Journal of Biomolecular NMR, **19:** 273–277, 2001.
KLUWER/ESCOM

Sensitive ^{1}H-^{31}P correlations with 5′ methylene protons of DNA via homonuclear double-quantum coherence

Andrei Kaikkonen & Gottfried Otting*
Department of Medical Biochemistry and Biophysics, Karolinska Institute, Tomtebodavägen 6, S-17177 Stockholm, Sweden

Received 19 October 2000; Accepted 10 January 2001

Key words: DNA assignment, double-quantum coherence, methylene protons, proton-phosphorus correlations

Abstract

A novel pulse sequence is presented for the correlation of 5′ and 5″ protons in DNA with phosphorus. Double-quantum coherence between the methylene protons is used to generate ^{1}H5′-^{31}P and ^{1}H5″-^{31}P cross peaks in an HMQC-type experiment. The resolution for these cross peaks is significantly improved over that of conventional HSQC experiments, as cross peaks between ^{1}H4′ and ^{31}P are largely suppressed and a 3D version of the experiment can be performed with little penalty in sensitivity. In addition, sensitivity is favoured by slower relaxation of the double-quantum coherence and a more favourable multiplet fine structure in the acquisition dimension.

The resonance assignment of backbone ^{1}H and ^{31}P resonances in DNA can be achieved by a number of different ^{1}H-^{31}P correlation experiments (Sklenár et al., 1986; Fu et al., 1988; Jones et al., 1988; Chary et al., 1993; Gorenstein, 1994). In all these experiments, the correlations with the 3′ and 4′ protons are much more intense than those with the 5′ and 5″ protons. Although cross peaks between the 5′ methylene protons and phosphorus are usually not required to assign the ^{31}P NMR spectrum, these cross peaks can be useful to assign the 5′ methylene protons, provided signals from overlapping H4′ resonances can be suppressed.

The weak cross-peak intensities between H5′/H5″ and ^{31}P are primarily caused by dipolar interaction between the methylene protons, which provides an efficient relaxation mechanism and compromises the excitation of antiphase coherence with respect to phosphorus via the small ^{1}H-^{31}P couplings. The problems of signal overlap with H4′ resonances and rapid relaxation are both alleviated by the use of double-quantum coherence.

*To whom correspondence should be addressed. E-mail: gottfried.otting@mbb.ki.se

In the slow motional regime, the highest order of the multiple-quantum coherence does not relax by dipolar interaction between the nuclei involved (Ernst et al., 1987; Griffey and Redfield, 1987). The remaining relaxation is primarily caused by dipolar interactions with nuclei that are not involved in the multiple-quantum coherence. Slow heteronuclear multiple-quantum relaxation of NH, CH, and CH_2 groups has been exploited in many applications involving proteins (Bax et al., 1989; Grzesiek and Bax, 1995; Grzesiek et al., 1995; Shang et al., 1997; Ponstingl and Otting, 1998; Larsson et al., 1999) and RNA (Marino et al., 1997). Homonuclear applications are less straightforward, as the generation of multiple-quantum coherences via ^{1}H-^{1}H couplings requires longer delays. Furthermore, zero-quantum (ZQ) coherence in a homonuclear two-spin system has no relaxation advantage over single-quantum (SQ) coherence, i.e. the benefits of slow relaxation are limited to double-quantum (DQ) coherence (Ernst et al., 1987). As an added benefit, however, double-quantum coherences evolve with the sum of *J*-couplings to a third spin (Sørensen et al., 1983), which is exploited in the present application to enhance the rate of coherence transfer from ^{1}H-^{1}H double-quantum coherences between the 5′ methylene protons of DNA to ^{31}P.

