

Electron Spin–Nuclear Spin Cross-Correlation Effects on Multiplet Splittings in Paramagnetic Proteins

Ranjeet Ghose and James H. Prestegard¹

Department of Chemistry, Yale University, New Haven Connecticut 06520

Received May 5, 1997; revised July 1, 1997

The effects of cross-correlation between Curie spin–nuclear dipole and nuclear dipole–nuclear dipole interactions on the line-widths and resonance frequencies of the individual lines of an AX multiplet in paramagnetic systems have been calculated. The implication of the relaxation-induced frequency shift of the lines (dynamic frequency shift) for the accurate measurement of residual dipolar couplings in field-oriented systems has been discussed. Our simulations indicate that these effects may play a role in the precise measurement of residual dipolar couplings in systems which belong to the small and intermediate tumbling regime, i.e., correlation times less than 5 ns. © 1997 Academic Press

Key Words: dynamic frequency shift; paramagnetic relaxation; Curie spin relaxation; dipolar coupling; cross-correlation.

INTRODUCTION

Recently, Tolman *et al.* (1) have shown that, for certain paramagnetic proteins, the measurement of dipolar contributions to the splitting of resonances for ¹⁵N–¹H scalar-coupled pairs of spins can provide structural information complementary to distance constraints obtained from NOESY data. This information could be particularly useful in determining the tertiary fold of proteins and other macromolecules when only a small number of distance constraints are available from NOESY type experiments. Tjandra *et al.* (2) have also demonstrated the utility of analogous information from diamagnetic systems in the refinement of NOE-based structures. In both cases, dipolar contributions to the splittings are small and are highly susceptible to errors due to neglect of other small contributions which can add to the primary scalar coupling components. Here we consider a contribution that can be of significance in paramagnetic systems, namely the dynamic frequency shift caused by cross-correlation effects between dipolar couplings and Curie relaxation from the electron spin of the paramagnetic center.

Dipolar coupling contributions to the splitting of reso-

nances are not normally observed in high-resolution spectra. The reason for this is that processes such as molecular tumbling, which occur on a time scale faster than the inverse of the dipolar coupling constant, reduce its contribution through dynamic averaging. Most diamagnetic molecules interact with the applied magnetic field in a nearly orientation-independent manner and space is sampled isotropically, causing the average dipolar interaction to be zero. However, the situation can be quite different in a paramagnetic molecule such as myoglobin, which has a paramagnetic susceptibility tensor that is highly anisotropic ($\Delta\chi_{\text{axial}} = 2198 \times 10^{-12} \text{ m}^3/\text{mol}$, $\Delta\chi_{\text{rhom}} = -573 \times 10^{-12} \text{ m}^3/\text{mol}$) (3). In cases such as this, the molecule has a preferred orientation in the applied magnetic field, and the effects of the dipolar couplings do not average to zero. The magnitude of the departure from normal scalar couplings, while small, can be significant at high fields, allowing residual dipolar couplings to be extracted. Several experimental modifications have been proposed in order to obtain very precise measurements of one-bond heteronuclear coupling constants (2, 4, 5). Some of these experiments allow couplings to be measured with a precision of 0.1 Hz, or better. However, several other factors need to be considered when interpreting such small contributions. Effects due to the differential relaxation of in-phase and antiphase terms (6) and those due to differential broadening of poorly resolved lines (2) have been previously discussed.

For paramagnetic systems in which couplings can be measured accurately, an additional effect comes into play. This is associated with the so-called Curie spin relaxation (7). Normally, one chooses a system with a very short electron spin relaxation time so that broadening of the nuclear spin lines is minimal. However, even under these conditions, an interaction remains with the electron moment averaged over a Boltzmann distribution of electronic spin states (7). This residual interaction is modulated by the same molecular tumbling which modulates the dipolar interaction between two nuclear spins attached to the molecular frame, a fact that can give rise to cross-correlation effects between the two

¹ To whom correspondence should be addressed. Fax: (203) 432-6144. E-mail: james.prestegard@yale.edu.

relaxation pathways. That cross-correlation effects can lead to shifts in line positions in addition to differential broadening of multiplet lines is well documented for several other types of interactions (8). These effects are known as dynamic frequency shifts. Scalar coupling-like transfer of coherence in COSY spectra of paramagnetic proteins has also been traced to cross-correlation effects between nuclear dipole–dipole and Curie relaxation (9, 10). It should not be surprising that such effects could be a concern in the accurate measurement of residual dipolar contributions to spin–spin couplings.

