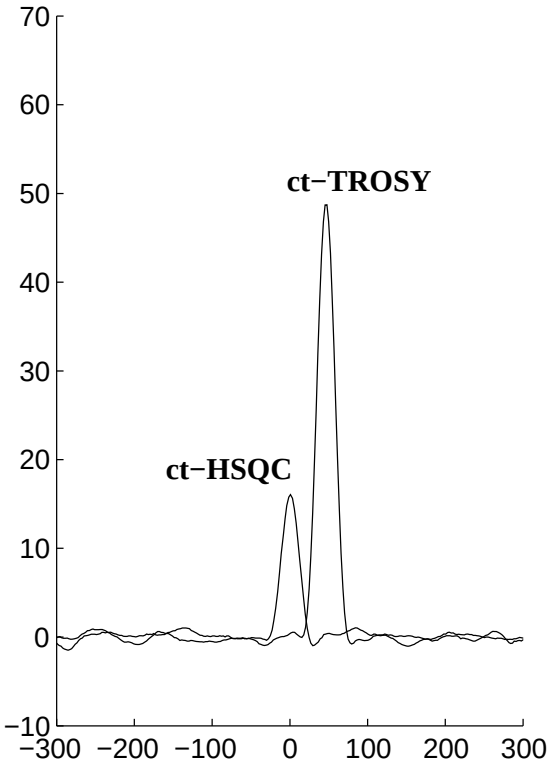
700 MHz



ct−HSQC	**1.0**
dec. ct−HSQC	**1.18 (0.05)**
ct−TROSY	**1.14 (0.10)**

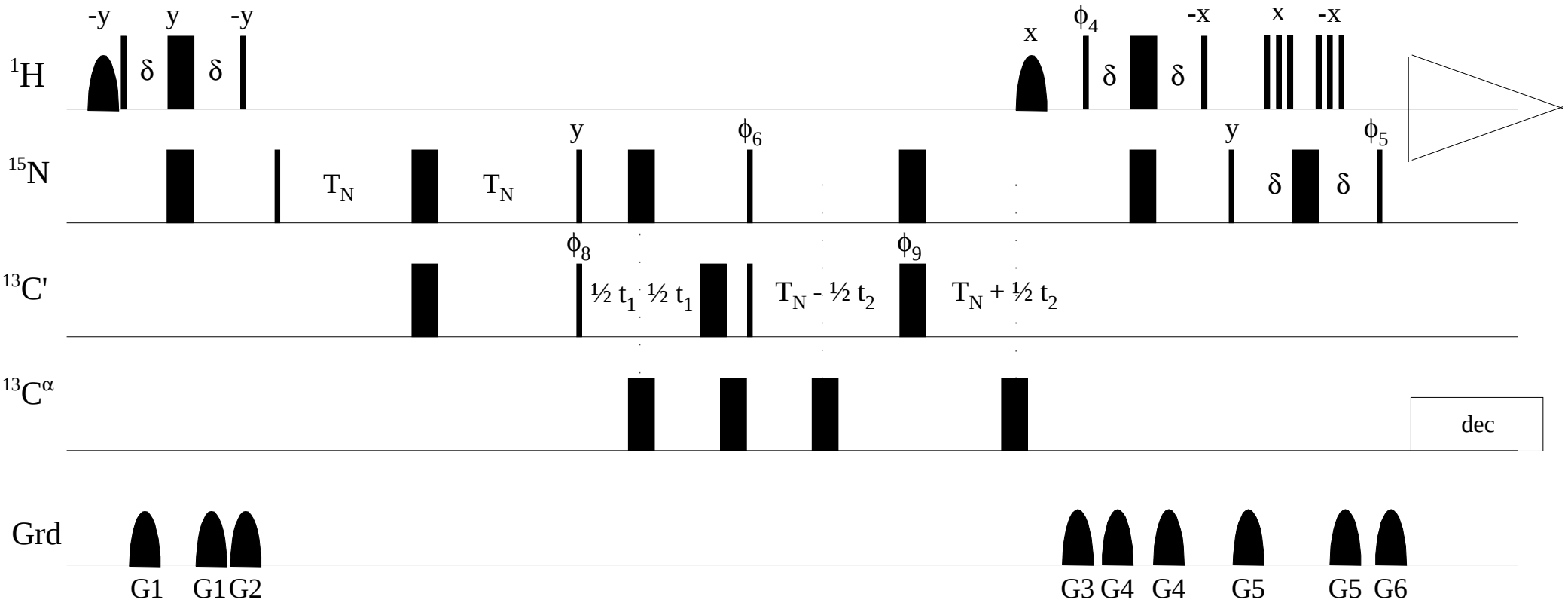
HSQC / TROSY Slices of 2H,15N labeled Tom70−16K at 800 MHz





