JOURNAL OF MAGNETIC RESONANCE **134,** 158–160 (1998)
ARTICLE NO. MN981483

Sensitivity Enhancement in the TROSY Experiment

M. Czisch[1] and R. Boelens

Bijvoet Center for Biomolecular Research, University Utrecht, 3584 CH Utrecht, The Netherlands

Received February 11, 1998; revised April 23, 1998

A modification of the TROSY experiment which allows for sensitivity enhancement without introduction of additional delays is presented. The method relies on the combination of two orthogonal pathways and results in a signal-to-noise gain of $\sqrt{2}$ independent of the size of the molecule. The sensitivity enhanced TROSY scheme can be combined with gradient coherence selection. The method has been applied to the 269 residue serine protease PB92. © 1998 Academic Press

Key Words: **HSQC; ^{15}N NMR; protein NMR; serine protease; gradient spectroscopy.**

Recently, a new heteronuclear correlation experiment (TROSY) was introduced by Pervushin *et al.* (*1*) who exploited constructive interference between dipolar relaxation and relaxation due to chemical shift anisotropy to drastically reduce the linewidth in both dimensions at higher field strengths. The method has clear advantages at high fields for large proteins when compared to the regular HSQC experiment (*2*). It has been shown that the regular HSQC experiment can be improved by exploiting the preservation of two orthogonal magnetization pathways (*3, 4*). This scheme was not used in the original experiment by Pervushin *et al.* Modification of the TROSY experiment allows for an additional sensitivity enhancement (SE) by separately storing the pathways which are phase modulated in t_1 as $-i \exp(i\omega_{12}t_1)$ and $i \exp(-i\omega_{12}t_1)$, as described below. These two pathways can be combined by simple addition or subtraction, leading to two independent spectral data sets. In full analogy to the sensitivity enhancement scheme by Kay *et al.* (*5*) the new pulse scheme can also be adapted with a gradient coherence selection scheme instead of water suppression by WATERGATE (*6*).

The original sequence by Pervushin *et al.* (*1*) is reproduced in Fig. 1. They showed that the magnetization pathway corresponding to the slowest relaxing coherence is selected in an eight-step phase cycle: $\psi_1 = \{y, -y, -x, x, y, -y, -x, x\}$; $\psi_2 = \{4(x), 4(-x)\}$; $\phi_1 = \{4(y), 4(-y)\}$; receiver $= \{x, -x, -y, y, x, -x, y, -y\}$. A detailed description of the pathway selection by the phase cycle can be found in the original paper (*1*) and will not be reproduced here. Only the major features shall be pointed out: even and uneven phase cycling steps serve for selecting the ^{15}N pathway. Phase cycling steps n and $n + 2$ select for the slowly relaxing magnetization component ω_{12} while keeping both the sine and the cosine component of the signal. The distinction between the latter components is made by adding the first four phase cycling steps to the last four steps. In the originally proposed experiment this leads to an inevitable loss of one of the components.

Sensitivity enhancement can be achieved in two ways:

(I) Two different experiments are performed with four scans each corresponding to the first half and the second half of the original experiment, respectively (Experiment 1: $\psi_1 = \{y, -y, -x, x\}$; $\psi_2 = x$; $\phi_1 = y$; receiver $= \{x, -x, -y, y\}$; Experiment 2: $\psi_1 = \{y, -y, -x, x\}$; $\psi_2 = -x$; $\phi_1 = -y$; receiver $= \{x, -x, y, -y\}$). Since frequency discrimination is achieved by incrementation of ψ_1 in a STATES-type manner (*7*) the time domain data of both independent experiments can be combined by addition (resulting in the sine component) and subtraction (resulting in the cosine component). The two data sets can be processed in the normal way and added afterward to give the sensitivity enhanced spectrum. The sine and the cosine spectra contain the same signal intensity since no additional delay had to be introduced in the original sequence. This results in a full signal-to-noise gain of $\sqrt{2}$ when added.