THEORY

In order to assess the importance of the effects of the cross-correlation between the nuclear dipolar and Curie spin interactions, we undertake an analysis of the relaxation behavior of the simplest system where these effects can occur, namely a three-spin system consisting of two dipolar-coupled nuclear spins and the electron spin of the paramagnetic center.

In the basis of the orthogonal spin operators B_k , i.e., in Liouville space, the matrix elements of the relaxation superoperator, Γ , may be written as

$$\Gamma_{kl} = \frac{1}{2} \sum_{q,i,j} (-1)^q [J_{ij}(\omega_i) - 2iQ_{ij}(\omega_i)] \times \frac{\langle B_k | [T_{2,j}^{(-q)}, [T_{2,i}^{(q)}, B_l]] \rangle}{\langle B_k | B_k \rangle}, \quad [1]$$

where the sum is over the indices of the second-rank spin tensors T_2^q and the pairs of interactions i, j . When $i = j$ the contribution is referred to as an auto-correlation contribution and when $i \neq j$ it is called a cross-correlation contribution. The spectral density components $J_{ij}(\omega)$ and $Q_{ij}(\omega)$ are given by

$$J_{ij}(\omega) = \int_{-\infty}^{+\infty} C_{ij}(t) e^{-i\omega t} dt$$

$$Q_{ij}(\omega) = \int_0^{+\infty} C_{ij}(t) \sin(\omega t) dt, \quad [2]$$

where $C_{ij}(t)$ is the molecular correlation function and the other terms in [1] and [2] have the usual definitions (11). $C_{ij}(t)$ may be represented by

$$C_{ij}(t) = K \exp(-t/\tau_c) \times \left\langle \sum_{q=-2}^{q=+2} c_i(0) c_j(t) Y_2^q(\Omega_i^{\text{mol}}(0)) Y_2^q(\Omega_j^{\text{mol}}(t)) \right\rangle, \quad [3]$$

where K is a constant, τ_c is the correlation time for overall

molecular tumbling, and the quantity within the brackets describes the reorientation of the interaction vector due to internal motion written in terms of second-order spherical harmonics Y_2^q . The $c_i(0)$ and $c_j(t)$ vary depending on the type of interaction being considered. For most of the commonly used motional models, [3] yields a $C_{ij}(t)$ which may be represented by a single exponential (for a rigid molecule) or as a sum of exponentials (when there is internal motion).

It is evident from [2] that the J terms are even functions of ω , i.e., $J_{ij}(\omega) = J_{ij}(-\omega)$, whereas the Q terms are odd functions of ω , i.e., $Q_{ij}(\omega) = -Q_{ij}(-\omega)$ and $Q_{ij}(0) = 0$. The functional dependencies of the J and Q terms on the molecular correlation time τ_c are quite different. The real part, i.e., the J terms in [1], lead to regular relaxation behavior whereas the imaginary part, i.e., the Q terms in [1], produce an oscillatory behavior that is more appropriately considered as resulting from a part of the static Hamiltonian (12).