Example of a TROSY-tripelresonance experiment

TROSY-HNCO



T_N = 12 ms

δ = 2.75 ms

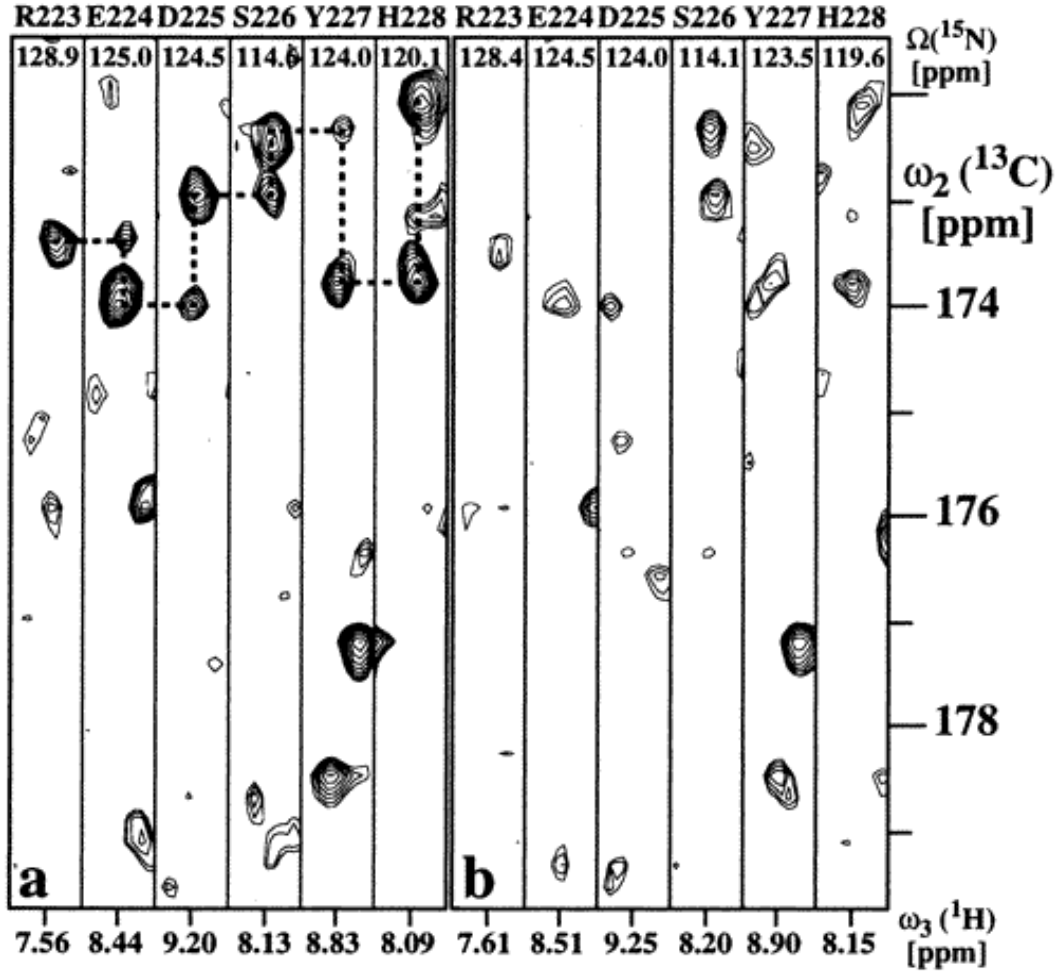
ϕ_4 = y
ϕ_5 = x
ϕ_6 = y, -y
ϕ_8 = x, x, -x, -x + States-TPPI
ϕ_9 = x, x, x, x, -x, -x, -x, -x
ϕ_{rec} = x, -x, -x, x

quadrature detection alaong F2 + TROSY selection:
Inversion of ϕ_4 and ϕ_5 and inversion von G2 und G3 for each second F2 data point

