Cross-Correlated Relaxation with Anisotropic Reorientation and Small Amplitude Local Motions

Michaël Deschamps*

Département de Chimie, Associé au CNRS, École Normale Supérieure, 24 rue Lhomond, F-75231 Paris Cedex 05, France

Received: September 5, 2001

Numerous molecules are asymmetric in shape and diffuse anisotropically in solution. Moreover, many local motions contribute to the nuclear magnetic relaxation process. Dipole−dipole cross-correlations depend on both geometrical (i.e., the average orientations of the dipolar vectors with respect to the principal axes of the diffusion tensor) and dynamical parameters (i.e., the local motions of the same dipolar vectors). We derived a new expression of the cross-correlation function that takes anisotropy of diffusion and local motions of small amplitude into account. The local motion of a unit dipolar vector is expressed in terms of the projection of the vector onto the plane that is perpendicular to its average position. Cross-correlations have been used to study the influence of dynamics on the determination of the angle between the two dipolar vectors.

1. Introduction

Nuclear magnetic resonance relaxation rates depend on the geometry of the average structure of the molecule and on its dynamics. To extract reliable geometrical constraints, one must take into account the fact that various dynamic processes may modify the expected result of the experiment. Usually, the lack of experimental data does not allow one to obtain a perfect knowledge of geometry and dynamics. An exhaustive study of a molecule is always possible but in most cases, its cost is too high. In fact, for each experimental result, the determination of geometrical data (such as distances,[1] angles between bond vectors, or the orientation of a bond vector with respect to the axes of an alignement tensor[2−4]) is not straightforward. In fact, there may be several associated pairs {geometry, dynamics} that may give the same experimental result. The effects of dynamics have been studied since the 60's, the spectral density functions appropriate for anisotropic rotational diffusion are well-known,[5−9] and several studies have taken internal motions into account.[10−20] A more realistic approach would be to consider that a given experimental result (considered with its associated uncertainty) will only provide an interval in which the geometrical parameter, such as the angle between the two considered interactions, should lie, rather than a unique value. A method to determine such an interval is to start with an average local structure, explore all possible dynamic processes and record the predicted rates obtained. One thus obtains a map indicating the allowed connections between an experimental result and the local structures. The analysis of the experimental results is achieved by using this map to determine the possible local structures.

In our case, the use of dipole−dipole cross-correlation rates[21−30] to obtain geometrical constraints in proteins may be hindered by rotational diffusion anisotropy and by local motions. A suitable knowledge of the anisotropy tensor requires calculations based on the knowledge (that may be unavailable) of the structure, and the uncertainties about the tensor cannot be neglected.[31] A new expression of the cross-correlation function including the effects of overall rotational anisotropy and local motion has been derived, using the method of Daragan and Mayo.[15,16,32] With such an expression, one may be able to determine the intervals of allowed cross-correlation rates, provided one assumes a upper limit of the overall rotational anisotropy and uses a specific model for local motions. We compared these results with the dipolar-dipolar $^{13}C_i^{\alpha}\ {}^{1}H_i^{\alpha} - {}^{13}C_{i+1}^{\alpha}\ {}^{1}H_{i+1}^{\alpha}$ cross-correlation rates in doubly enriched human ubiquitin measured by Chiarparin et al.,[28] using 10 solution-state structures determined by Cornilescu et al.[33] for the angles between the dipolar interactions. To limit the motions of the internuclear vectors, we considered rotations about the two neighboring bond vectors characterized by a small amplitude and a short correlation time, when required.[16,34] For the overall rotational diffusion tensor, we used a calculated value of the anisotropy.[34] Using the same procedure, one may be able to determine to what extent the S^2 model is suitable for cross-correlations, or when overall rotational anisotropy has to be taken into account for a given local motion.