The coefficients that result from the evaluation of the spin tensor commutators in [1] and the $c_i(t)$ in [3] depend on the exact nature of the Hamiltonians describing the particular interaction under consideration. Working in the spherical tensor basis, the nuclear dipole–dipole interaction Hamiltonian for an AX spin system can be written as

$$\mathcal{H}_{\text{DD}} = \frac{\xi_{\text{DD}}}{r_{\text{AX}}^3} \sum_{q=-2}^{q=+2} (-1)^q Y_2^{(-q)}(\Omega_{\text{DD}}^{\text{lab}}(t)) T_2^q(I, S), \quad [4]$$

where r_{AX} is the AX internuclear distance, the I and S are the A and X spin operators, respectively, and ξ_{DD} is given by

$$\xi_{\text{DD}} = -(24\pi/5)^{1/2} (\mu_0/4\pi) \hbar \gamma_A \gamma_X. \quad [5]$$

The irreducible spin operators, $T_2^q(I, S)$, in [4] for the dipole–dipole interaction, are given by

$$T_2^0(I, S) = \frac{1}{\sqrt{6}} \left[2I_z S_z - \frac{1}{2} (I^+ S^- + I^- S^+) \right]$$

$$T_2^{\pm 1}(I, S) = \mp \frac{1}{2} [I^{\pm} S_z + I_z S^{\pm}]$$

$$T_2^{\pm 2}(I, S) = \pm I^{\pm} S^{\pm}. \quad [6]$$

The $c_i(t)$ in [3] are time-independent if the distance r_{AX} is fixed and only angular reorientation is considered. The c_{DD} in this case ($i = \text{DD}$) is equal to $\xi_{\text{DD}}/r_{\text{AX}}^3$.

The Curie spin interaction Hamiltonian may be written in a form similar to [4]

$$\mathcal{H}_{\text{CSR}} = \frac{\xi_{\text{CSR}}}{r_e^3} \sum_{q=-2}^{q=+2} (-1)^q Y_2^{-q}(\Omega_{\text{CSR}}^{\text{lab}}(t)) T_2^q(I, S_e), \quad [7]$$

where r_e is the fixed distance of the A nucleus from the unpaired electron, the I and S_e are the A and electronic spin operators, respectively, and ξ_{CSR} is given by

$$\xi_{\text{CSR}} = -(24\pi/5)^{1/2}(\mu_0/4\pi)\hbar\gamma_A\gamma_e, \quad [8]$$

where γ_e is gyromagnetic ratio of the electron. The c_{CSR} ($i = \text{CSR}$) in this case is also time-independent and is equal to ξ_{CSR}/r_e^3 . The nuclear spin operators in the irreducible spin tensors of [7] appear just as they do in [6]. However, the electronic spin operators should be replaced by their values averaged over the electronic spin states in a Boltzmann fashion. This averaging results in

$$\begin{aligned} \langle S_{ez} \rangle &= \frac{g_e\mu_e B_0}{3kT} S_e(S_e + 1) \\ \langle S_e^\pm \rangle &= 0, \end{aligned} \quad [9]$$

where g_e is the electronic g factor (assumed to be isotropic), μ_e is the electronic Bohr magneton, and S_e is the total electronic spin of the system. Using [9] we may write down the irreducible spin operators for the Curie spin interaction as

$$\begin{aligned} T_2^0(I, S_e) &= \frac{2}{3\sqrt{6}} \frac{g_e\mu_e B_0}{kT} S_e(S_e + 1)I_z \\ T_2^{\pm 1}(I, S_e) &= \mp \frac{1}{6} \frac{g_e\mu_e B_0}{kT} S_e(S_e + 1)I^\pm \\ T_2^{\pm 2}(I, S_e) &= 0. \end{aligned} \quad [10]$$

It is evident from [10] that the irreducible spin tensors for the Curie spin interaction have transformation properties which are similar to those of the CSA interaction (9).

Having defined the dipole–dipole and Curie spin Hamiltonians in terms of the irreducible spin tensors, the latter can be used in [1] to estimate relaxation effects on the shape and position of the lines of an AX multiplet. Linewidths will come from the J_{ij} parts of [1] and the frequency shifts from the Q_{ij} parts. The individual lines of a doublet may be represented as a linear combination of the basis set elements (B_k of [1]) as

$$\begin{aligned} I^\alpha &= \frac{1}{2} [I^+ + 2I^+ S_z] \\ I^\beta &= \frac{1}{2} [I^+ - 2I^+ S_z], \end{aligned} \quad [11]$$

where the α and β signify the spin states of the X spin.