(II) A phase modulated data set can be produced by storing the first FID recorded with the same phase cycle as that in method I. The second FID will have a slightly modified phase cycle: $\psi_1 = \{-x, x, y, -y\}$; $\psi_2 = -x$; $\phi_1 = -y$; receiver $= \{x, -x, -y, y\}$. The resulting data can be treated like an echo–antiecho data set and processed accord-

[1] To whom correspondence should be addressed.

1090-7807/98 $25.00
Copyright © 1998 by Academic Press
All rights of reproduction in any form reserved.

ingly. The echo–antiecho modification also allows for additional implementation of coherence selection gradients which can drastically improve the performance of the experiment by better artifact suppression. In principle, the introduction of additional gradient delays will allow for the full S/N gain of $\sqrt{2}$. In practice, introduction of additional gradient delays might reduce this gain slightly. In our case the S/N gain was found to be 1.35. For molecules with a molecular weight beyond 30 kDa approach (I) or approach (II) without gradient coherence selection might therefore be advantageous.

The sensitivity-enhanced TROSY experiment was tested on a ^{15}N-labeled mutant of serine protease PB92 (*8*), a

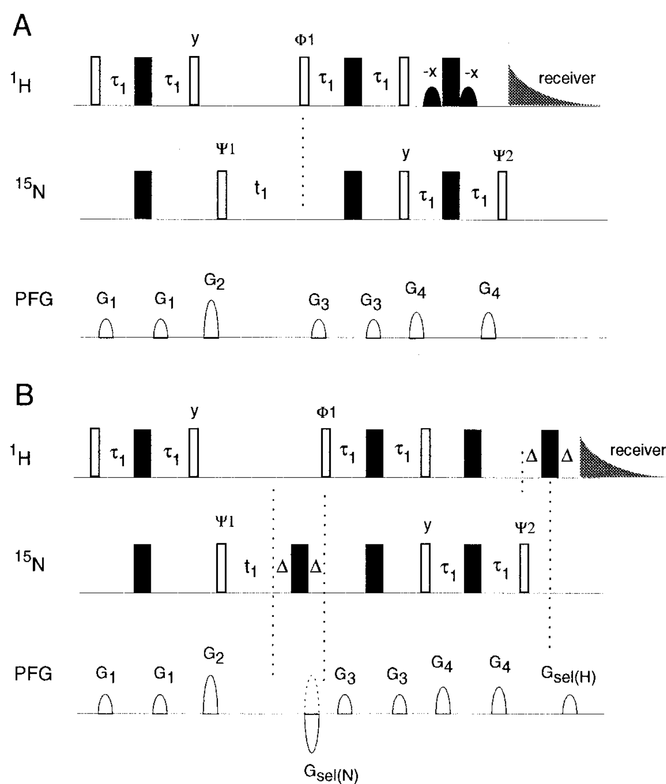

FIG. 1. (A) TROSY sequence as described by Pervushin *et al.* (*1*). Open and filled rectangles correspond to 90 and 180 degree pulses, respectively. τ_1 corresponds to $1/(4\ J_{NH})$. All pulses are applied out of the x direction if not stated otherwise. Phase cycling is as described in the text. Water suppression is achieved by the WATERGATE sequence, and frequency discrimination in F_1 by shifting the phase ψ_1 according to the STATES method. (B) Sensitivity-enhanced TROSY with gradient coherence selection. The gradients $G_{sel(H)}$ and $G_{sel(N)}$ are placed in spin-echo blocks ($\Delta = 800\ \mu s$) to avoid chemical shift evolution. Phase cycling is as described in the text. Frequency discrimination in F_1 is achieved by echo–antiecho combination. Gradient pulses had a duration of 400 μs with a relative gradient strength of $G_1 = 0$, $G_2 = 27$, $G_3 = 15$, $G_4 = 73$, $G_{sel(H)} = 10.134$, $G_{sel(N)} = -100$ for uneven FIDs, $G_{sel(N)} = +100$ for even FIDs.