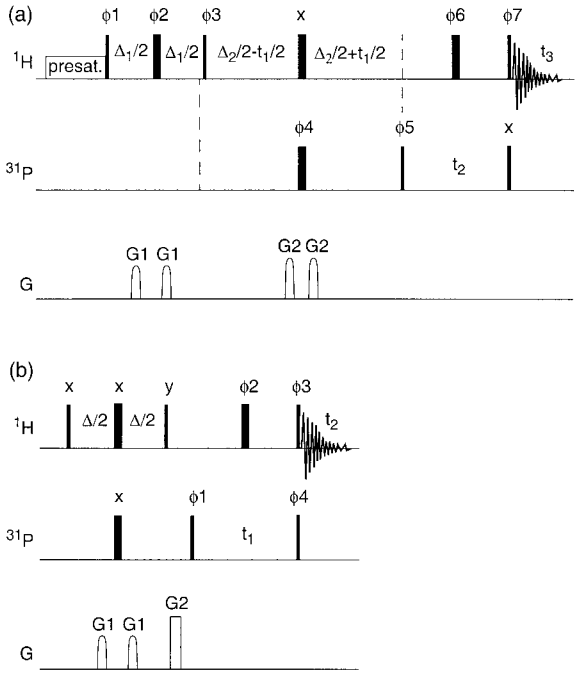

Figure 1. Pulse sequences for ^{1}H-^{31}P correlations. Narrow and wide bars represent 90° and 180° pulses, respectively. Rounded shapes indicate sine-shaped field gradients. (a) DQ-^{31}P experiment. $\Delta_1 = 22$ ms, $\Delta_2 = 30$ ms. $\Phi_1 = \Phi_2 = 45°$, 135°,225°,315°; $\Phi_3 = 8(45°,135°,225°,315°),8(225°,315°,45°,135°)$; $\Phi_4 = x$; $\Phi_5 = 8(x),8(-x)$; $\Phi_6 = 16(x),16(-x)$; $\Phi_7 = 4(x),4(-x)$; receiver = 2(x,−x,x−x,−x,x,−x,x,−x,x,x,−x,x,−x),2(−x,x,−x,x,x,−x,x,−x,x,−x,x,−x,x,−x). Quadrature detection in the t_1 dimension is achieved by incrementing Φ_1, Φ_2, and Φ_3 in steps of 45° according to States-TPPI; quadrature detection in the t_2 dimension is achieved by incrementing the phases Φ_4 and Φ_5 in steps of 90°. Maximum gradient amplitudes (durations): G1,2 = 10(1), 12(0.5) G/cm (ms). (b) ^{31}P-HSQC experiment (Chary et al., 1993). $\Delta = 20$ ms. Φ_1 = x,−x; Φ_2 = x,x,−x,−x; $\Phi_3 = 4(x),4(-x)$; $\Phi_4 = 8(x),8(-x)$; receiver = x,−x,x,−x,2(−x,x,−x,x),x,−x,x,−x. Φ_1 was incremented according to States-TPPI. Maximum gradient amplitudes (durations): G1,2 = 10(0.5), 17.5(1) G/cm (ms).

The relaxation advantage of DQ over SQ coherence for 5′ methylene protons of DNA can be estimated by calculating the dipolar relaxation between the methylene protons, I_1 and I_2, and all other protons, K. In the slow motional regime and disregarding cross-correlation effects, the dipolar relaxation rate of DQ coherence between I_1 and I_2 is

$$R^{DQ}_{I1,I2} = \tfrac{1}{20} J(0) \sum_K \left(5D^2_{I1,K} + 5D^2_{I2,K}\right), \quad (1a)$$

and the relaxation rate of SQ coherence of the I_1 spin is

$$R^{SQ}_{I1} = \tfrac{1}{20} J(0) \left(5D^2_{I1,I2} + \sum_K 5D^2_{I1,K}\right), \quad (1b)$$

with a similar equation for R^{SQ}_{I2}. $D_{ij} = h\gamma_i\gamma_j/(2\pi r^3_{ij})$, where h is Planck's constant, γ_i and γ_j the gyromagnetic ratios of spins i and j respectively, and r_{ij} is the distance between spins i and j. $J(0)$ is the predominant spectral density contribution, and equal to the rotational correlation time τ_c.