The auto-correlated effects of both the dipole–dipole interaction (11, 13) and the Curie spin interaction (7) have been well documented in the literature; they will not be repeated here. We instead concentrate on the effects of the cross-correlation between the two relaxation pathways. For

simplicity, we consider the case where the overall tumbling of the molecule is isotropic and there is no internal motion. Since we have only a single dipolar and a single Curie interaction, the indices i and j in [3] can be replaced by DD for the dipole–dipole interaction and CSR for the Curie spin interaction. Under these conditions,

$$C_{ij}(t) = \frac{1}{4\pi} \frac{\xi_{\text{DD}}\xi_{\text{CSR}}}{r_{AX}^3 r_e^3} \frac{(3 \cos^2\theta - 1)}{2} e^{-|t|/\tau_c}, \quad [12]$$

where θ is the angle between the A -electron and AX vectors and τ_c is the correlation time for molecular tumbling. Using [12] in [2] we obtain expressions for $J_{ij}(\omega)$ and $Q_{ij}(\omega)$,

$$\begin{aligned} J_{ij}(\omega) &= \frac{1}{4\pi} \frac{\xi_{\text{DD}}\xi_{\text{CSR}}}{r_{AX}^3 r_e^3} \frac{(3 \cos^2\theta - 1)}{2} \frac{2\tau_c}{1 + \omega^2\tau_c^2} \\ Q_{ij}(\omega) &= \frac{1}{4\pi} \frac{\xi_{\text{DD}}\xi_{\text{CSR}}}{r_{AX}^3 r_e^3} \frac{(3 \cos^2\theta - 1)}{2} \frac{\tau_c^2\omega}{1 + \omega^2\tau_c^2}. \end{aligned} \quad [13]$$

We first look at the effects of the real part of the spectral density function, i.e., $J_{ij}(\omega)$. Calculating the relaxation rates of I^α and I^β using [1] we find that these rates differ in a manner similar to that caused by CSA dipolar cross-correlation (14). This difference in relaxation rates is related to $\Delta\nu$, where $\Delta\nu$ is the difference in the linewidths of the two lines of the A multiplet. $\Delta\nu$ is given by

$$\Delta\nu = K_e \left[\frac{1}{9} J_{ij}(0) + \frac{1}{12} J_{ij}(\omega_I) \right], \quad [14]$$

where $K_e = g_e\mu_e B_0 S_e(S_e + 1)/kT$. In the slow motion limit, this is identical to the expression obtained by Bertini *et al.* (9).

The imaginary part of the spectral density function, i.e., $Q_{ij}(\omega)$, as mentioned previously, leads to an oscillatory behavior due to the fact that it causes a pure unitary time evolution. This contribution can also be evaluated using [1]. Using the methods outlined by Brüsweiler (12), this contribution is found to be equivalent to the addition of the following term to the static Hamiltonian,

$$\mathcal{H}_{\text{DFS}} = -K_e Q_{ij}(\omega_I) I_z S_z. \quad [15]$$

Note that [15] is identical in form to the truncated scalar coupling Hamiltonian. It is therefore evident that the dynamic frequency shift results in a change in the position of the individual lines of the A multiplet causing each line to be shifted by an equal amount in opposite directions. The magnitude of this additional shift is given by

