Journal of Magnetic Resonance **139,** 439–442 (1999)
Article ID jmre.1999.1788, available online at http://www.idealibrary.com on IDEAL®

# The Role of Coherence Transfer Efficiency in Design of TROSY-Type Multidimensional NMR Experiments

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Received January 19, 1999; revised April 1, 1999

**An improved method for TROSY-type (Pervushin *et al., Proc. Natl. Acad. Sci. USA* 94, 12366–12371 (1997)) heteronuclear two-dimensional correlation involving protons of negligible CSA is presented. Rather than applying a simple INEPT sequence for back-transfer to protons (Pervushin *et al., J. Am. Chem. Soc.* 120, 6394–6400 (1998)), we replace the $\pi/2$ proton pulse in INEPT by a spin-state-selective coherence transfer element (Sørensen *et al., J. Biomol. NMR* 10, 181–186 (1997)) and maintain broadband decoupling during acquisition. Theoretically that results in a sensitivity enhancement of a factor of 2. The new method is demonstrated using a $^{13}C,^{15}N$-labeled protein sample, RAP 18-112 (N-terminal domain of $\alpha_2$-macroglobulin receptor associated protein), at 750 MHz.** 

*Key Words:* **multidimensional NMR; $S^3CT$; TROSY.**

In the design of multidimensional NMR experiments it is sometimes of interest for various reasons to restrict coherence transfer processes so that only a subset of the multiplet components occurs in the cross peaks. One reason could be the necessity to simplify complex multiplet patterns with extensive overlap in order to make possible extraction of interesting information. An example is E. COSY (*1–3*) and its many variants in protein NMR where the simplified cross-peak multiplets allow accurate measurement of *J* coupling constants. Somewhat related to this is the notion of TROSY (transverse-relaxation-optimized spectroscopy) (*4*), which for larger proteins at high fields provides sensitivity enhancement in experiments involving the two spins of amide or aromatic $^1H$–$^{13}C$ groups.

Normally, one would decouple the $^1H$ and, e.g., $^{15}N$ spins from each other when it is of interest only to correlate the chemical shifts. However, very different transverse relaxation times of the two resonances in the doublets make this unfavorable sensitivity-wise. Hence decoupling is dispensed with but that results in two peaks in the multiplets, so a restricted coherence transfer sequence is applied to eliminate the peak of lowest intensity and thereby simplify the multiplets. Thus a spectrum with the same appearance as the decoupled one is obtained, only with higher sensitivity. In other words, the motivation for restricting coherence transfer in TROSY is enhancement of sensitivity and resolution due to narrower resonances.

In a recent publication (*5*), Pervushin *et al.* draw the conclusion that the absence of differential transverse relaxation of aromatic protons *makes the TROSY method unattractive for these nuclei.* As a consequence, a pulse sequence without $^1H$ decoupling in the indirect $^{13}C$ dimension to exploit large differential relaxation and a simple INEPT-type coherence transfer mixing sequence is proposed.

There are several aspects of this pulse sequence design problem to consider in order to arrive at a favorable solution. While it is obvious that the TROSY method is unattractive for small differential relaxation on both spins of an IS system because part of the initial spin order is eliminated by the pulse sequence in contrast to decoupled HSQC (*6, 7*), that is not so in the case of large differential relaxation of one of the spins as in aromatic $^1H$–$^{13}C$ systems. The spin-engineering problem is one of transferring the coherence on one of the S-spin ($^{13}C$) transitions to the I-spin ($^1H$) so as to obtain maximum signal intensity. That amounts to a coherence transfer process of the type

$$I^{\alpha/\beta}S^{\pm} \rightarrow aI^{-}S^{\alpha} + bI^{-}S^{\beta} + Q, \qquad [1]$$

where the starting operator is one of the four combinations indicated and $Q$ is a residual operator that ideally should be zero. Then there are two strategies: The first one is to maximize the sum, $a + b$, in which case S-spin decoupling should be applied when neither $a$ nor $b$ is zero. The second one is to maximize either $a$ or $b$, which would be interesting if a higher value could be obtained than for $a + b$. Anyway, for the optimization in Eq. [1] there is a multitude of solutions, namely all combinations of $|a + b| = 1$. Planar mixing (*6–8*) or double spin-state-selective coherence transfer $(S^3CT)^2$ (*9*) will produce the desired result of $a = 1$ and $b = 0$ or vice versa. Hence S-spin decoupling is an



