TROSY NMR with partially deuterated proteins

Alexander Eletsky[a], Alexander Kienhöfer[b] & Konstantin Pervushin[a,*]

[a]*Laboratorium für Physikalische Chemie, and* [b]*Laboratorium für Organische Chemie, Eidgenössische Technische Hochschule Hönggerberg, CH-8092 Zürich, Switzerland*

Abstract

TROSY-type optimization of liquid-state NMR experiments is based on the preservation of unique coherence transfer pathways with distinct transverse relaxation properties. The broadband decoupling of the ^{1}H spins interchanges the TROSY and anti-TROSY magnetization transfer pathways and thus is not used in TROSY-type triple resonance experiments or is replaced with narrowband selective decoupling. To achieve the full advantage of TROSY, the uniform deuteration of proteins is usually required. Here we propose a new and general method for ^{1}H broadband decoupling in TROSY NMR, which does not compromise the relaxation optimization in the ^{15}N–^{1}H moieties, but uniformly and efficiently refocuses the $^1J_{CH}$ scalar coupling evolution in the ^{13}C–^{1}H moieties. Combined with the conventional ^{2}H decoupling, this method enables obtaining high sensitivity TROSY-type triple resonance spectra with partially deuterated or fully protonated ^{13}C,^{15}N labeled proteins.

Abbreviations: TROSY, transverse relaxation-optimized spectroscopy; DD, dipole–dipole coupling; CSA, chemical shift anisotropy; 2D, two-dimensional.

Uniform or partial replacement of nonlabile protons with deuterons significantly reduces transverse relaxation by scaling down dipole–dipole interactions with remote hydrogens (LeMaster, 1994; Yamazaki et al., 1994; Grzesiek et al., 1995; Shan et al., 1996; Gardner and Kay, 1998). In all cases transverse relaxation of the amide protons and remaining aliphatic and aromatic protons is substantially reduced (LeMaster, 1994; Gschwind et al., 1999). Transverse relaxation-optimized spectroscopy (Pervushin et al., 1997) is shown to be effective in further improving sensitivity of the triple resonance experiments performed with both protonated and uniformly deuterated ^{13}C,^{15}N labeled proteins (Salzmann et al., 1998). TROSY-type optimization of NMR experiments is based on the preservation of the unique magnetization transfer pathways with distinct transverse relaxation properties (Pervushin et al., 1997). The broadband decoupling of the ^{1}H spins interchanges the TROSY and anti-TROSY coherence transfer pathways and thus is avoided in the TROSY-type triple resonance experiments or is replaced with a narrowband selective decoupling (Salzmann et al., 1998). To achieve the full advantage of TROSY the uniform deuteration of proteins was usually required. We propose a new and general method for the ^{1}H broadband decoupling in TROSY NMR, which does not compromise the relaxation optimization in the ^{15}N-^{1}H moieties, but uniformly and efficiently refocuses the $^1J_{CH}$ scalar coupling evolution in the ^{13}C-^{1}H moieties. This method makes the TROSY-type triple resonance experiments suitable for obtaining backbone resonance assignment of partially deuterated and fully protonated ^{13}C,^{15}N labeled proteins.
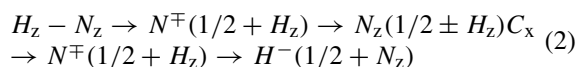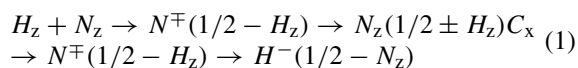
The two relevant magnetization transfer pathways in TROSY-type triple resonance experiments HNCA and HNCACB shown in Figure 1a and c with the most and the least favorable transverse relaxation properties can be schematically represented as (Pervushin et al.,