Since the distance between the 5′ methylene protons of DNA is much shorter than the distance between the methylene protons and any of the other protons, the combined effect of the other protons can be approximated by a single hypothetical pseudospin S located at a distance r_S from the spins I_1 and I_2, so that

$$\left(\sum_K r^{-6}_{I1,K}\right)^{-1/6} \cong \left(\sum_K r^{-6}_{I2,K}\right)^{-1/6} \cong r_S.$$

With this approximation, the relative relaxation rates are

$$R^{SQ}_{I1,2}/R^{DQ}_{I1,I2} = \tfrac{1}{2} + \tfrac{1}{2}\left(\frac{r_S}{r_{I1,I2}}\right)^6. \quad (2)$$

For the Dickerson-Drew dodecamer (Drew et al., 1981), r_S is about 2.15 Å, while the distance $r_{I1,I2}$ between H5′ and H5″ is about 1.75 Å. With these parameters, the relaxation of DQ coherence between H5′ and H5″ is predicted to be 2.2 times slower than the relaxation of the SQ coherence of either H5′ or H5″.

The pulse sequence of the new DQ-^{31}P experiment is shown in Figure 1a. Following the DQ excitation period Δ_1, DQ coherence between H5′ and H5″, $(I_{1x}I_{2x} - I_{1y}I_{2y})$, evolves during the delay Δ_2 into antiphase coherence with respect to phosphorus, $2(I_{1x}I_{2y} + I_{1y}I_{2x})P_z$. The subsequent 90°(^{31}P) pulse generates transverse ^{31}P coherence for frequency labelling of the ^{31}P chemical shifts during the evolution time t_2. The final 90°(^{1}H) and 90°(^{31}P) pulses generate doubly antiphase magnetization, $2(I_{1x}I_{2z} + I_{1z}I_{2x})P_z$, which is detected during the acquisition time t_3.

It is instructive to compare the DQ-^{31}P experiment with the previously published ^{31}P-HSQC experiment (Figure 1b), where the magnetization observed during the acquisition time, $2I_{1y}P_z + 2I_{2y}P_z$, is singly antiphase with respect to phosphorus (Chary et al., 1993). The typical combination of coupling constants for H5′/H5″/^{31}P spin systems in DNA leads to the same peak heights of singly and doubly antiphase coherences when the signals are narrow. For broader lines, however, signal cancellation effects reduce the maximum peak height of the singly antiphase multiplet more than that of the doubly antiphase multiplet (Figure 2).

For comparison between the DQ-^{31}P and ^{31}P-HSQC experiments, optimum delay settings were numerically simulated for both experiments using the GAMMA C++ library (Smith et al., 1994) with the following coupling constants: $^{2}J_{\mathrm{H5'},\mathrm{H5''}} = -12$ Hz, $^{3}J_{\mathrm{H4'},\mathrm{H5'}} = {}^{3}J_{\mathrm{H4'},\mathrm{H5''}} = 3$ Hz, $^{3}J_{\mathrm{P},\mathrm{H5'}} = {}^{3}J_{\mathrm{P},\mathrm{H5''}} = 5$ Hz, and $^{3}J_{\mathrm{P},\mathrm{H4'}} = 4$ Hz. Dipolar relaxation was taken into account by mutual relaxation between the H5′ and H5″ spins, which were separated by 1.75 Å, and a pseudo spin placed at a distance of 2.15 Å from the H5′ and H5″ spins. In addition, CSA relaxation of the ^{31}P spin was included, assuming a chemical shift anisotropy value of 225 ppm. An isotropic rotational correlation time of 3.6 ns was assumed for the DNA. The optimum delay settings found were $\Delta_1 = 22$ ms and $\Delta_2 = 30$ ms for the DQ-^{31}P experiment, and $\Delta = 20$ ms for the ^{31}P-HSQC experiment. With these delays and for zero ^{31}P-evolution times, 19% of the equilibrium magnetization in the DQ-^{31}P experiment was transformed into observable signal, while the yield of the ^{31}P-HSQC experiment was only 13%. The yields were not sensitive to small changes in the delays. For a rotational correlation time of 10 ns, the respective values were 5 and 6% for optimum delays ($\Delta_1 = 22$ ms, $\Delta_2 = 20$ ms, and $\Delta = 18$ ms). In this situation, the DQ-^{31}P experiment becomes more sensitive only due to the favourable doubly antiphase multiplet fine structure (Figure 2), which becomes significantly more advantageous for larger linewidths.