2. Theory

The Hamiltonian for the dipolar interaction between spins k and l is given by[35]

$$\hat{H}_{\mathrm{kl}}^{\mathrm{DD}} = b_{\mathrm{kl}} \sum_{\mathrm{q}=-2}^{+2} F_{\mathrm{kl}}^{(\mathrm{q})}(\Omega_{\mathrm{kl}}) \hat{A}_{\mathrm{kl}}^{(-\mathrm{q})}(I_{\mathrm{k}}, I_{\mathrm{l}}) \tag{1}$$

with $b_{kl} = -\mu_0\ \gamma_k\gamma_l\hbar/4\pi r_{kl}^3$ and where Ω_{kl} corresponds to the orientation angles of $\boldsymbol{r}_{kl}$: $\Theta_{kl}(t)$, $\Phi_{kl}(t)$ with respect to the laboratory frame. For cross-correlations, we have to consider two pairs of spins (k,l) and (k',l') and the corresponding internuclear vectors **v** and **w**. The behavior of the spin system is described by the Liouville equation

* To whom correspondence should be addressed: E-mail: Michael.Deschamps@ens.fr. Phone: +33 1 44 32 33 44. Fax: +33 1 44 32 33 97.

10.1021/jp013407e CCC: $22.00
Published on Web 02/12/2002

$$\frac{d\hat{\sigma}(t)}{dt} = -i[\hat{H}_{(0)}, \hat{\sigma}(t)] - \sum_{\mathbf{v,w}} \hat{\Gamma}_{\mathbf{v,w}}(\hat{\sigma}(t) - \hat{\sigma}_{\mathrm{eq}}) \qquad (2)$$

where

$$\hat{\Gamma}_{\mathbf{v,w}}\hat{\sigma} = b_{\mathrm{v}} b_{\mathrm{w}} \sum_{\mathrm{q}=-2}^{+2} [\hat{A}_{\mathrm{v}}^{(-\mathrm{q})}[\hat{A}_{\mathrm{w}}^{(+\mathrm{q})}, \hat{\sigma}]] j^{\mathrm{q}}_{\mathrm{vw}}(\omega_{\mathrm{q}}) \qquad (3)$$

For dipole−dipole cross-correlated relaxation, the relaxation superoperator contains contributions from autocorrelated relaxation ($\mathbf{v} = \mathbf{w}$)

$$[\hat{\Gamma}^{\mathrm{a}}_{\mathbf{v,v}} + \hat{\Gamma}^{\mathrm{a}}_{\mathbf{w,w}}]\hat{\sigma} = b^2_{\mathrm{v}} \sum_{\mathrm{q}} [\hat{A}_{\mathrm{v}}^{(-\mathrm{q})}, [\hat{A}_{\mathrm{v}}^{(+\mathrm{q})}, \hat{\sigma}]] j^{\mathrm{q}}_{\mathrm{v,v}}(\omega_{\mathrm{q}}) + b^2_{\mathrm{w}} \sum_{\mathrm{q}} [\hat{A}_{\mathrm{w}}^{(-\mathrm{q})}, [\hat{A}_{\mathrm{w}}^{(+\mathrm{q})}, \hat{\sigma}]] j^{\mathrm{q}}_{\mathrm{w,w}}(\omega_{\mathrm{q}}) \qquad (4)$$

and from cross-correlated relaxation ($\mathbf{v} \neq \mathbf{w}$)

$$[\hat{\Gamma}^{\mathrm{c}}_{\mathbf{v,w}} + \hat{\Gamma}^{\mathrm{c}}_{\mathbf{w,v}}]\hat{\sigma} = b_{\mathrm{v}} b_{\mathrm{w}} \sum_{\mathrm{q}} [\hat{A}_{\mathrm{v}}^{(-\mathrm{q})}, [\hat{A}_{\mathrm{w}}^{(+\mathrm{q})}, \hat{\sigma}]] j^{\mathrm{q}}_{\mathrm{v,w}}(\omega_{\mathrm{q}}) + b_{\mathrm{v}} b_{\mathrm{w}} \sum_{\mathrm{q}} [\hat{A}_{\mathrm{w}}^{(-\mathrm{q})}, [\hat{A}_{\mathrm{v}}^{(+\mathrm{q})}, \hat{\sigma}]] j^{\mathrm{q}}_{\mathrm{w,v}}(\omega_{\mathrm{q}}) \qquad (5)$$

