Single Scan, Sensitivity- and Gradient-Enhanced TROSY for Multidimensional NMR Experiments

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The TROSY experiment[1] has been shown to increase the sensitivity of ^{1}H−^{15}N and ^{1}H−^{13}C correlation experiments by selecting the multiplet component which relaxes most slowly due to cross-correlation between dipole−dipole and chemical shift anisotropy relaxation.[2] This enhancement is most pronounced for slowly tumbling macromolecules at high magnetic field strength.[1−3] As in sensitivity-enhanced HSQC experiments,[4] the TROSY experiment can be conducted in a way where all of the magnetization components precessing during the evolution time t_1 are turned into observable magnetization, increasing the sensitivity by $\sqrt{2}$ compared to the original experiment.[5] This sensitivity-enhancement scheme requires the separate storage of at least 4 scans per t_1 value and some nonstandard data rearrangement before Fourier transformation. Here a gradient-enhanced version of the TROSY experiment is presented which achieves the sensitivity-enhancement with a single scan per FID (two scans per t_1 value), and thus, it is an attractive building block for 3D and 4D NMR as well as for amide proton-exchange experiments. Data processing is identical to that of established echo−antiecho experiments.[4]

Figure 1a shows the pulse sequence of the sensitivity- and gradient-enhanced ^{15}N TROSY experiment (^{15}N SG-TROSY). Compared to the original TROSY pulse sequence,[1] a 180°(^{15}N) and a 180°(^{1}H) pulse with delays δ and ϵ have been added to accommodate the pulsed field gradients (PFG) for coherence order selection. Any of the four different multiplet components of a ^{15}N−^{1}H cross-peak can be selected by different initial settings of the phases ϕ_1 and ϕ_2,[6] while signals from NH_2 and NH_3 groups are suppressed.[5] Starting from proton magnetization H_z, a description in terms of Cartesian product operators[7] yields the following terms by the end of the evolution period t_1:

$$\cos(\omega_N t_1)\cos(\pi J_{HN} t_1)2N_xH_z + \sin(\omega_N t_1)\cos(\pi J_{HN} t_1)2N_yH_z + \cos(\omega_N t_1)\sin(\pi J_{HN} t_1)N_y - \sin(\omega_N t_1)\sin(\pi J_{HN} t_1)N_x \quad (1)$$

This magnetization is defocused by the gradients g_1. All terms are transferred to observable ^{1}H magnetization by the following pulse sequence.[5] The gradient g_4 refocuses the ^{1}H magnetization, leading to the following terms before detection:

For $\phi_1 = y$, $\phi_2 = x$, $g_1 = |\gamma_H/2\gamma_N|g_4$:

$$-0.5\sin[(\omega_N - \pi J_{HN})t_1](H_x - 2H_xN_z) - 0.5\cos[(\omega_N - \pi J_{HN})t_1](H_y - 2H_yN_z) \quad (2)$$

and for $\phi_1 = -y$, $\phi_2 = -x$, $g_1 = -|\gamma_H/2\gamma_N|g_4$:

$$-0.5\sin[(\omega_N - \pi J_{HN})t_1](H_x + 2H_xN_z) + 0.5\cos[(\omega_N - \pi J_{HN})t_1](H_y + 2H_yN_z) \quad (3)$$

Expressions 2 and 3 represent the P and N peaks of the cross-peak multiplet component which is low-field in the ^{15}N dimension and high-field in the ^{1}H dimension. Recording the P- and N-type signal for each t_1 value of the 2D experiment allows a phase-sensitive representation of the spectrum.[4]

Since the PFGs provide coherence order selection, a single scan per FID is sufficient to record a clean spectrum (Figure 2). If the ^{1}H pulses of the initial INEPT sequence are not phase-cycled, the steady-state ^{15}N magnetization contributes to the signal intensity, too.[3] The phase settings of Figure 1 ensure the constructive interference with the narrow low-field ^{15}N doublet component.[8]