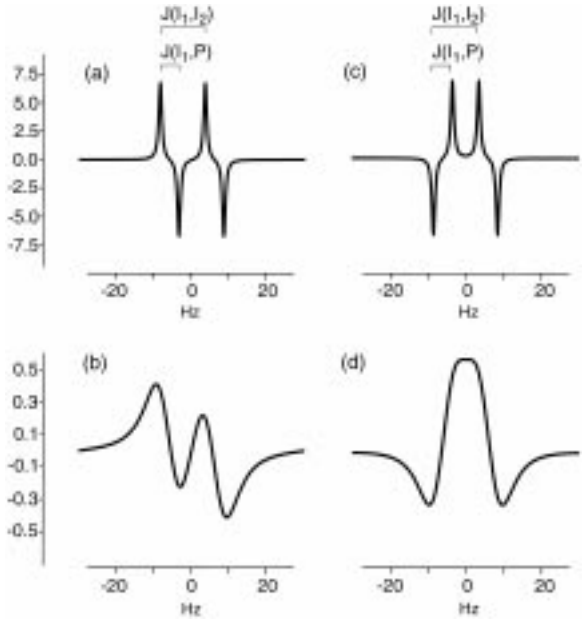

Figure 2. Multiplet fine structures of singly and doubly antiphase ^{1}H NMR signals, simulated for a system of three spins I_1, I_2, and P, using $J(I_1,I_2) = -12$ Hz and $J(I_1,P) = J(I_2,P) = 5$ Hz. (a and b) Multiplet corresponding to $2I_{1x}P_z$. (c and d) Multiplet corresponding to $4I_{1x}I_{2z}P_z$. The multiplets in the top and bottom panel were simulated using line widths of 1 Hz and 10 Hz, respectively. Further linebroadening would enhance the sensitivity advantage of the $4I_{1x}I_{2z}P_z$ term even further.

The sensitivity advantage of the DQ-^{31}P experiment is reduced by relaxation of the DQ coherence during the ^{31}P evolution time, which is probably faster than the relaxation during the corresponding evolution time of the ^{31}P-HSQC experiment. Furthermore, water magnetization cannot be suppressed as elegantly in the DQ-^{31}P experiment as in the ^{31}P-HSQC experiment (Figure 1). Yet, it is remarkable that the basic sensitivity of the longer DQ-^{31}P pulse sequence is competitive with that of the ^{31}P-HSQC experiment. As an additional benefit, the long delay Δ_2 lends itself to the design of a three-dimensional experiment, where the DQ coherences are frequency labelled in a constant-time manner (Figure 1a) without much penalty in sensitivity.

For experimental verification, spectra were recorded of the Dickerson–Drew dodecamer (Drew et al., 1981). The ^{31}P-HSQC spectrum shows strong ^{1}H-^{31}P cross peaks for the H4′ (Figure 3a) and H3′ protons (not shown), while the cross peaks with the H5′ and H5″ protons are much weaker. In contrast,