Only the contribution for $q = 0$ (i.e., $\omega_{\mathrm{q}} = 0$) is significant for the conversion of double-quanta terms into antiphase double-quanta terms.[29] Thus, the interesting term of the cross-correlated part of the relaxation superoperator can be written, knowing that

$$[\hat{A}_{\mathrm{w}}^{(0)}, [\hat{A}_{\mathrm{v}}^{(0)}, \hat{\sigma}]] = [\hat{A}_{\mathrm{v}}^{(0)}, [\hat{A}_{\mathrm{w}}^{(0)}, \hat{\sigma}]]:$$

$$[\hat{\Gamma}^{\mathrm{c}}_{\mathbf{v,w}} + \hat{\Gamma}^{\mathrm{c}}_{\mathbf{w,v}}]\hat{\sigma} = 2 b_{\mathrm{v}} b_{\mathrm{w}} [\hat{A}_{\mathrm{v}}^{(0)}, [\hat{A}_{\mathrm{w}}^{(0)}, \hat{\sigma}]](j^0_{\mathrm{v,w}}(0) + j^0_{\mathrm{w,v}}(0)) \qquad (6)$$

We have to focus on the spectral density function. It is defined through the correlation function

$$j^{\mathrm{q}}_{\mathrm{v,w}}(\omega_{\mathrm{q}}) = \int_0^{\infty} d\tau \langle F_{\mathrm{v}}^{(+\mathrm{q})}(t') F_{\mathrm{w}}^{(+\mathrm{q})*}(t' + \tau)\rangle e^{(-i\omega_q\tau)} \qquad (7)$$

where the angular brackets correspond to a time average over t'. The correlation function $c_{\mathbf{vw}}(\tau) = \langle F_{\mathrm{v}}^{(+\mathrm{q})}(t') F_{\mathrm{w}}^{(+\mathrm{q})*}(t' + \tau)\rangle$ may be expressed in terms of Wigner rotation elements

$$\begin{aligned} F_{\mathrm{v}}^{(+\mathrm{q})}(\Omega_{\mathbf{v}}(t')) &= F_{\mathrm{v}}^{(-\mathrm{q})*}(\Omega_{\mathbf{v}}(t')) \\ &= D^{(2)}_{\mathrm{q}0}(\Phi_{\mathbf{v}}(t'), \Theta_{\mathbf{v}}(t'), 0) \\ &= d^{(2)}_{\mathrm{q}0}(\Theta_{\mathbf{v}}(t')) e^{-\mathrm{iq}\Phi_{\mathbf{v}}(t')} \end{aligned} \qquad (8)$$

To express the correlation function properly, we have to split the motion into rotations about well-defined axes. Thus, we define different steps for the motion of a defined vector: First, the orientation of the diffusion tensor with respect to the magnetic field is defined by $\Omega^D(t')$. The average position of $\mathbf{v}$ with respect to the principal axes of the rotational diffusion tensor is defined by $\Omega^A_{\mathbf{v}}$. Second, the motion around the average position may be represented by $\Omega^L_{\mathbf{v}}(t')$, which characterizes local fluctuations (See Figure 1). Then we can express $F_{\mathrm{v}}^{(+\mathrm{q})}(\Omega_{\mathbf{v}}(t'))$

$$D^{(2)}_{\mathrm{q}0}(\Omega_{\mathbf{v}}(t')) = \sum_{\mathrm{r,s}} D^{(2)}_{\mathrm{qr}}(\Omega^D(t'))\, D^{(2)}_{\mathrm{rs}}(\Omega^A{}_{\mathbf{v}})\, D^{(2)}_{\mathrm{s}0}\,(\Omega^{\mathrm{L}}_{\mathbf{v}}(t')) \qquad (9)$$

