Supporting Information for Yang and Kay, " TROSY Triple Resonance Four Dimensional Spectroscopy of a 46 ns Tumbling Protein"

Figure 1: TROSY-based triple resonance pulse schemes, CT-HNCA, CT-HN(CO)CA, CT-HN(CA)CB, CT-HN(COCA)CB, which make use of the enhanced sensitivity pulsed field gradient implementation for the transfer of magnetization from ^{15}N to HN prior to detection[1]. The shaded region of each of sequences A - D can be replaced with either schemes (a) or (b). In scheme (a) $\phi o = x$ and suppression of the undesired crosspeak derived from the pathway, $N_{TR}(1 + 2HN_Z) \dashrightarrow HN_{TR}(1 - 2N_Z)$, is achieved by efficient relaxation occurring during the τ_b and TN delays (totaling $\approx$ 50 ms) and the broad ^1HN linewidth[1]. In sequence (b) active suppression of the undesired crosspeak occurs by actively selecting only the slowly relaxing component[2]. The value of τ_e is set to $1/(8J_{NH})$, while $|\phi o| = 45^o$. The sign of ϕo must be adjusted on each brand of spectrometer to give optimum sensitivity. Note that the phase of the final ^1H 90° of the INEPT[3] (y or -y) must also be adjusted for optimum signal and can vary between spectrometers. (The phase values listed were calibrated on a Varian spectrometer). The ^1H 180° pulse applied after a delay τ_e from the start of ^{15}N evolution is of the composite variety $(90_x180_y90_x)$. The shaped ^1H 90° pulse is a water selective pulse having the EBURP-1 profile[4] (1.05 kHz). Use of scheme (b) does result in a slight attenuation of the signal relative to sequence (a) since for the first τ_e delay the fast relaxing ^{15}N component is the one which ultimately contributes to the signal of interest. Nevertheless a comparison of TROSY based triple resonance experiments recorded with scheme (b) using the enhanced sensitivity pulsed field gradient approach[1] was 3% more sensitive than the implementation of Pervushin et al.[5] for an MBP sample (600 MHz) at 37 °C (τ_C = 17 ns) and at 25 °C (τ_C = 23 ns). It is difficult to provide a definitive statement regarding when to use scheme (a) or (b). Figure 1 of Yang and Kay[1] illustrates the relative intensity ratio of the crosspeaks derived from the two pathways [$N_{TR}(1 \pm 2HN_Z) \dashrightarrow HN_{TR}(1 \mp 2N_Z)$] which can be used as a guideline. For

spectra recorded on MBP at 25 °C (τ_C = 23 ns) using scheme (a) the intensity of the undesired component was no more than 5-6% of the slowly relaxing crosspeak and is predicted to be under 2% for spectra recorded at 800 MHz[1]. The undesired crosspeak is below the noise level in spectra recorded on MBP at 5 °C and at 600 MHz. In order to establish whether scheme (a) or (b) should be employed a 2D ^{15}N-^{1}HN plane of an HNCO can be recorded to evaluate the size of the undesired crosspeak when sequence a is employed. In what follows only a brief description of the pulse schemes is provided; details can be found in the original references[6-8]. A. CT-TROSY-HNCA. The ^{13}C carrier is centered at 58 ppm and the rectangular $^{13}C^{\alpha}$ 90° ($^{13}C^{\alpha}/^{13}C'$ 180°) pulses are applied with a field strength of $\Delta/\sqrt{15}$ ($\Delta/\sqrt{3}$)[9], where Δ is defined as in the legend to Figure 1. The shaped non-selective $^{13}C^{\alpha}$ refocusing