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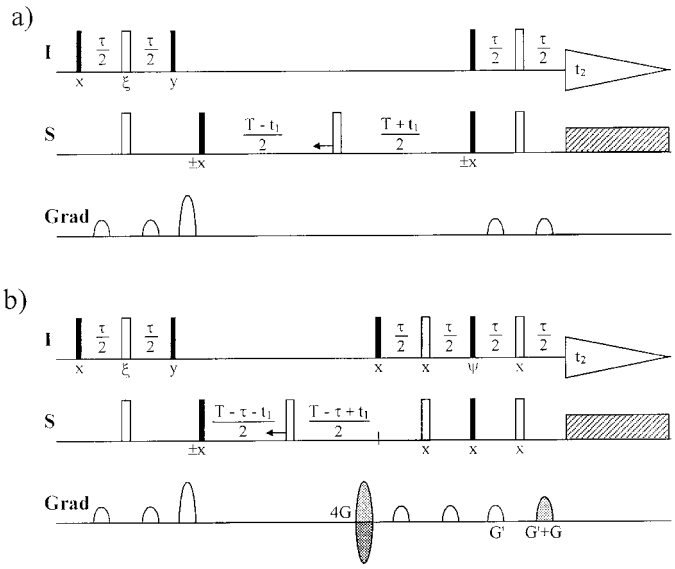


**FIG. 1.** (a) $^{1}H$–$^{13}C$ (I–S) TROSY pulse sequence similar to the one proposed by Pervushin *et al.* (*5*) and (b) the new constant-time $S^3CT$ TROSY pulse sequence. Filled and open bars represent $\pi/2$ and $\pi$ pulses, respectively. Phases are indicated below the pulses and the delays $\tau$ and $T$ are $(2J_{IS})^{-1}$ and $(J_{SS})^{-1}$, respectively. Pulse phases with the prefix $\pm$ indicate independent two-step phase cycles with alternating receiver phase. The phase cycle of the $\pi$ pulse in the constant time delay is $\{x, y, -x, -y\}$ with alternating receiver phase $\{x, -x\}$. In order to retain the native S-spin magnetization in the TROSY resonance the phase $\zeta$ should be $y$ on our Varian Unity Inova spectrometers while it must be $x$ on our Bruker DRX instrument. In combination with the shaded pulsed field gradients the phase $\psi$ is $-y$ and $y$, for echo and antiecho, respectively, on our Varian instruments. Interchanging these results in selection of the $^{13}C$ anti-TROSY resonance.

option that does not alter the sensitivity in case of negligible CSA of the I spins. However, with the applications in mind we consider in the remainder of the paper only the case employing S-spin decoupling.

The above result is to be compared with the transfer employed in Ref. (*5*) and involving simultaneously both echo and antiecho:

$$I^{\alpha/\beta}S^{+} \rightarrow \tfrac{1}{4}I^{-}S^{\alpha} + \tfrac{1}{4}I^{-}S^{\beta} + Q' \qquad [2a]$$

$$I^{\alpha/\beta}S^{-} \rightarrow -\tfrac{1}{4}I^{-}S^{\alpha} - \tfrac{1}{4}I^{-}S^{\beta} + Q''. \qquad [2b]$$

Upon S-spin decoupling the two doublet line intensities add up but this experiment is a factor of 2 less sensitive than the optimized one in Eq. [1]. In other words, it is worthwhile evaluating coherence transfer efficiencies for the types of pulse sequences relevant. The two pulse sequences are outlined in Fig. 1. The experiment based on Eq. [1] must employ so-called echo–antiecho Fourier transformation (*6, 7*) whereas the one based on Eq. [2] must employ the TPPI or hypercomplex (States) method.

The new constant-time $S^3CT$ TROSY pulse sequence in Fig. 1b can be understood as the $\pi/2$ proton pulse in the INEPT back-transfer (Fig. 1a) having been replaced by a spin-state-selective coherence transfer element $(\pi/2)2I_yS^{\alpha/\beta}$ (*10*). In short-hand product operator terms, the transformations amount to

$$I^{\alpha/\beta}S^{\pm} \xrightarrow{\pi/2\ 2I_yS^{\alpha/\beta}} I^{+}S^{\pm} \xrightarrow{(\pi/2)^{S}} 2I^{+}S_z \xrightarrow{\pi^{I,S}} 2I^{-}S_z \xrightarrow{1/2J} I^{-}, \qquad [13]$$

where only relevant terms are included. When the S-spin TROSY resonances are in the echo, the anti-TROSY resonances are in the antiecho and vice versa. Alternatively, the new pulse sequence may also be described as a truncated version of two $S^3CT$ elements as used when no decoupling is applied during acquisition (*4, 9*). Finally, it should be mentioned that additional benefits of the new constant-time $S^3CT$ TROSY pulse sequence in Fig. 1b are that cross talk from the $^{13}C$ coherence of short $T_2$ in the $t_1$ dimension is suppressed by the mixing sequence and that gradient echoes can be employed without sensitivity loss.

