JOURNAL OF MAGNETIC RESONANCE **132,** 159–161 (1998)
ARTICLE NO. MN981381

A Spectral Window in Protein NMR Revealing Cross-Relaxation between Amide Protons

Pierre Mutzenhardt* and Geoffrey Bodenhausen†,[1]

*Laboratoire de méthodologie RMN, Université Henri Poincaré, Faculté des Sciences, B.P. 239, 54506 Vandoeuvre-lès-Nancy Cedex, France; and †Département de chimie, Ecole Normale Supérieure, 24 rue Lhomond, 75231 Paris Cedex 05, France

Received October 22, 1997; revised January 13, 1998

The principle of *q*uenching *u*ndesirable *i*ndirect *e*xternal *t*rouble in *n*uclear *O*verhauser *e*ffect *s*pectroscop*y* (QUIET-NOESY) relies on a doubly selective inversion of the longitudinal magnetization components of a source spin *A* and a target spin *X* to measure the cross-relaxation rate (Overhauser effect) between *A* and *X* without significant perturbation by spin diffusion. In ^{15}N-enriched proteins, this can be achieved by using a *bi*linear *r*otation *d*ecoupling (BIRD) sequence for the selective inversion of amide protons that have a scalar coupling to nitrogen-15. The procedure can be improved by using editing techniques to simplify the resulting NOESY spectra. © 1998 Academic Press

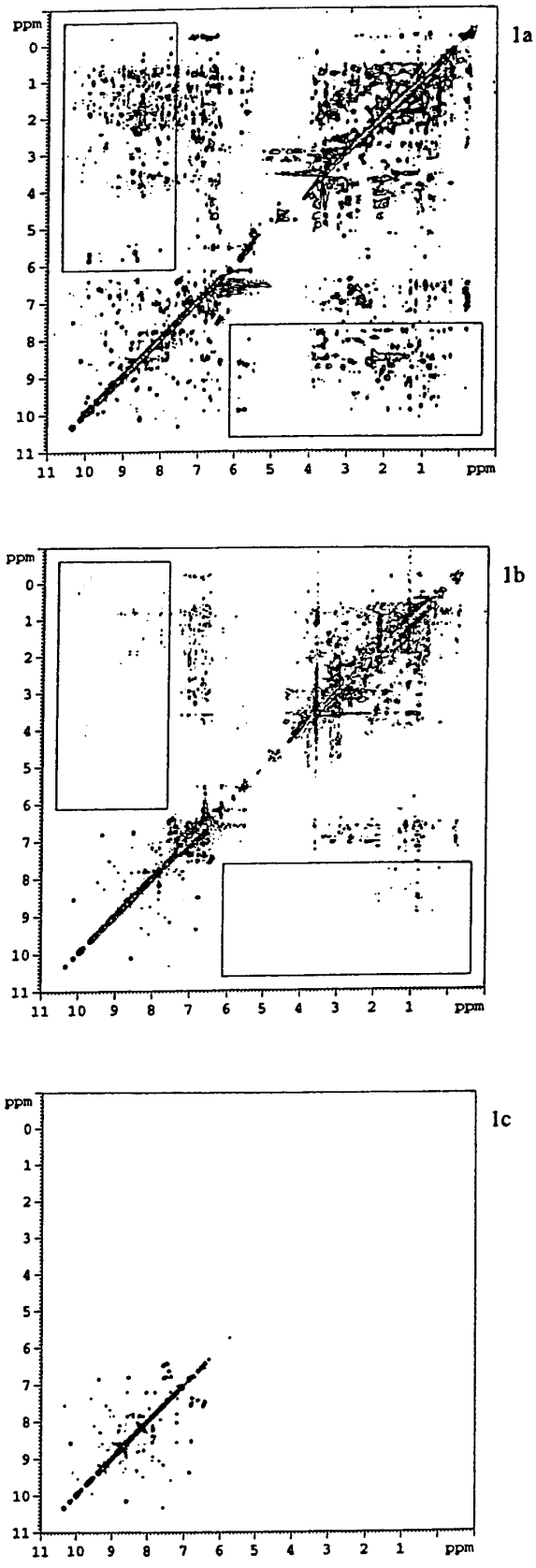
Measuring accurate cross-relaxation rates or Overhauser effects is the key to the determination of molecular structures by NMR (*1*). In practice, spin-diffusion processes greatly complicate data analysis (*2, 3*), but the principle of *q*uenching *u*ndesirable *i*ndirect *e*xternal *t*rouble (QUIET) (*4–6*) and related methods (*7*) permits one to isolate Overhauser effects between a limited number of spins. We present an improvement which allows one to focus attention on interactions between protons that have a scalar coupling to a heteronucleus such as nitrogen-15.