TROSY tripleresonance experiment
(M. Salzmann et al. (1999), J. Am. Chem. Soc. 121, 844-848)

NMR sample: ^{2}H, ^{13}C, ^{15}N labeled Gyrase 23B, 1 mM, 20°C, 750 MHz

TROSY-HN(CA)CO

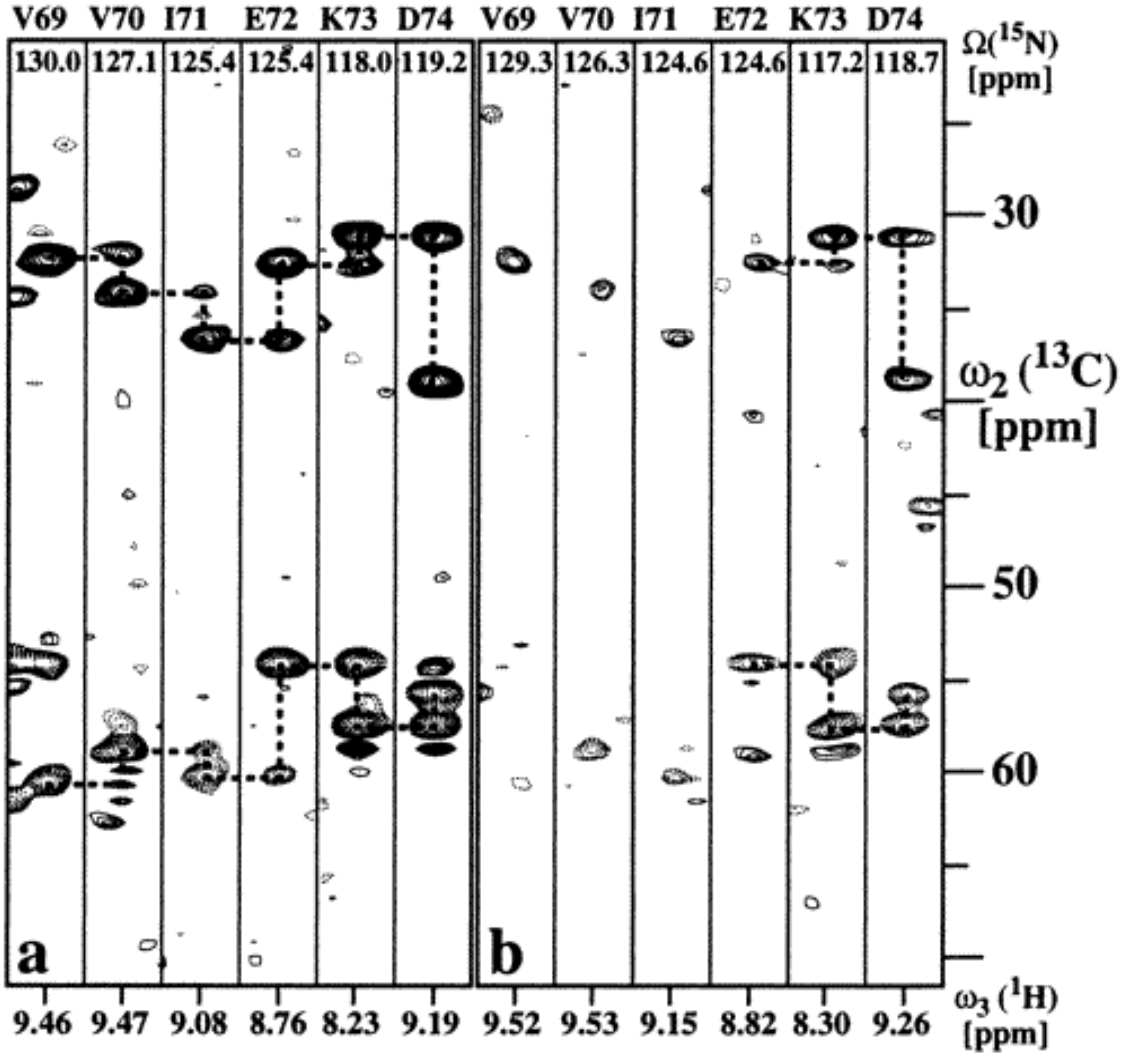


classical HN(CA)CO

TROSY tripleresonance experiments
(M. Salzmann et al. (1999), J. Am. Chem. Soc. 121, 844-848)