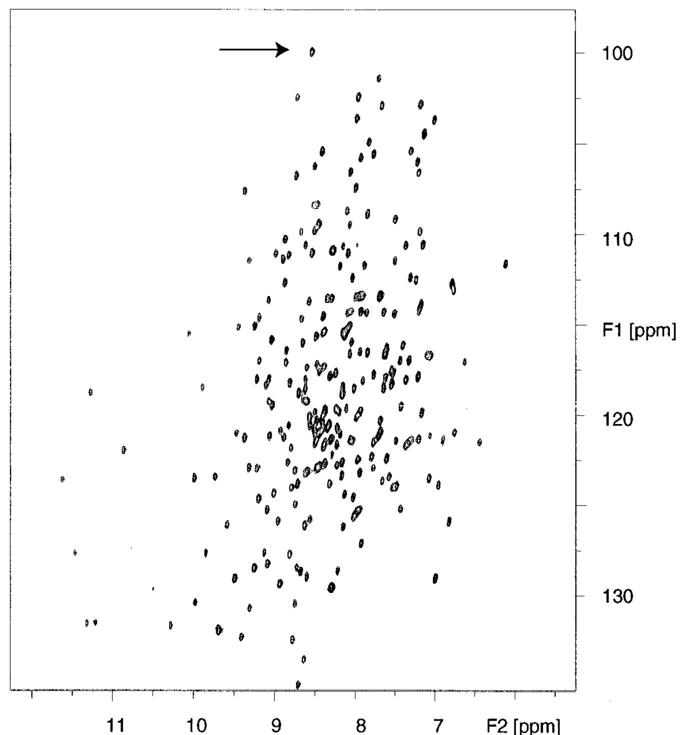

FIG. 2. N–H correlation spectrum of serine protease PB92 as obtained by pulse sequence 1B with eight scans per FID. The position of Gly 223 is indicated by an arrow at $F_1 = 100.57$ ppm.

protein containing 269 residues, at 600 MHz on a BRUKER AMXII spectrometer equipped with a BGU-II z-gradient accessory. Sample conditions were 50 m*M* NaAc buffer, pH 5.0 and 315 K. Spectra were acquired accumulating eight scans per FID for the reference TROSY (*1*) and SE HSQC (*5*), and for the SE TROSY acquired using method II. Two times four scans were measured for SE TROSY with method II, giving the same total experimental time. In all cases 160 complex points in F_1 and 1 K complex points in F_2 were recorded. The spectral width was 10 kHz and 2227 Hz in F_2 and F_1, respectively. Figure 2 shows the heteronuclear correlation acquired with the pulse sequence of Fig. 1B. Figure 3 shows traces through the correlation spectra at the F_1 position of Gly 223. It is obvious that our proposed sensitivity enhanced pulse sequence (method I) gives the theoretically expected sensitivity gain as compared to the original sequence. At 600 MHz the signal-to-noise ratio of the SE TROSY comes close to the values as obtained by the classical SE HSQC scheme. The advantage of the TROSY experiment at higher field strength was predicted by Pervushin *et al.* (*1*). Our modification will result in a further sensitivity gain of $\sqrt{2}$ in this promising TROSY technique, independent

