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Gradient and sensitivity enhancement of 2D TROSY with water flip-back, 3D NOESY-TROSY and TOCSY-TROSY experiments

Guang Zhu*, Xiang Ming Kong & Kong Hung Sze
Department of Biochemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

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Abstract

Previously we demonstrated a sensitivity enhancement of the original TROSY experiment by a factor of $\sqrt{2}$ by the use of the sensitivity enhanced TROSY (en-TROSY) scheme. Here, we develop a gradient and sensitivity enhanced TROSY experiment (gs-TROSY), which is designed to select magnetization transfer pathways that suppress spectral artifacts and reduce the number of required phase cycles while having minimal loss of sensitivity. Both of these experimental methods (en-TROSY and gs-TROSY) have been combined with a water flip-back scheme which provides a further increase in sensitivity for labile NH groups by avoiding water saturation. We also apply these TROSY schemes to 3D NOESY-TROSY and 3D TOCSY-TROSY experiments.

The introduction of multidimensional heteronuclear techniques for proteins labeled with ^{13}C and ^{15}N has greatly facilitated NMR studies of the structure and function of proteins with molecular weights up to 30 kD. However, applications of these techniques to the study of larger proteins and their interactions with ligands are hampered due to the decrease in the molecular tumbling rate, which introduces larger spectral line widths and lower sensitivity into these experiments (Wagner, 1993). It has been recognized that in a high magnetic field, the interference between the dipole-dipole interaction and chemical shift anisotropy can greatly reduce the transverse relaxation rates of ^{1}H and ^{15}N. This line narrowing effect is utilized in transverse relaxation-optimized spectroscopy (TROSY) in which only one component, $(\omega_N + \pi^1 J_{NH}, \omega_H - \pi^1 J_{NH})$, of the four multiplets, $(\omega_N \pm \pi^1 J_{NH}, \omega_H \pm \pi^1 J_{NH})$, within a ^{15}N-^{1}H moiety is observed. The introduction of the TROSY experiment opened a new avenue to the study of very large biomolecules by NMR spectroscopy. Experimental results have demonstrated a striking reduction of spectral line widths in a TROSY ^{15}N-^{1}H spectrum compared to those in an HSQC spectrum at 750 MHz ^{1}H frequency (Pervushin et al., 1997). The much narrower line widths in both dimensions afforded by TROSY make this experiment especially attractive for NMR studies of larger biomolecules.

Previously we demonstrated a sensitivity enhancement of the original TROSY experiment by a factor of $\sqrt{2}$ by the use of the sensitivity enhanced TROSY scheme (en-TROSY) (Zhu et al., 1998a). Here, we develop a gradient and sensitivity enhanced TROSY experiment (gs-TROSY), which is designed to select magnetization transfer pathways that suppress spectral artifacts and reduce the number of the required phase cycles. In addition, the arrangement of the coherence selection gradients in the proposed gs-TROSY experiment avoids sensitivity loss, in a way similar to that proposed for the gradient and sensitivity enhanced HSQC (Kay et al., 1992) and HMQC (Zhu et al., 1998b) experiments. Both of these experimental methods (en-TROSY and gs-TROSY) have been combined with a water flip-back scheme to provide further sensitivity gain for labile NH groups by avoiding water saturation (Grzesiek and Bax, 1993; Stonehouse et al.,

*To whom correspondence should be addressed.

1994; Mori et al., 1995). In our case, an additional 15% on average over all resonance peaks and a maximum of 82% enhancement in sensitivity was obtained when compared with the non-water flip-back version.

To fully take advantage of the line narrowing effect afforded by the 2D TROSY experiment in NMR studies of large proteins, it is preferable to design the corresponding multidimensional experiments to alleviate spectral overlap. Three-dimensional ^{15}N-edited NOESY experiments have been an invaluable tool for obtaining information on inter-proton distances by taking advantage of well dispersed ^{15}N resonances. It has long been recognized that NOE intensities measured in this experiment can be attenuated due to the chemical-exchange processes between water protons and amide protons if water is saturated before acquisition. In this report, we apply the water flip-back en-TROSY to a 3D NOESY-TROSY experiment. This will allow more accurate quantitative analysis of the NOEs of large biomolecules labeled with ^{15}N, because the attenuation of the peak intensities caused by exchange processes is minimized (Jahnke et al., 1995). To further facilitate chemical shift assignment, we also propose a 3D TOCSY-TROSY experiment which is a combination of the TOCSY and en-TROSY experiments.