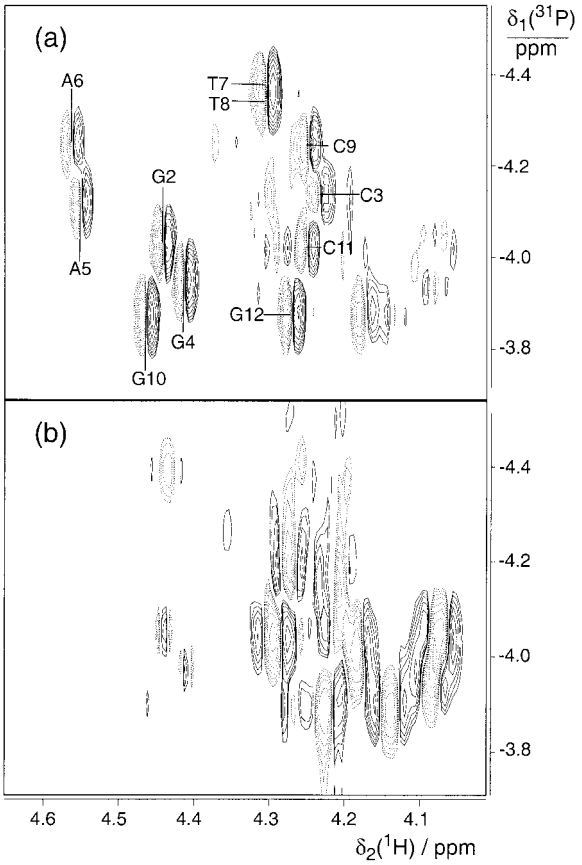

Figure 3. Two-dimensional ^{31}P-^1H correlation spectra recorded of a 5 mM solution of d-(GCGCAATTGCGC)$_2$ in 90% H_2O/10% D_2O at 25 °C and pH = 6.9. The spectral region shown contains the cross peaks between ^{31}P and the protons H4′, H5′, and H5″. All spectra were recorded at a ^1H NMR frequency of 500 MHz on a Bruker DRX-500 NMR spectrometer. The contour levels were plotted on an exponential scale, where each level is 1.4-fold higher than the preceding one. (a) ^{31}P-HSQC spectrum recorded with the pulse sequence of Figure 1b, using $\Delta = 22$ ms, $t_{max}(^{31}P) = 37$ ms, $t_{max}(^1H) = 205$ ms and a total recording time of about 9 h. The ^{31}P-H4′ cross peaks are labeled. For improved visual presentation, three times higher contour levels were plotted than in (b). (b) 2D DQ-^{31}P spectrum recorded with the pulse sequence of Figure 1a, except that $\Delta_1 = 25$ ms and $\Delta_2 = 31$ ms. All other parameters were the same as in (a).