In proteins, the transfer of longitudinal magnetization from one amide proton to another, e.g., $I_z(\mathrm{H}^{\mathrm{N}}_n) \rightarrow I_z(\mathrm{H}^{\mathrm{N}}_{n+1})$, is often overshadowed by spin diffusion effects. Typically, two-step processes such as $I_z(\mathrm{H}^{\mathrm{N}}_n) \rightarrow I_z(\mathrm{H}^{\alpha}_n) \rightarrow I_z(\mathrm{H}^{\mathrm{N}}_{n+1})$ may contribute to the amplitude of the corresponding cross peak. It is possible to invert the longitudinal magnetization of all protons that are coupled to nitrogen-15 by using a *bi*linear *r*otation *d*ecoupling (BIRD) (*5, 8–10*) sequence in the middle of the mixing time τ_{m}. As a result, the flow of longitudinal magnetization between the amide protons remains unaffected (except for some attenuation due to transverse relaxation during the BIRD sequence), but two-step spin diffusion processes involving ''clandestine'' protons that are *not* scalar-coupled to nitrogen-15 are quenched to a very good approximation. These principles are illustrated by applications to an ^{15}N-labeled C22A mutant of the FK506 binding protein (FKBP), which contains 107 amino acids, has a molecular mass of 11.8 kD, and has an overall correlation time of about 9 ns at 303 K (*11–14*). In both conventional NOESY and QUIET-BIRD-NOESY of Figs. 1a and 1b, the ^{15}N spins have been decoupled by a π pulse in the middle of t_1 and by a CHIRP-95 sequence (*15, 16*) during t_2. Most cross-peaks that correlate amide and nonamide protons (framed in boxes in Figs. 1a and 1b) have been greatly attenuated in Fig. 1b, indicating that the flow of magnetization between amide and nonamide protons has been largely canceled. Some cross-peaks that survive in these boxes are due to cross-relaxation between aromatic and aliphatic protons, which is confirmed by their opposite signs compared to cross-peaks between amide protons. Cross-peaks that correlate two different amide protons (near the diagonal) must therefore arise solely from *direct* cross-relaxation between these amide protons, without significant contamination by spin diffusion.

Since the main goal of the experiment is to focus on interactions between pairs of amide protons, all resonances of nonamide protons may be suppressed by nitrogen-15 half-filters (*17–23*). In Fig. 2, a first half-filter is used at the beginning of the t_1 period to excite only transverse magnetization of the amide protons. After the mixing interval, a modified WATERGATE (*24–27*) scheme is used to suppress the water signal *and* to eliminate all magnetization components except those arising from amide protons. For this purpose, the δ delays in the

[1] To whom correspondence should be addressed. E-mail: Geoffrey.Bodenhausen@ens.fr. Fax: +33 1 44 32 33 97.