*To whom correspondence should be addressed. E-mail: kope@phys.chem.ethz.ch
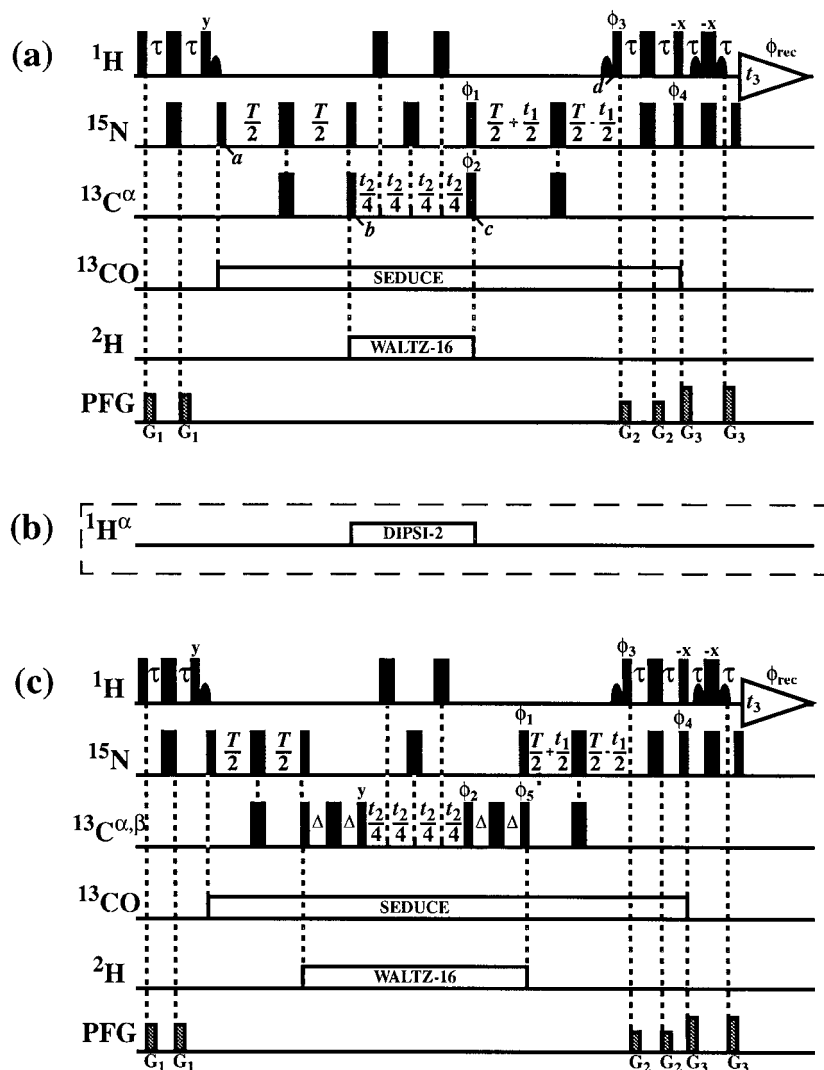
Figure 1. Experimental scheme for the $[^{15}\text{N},^1\text{H}]$-TROSY-HNCA (a) and $[^{15}\text{N},^1\text{H}]$-TROSY-HNCACB (c) experiments. In (a) the ra-dio-frequency pulses on ^1H, ^{15}N, ^{13}C, ^{13}CO, ^2H and $^1\text{H}^\alpha$ are applied at 4.7, 118, 55, 174, 3.6 and 4.7 ppm, respectively. The same frequencies are used in (c) except for ^{13}C, which is placed at 46 ppm. Narrow and wide black bars indicate non-selective 90° and 180° pulses, respectively. Sine bell shapes on the line marked ^1H indicate selective 90° pulses. The line marked PFG indicates the duration and strength of pulsed magnetic field gradients applied along the z-axis: G_1: 800 μs, 50 G/cm; G_2: 800 μs, 40 G/cm; G_3: 800 μs, 70 G/cm. The delays are τ=2.4 ms and T=44 ms. The phase cycle is: $\phi_1 = \{y, -y, -x, x\}$; $\phi_2 = \{4x, 4(-x)\}$; $\phi_3 = \{-y\}$; $\phi_4 = \{-y\}$; $\phi_5 = \{4y, 4(-y)\}$; $\phi_{rec} = \{y, -y, -x, x, -y, y, x, -x\}$. All other radio-frequency pulses are applied with phase x. A phase-sensitive spectrum in the $^{15}\text{N}(t_1)$ dimension is obtained by recording a second FID for each t_1 value, with $\phi_1 = \{y, -y, x, -x\}$, $\phi_3 = \{y\}$ and $\phi_4 = \{y\}$, and data processing as described by Kay et al. (1992). Quadrature detection in the $^{13}\text{C}^\alpha(t_2)$ dimension is achieved by the States-TPPI method (Marion et al., 1989) applied to the phases ϕ_2 and ϕ_5. The use of water flip-back pulses (Grzesiek and Bax, 1993) ensures that the water magnetization stays aligned along the $+Z$ axis throughout both the constant-time period T and the data acquisition period $^1\text{H}(t_3)$. ^2H-decoupling during t_2 is achieved with WALTZ-16 (Shaka et al., 1983) at the field strength of $\gamma B_2 = 2.5$ kHz. The reference TROSY-HNCA experiment with selective $^1\text{H}^\alpha$-decoupling during the $^{13}\text{C}^\alpha(t_2)$ evolution period (Salzmann et al., 1998) is constructed by performing the DIPSI-2 sequence (Cavanagh and Rance, 1992) with $\gamma B_2 = 0.51$ kHz as indicated on the insert (b).