An experimental comparison of the two methods is shown in Fig. 2 with an excerpt from a contour plot of a $^{13}C$–$^{1}H$ 2D spectrum of a $^{13}C$,$^{15}N$-labeled protein, RAP 18-112 (N-terminal domain of $\alpha_2$-macroglobulin receptor associated protein) (*11*), recorded on a Varian Unity Inova 750-MHz spectrometer with the constant-time $S^3CT$ TROSY sequence in Fig. 1b. $F_2$ sections through the peaks are shown together with the corresponding sections (dashed) from a spectrum recorded with the sequence of Pervushin *et al.* in Fig. 1a. On average a sensitivity enhancement of 73% is observed.

Figure 3 shows $F_1$ sections through peaks in the same spectra as above. It is clear that suppression of cross talk from the anti-TROSY resonance on $^{13}C$ is an additional benefit of the new constant-time $S^3CT$ TROSY pulse sequence relative to the earlier sequence.

In conclusion, we have provided an example of the value of considering coherence transfer efficiency in designing a TROSY-type pulse sequence. The new constant-time $S^3CT$ TROSY sequence almost doubles the sensitivity enhancement factors of 4–10 reported by Pervushin *et al.* (*5*) relative to sequences employing conventional heteronuclear decoupling in the $t_1$ period.