NMR sample: ^{2}H, ^{13}C, ^{15}N labeled Gyrase 23B, 1 mM, 20°C, 750 MHz

TROSY-HNCACB



classical HNCACB

HN(CO)CA

TROSY

classical

HN(CA)CO

TROSY

classical

HNCACB

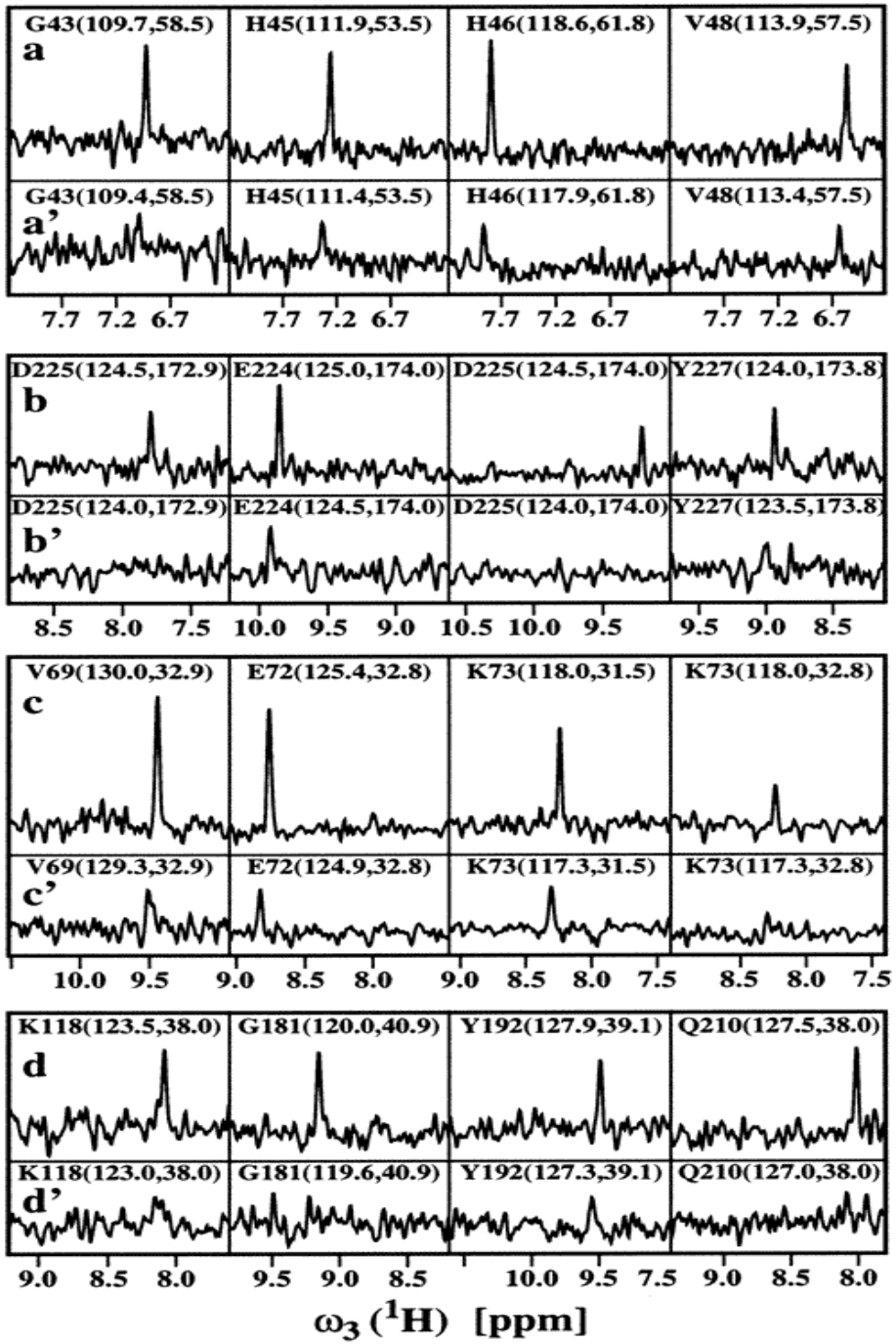
TROSY

classical