1998):

$$H_z + N_z \rightarrow N^{\mp}(1/2 - H_z) \rightarrow N_z(1/2 \pm H_z)C_x$$
$$\rightarrow N^{\mp}(1/2 - H_z) \rightarrow H^-(1/2 - N_z) \quad (1)$$

$$H_z - N_z \rightarrow N^{\mp}(1/2 + H_z) \rightarrow N_z(1/2 \pm H_z)C_x$$
$$\rightarrow N^{\mp}(1/2 + H_z) \rightarrow H^-(1/2 + N_z) \quad (2)$$

The first arrow designates coherence transfer from the combined ^{1}H and ^{15}N steady state polarizations to the individual transition of the ^{15}N spin, the second arrow represents the coherence transfer from ^{15}N to ^{13}C and the evolution of magnetization on the ^{13}C spins, and the last two arrows designate the return of magnetization to the individual transition of the ^{1}H spin for detection (Salzmann et al., 1998, 1999). The two pathways are distinguished by significantly different relaxation properties of the $N^{\mp}(1/2 - H_z)$ and $N^{\mp}(1/2 + H_z)$ operators due to the interference between ^{15}N-^{1}H DD and ^{15}N CSA interactions (Shimizy, 1964; Goldman, 1984) during the coherence transfer phase and by the relaxation properties of the $H^-(1/2 - N_z)$ and $H^-(1/2 + N_z)$ operators during signal acquisition. Note that the two ^{15}N and ^{1}H operators and the corresponding magnetization transfer pathways can be mutually interchanged if the longitudinal spin in each operator is inverted by the rf-pulse. Thus, during the coherence transfer periods it is important not to perturb corresponding longitudinal spins, which happens for example during the long constant time periods T between time points a and b and c and d in the experimental schemes of Figure 1 and during signal acquisition. On the other hand, during the ^{13}C evolution period between time points b and c, the ^{15}N magnetization is stored in the form of magnetization modes $N_z(1/2 + H_z)$ and $N_z(1/2 - H_z)$, which can be interchanged without any significant effect on relaxation (Canet, 1989).