Since the pulse sequence element of Figure 1a does not require phase cycling, it is a convenient building block in multidimensional NMR experiments. We have tested implementations in a 3D HNHB (Figure 1b) and a 3D HNCA experiment (Figure 1c). In the original HNHB experiment, magnetization is transferred from the amide nitrogen to the Hβ protons during a long dephasing delay, typically 38 ms.[9] Since the $^3J_{NH\beta}$ coupling is at most about 5 Hz, a longer coupling evolution delay would be advantageous. The HNHB experiment of Figure 1b uses selective pulses to excite the Hα and Hβ resonances and a selective inversion pulse to refocus the $^3J_{NH\beta}$ couplings during the constant time evolution period. Since these pulses do not disturb the spin-state of the amide protons, the slowly and rapidly relaxing doublet components of the nitrogen spins are not interchanged. The slowly relaxing component is transferred to ^{1}H magnetization by the spin-state selective coherence transfer sequence. As an additional benefit of spin-state selectivity, passive $^1J_{HN}$ couplings during the dephasing delay, T, no longer interfere with the magnetization transfer.[9] The intensities of the HNHB cross-peaks are proportional to $\sin(\pi J_{NH\beta}T)\sin[\pi J_{NH\beta}(T + \epsilon')]$, where ϵ' corresponds to the effective coupling evolution time during the delay, 2ϵ, and the adiabatic inversion pulse.[10] To evaluate the $^3J_{NH\beta}$ coupling constants quantitatively, a reference 2D experiment can be recorded,[11] omitting the selective 90°(^{1}H) pulses and the corresponding phase cycle of the receiver phase. In this reference experiment, t_1 can be incremented together with the delay, t_2, so that the maximum $t_1 + t_2$ value is identical to that of the 3D experiment.

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(8) Because of the different signs of the one-bond J_{HN} and J_{HC} couplings, the phase of the first or second 90° (^{1}H) pulse must be inverted to obtain constructive interference in a ^{13}C TROSY experiment (ref 3). Yet, the same sign combination in ϕ_1, ϕ_2, and g_1 selects the same multiplet component in both dimensions in ^{13}C SG-TROSY as in ^{15}N SG-TROSY. The phase cycle given in ref 5 eliminates the contribution of the ^{15}N equilibrium magnetization; including the ^{15}N steady-state magnetization would increase the sensitivity for the low (high)-field ^{15}N doublet component and decrease it for the high (low)-field one. Maximum sensitivity in the β-HSQC-β subspectrum of the generalized ^{15}N TROSY experiment of ref 5 would be obtained with the phase settings of Figure 1a, except that the phases of the last two 90° (^{1}H) pulses have to be inverted to preserve the water flip-back effect and the data processing protocol.
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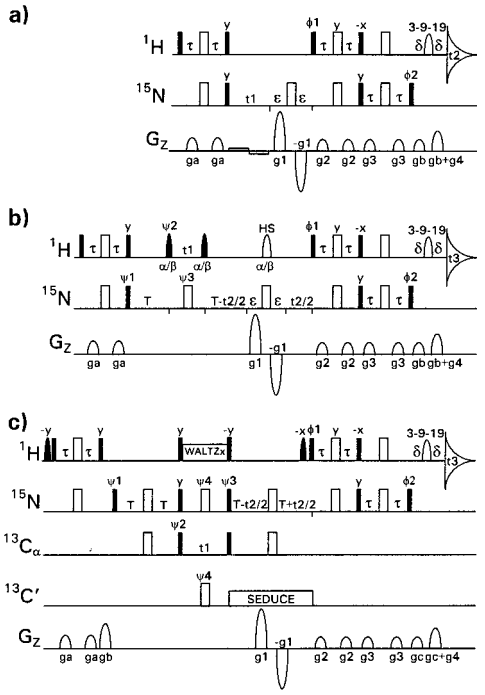

Figure 1. Pulse schemes with spin-state selective-, sensitivity-, and gradient-enhanced ^{15}N−^{1}H magnetization transfer. Filled (open) pulses are applied with flip angles of 90° (180°). Bell-shaped pulses are selective. Pulse phases are *x* unless indicated otherwise. P- and N-type signals of the most slowly relaxing cross-peak component is selected with $\phi_1/\phi_2 = y/x$ and PFG signs as indicated (P-peak), and with $\phi_1/\phi_2 = -y/-x$ and inverted sign of the PFGs g_1 (N-peak). PFGs are applied with a duration of 1 ms and a sine-bell-shaped envelope. (a) ^{15}N SG-TROSY. Parameters: $\epsilon = \delta = 1.4$ ms (PFG duration + recovery delay), $\tau = 1/(4^1J_{NH}) = 2.7$ ms, $g_{1,2,3,4,a,b} = 15, 1.5, 1.5, 3.05, 1.5, 3.0$ G/cm, bipolar gradients[16] 0.5 G/cm. The phase of the first 90° (^{15}N) pulse may be phase-alternated together with the receiver phase. Axial peak artifacts are shifted to the side of the spectrum by inverting the phases of all ^{15}N pulses before t_1 and the receiver phase with each t_1 increment. The pulse sequence provides water flip-back. For enhanced water suppression, the last 180° (^{1}H) pulse is implemented as a 3−9−19 pulse.[15] With magic angle PFGs,[17] acceptable water suppression was also obtained with a hard 180° pulse as the last ^{1}H pulse. This requires, in addition, phase inversion of the last two 90° (^{1}H) pulses to maintain the water flip-back effect.[14] (b) HNHB experiment with spin-state selection. The ^{1}H pulses immediately preceding and following t_1 are 1.6 ms E-BURP-2 and time reversed E-BURP-2 pulses, respectively.[18] The 180° (^{1}H) pulse, labeled HS, is a hyperbolic secant pulse[19] of 3.4 ms duration. The delay *T* can be adjusted for optimum sensitivity and is typically 30−70 ms. PFGs as in (a), except that $g_b = 10$ G/cm. Phase cycle: $\psi_1 = y, -y$; $\psi_2 = 2(x), 2(-x)$; $\psi_3 = 4(x), 4(-x)$; receiver = $x, -x, -x, x$. (c) HNCA experiment with spin-state selection. Selective 90° (^{1}H) pulses are 2 ms long and applied to the water resonance. A 12.5-kHz RF field was used for WALTZ decoupling. $T = 12.8$ ms. PFGs as in (b), except that $g_{b,c} = 5, 8$ G/cm. Phase cycle: $\psi_1 = x, -x$; $\psi_2 = 2(x), 2(-x)$; $\psi_3 = 4(x), 4(-x)$; $\psi_4 = 8(x), 8(-x)$; receiver = $x, -x, -x, x, -x, x, x, -x$.