$$\Delta\nu_{\text{DFS}} = \frac{1}{2} K_e Q_{ij}(\omega_I). \quad [16]$$

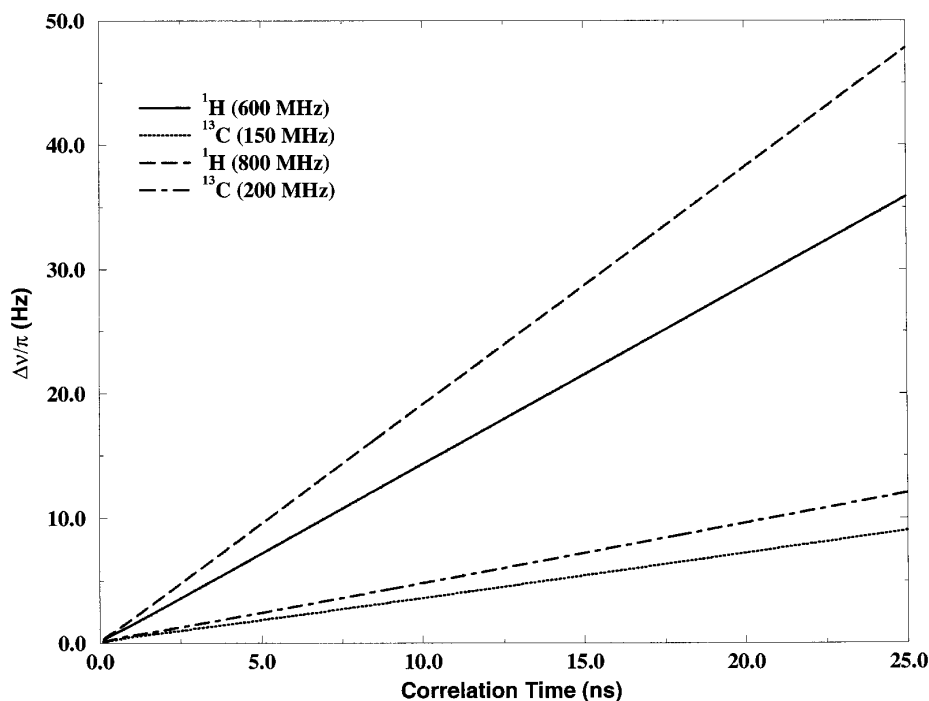


FIG. 1. Plot of the variation in the magnitude of the differential broadening of the lines of an AX doublet with a change in correlation time. A -electron distance = 10 Å. Effects in both the ^1H and ^{13}C dimensions are plotted for ^1H frequencies of 600 and 800 MHz.

RESULTS AND DISCUSSION

We have calculated the influence of the above cross-correlation effects on the differential line broadening and the shift in the resonance positions of the lines of a ^{13}C - ^1H multiplet at two magnetic field strengths corresponding to ^1H Larmor frequencies of 600 and 800 MHz. Figure 1 shows the variation in the differential line broadening in a ^1H - ^{13}C spin system as observed in both proton and carbon dimensions of a 2D experiment (such as a coupled ^1H - ^{13}C HSQC). The length of the C-H internuclear vector has been taken to be 1.1 Å, the A -electron distance has been taken to be 10 Å, the temperature $T = 298$ K, the electron spin $S_e = 3/2$, and θ has been taken to be 0 (the effects are maximal at $\theta = 0$). It can be seen that the predicted effects are larger in the proton dimension than in the carbon dimension. The effects become much larger as the A -electron distance decreases. In a protein, however, any process such as rapid spin flips of the coupling partner, which exchanges the two components of the doublet, would diminish the magnitude of this effect, if these effects are measured directly in the frequency domain (15).

The variation in the magnitude of the shift in the resonance position of the individual lines of the AX doublet due to the dynamic frequency shift is plotted in Fig. 2, the parameters used being the same as above. It is seen that there is a sharp increase in the magnitude of this effect with an increase in

correlation time until a maximum is reached. This behavior is quite similar to that seen for cross-correlation between CSA and dipole-dipole interactions (2, 16). The effect described can clearly become very large for short electron-nuclear distances and when the electron spin quantum number is high ($\Delta\nu_{\text{DFS}}$ depends on $S_e(S_e + 1)$). Another curious result of this cross-correlated dynamic frequency shift is that up to a correlation time of roughly 5 ns, the magnitude of the dynamic frequency shift is different in the carbon and proton dimensions (even if the distance of the electron from both nuclei is the same). This is because of a dependence on the nuclear gyromagnetic ratio. As a result, the C-H splitting measured from the carbon dimension can be different from the same splitting measured from the proton dimension. Thus caution must be exercised in interpreting the multiplet splitting for smaller molecules simply in terms of scalar and dipolar contributions, and one must be cognizant of how the splitting was measured.