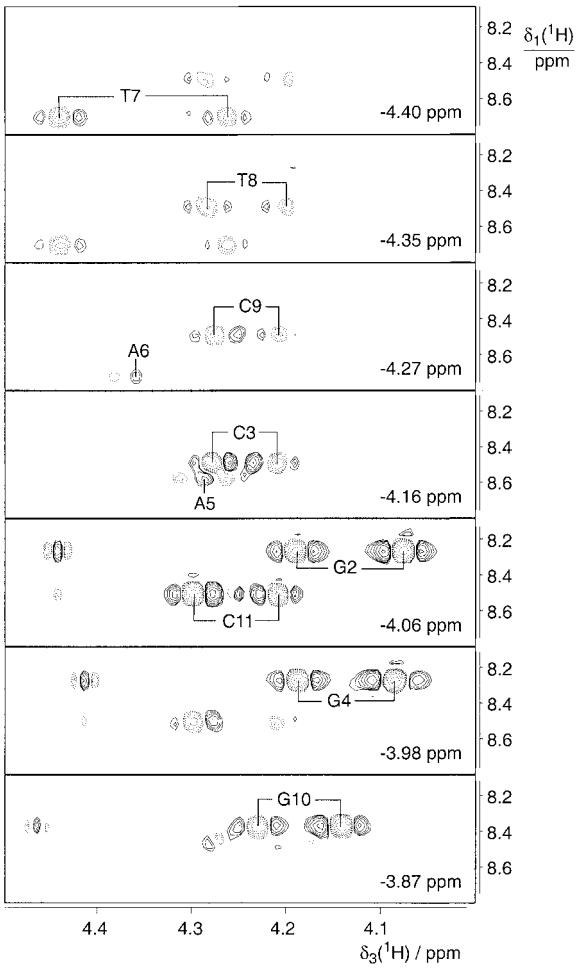

Figure 4. 3D DQ-^{31}P spectrum recorded of d-(CGCGAATTCGCG)$_2$. The sample and conditions were identical to those of Figure 3. The cross sections shown were taken at the ^{31}P chemical shifts (Sklenář et al., 1986) indicated. The spectrum was recorded with the pulse sequence of Figure 1a, using $\Delta_1 = 25$ ms, $\Delta_2 = 23$ ms, $t_{1max} = 20$ ms, $t_{2max} = 46$ ms, $t_{3max} = 205$ ms, and a total acquisition time of 32 h. The ^{31}P-H5′ and ^{31}P-H5″ cross peaks are labeled. δ_1 is the double-quantum dimension.

the DQ-^{31}P spectrum shows good intensities for the H5′-^{31}P and H5″-^{31}P cross peaks, but only weak cross peaks with H4′ (Figure 3b).

The overlap between the cross peaks in the two-dimensional spectrum of Figure 3b is alleviated in the three-dimensional DQ-^{31}P spectrum, where the cross peaks are further separated by the DQ frequencies $\Omega_{H5'} + \Omega_{H5''}$ (Figure 4). Ten out of 11 possible 5′ methylene-phosphorus correlations were observed. The absence of cross peaks with the 5′ methylene protons of C12 can be explained by the degeneracy of these protons (Nerdal et al., 1989), which prohibits the generation of DQ coherence. Notably, degenerate chemical shifts were also reported for the 5′ methylene protons of A5 and A6. The presence of cross peaks for those protons in Figure 4 indicates incomplete degeneracy.

The apparent sign of the cross peaks of A5 and A6 is opposite to that of all other 5′ methylene-^{31}P correlations. This effect was also observed in a conventional homonuclear DQ experiment, where the direct

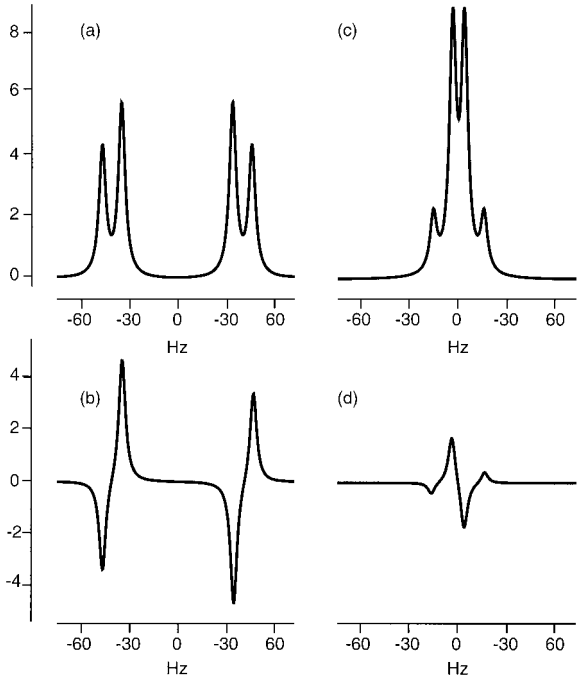

Figure 5. Simulations of in-phase and antiphase line shapes in the ^{1}H NMR spectrum of an AB-spin system with $J_{AB} = 12$ Hz and a line width of 5 Hz. (a and b) $\delta_A - \delta_B = 80$ Hz. (c and d) $\delta_A - \delta_B = 15$ Hz. The top and bottom panels display the in-phase and antiphase multiplets, respectively. Note that the intensities of the two most intense lines in (d) assume an apparent +/− pattern, although the real multiplet pattern is −/+ as in (b).

peaks between the 5′ methylene protons of A5 and A6 overlap with the double-quantum diagonal (data not shown). It is simply explained by the strong coupling effect in an AB spin system, which can reduce the signal intensity of the outer multiplet components to a level below the white noise (Figure 5).