Figure 1a is the pulse scheme of the gradient and sensitivity enhanced TROSY experiment. The application of gradients G5 and G6 serves to select magnetization transfer. For each t_1 value, two transients are recorded and stored separately as suggested for the sensitivity enhancement technique. As noted in our previous report (Zhu et al., 1998a), the signal intensities of the detectable magnetization of the two transients corresponding to the two phase settings can be expressed as

$$\begin{aligned} M_1 &= 4i \exp(-i\omega_s^{12} t_1 - \theta_1) \exp(-R_{12} t_1) \\ &\quad \exp(i\omega_I^{24} t_2 + \theta_2 - R_{24} t_2) \\ M_2 &= -4i \exp(i\omega_s^{12} t_1 + \theta_1) \exp(-R_{12} t_1) \\ &\quad \exp(i\omega_I^{24} t_2 + \theta_2 - R_{24} t_2) \end{aligned}$$

where R_{12} and R_{24} are the relaxation rates of the individual components ω_s^{12} of ^{15}N spins and ω_I^{24} of ^{1}H spins, $(\omega_s^{12}, \omega_I^{24}) = (\omega_N + \pi^1 J_{NH}, \omega_H - \pi^1 J_{NH})$, $\theta_1 = \gamma_N B^N(z)\tau^N$, and $\theta_2 = \gamma_H B^H(z)\tau^H$, with τ^N and τ^H being the durations of the selection gradient pulses. The gradient settings for signal selection are