In partially deuterated proteins the evolution of the ^{13}C spins under the scalar coupling to the directly attached hydrogen spin in both ^{13}C-^{1}H and ^{13}C-^{2}H moieties has to be simultaneously refocused. This is achieved by an application of the conventional broadband ^{2}H decoupling and two 180° ^{1}H hard pulses as is shown in Figure 1a and c. Such a combination interchanges the ^{15}N magnetization modes twice, thus resulting in no mixing of the TROSY and 'anti-TROSY' magnetization transfer pathways represented by Equations 1 and 2, respectively. Another advantage of the proposed experimental scheme of Figure 1 is the minimal saturation of the water signal during the ^{1}H$^{\alpha,\beta}$ decoupling due to the absence of net rotation of
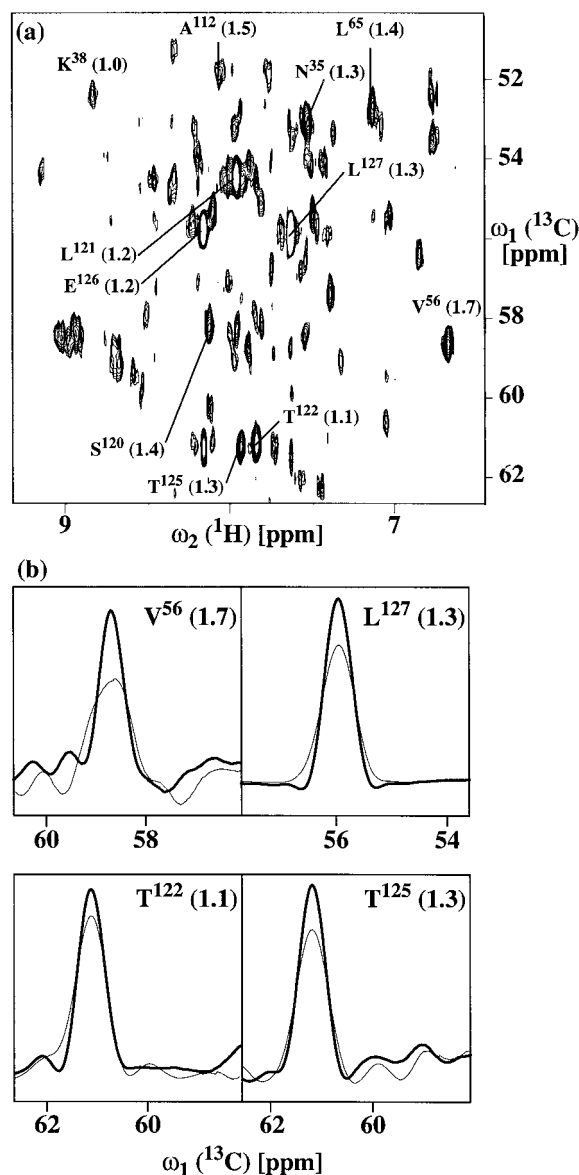
Figure 2. Comparison of broadband ^{1}H decoupling versus ^{1}H$^\alpha$-selective narrowband decoupling in TROSY-HNCA experiments. Two spectra were recorded for ^{15}N,^{13}C-labeled BsCM protein with 30–70% random deuteration free in solution. (a) Contour plot of the broadband ^{1}H decoupled 2D [^{13}C,^{1}H]-correlation projection of the 3D TROSY-HNCA spectrum measured with the experimental scheme of Figure 1a. Values of relative improvement in signal amplitude as compared to the experiment with ^{1}H$^\alpha$-selective decoupling are given in parentheses together with the sequence-specific resonance assignments of the selected intraresidual cross peaks. (b) 1D cross-sections of the 2D TROSY-HNCA spectra along the ^{13}C dimension at the intraresidual [^{13}C,^{1}H]-correlation cross peak positions of residues V^{56}, T^{122}, T^{125} and L^{127}. Thick and thin lines correspond to the broadband ^{1}H-decoupled and the ^{1}H$^\alpha$-selective decoupled experiments, respectively. The size of the acquired time domain data was 80×2048 complex points. Forty-eight scans per increment were accumulated, with a total measuring time of 1 h for both experiments.

the water magnetization between time points b and c. Since the ^{13}C t_{2max} delay in both TROSY-HNCA and TROSY-HNCACB experiments is usually limited by $^1J_{C\alpha C\beta}$ coupling to a few milliseconds, the loss of the water signal due to radiation damping between 180° ^{1}H pulses can be safely neglected (Sobol et al., 1998).

The 44 kDa 30–70% randomly deuterated and uniformly ^{13}C,^{15}N-labeled water-soluble trimeric enzyme *B. subtilis* Chorismate Mutase (EC 5.4.99.5) (Chook et al., 1994; Ladner et al., 2000) was used in the setup and evaluation of the proposed experiments. Figure 2 compares the 2D [^{13}C,^{1}H]-correlation projection of the 3D TROSY-HNCA spectrum measured with the experimental scheme of Figure 1a with the corresponding spectrum obtained using the narrowband ^{1}H decoupling as described by Salzmann et al. (1998, 1999). A significant improvement of the line shape along the ^{13}C dimension for the majority of resonances with a concomitant increase of the sensitivity in the range between 1.1 and 1.8 are observed. This is demonstrated in Figure 2b by superimposing 1D ^{13}C cross-sections taken from the conventional TROSY-HNCA spectrum (thin lines) and the TROSY-HNCA spectrum measured with the experimental scheme of Figure 1a (thick lines) at the positions of the resonances of V^{56}, T^{122}, T^{125} and L^{127}.