pulse has the REBURP[4] profile and is centered at 45 ppm. The phase cycle used is: $\phi1$ = 2(x),2(-x); $\phi2$ = (y,-y); $\phi3$ = 4(x),4(-x); $\phi4$ = x; rec=x,2(-x),x. B. CT-TROSY-HN(CO)CA. The ^{13}C carrier is initially set to 176 ppm, jumped to 58 ppm prior to the application of the $^{13}C^{\alpha}$ 90° pulse of phase $\phi2$ and set to 176 ppm after the subsequent $^{13}C^{\alpha}$ 90° pulse. All $^{13}C'$ and $^{13}C^{\alpha}$ 90° pulses are applied with a field of $\Delta/\sqrt{15}$; the $^{13}C^{\alpha}$ and $^{13}C'$ rectangular 180° pulses have a strength of $\Delta/\sqrt{3}$; the $^{13}C^{\alpha}$ shaped pulse during the CT-$^{13}C^{\alpha}$ evolution period has the REBURP profile[4] with the center of excitation at 45 ppm. Vertical arrows indicate the position of the Bloch-Siegert compensation pulses[10]. Note that the $^{13}C^{\alpha}$ 180° pulse during the first (second) $2\tau_C$ period is applied after (before) the $^{13}C'$ pulse. The phase cycle is: $\phi1$ = (x,-x); $\phi2$ = 2(x),2(-x); $\phi3$ = y; $\phi4$ = 4(x),4(-x); $\phi5$ = x; rec = x,2(-x),x. C. CT-TROSY-HN(CA)CB. The ^{13}C carrier is at 43 ppm and the $^{13}C^{\alpha}$ pulse (of schemes a and b) and the final $^{13}C^{\alpha/\beta}$ 180° pulse labeled α are applied with the center of excitation at 58 ppm by phase modulation of the carrier[11,12]. All $^{13}C^{\alpha/\beta}$ 90° pulses are applied at the highest field possible, while the shaped (non-selective for aliphatic carbons) $^{13}C^{\alpha/\beta}$ refocusing pulses have the REBURP profile[4]. The phases of these pulses are carefully adjusted for optimum sensitivity. The $^{13}C'$ pulse labeled with * is applied with the G3 profile[13] (≈ 300 μs at 600 MHz) centered

at 150 ppm. The phase cycle employed is: ϕo = (x,-x) for scheme (a) and either (45°, 225°) or (-45°, 135°) for scheme (b), depending on the brand of spectrometer; $\phi 1$ = 2(x),2(-x); $\phi 2$ = 4(y),4(-y); $\phi 3$ = y; $\phi 4$ = 4(x),4(-x); $\phi 5$ = x; rec = x,2(-x),x. D. CT-TROSY-HN(COCA)CB. The ^{13}C carrier is initially set to 176 ppm, jumped to 43 ppm prior to the application of the $^{13}C^{\alpha/\beta}$ 90° pulse of phase $\phi 2$ and set back to 176 ppm prior to the $^{13}C'$ 90_y pulse. All $^{13}C^{\alpha/\beta}$ 90° pulses are applied with a field of $\Delta'/\sqrt{15}$; $^{13}C'$ 90° pulses are applied with a field stregnth of $\Delta/\sqrt{15}$; the $^{13}C'$ rectangular 180° pulses have a strength of $\Delta/\sqrt{3}$; the $^{13}C^{\alpha/\beta}$ shaped pulses during the CT-$^{13}C^{\beta}$ evolution period and the two $2x\tau_d$ periods have REBURP[4] profiles with the center of excitation at 43 ppm; the ^{13}C pulses labeled α [and the first $^{13}C^{\alpha}$ pulse of either scheme (a) or (b)] are centered at 58 ppm by phase modulation of the carrier[11,12] and applied with a field of $\Delta/\sqrt{3}$. The value of Δ' is set to the difference in Hz between the centers of the $^{13}C'$ (176 ppm) and $^{13}C^{\alpha/\beta}$ (43 ppm) spectral regions. Vertical arrows indicate the position of the Bloch-Siegert compensation pulses[10]. Other pulses are as defined above. The phase cycle is: $\phi 1$ = (x,-x); $\phi 2$ = 2(x),2(-x); $\phi 3$ = 4(y);4(-y); $\phi 4$ = y; $\phi 5$ = 4(x),4(-x); $\phi 6$ =x; rec = x,2(-x),x.