Thus, the correlation function $c_{\mathbf{vw}}(\tau)$ may be calculated

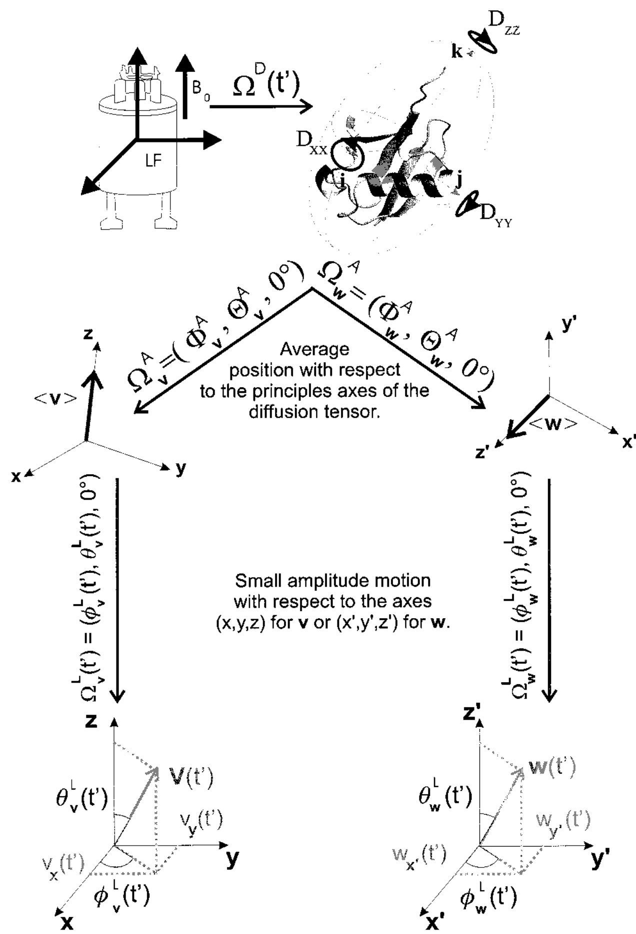

Figure 1. Scheme of the different rotations needed to describe the motion of the dipolar vector.

$$\begin{aligned} c_{\mathbf{vw}}(\tau) &= \langle D^{(2)}_{\mathrm{q}0}(\Omega_{\mathbf{v}}(t')) D^{(2)}_{+\mathrm{q}0}{}^*\,(\Omega_{\mathbf{w}}(t'+\tau))\rangle \\ &= \langle \sum_{\mathrm{r,s}} D^{(2)}_{\mathrm{qr}}(\Omega^D(t')) D^{(2)}_{\mathrm{rs}}(\Omega^A_{\mathbf{v}}) D^{(2)}_{\mathrm{s}0}(\Omega^{\mathrm{L}}_{\mathbf{v}}(t')) \times \sum_{\mathrm{r's'}} D^{(2)}_{+\mathrm{qr'}}{}^*\,(\Omega^D(t'+\tau)) D^{(2)}_{\mathrm{r's'}}{}^*\,(\Omega^A_{\mathbf{w}}) D^{(2)}_{\mathrm{s'}0}{}^*\,(\Omega^L_{\mathbf{w}}(t'+\tau))\rangle \\ &= \sum_{\mathrm{r,s,r',s'}} \langle D^{(2)}_{\mathrm{qr}}(\Omega^D(t')) D^{(2)}_{\mathrm{qr'}}{}^*\,(\Omega^D(t'+\tau))\rangle \times D^{(2)}_{\mathrm{rs}}(\Omega^A_{\mathbf{v}}) D^{(2)}_{\mathrm{r's'}}{}^*\,(\Omega^A_{\mathbf{w}}) \times \langle D^{(2)}_{\mathrm{s}0}(\Omega^{\mathrm{L}}_{\mathbf{v}}(t')) D^{(2)}_{\mathrm{s'}0}{}^*\,(\Omega_{\mathbf{w}}{}^L(t'+\tau))\rangle \end{aligned} \qquad (10)$$

because overall and local motions are assumed to be independent. Let us consider the overall rotational diffusion, and assume that the rotational diffusion tensor is symmetric. Then[36]

$$\langle D^{(2)}_{\mathrm{qr}}(\Omega^D(t')) D^{(2)}_{\mathrm{qr'}}{}^*\,(\Omega^D(t'+\tau))\rangle = \frac{1}{5}\,\delta_{rr'}\, e^{-[6D_\perp + r^2(D_\parallel - D_\perp)]\tau} \qquad (11)$$