1090-7807/98 $25.00



WATERGATE scheme were increased to $(2J_{NH})^{-1} = 5.5$ ms (instead of about 1 ms required for a typical gradient pulse) while the nitrogen-15 magnetization was inverted in every other scan. This was achieved by alternating between $90^\circ_x 90^\circ_x$ and $90^\circ_x 90^\circ_{-x}$. Similar schemes have been used in NMR of protein dimers or protein/peptide complexes to separate inter- and intramolecular Overhauser effects (*20, 28–30*). The resulting NOESY map of Fig. 1c shows that only the signals of amide protons remain. The intensities of the cross-peaks solely reflect interactions between amide protons, since all other protons have been ''reduced to silence'' through the QUIET principle. The peak intensities are attenuated by relaxation of the (transverse) magnetization during the two half-filters and during the BIRD sequence. This attenuation does not exceed a factor two compared to conventional NOESY. In our opinion, this loss is largely compensated by the clarity of the QUIET spectra. The attenuation can be taken into account by comparing all cross-peak amplitudes with diagonal peaks recorded with very short mixing times.

A potentially useful variation of the method combines QUIET-BIRD-NOESY with HSQC to label the magnetization by nitrogen-15 shifts in the evolution period, so that cross-peaks can be separated even if the amide protons are degenerate. However, it is advisable to use the homonuclear version of Fig. 1c to check the efficiency of the QUIET-BIRD sequence before using an HSQC variant. All techniques can be transposed to carbon-13-labeled macromolecules including proteins, DNA, RNA, and polysaccharides.

ACKNOWLEDGMENTS

We are indebted to Dr Timothy Logan, Department of Chemistry, Florida State University, Tallahassee, for the preparation and purification of a sample of nitrogen-15-enriched FKBP, and to the Center of Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Tallahassee, Florida, for the use of 300- and 720-MHz NMR spectometers.

FIG. 1. (a) Conventional NOESY spectrum of an ^{15}N-labeled C22A mutant of FK506 binding protein (FKBP). (b) Conventional QUIET-BIRD-NOESY and (c) filtered QUIET-BIRD-NOESY, recorded at 300 K and 7 T on a Bruker DMX 300 spectrometer with an inverse 5-mm probe and a triple-axis gradient system. The spectral width was 4500 Hz (512 points) in both dimensions, acquired with 64 scans. The experiment included the following parameters: mixing time τ_m = 200 ms, delays δ = 5.5 ms ($= 1/2J_{NH}$), sine-shaped pulsed field gradients of 1 ms duration with amplitudes G_x = 10 G/cm and G_z = 20 G/cm. Selective 90° Gaussian pulses of 3 ms were used during the WATERGATE scheme (*24–27*). Decoupling during t_2 was achieved by a CHIRP-95 sequence (*15, 16*) with 1 ms CHIRP units of approximately 1.5 kHz amplitude, 15 kHz sweep width, and an 80-step phase cycle. The contour levels in (c) were lower by a factor 2 with respect to (a) and (b).

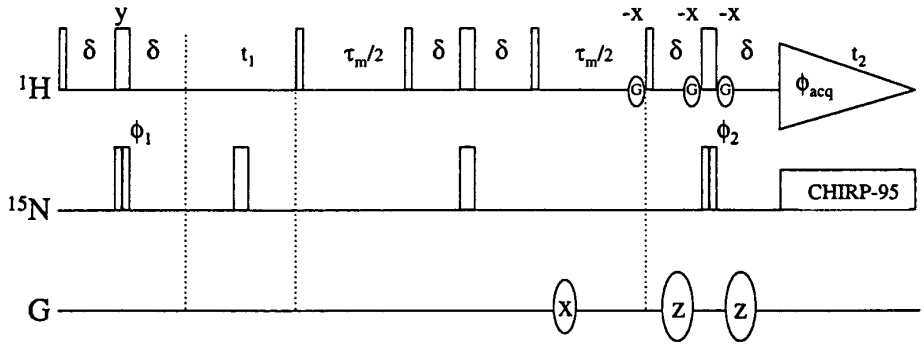


FIG. 2. Pulse sequence for filtered QUIET-BIRD-NOESY with a bilinear rotation decoupling (BIRD) sequence in the middle of the mixing time and two half-filters, the latter combined with water suppression. Small ellipses represent selective 90° Gaussian radiofrequency pulses applied to the water resonance; larger ellipses represent G_x and G_z gradient pulses. The phase cycle is $\phi_1 = x, -x, x, -x; \phi_2 = x, x, -x, -x; \phi_{acq} = x, -x, -x, x$.