It is noteworthy that many of the original schemes used a $^{13}C'$ 180° pulse having the seduce-1 profile[14] rather than rectangular pulses with nulls at the center of the $^{13}C^{\alpha}$ region. We have found these different pulses make little difference to the quality of the spectra. Note that on systems where a power change is accompanied by phase changes the phase adjustments needed may vary depending on whether the seduce profile or a rectangular pulse is employed.

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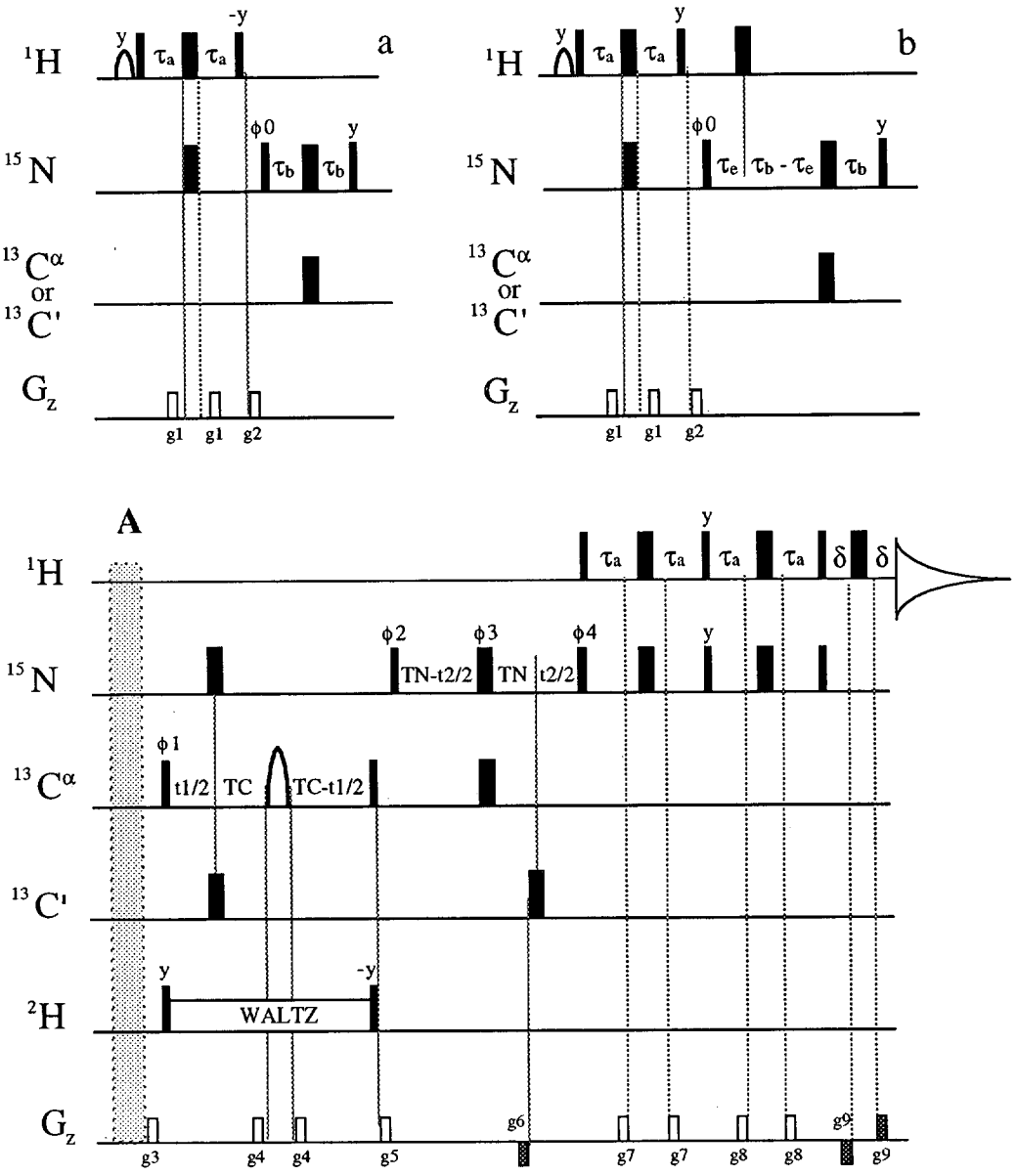