The HNCA pulse sequence of Figure 1c was derived from the experiment by Grzesiek and Bax.[12] To minimize relaxation of the Cα spins during t_1, the proton magnetization is flipped to the *x*-axis, spin-locked during t_1 and flipped back to the *z*-axis afterward. The spin-state of the amide protons is preserved during the first two delays, *T*, restored after t_1, and again preserved during the following constant time evolution period. In this way, the most slowly relaxing ^{15}N doublet component is maintained throughout most of the pulse sequence.

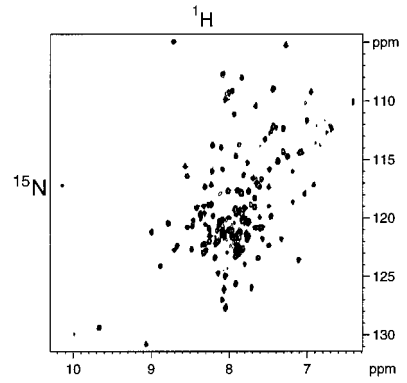

Figure 2. ^{15}N SG-TROSY spectrum of a 0.7 mM sample of ^{2}H/^{13}C/^{15}N-labeled N-terminal domain of *E. coli* DnaB DnaB(1-161),[20] recorded at 32 °C and pH 7.5 on a Bruker DMX 600 NMR spectrometer using the scheme of Figure 1a, except that an adiabatic 180° (^{13}C) inversion pulse was applied in the middle of t_1 to refocus ^{15}N−^{13}C couplings. The spectrum was recorded in 8 min with t_{1max} (t_{2max}) of 65 (114) ms (1024 (t_2)*200 (t_1) complex points), using one scan per FID. Average amplitudes were 1.5 ± 0.2 higher than in a standard FHSQC experiment,[21] and 1.05 ± 0.1 higher than in a corresponding sensitivity-enhanced HSQC experiment with WATERGATE and a selective water flip-back pulse preceding the pulse sequence.[14,15,22] For this protein sample, $\tau_c = 14$ ns as determined from ^{15}N T_1 and T_2 relaxation measurements.

The experimental schemes of Figure 1b and 1c were tested at 600 MHz ^{1}H frequency with a 2.3 mM sample of ^{15}N-labeled *Escherichia coli* DnaB(24-136) at 32 °C and pH 7.5. With this sample, for which a rotational correlation time, τ_c, of 10 ns was determined from ^{15}N T_1 and T_2 relaxation times,[13] the HNHB experiment showed maximum sensitivity at $T = 50$ ms. Compared to an HNHB spectrum recorded with the experiment of Madsen et al.,[9] ($T = 37$ ms), the average sensitivity was improved 1.4-fold, with a standard deviation of ±0.4. On the other hand, the HNCA experiment, recorded with $2T = 25.6$ ms, was 5−25% less sensitive than a standard ^{1}H decoupled HNCA experiment recorded with identical delays, water flip-back and WATERGATE, but without sensitivity enhancement.[4,12,14,15] The HNCA experiment of Figure 1c and related spin-state selective "out-and-back" type experiments would be attractive at higher magnetic fields.

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Note Added in Proof. A very similar TROSY scheme has recently been published: Pervushin, K. V.; Wider, G.; Wüthrich, K. *J. Biomol. NMR* **1998**, *12*, 345−348.

Supporting Information Available: Product operator analysis for SG-TROSY experiments selecting different multiplet components (1 page, print/PDF). See any current masthead page for ordering information and Web access instructions.

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