Fortunately, for our problem of initial concern, extraction of residual dipolar couplings in field-oriented macromolecules, effects of dynamic frequency shifts may be minimal. For very large correlation times ($\omega\tau_c \gg 1$) the effects become independent of both the correlation time and the nucleus observed. This can be seen in [13] which becomes independent of τ_c for large τ_c and becomes proportional to ξ_{DD}/ω . The proportionality leads to the cancellation of the γ_A which appears in both ξ_{DD} and ω . Field dependence also

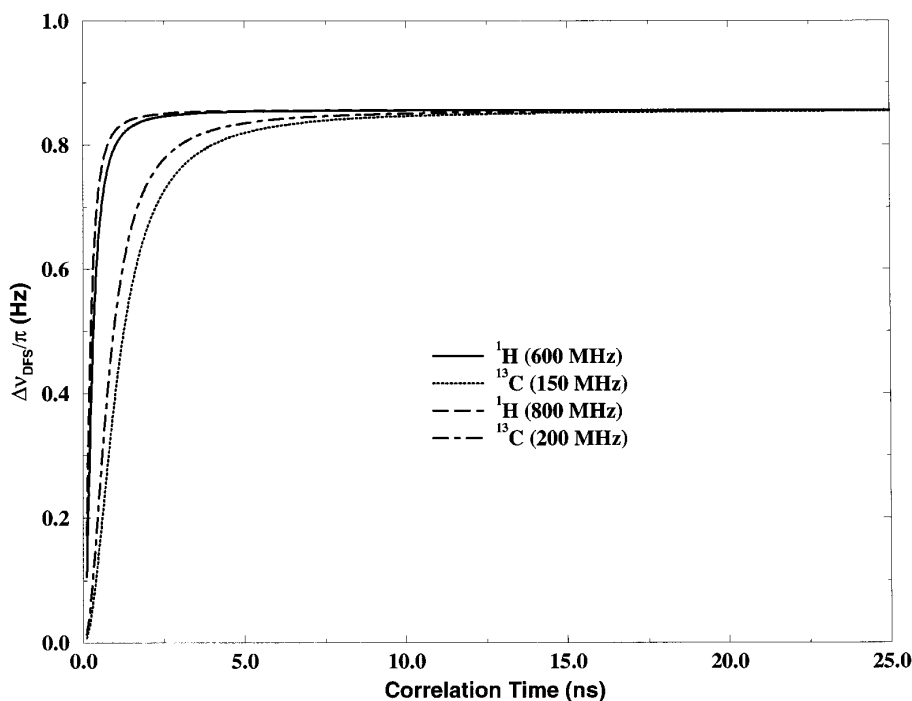


FIG. 2. Plot of the variation in the magnitude of the shift of the resonance position of each of the components of an AX doublet with a change in correlation time. A -electron distance = 10 Å. Effects in both the ^1H and ^{13}C dimensions are plotted for ^1H frequencies of 600 and 800 MHz.

disappears because of its appearance in both ω^{-1} and K_e of [16]. Thus, in most biological systems of interest (which belong to this slow tumbling regime), the dynamic frequency shift contribution to the splitting between the lines is independent of the static magnetic field. This latter property contrasts with the effect from residual dipolar coupling where a field-squared dependence is frequently used to separate it from normal scalar coupling. The lack of field dependence of the dynamic frequency shift automatically separates it from dipolar contributions, when field dependence is used to extract the dipolar part.

ACKNOWLEDGMENTS

We thank J. R. Tolman for helpful discussions. This work has been supported by a grant from the National Institutes of Health, GM 33225.