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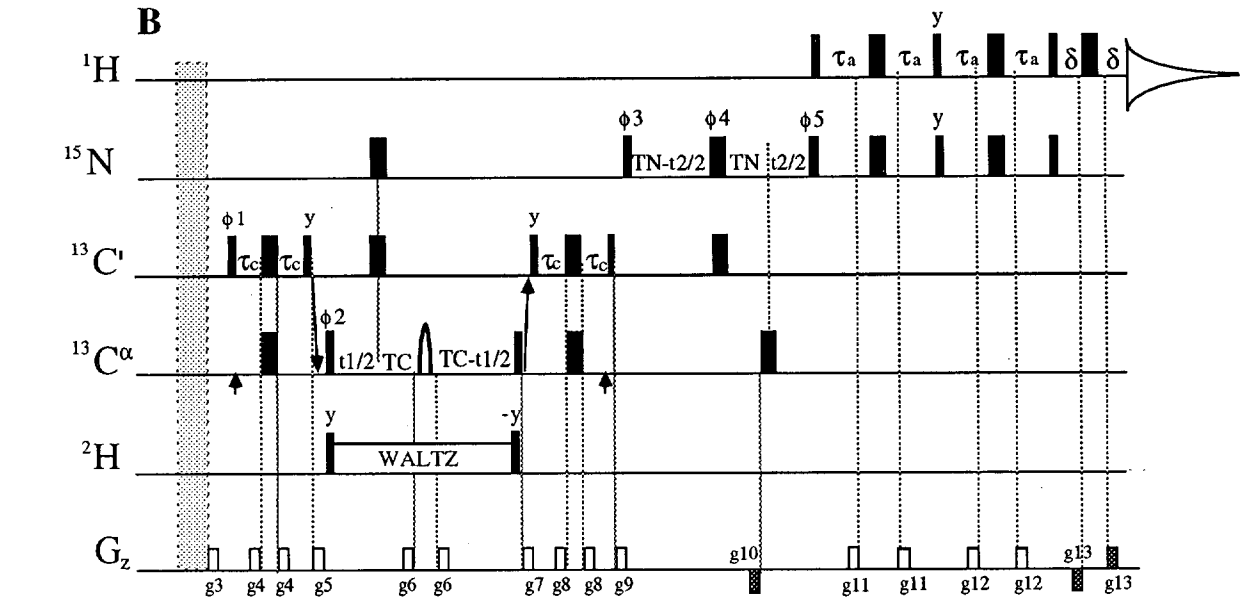
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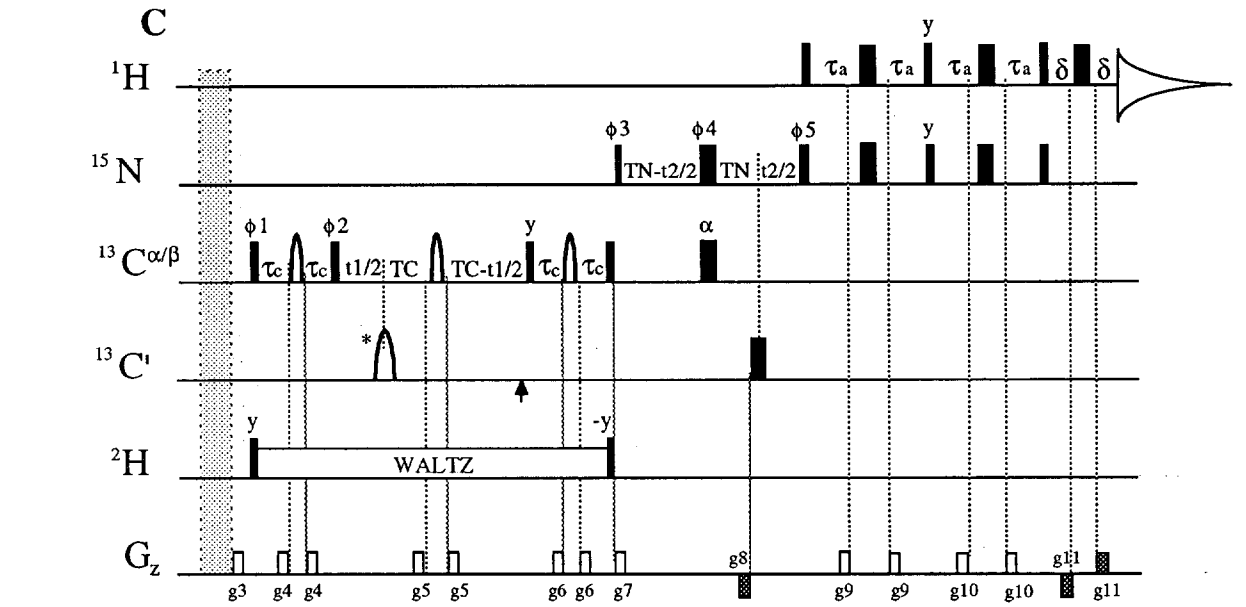
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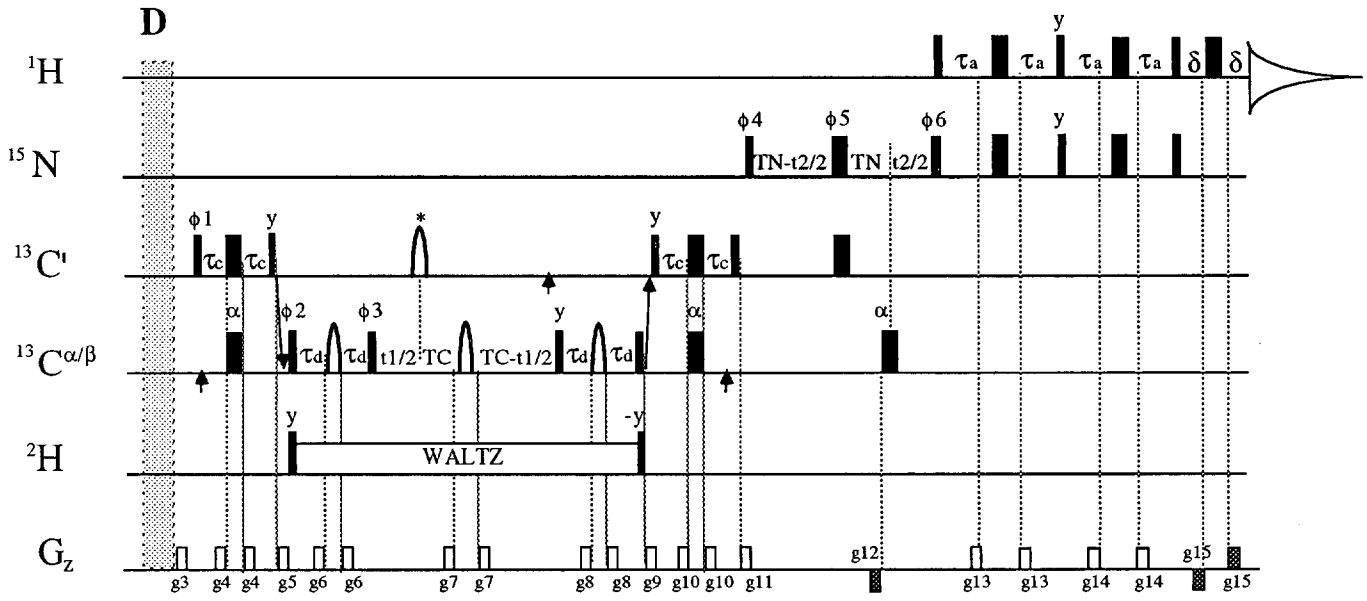
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