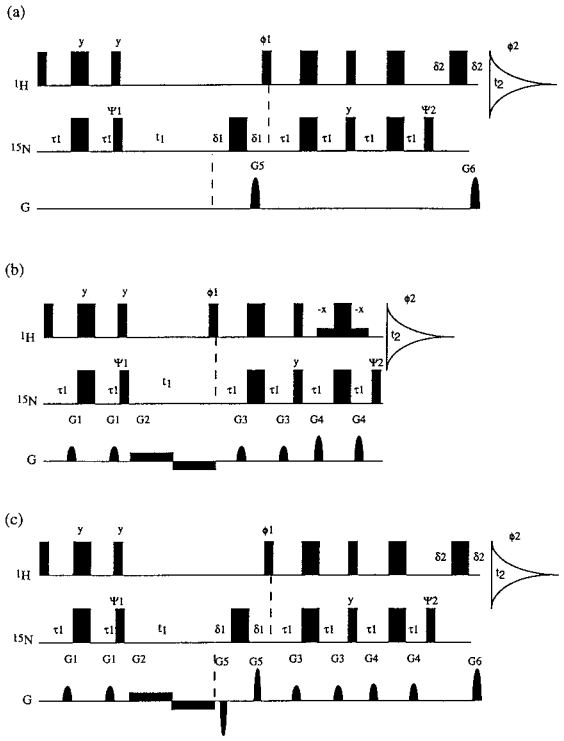

Figure 1. Pulse schemes of ^{15}N-^{1}H TROSY correlation experiments: (a) gs-TROSY, (b) water flip-back en-TROSY, and (c) water flip-back gs-TROSY. The narrow and wide bars represent nonselective 90° and 180° pulses, respectively. Pulses are applied along the x direction unless indicated otherwise. WATERGATE uses a 2.1 ms RF pulse with a power of 110 Hz. The delay τ_1 equals $1/(4\ ^1J_{NH})$ = 2.7 ms, $\delta 1$ = 1.6 ms, $\delta 2$ = 0.25 ms. All gradients are applied along the z-axis. The magnitudes of G1, G3, G4, G5 and G6 are 0.9, 1.1, 21.0, 23.0 and 23.0 G/cm with durations of 0.4, 0.4, 0.5, 1.5 and 0.15 ms, respectively. The bipolar gradient G2 is 0.5 G/cm. Two transients are acquired and stored separately for each increment of t_1. The final absorption spectrum is obtained by the method stated in the text. The first transient is obtained with the use of the phase cycle $\Psi 1$ = y, x, −y, −x; $\phi 1$ = y; $\Psi 2$ = x; $\phi 2$(Rec) = x, −y, −x, y, and the gradient setting is $\theta 1 = \theta 2$. For the second transient, the phase cycle $\Psi 1$ = y, x, −y, −x; $\phi 1$ = −y; $\Psi 2$ = −x; $\phi 2$(Rec) = x, y, −x, −y is used, and the gradient setting is $\theta 1 = -\theta 2$. In (c) the duration of each G5 gradient is 0.75 ms. Please see the Note added in proof.

$\theta_1 = \theta_2$ for the first transient and $\theta_1 = -\theta_2$ for the second transient. After data acquisition, the two transients are, respectively, added and subtracted to form the corresponding real and imaginary parts of FIDs with a 90° phase shift being added to one of the two resultant FIDs. The final absorption spectrum can be obtained by the use of the States data processing method (States et al., 1982). Water suppression in the pulse scheme is achieved by the use of selection gradients G5 and G6 which dephase the water magnetization. Compared

to the non-gradient selection experiment (en-TROSY), two 180° pulses and additional delays, $2\delta_1$ and $2\delta_2$, are needed and this introduces a sensitivity loss due to relaxation and imperfections in the 180° pulses. An additional sensitivity loss, when using selection gradients, is due to non-negligible relaxation (T_{1H}) induced by ^{1}H-^{1}H homonuclear dipole-dipole interactions (Pervushin et al., 1997) which can be minimized by complete deuteration of all but the amide protons. Despite a sensitivity loss (13% in the experiment shown below) when compared with the en-TROSY a further advantage of the gs-TROSY experiment is that no water selection pulse is needed, hence, NH peaks close to or overlapping with the water signal can be observed.

Figure 1b depicts the pulse sequences of a water flip-back sensitivity enhanced TROSY experiment. In this pulse sequence, the gradients G1 and G3 not only serve to eliminate imperfections in the 180° pulse, but also minimize radiation damping of the water magnetization due to its dephasing and rephasing by the pulsed field gradients. In the t_1 evolution period, bipolar gradients are applied to suppress radiation damping of the water magnetization (Sklenar, 1995; Andersson et al., 1998). These gradients must be weak to avoid loss of magnetization and line-broadening by diffusion. The phases of the 90° and 180° ^{1}H pulses are designed so that the water magnetization is flipped back to the +z-axis before the WATERGATE scheme. This approach minimizes the number of water selection pulses that could introduce artifacts and reduce sensitivity. The WATERGATE scheme is then used to eliminate any residual water magnetization on the transverse plane introduced by pulse imperfection and residual radiation damping while leaving the major portion of water magnetization in the +z direction. Figure 1c shows a gradient and sensitivity enhanced TROSY experiment with a water flip-back, similar to the pulse sequence in Figure 1b. In this pulse sequence, the ^{15}N selection gradient G5 is arranged in such a way that it leaves the water magnetization unaffected. The ^{1}H selection gradient G6 refocuses the signal and dephases any residual water magnetization on the transverse plane.