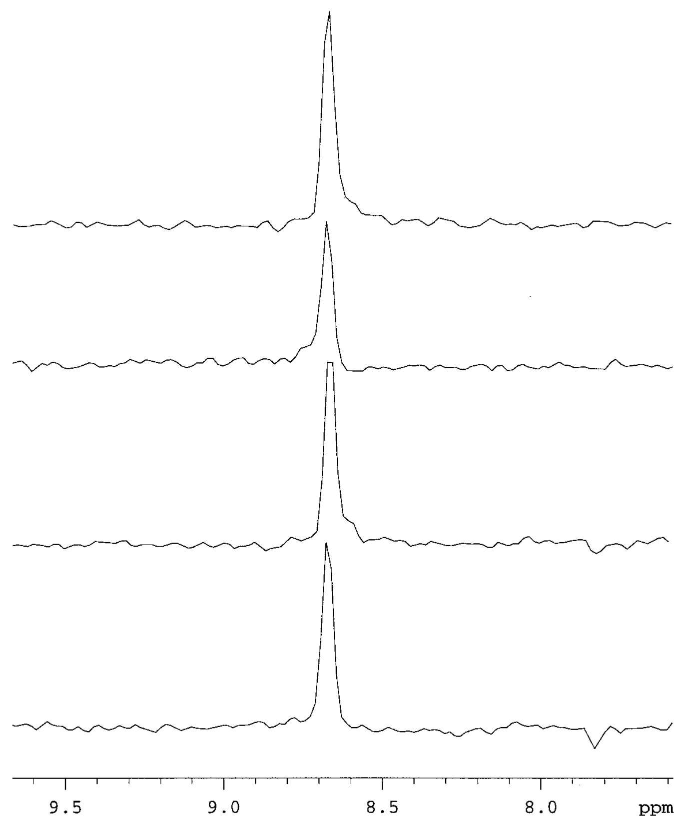

FIG. 3. One-dimensional extractions at the position of Gly 223 of the correlation maps acquired with SE HSQC, TROSY, and SE TROSY of method I, and SE TROSY of method II, from top to bottom, respectively. The signal-to-noise ratios relative to the SE HSQC are 1, 0.7, 0.98, and 0.95, respectively.

of the molecular weight. The modification can be implemented in many NMR experiments that will be based on the TROSY principle.

ACKNOWLEDGMENTS

The spectra were recorded at the SON NMR Large-Scale Facility in Utrecht, The Netherlands. We thank the EC for support (Contract ERBFMGECT-950032, to M.C.). We thank Dr. R. Bott for making available the ^{15}N-labeled serine protease sample.

REFERENCES

1. K. Pervushin, R Riek, G. Wider, and K. Wüthrich, Attenuated T2 relaxation by mutual cancellation of dipole–dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules, *Proc. Natl. Acad. Sci. U.S.A.* **94,** 12366–12371 (1997).
2. G. Bodenhausen and D. J. Ruben, Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy, *Chem. Phys. Lett.* **69,** 185–189 (1980).
3. J. Cavanagh, A. G. Palmer III, P. E. Wright, and M. Rance, Sensitivity improvement in proton-detected two-dimensional heteronuclear relay spectroscopy, *J. Magn. Reson.* **91,** 429–436 (1991).
4. A. G. Palmer III, J. Cavanagh, P. E. Wright, and M. Rance, Sensitivity improvement in proton-detected two-dimensional heteronuclear correlation NMR spectroscopy, *J. Magn. Reson.* **93,** 151–170 (1991).
5. L. E. Kay, P. Keifer, and T. Saarinen, Pure absorption gradient enhanced heteronuclear single quantum correlation spectroscopy with improved sensitivity, *J. Am. Chem. Soc.* **114,** 10663–10665 (1992).
6. M. Piotto, V. Saudek, and V. Sklenář, Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions, *J. Biomol. NMR* **2,** 661–665 (1992).
7. D. J. States, R. A. Haberkorn, and D. J. Ruben, A two-dimensional nuclear Overhauser experiment with pure absorption phase in four quadrants, *J. Magn. Reson.* **48,** 286–292 (1982).
8. J. R. Martin, F. A. A. Mulder, Y. Karimi-Nejad, J. van der Zwan, M. Mariani, D. Schipper, and R. Boelens, The solution structure of serine protease PB92 from *bacillus alcalophilus* presents a rigid fold with a flexible substrate-binding site, *Structure* **5,** No. 4, 521–532 (1997).