Overall, the sensitivity improvement of the proposed TROSY-type triple resonance experiments for partially deuterated proteins stems from several factors such as removal of the off-resonance effects of the narrowband ^{1}H$^{\alpha,\beta}$ decoupling, which might result in the interchange of the TROSY and 'anti-TROSY' pathways, removal of the intrinsic problems of the narrowband decoupling such as long supercycles and arbitrary truncation of the selective pulses due to the time delay incrementation (Matsuo et al., 1996; Van der Kooi et al., 1999), and finally the minimization of the water saturation (Stonehouse et al., 1995). The proposed method of ^{1}H$^{\alpha,\beta}$ decoupling further broadens the scope of applicability of the TROSY-type experiments, which renders the TROSY line of experiments an attractive choice for resonance assignment of partially deuterated or fully protonated proteins.

References

Canet, D. (1989) *Prog. NMR Spectrosc.*, **21**, 237–291.
Cavanagh, J. and Rance, M. (1992) *J. Magn. Reson.*, **96**, 670–678.
Chook, Y.M., Gray, J.V., Ke, H.M. and Lipscomb, W.N. (1994) *J. Mol. Biol.*, **240**, 476–500.
Gardner, K.H. and Kay, L.E. (1998) *Annu. Rev. Biophys. Biomol. Struct.*, **27**, 357–406.
Goldman, M. (1984) *J. Magn. Reson.*, **60**, 437–452.
Grzesiek, S. and Bax, A. (1993) *J. Am. Chem. Soc.*, **115**, 12593–12594.
Grzesiek, S., Wingfield, P., Stahl, S., Kaufman, J.D. and Bax, A. (1995) *J. Am. Chem. Soc.*, **117**, 9594–9595.
Gschwind, R.M., Kessler, H. and Gemmecker, G. (1999) *J. Magn. Reson.*, **137**, 285–288.
Kay, L.E., Keifer, P. and Saarinen, T. (1992) *J. Am. Chem. Soc.*, **114**, 10663–10665.
Ladner, J.E., Reddy, P., Davis, A., Tordova, M., Howard, A.J. and Gilliland, G.L. (2000) *Acta Crystallogr.*, **D56**, 673–683.
LeMaster, D.M. (1994) *Prog. NMR Spectrosc.*, **26**, 371–419.
Marion, D., Ikura, M., Tschudin, R. and Bax, A. (1989) *J. Magn. Reson.*, **85**, 393–399.
Matsuo, H., Kupce, E., Li, H.J. and Wagner, G. (1996) *J. Magn. Reson.*, **113**, 91–96.
Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12366–12371.
Pervushin, K., Wider, G. and Wüthrich, K. (1998) *J. Biomol. NMR*, **12**, 345–348.
Salzmann, M., Pervushin, K., Wider, G., Senn, H. and Wüthrich, K. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 13585–13590.
Salzmann, M., Wider, G., Pervushin, K., Senn, H. and Wüthrich, K. (1999) *J. Am. Chem. Soc.*, **121**, 844–848.
Shaka, A.J., Keeler, J., Frenkiel, T. and Freeman, R. (1983) *J. Magn. Reson.*, **52**, 335–338.
Shan, X., Gardner, K.H., Muhandiram, D.R., Rao, N.S., Arrowsmith, C.H. and Kay, L.E. (1996) *J. Am. Chem. Soc.*, **118**, 6570–6579.
Shimizy, H. (1964) *J. Phys. Chem.*, **40**, 3357–3364.
Sobol, A.G., Wider, G., Iwai, H. and Wüthrich, K. (1998) *J. Magn. Reson.*, **130**, 262–271.
Stonehouse, J., Clowes, R.T., Shaw, G.L., Keeler, J. and Laue, E.D. (1995) *J. Biomol. NMR*, **5**, 226–232.
Van der Kooi, C.W., Kupce, E., Zuiderweg, E.R.P. and Pellecchia, M. (1999) *J. Biomol. NMR*, **15**, 335–338.
Yamazaki, T., Lee, W., Arrowsmith, C.H., Muhandiram, D.R. and Kay, L.E. (1994) *J. Am. Chem. Soc.*, **116**, 11655–11666.