Note that this part does not depend on q, and consequently, the same is true for the whole correlation function. Only terms where $r = r'$ do not vanish. We can write the correlation function

$$c_{\mathbf{vw}}(\tau) = \frac{1}{5}\sum_{\mathrm{r}=-2}^{+2} e^{[6\mathrm{D}_\perp + \mathrm{r}^2(\mathrm{D}_\parallel - \mathrm{D}_\perp)]\tau} \times \sum_{\mathrm{s,s'}} D^{(2)}_{\mathrm{rs}}(\Omega^{\mathrm{A}}_{\mathbf{v}})\, D^{(2)}_{\mathrm{rs'}}{}^*\,(\Omega^{\mathrm{A}}_{\mathbf{w}}) \times \langle D^{(2)}_{\mathrm{s}0}(\Omega^{\mathrm{L}}_{\mathbf{v}}(t')) D^{(2)}_{\mathrm{s'}0}{}^*\,(\Omega^{\mathrm{L}}_{\mathbf{w}}(t'+\tau))\rangle \qquad (12)$$

If one looks carefully at the last terms, one notices that it is possible to make simplifications.[16] First

$$D^{(2)}_{s0}(\Omega^{L}_{\mathbf{v}}(t'))D^{(2)*}_{s'0}(\Omega^{L}_{\mathbf{w}}(t'+\tau)) = d^{(2)}_{s0}(\theta_{\mathbf{v}}(t'))d^{(2)}_{s'0}(\theta_{\mathbf{w}}(t'+\tau))e^{-\mathrm{is}\phi_{\mathbf{v}}(t')+\mathrm{is}'\phi_{\mathbf{w}}(t'+\tau)} \quad (13)$$

Second, we define

$$\mathscr{F}^{\mathbf{vw}}_{ss'}(\tau) = \langle d^{(2)}_{s0}(\theta_{\mathbf{v}}(t'))\, d^{(2)}_{s'0}(\theta_{\mathbf{w}}(t'+\tau))\rangle \quad (14)$$

Assuming that the amplitudes of the fluctuations are small and symmetric with respect to the average position, we can approximate the functions $\mathscr{F}^{\mathbf{vw}}_{ss'}(\tau)$ with a second-order Taylor expansion (the angular brackets have been dropped to simplify the expressions)

$$\mathscr{F}^{\mathbf{vw}}_{00}(\tau) \approx 1 - \frac{3}{2}\,\theta^2_{\mathbf{v}}(t') - \frac{3}{2}\,\theta^2_{\mathbf{w}}(t'+\tau)$$

$$\mathscr{F}^{\mathbf{vw}}_{02}(\tau) = \mathscr{F}^{\mathbf{vw}}_{0-2}(\tau) \approx \sqrt{\frac{3}{8}}\theta^2_{\mathbf{w}}(t'+\tau)$$

$$\mathscr{F}^{\mathbf{vw}}_{11}(\tau) = -\mathscr{F}^{\mathbf{vw}}_{-11}(\tau) = -\mathscr{F}^{\mathbf{vw}}_{1-1}(\tau) = \mathscr{F}^{\mathbf{vw}}_{-1-1}(\tau) \approx \frac{3}{2}\theta_{\mathbf{v}}(t')\theta_{\mathbf{w}}(t'+\tau)$$

$$\mathscr{F}^{\mathbf{vw}}_{20}(\tau) = \mathscr{F}^{\mathbf{vw}}_{-20}(\tau) \approx \sqrt{\frac{3}{8}}\theta^2_{\mathbf{v}}(t')$$

$$\mathscr{F}^{\mathbf{vw}}_{0\pm1}(\tau) = \mathscr{F}^{\mathbf{vw}}_{\pm10}(\tau) = \mathscr{F}^{\mathbf{vw}}_{\pm1\pm2}(\tau) = \mathscr{F}^{\mathbf{vw}}_{\pm2\pm1}(\tau) = \mathscr{F}^{\mathbf{vw}}_{\pm2\pm2}(\tau) \approx 0 \quad (15)$$