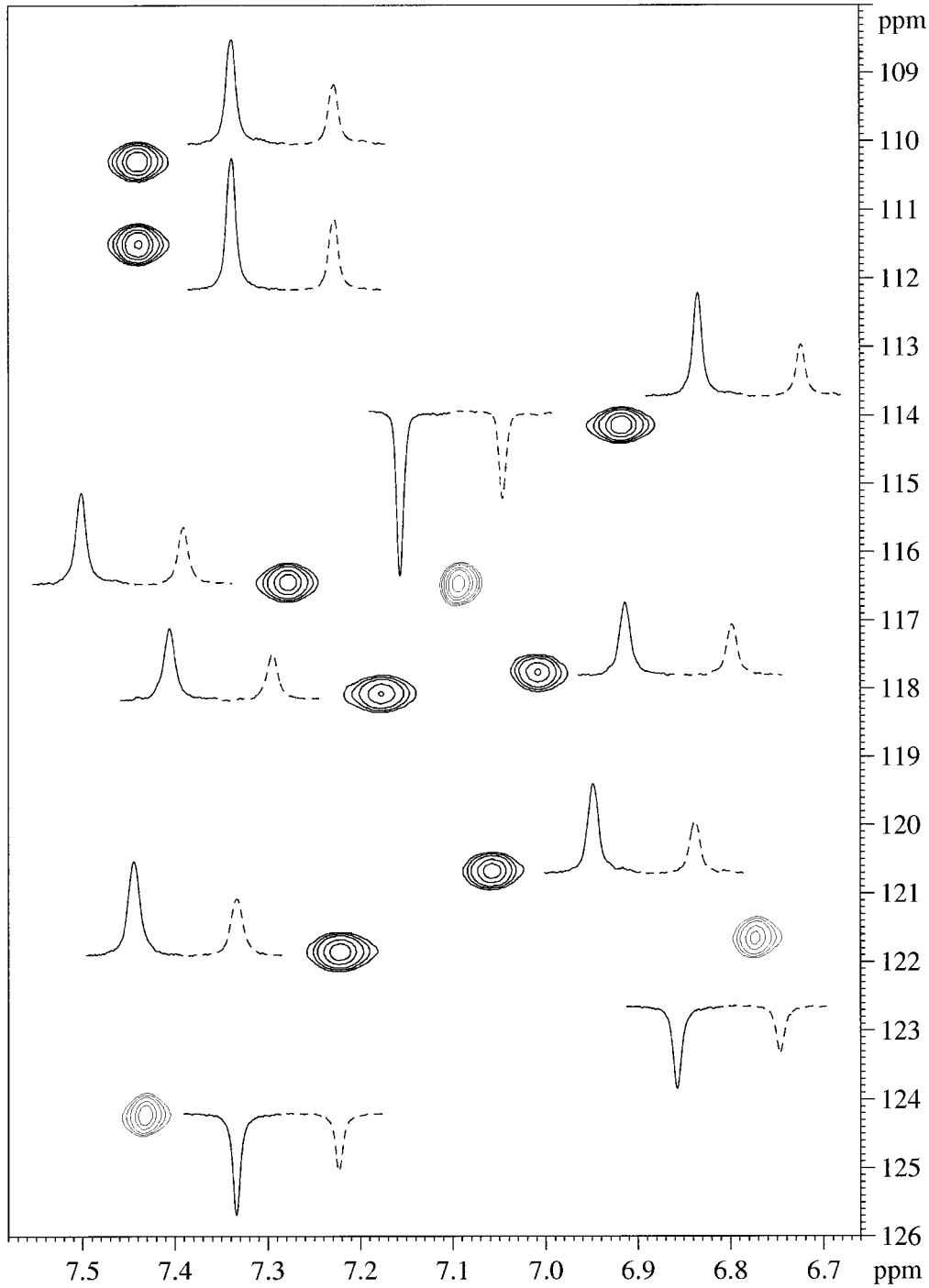


**FIG. 2.** Excerpt from the aromatic region of a $^{1}H–^{13}C$ 2D spectrum of $^{15}N,^{13}C$-labeled RAP 18-112 (90% $H_2O$/10% $D_2O$, 25°C, pH 6.4) recorded with the new constant-time $S^3CT$ TROSY pulse sequence in Fig. 1b on a Varian Unity Inova 750-MHz spectrometer. Sections through the peaks together with the corresponding sections from a spectrum recorded with the sequence in Fig. 1a (dashed lines) under identical conditions are shown. Parameters: relaxation delay 1.5 s with water presaturation, $T = 17.6$ ms; $\tau = 3.21$ ms; $t_1(\max) = 14.25$ ms; 32 scans. GARP was used for $^{13}C$ decoupling in $t_2$. Data matrices of 230 × 4096 points covering 8000 × 10000 Hz were zero-filled to 2048 × 8192 prior to Fourier transformation while the window functions were cosine squared in $t_1$ and cosine in $t_2$.

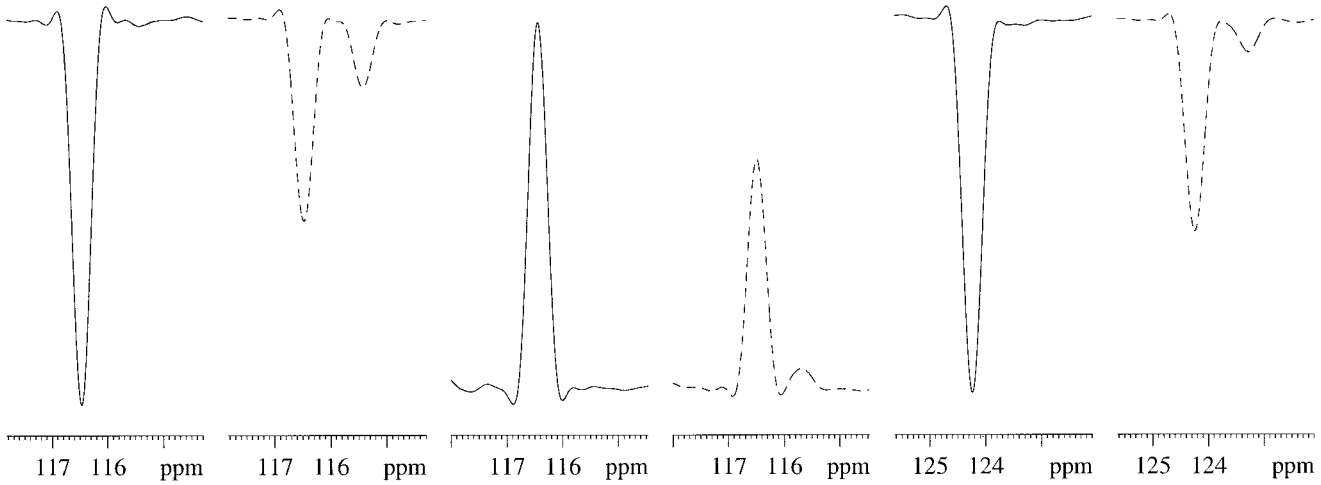


**FIG. 3.** Three $F_1$ sections from the spectrum recorded with the new constant-time $S^3CT$ TROSY pulse sequence in Fig. 1b (solid lines) and the corresponding ones from a spectrum recorded with the sequence in Fig. 1a (dashed lines) where cross talk is clearly visible.

## ACKNOWLEDGMENTS

The spectra presented have been recorded on the Varian Unity Inova 750-MHz NMR spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules at Carlsberg Laboratory. We thank Flemming M. Poulsen for the loan of the $^{13}C,^{15}N$-RAP sample. A.M. is supported by a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft.

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