HN(CO)CACB

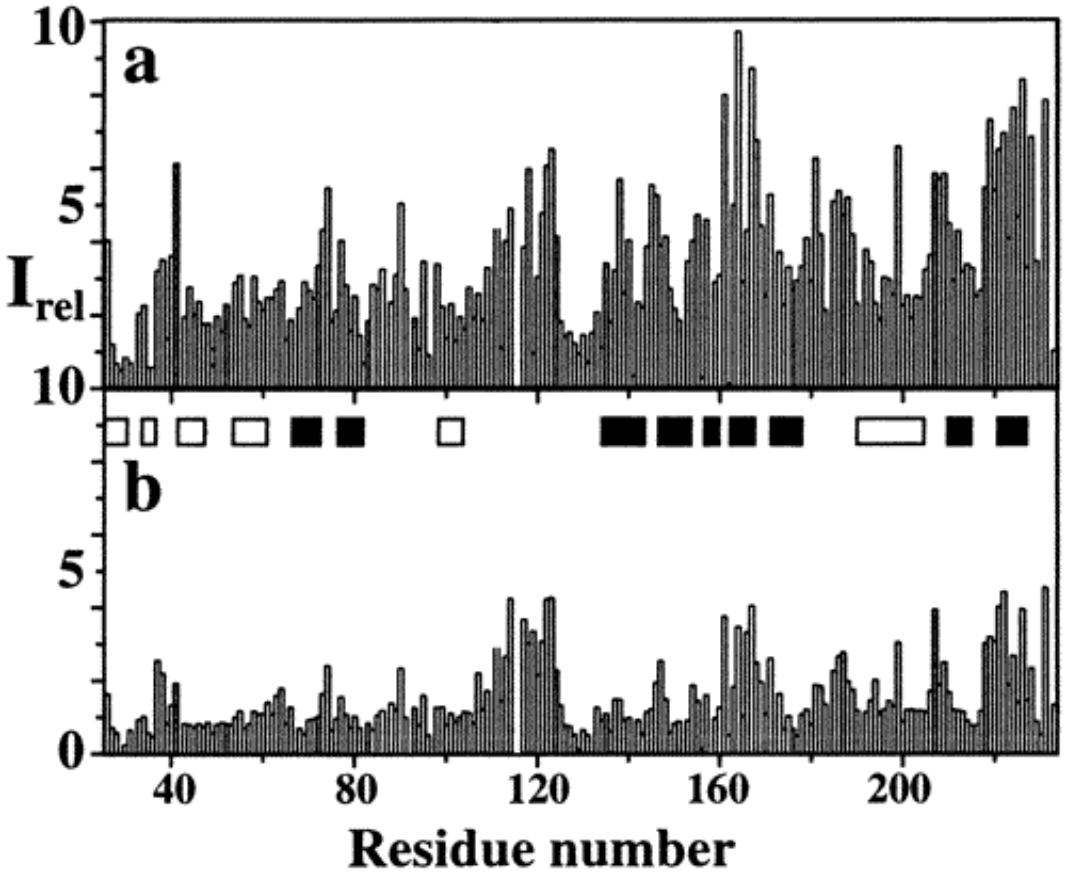
TROSY

classical



relative intensity in a TROSY-HN(CO)CA

$I_{rel} = I_{TROSY} / I_{classical}$



→ TROSY in triple resonance experiments: sensitivity gain by a factor of 3