Weak H4′-^{31}P cross peaks were observed in the DQ-^{31}P spectrum for G2, G4 and G10 (Figure 4). They occurred at the same double-quantum frequencies as the direct cross peaks of the 5′ methylene-^{31}P correlations and had the opposite apparent sign, which are the hallmarks of remote cross peaks (Otting and Wüthrich, 1986). Their weak intensities illustrate the selective power of the DQ-^{31}P experiment for cross peaks with 5′ methylene groups.

In conclusion, the new DQ-^{31}P experiment provides a remarkably straightforward route for singling out the resonances from H5′ and H5″ in unlabelled DNA. The concept may be useful for any experiment, where methylene protons are correlated with a third spin and the proton density is low.

Acknowledgements

We thank Edvards Liepinsh for stimulating discussions, the Wenner-Gren Foundation for a postdoctoral fellowship (to A.K.), and the Swedish research councils NFR and FRN for financial support.

References

Bax, A., Kay, L.E., Sparks, S.W. and Torchia, D.A. (1989) *J. Am. Chem. Soc.*, **111**, 408–409.

Chary, K.V.R., Rastogi, V.K. and Govil, G. (1993) *J. Magn. Reson.*, **B102**, 81–83.

Drew, H.R., Wing, R.M., Takano, T., Broka, C., Takana, S., Itakura, K. and Dickerson, R.E. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 2179–2183.

Ernst, R.R., Bodenhausen, G. and Wokaun, A. (1987) *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford.

Fu, J.M., Schroeder, S.A., Jones, C.R., Santini, R. and Gorenstein, D.G. (1988) *J. Magn. Reson.*, **77**, 577–582.

Gorenstein, D.G. (1994) *Chem. Rev.*, **94**, 1315–1338.

Griffey, R.H. and Redfield, A.G. (1987) *Quart. Rev. Biophys.*, **19**, 51–82.

Grzesiek, S. and Bax, A. (1995) *J. Biomol. NMR*, **11**, 335–339.

Grzesiek, S., Kuboniwa, H., Hinck, A.P. and Bax, A. (1995) *J. Am. Chem. Soc.*, **117**, 5312–5315.

Jones, C.R., Schroeder, S.A. and Gorenstein, D.G. (1988) *J. Magn. Reson.*, **88**, 370–374.

Larsson, G., Wijmenga, S.S. and Schleucher, J. (1999) *J. Biomol. NMR*, **14**, 169–174.

Marino, J.P., Diener, J.L., Moore P.B. and Griesinger, C. (1997) *J. Biol. Chem.*, **119**, 7361–7366.

Nerdal, W., Hare, D.R. and Reid, B.R. (1989) *Biochemistry*, **28**, 10008–10021.

Otting, G. and Wüthrich, K. (1986) *J. Magn. Reson.*, **66**, 359–363.

Ponstingl, H. and Otting, G. (1998) *J. Biomol. NMR*, **12**, 319–324.

Shang, Z., Swapna, G.V.T., Rios, C.B. and Montelione, G.T. (1997) *J. Am. Chem. Soc.*, **119**, 9274–9278.

Sklenář, V., Miyashiro, H., Zon, G., Miles, H.T. and Bax, A. (1986) *FEBS Lett.*, **208**, 94–98.

Smith, S.A., Levante, T.O., Meier, B.H. and Ernst, R.R. (1994) *J. Magn. Reson.*, **A106**, 75–105.

Sørensen, O.W., Eich, G.W., Levitt, M.H., Bodenhausen, G. and Ernst, R.R. (1983) *Prog. NMR Spectrosc.*, **16**, 163–192.