REFERENCES

1. D. Neuhaus and M. Williamson, ''The Nuclear Overhauser Effect in Structural and Conformational Analysis,'' VCH, New York (1989).
2. Anil Kumar, G. Wagner, R. R. Ernst, and K. Würtrich, *J. Am. Chem. Soc.* **103,** 3654 (1981).
3. A. N. Lane, *J. Magn. Reson.* **78,** 425 (1988).
4. C. Zwahlen, S. J. F. Vincent, L. Di Bari, M. H. Levitt, and G. Bodenhausen, *J. Am. Chem. Soc.* **116,** 362 (1994).
5. S. J. F. Vincent, C. Zwahlen, P. H. Bolton, T. M. Logan, and G. Bodenhausen, *J. Am. Chem. Soc.* **118,** 3531 (1996).
6. M. Schwager and G. Bodenhausen, *J. Magn. Reson. B* **111,** 40 (1996).
7. Z. Zolnai, N. Juranic, J. L. Markley, and S. Macura, *Chem. Phys.* **200,** 161 (1995).
8. J. R. Garbow, D. P. Weitekamp, and A. Pines, *Chem. Phys. Lett.* **93,** 504 (1982).
9. A. Bax, *J. Magn. Reson.* **53,** 512 (1983).
10. V. Rutar, *J. Magn. Reson.* **56,** 87 (1984).
11. D. A. Egan, T. M. Logan, H. Liang, E. Matayoshi, S. W. Fesik, and T. F. Holzman, *Biochemistry* **32,** 1920 (1993).
12. M. K. Rosen, S. W. Michnik, M. Karplus, and S. L. Schreiber, *Biochemistry* **30,** 4774 (1991).
13. J. W. Cheng, C. A. Lepre, S. P. Chambers, J. R. Fulghum, J. A. Thomson, and J. M. Moore, *Biochemistry* **32,** 9000 (1993).
14. J. W. Cheng, C. A. Lepre, and J. M. Moore, *Biochemistry* **33,** 4013 (1994).
15. R. Fu and G. Bodenhausen, *Chem. Phys. Lett.* **245,** 415 (1995).
16. R. Fu and G. Bodenhausen, *J. Magn. Reson. A.* **117,** 324 (1995).
17. R. Freeman, T. H. Mareci, and G. A. Morris, *J. Magn. Reson.* **42,** 341 (1981).
18. G. Otting, H. Senn, G. Wagner, and K. Wütrich, *J. Magn. Reson.* **70,** 500 (1986).
19. A. Bax and M. Weiss, *J. Magn. Reson.* **71,** 571 (1987).
20. S. W. Fesik, R. T. Gampe, and T. W. Rockaway, *J. Magn. Reson.* **74,** 366 (1987).
21. S. W. Fesik, *Nature* **332,** 865 (1988).
22. G. Otting and K. Wütrich, *J. Magn. Reson.* **85,** 586 (1989).
23. G. Wider, C. Weber, R. Traber, H. Widmer, and K. Wütrich, *J. Am. Chem. Soc.* **112,** 9015 (1990).
24. M. Piotto, V. Saudek, and V. L. Sklenar, *J. Biomol. NMR* **2,** 661 (1992).
25. V. L. Sklenar, M. Piotto, R. Leppik, and V. Saudek, *J. Magn. Reson. A* **102,** 241 (1993).
26. S. Grzesiek and A. Bax, *J. Am. Chem. Soc.* **115,** 12,593 (1993).
27. G. Lippens, C. Dhalluin, and J.-M. Wieruszeski, *J. Biomol. NMR* **5,** 327 (1995).
28. S. W. Fesik, R. T. Gampe, and T. F. Holzman, *Science* **250,** 1406 (1990).
29. S. W. Fesik, R. T. Gampe, and H. L. Eaton, *Biochemistry* **30,** 6574 (1991).
30. M. Slijper, R. Kaptein, and R. Boelens, *J. Magn. Reson. B* **111,** 199 (1996).