We also propose 3D NOESY-TROSY and 3D TOCSY-TROSY experiments with the use of the sensitivity enhanced TROSY scheme as a basic building block. The pulse sequence for the 3D NOESY-TROSY experiment is $90°(\phi3) - t_1 - 90°(x) - \tau_m -$ (water flip-back en-TROSY) with the phase cycle $\phi3 = 4(x), 4(-x)$; $\phi2(\text{Rec}) = x, -y, -x, y, -x, y, x, -y$. It is well known that for a high field spectrometer with a high-Q probe, radiation damping is very effective. This will restore a major portion of the water magnetization from the −z-axis or the transverse plane to the +z-axis by the end of a typical NOE mixing period (Talluri and Wagner, 1996). Hence, a 3D NOESY-TROSY experiment with optimized sensitivity can be achieved by a simple extension of the water flip-back en-TROSY scheme described above. Similarly, a 3D NOESY-TROSY experiment can also be designed with the use of the water flip-back gs-TROSY scheme. For ^{15}N labeled large biomolecules, relaxation of aliphatic ^{1}H magnetization is dominated by the homonuclear dipole-dipole interaction. This relaxation time can be increased by partial random deuteration resulting in even narrower linewidths in the proposed 3D NOESY-TROSY experiment (LeMaster and Richards, 1988; Torchia et al., 1988; Reisman et al., 1991; Nietlispach et al., 1996), which would greatly facilitate the study of large proteins. The corresponding pulse sequence for the 3D TOCSY-TROSY experiment is $90°(\phi3) - t_1 -$ DIPSI-2(y) − (en-TROSY*) with the phase cycle $\phi3 = 4(x), 4(-x)$; $\phi2(\text{Rec}) = x, -y, -x, y, -x, y, x, -y$. Note that en-TROSY* means the en-TROSY sequence with the first 90° ^{1}H pulse removed.

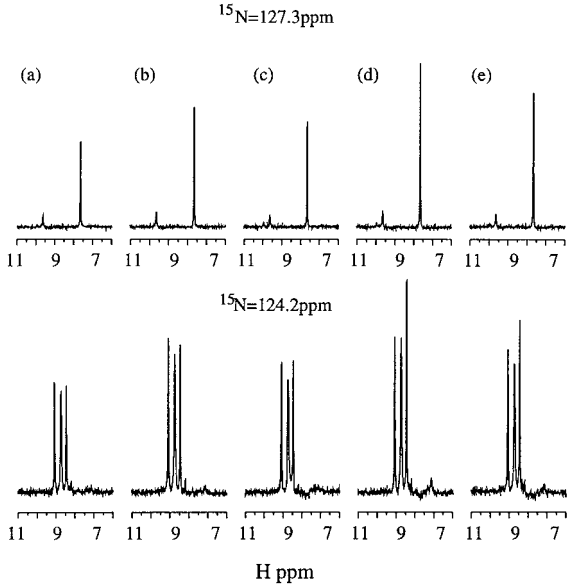

Figure 2. 1D cross-sections taken at ^{15}N = 127.3 ppm and 124.2 ppm from 2D TROSY experiments, recorded under the same conditions: (a) the original TROSY, (b) en-TROSY, (c) gs-TROSY, (d) water flip-back en-TROSY and (e) water flip-back gs-TROSY. The noise levels of the spectra are scaled to be the same. All experimental parameters are set to be the same except for those that are specific to a particular pulse sequence.