REFERENCES

1. J. R. Tolman, J. M. Flanagan, M. A. Kennedy, and J. H. Prestegard, Nuclear magnetic dipole interactions in field-oriented proteins: Information for structure determination in solution, *Proc. Natl. Acad. Sci. USA* **92**, 9279–9283 (1995).
2. (a) N. Tjandra, S. Grzesiek, and A. Bax, Magnetic field dependence of nitrogen-proton J splittings in ^{15}N -enriched human ubiquitin resulting from relaxation interference and residual dipolar coupling, *J. Am. Chem. Soc.* **118**, 6264–6272 (1996); (b) N. Tjandra and A. Bax, Measurement of dipolar contributions to $^1J_{\text{CH}}$ splittings from magnetic-field dependence of J modulation in two-dimensional NMR spectra, *J. Magn. Reson.* **124**, 512–515 (1997).
3. S. D. Emerson and G. N. La Mar, NMR determination of the orientation of the magnetic susceptibility tensor in cyanometmyoglobin: A new probe of steric tilt of bound ligand, *Biochemistry* **29**, 1556–1566 (1990).
4. J. R. Tolman and J. H. Prestegard, A quantitative J -correlation experiment for the accurate measurement of one-bond amide ^{15}N – ^1H couplings in proteins, *J. Magn. Reson. B* **112**, 245–252 (1996).
5. J. R. Tolman and J. H. Prestegard, Measurement of amide ^{15}N – ^1H one-bond couplings in proteins using accordion heteronuclear-shift-correlation experiments, *J. Magn. Reson. B* **112**, 269–274 (1996).
6. G. S. Harbison, Interference between J -couplings and cross-relaxation in solution NMR spectroscopy: Consequences for macromolecular structure determination, *J. Am. Chem. Soc.* **115**, 3026–3027 (1993).
7. L. Banci, I. Bertini, and C. Luchinat, "Nuclear and Electron Relaxation," VCH, Weinheim (1991).
8. (a) R. E. London, D. M. LeMaster, and L. G. Werbelow, Unusual NMR multiplet structures of spin-1/2 nuclei coupled to spin-1 nuclei, *J. Am. Chem. Soc.* **116**, 8400 (1994); (b) S. Grzesiek and A. Bax, Interference between dipolar and quadrupolar interactions in the slow tumbling limit: A source of line shift and relaxation in ^2H -labeled compounds, *J. Am. Chem. Soc.* **116**, 10196–10201 (1994).
9. I. Bertini, C. Luchinat, and D. Tarchi, Are true scalar proton–proton couplings ever measured in COSY spectra of paramagnetic molecules? *Chem. Phys. Lett.* **203**, 445–449 (1993).
10. J. Qin, F. Delaglio, G. La Mar, and A. Bax, Distinguishing the effects

- of cross-correlation and J coupling in COSY spectra of paramagnetic proteins, *J. Magn. Reson. B* **102**, 332–336 (1993).
11. R. R. Ernst, G. Bodenhausen, and A. Wokaun, "Principles of Nuclear Magnetic Resonance in One and Two Dimensions," Oxford Univ. Press, Oxford (1990).
 12. R. Brüschweiler, Three-spin interaction in liquid state nuclear magnetic resonance, *J. Chem. Phys.* **105**, 6164–6167 (1996).
 13. (a) T. E. Bull, Cross-correlation and 2D NOE spectra, *J. Magn. Reson.* **72**, 397–413 (1987); (b) T. E. Bull, Relaxation in the rotating frame in liquids, *Prog. NMR Spectrosc.* **24**, 377–410 (1992).
 14. M. Goldman, Interference effects in the relaxation of a pair of unlike spin-1/2 nuclei, *J. Magn. Reson.* **60**, 437–452 (1984).
 15. L. E. Kay, L. K. Nicholson, F. Delaglio, A. Bax, and D. A. Torchia, Pulse sequences for removal of the effects of cross-correlation between dipolar and chemical-shift anisotropy relaxation mechanisms on the measurement of heteronuclear T_1 and T_2 values in proteins, *J. Magn. Reson.* **97**, 359–375 (1992).
 16. R. Brüschweiler, Cross-correlation-induced J coupling, *Chem. Phys. Lett.* **257**, 119–122 (1996).