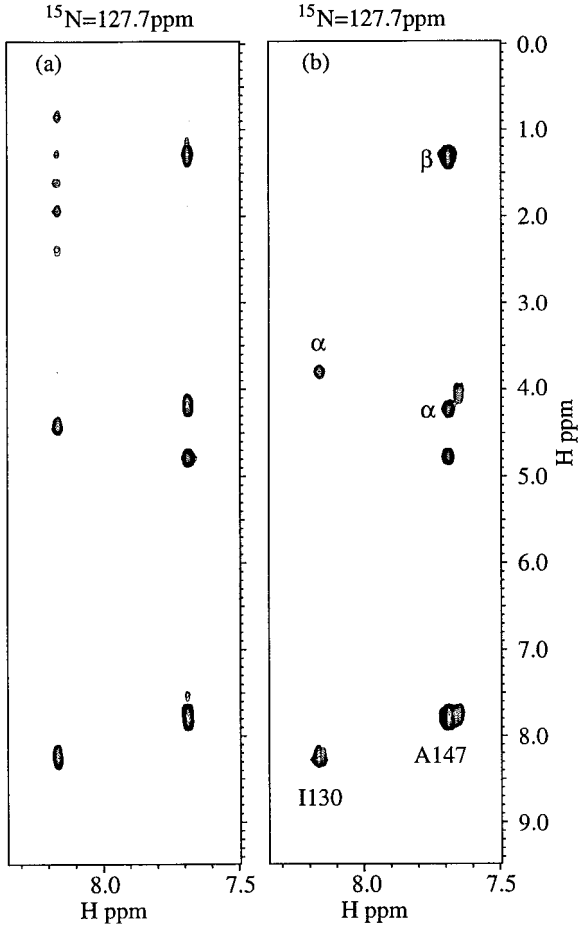

Figure 3. (a, b) 2D cross-sections taken from 3D spectra (at ^{15}N = 127.7 ppm) recorded with the proposed 3D NOESY-TROSY and 3D TOCSY-TROSY experiments. Quadrature detection in the F1 dimension was obtained via the States-TPPI method by increasing phase $\phi 3$. Absorption mode in the t_2 domain is obtained by the sensitivity enhanced TROSY experiment as described in the text. The NOE mixing time is 100 ms and the TOCSY mixing time is 55 ms with a power of 3.2 KHz. The peak at ^{1}H = 4.8 ppm is the exchange peak between water and NH of A147 at the C terminus of the protein.

The effectiveness of all the experiments is demonstrated on a ^{15}N labeled calmodulin sample. Recordings were made at 15 °C on a Varian Inova 500 MHz NMR spectrometer, with the protein concentration being 1 mM in 90% H_2O, 10% D_2O at pH 6.8. Figures 2a-e display 1D cross-sections of the 2D ^{15}N-^{1}H correlation spectra of calmodulin recorded with the original, en-TROSY experiments and the pulse sequences shown in Figures 1a-c, respectively. Based on a comparison of the S/N (signal-to-noise ratio) of 116 resolved peaks, the gs-TROSY is about 13% less sensitive than the en-TROSY experiment. The reasons for the sensitivity loss were given above. The en-TROSY, and gs-TROSY experiments with water flip-back (Figures 1b-c) are, on average over all resonance peaks, 14% (maximum 72%) and 15% (maximum 82%) more sensitive than their corresponding non-water flip-back versions. Figure 3 displays 2D cross-sections of (a) 3D NOESY-TROSY and (b) 3D TOCSY-TROSY spectra taken at ^{15}N = 127.7 ppm. The 3D FID matrices for both experiments are $64^* \times 32^* \times 2048^*$, with * denoting complex numbers. The spectra were processed with the use of linear prediction in the t_1 and t_2 time domains to extend the size of the FIDs by 50% (Zhu and Bax, 1992; Delaglio et al., 1995). A sine square bell window function was used before Fourier transformation.

In conclusion, the introduction of the TROSY experiment makes NMR a promising technique for the study of the structure and function of larger biomolecules. We have introduced a gradient and sensitivity enhanced TROSY experiment (gs-TROSY) with minimal sensitivity loss being caused by the application of a gradient selection scheme. This experiment can be used to study amide protons that are close to the water resonance. It can also be applied effectively to triple resonance experiments. The en-TROSY and the gs-TROSY experiments with water flip-back have also been introduced to minimize signal loss due to gradient saturation, and have resulted in significant sensitivity enhancements over the original TROSY experiment. In addition, we report 3D NOESY-TROSY and 3D TOCSY-TROSY experiments. These experiments will certainly be very useful in a wide range of applications involving NMR studies of large biomolecules.

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Note added in proof

After this manuscript had been submitted for publication, a paper describing a similar gradient-enhanced TROSY experiment has appeared (Pervushin, K., Wider, G. and Wüthrich, K. (1998) *J. Biomol. NMR*, **12**, 345–348). When converting pulse sequences from Bruker to Varian and vice versa it is necessary to invert the y-axis receiver and pulse phases. Due to phase cycling and decoupling schemes, the inversion of the y-axis pulse phases does not have an effect in most pulse sequences. In the case of TROSY based sequences this phase inversion is significant. For the pulse sequences in Figure 1 the phase of the second 90° proton pulse must be +y for Varian and −y for Bruker. If this phase is inverted then the 'steady-state' enhancement, described by Pervushin et al. [(1988) *J. Am. Chem. Soc.*, **120**, 6394–6400] will be lost.

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