These expression are valid for angular fluctuations smaller than $\pi/6$. The correlation function

$$c_{\mathbf{vw}}(\tau) = \frac{1}{5}[\mathscr{G}_0(\tau)e^{-6D_\perp\tau} + \mathscr{G}_1(\tau)e^{-(5D_\perp+D_\parallel)\tau} + \mathscr{G}_2(\tau)e^{-(2D_\perp+4D_\parallel)\tau}] \quad (16)$$

Where the detailed functions $\mathscr{G}_i(\tau)$ are shown in Appendix A

$$\mathscr{G}_0(\tau) = \sum_{ss'} d^{(2)}_{0s}(\Theta^A_{\mathbf{v}})d^{(2)}_{0s'}(\Theta^A_{\mathbf{w}})\mathscr{F}^{\mathbf{vw}}_{ss'}(\tau)e^{\mathrm{is}\Phi_{\mathbf{v}}(t')-\mathrm{is}'\Phi_{\mathbf{w}}(t'+\tau)}$$

$$\mathscr{G}_1(\tau) = \sum_{ss'}[d^{(2)}_{1s}(\Theta^A_{\mathbf{v}})d^{(2)}_{1s'}(\Theta^A_{\mathbf{w}})e^{\mathrm{i}\Phi^A_{\mathbf{v}}-\mathrm{i}\Phi^A_{\mathbf{w}}} + d^{(2)}_{-1s}(\Theta^A_{\mathbf{v}})d^{(2)}_{-1s'}(\Theta^A_{\mathbf{w}})e^{-\mathrm{i}\Phi^A_{\mathbf{v}}+\mathrm{i}\Phi^A_{\mathbf{w}}}] \times \mathscr{F}^{\mathbf{vw}}_{ss'}(\tau)e^{\mathrm{is}\Phi_{\mathbf{v}}(t')-\mathrm{is}'\Phi_{\mathbf{w}}(t'+\tau)}$$

$$\mathscr{G}_2(\tau) = \sum_{ss'}[d^{(2)}_{2s}(\Theta^A_{\mathbf{v}})d^{(2)}_{2s'}(\Theta^A_{\mathbf{w}})e^{2\mathrm{i}\Phi^A_{\mathbf{v}}-2\mathrm{i}\Phi^A_{\mathbf{w}}} + d^{(2)}_{-2s}(\Theta^A_{\mathbf{v}})d^{(2)}_{-2s'}(\Theta^A_{\mathbf{w}})e^{-2\mathrm{i}\Phi^A_{\mathbf{v}}+2\mathrm{i}\Phi^A_{\mathbf{w}}}] \times \mathscr{F}^{\mathbf{vw}}_{ss'}(\tau)e^{\mathrm{is}\Phi_{\mathbf{v}}(t')-\mathrm{is}'\Phi_{\mathbf{w}}(t'+\tau)} \quad (17)$$

Now we must consider the sum of the two contributions stemming from $c_{\mathbf{vw}}(\tau)$ and $c_{\mathbf{wv}}(\tau)$. This will give a new correlation function, with different internal (cross-)correlation functions

$$c_{\mathbf{vw}}(\tau) + c_{\mathbf{wv}}(\tau) = \frac{1}{5}[\mathscr{K}_0(\tau)e^{-6D_\perp\tau} + \mathscr{K}_1(\tau)e^{-(5D_\perp+D_\parallel)\tau} + \mathscr{K}_2(\tau)e^{-(2D_\perp+4D_\parallel)\tau}] \quad (18)$$

where

$$\begin{aligned}\mathscr{K}_0(\tau) = {} & 2\, d^{(2)}_{00}(\Theta^A_{\mathbf{v}})d^{(2)}_{00}(\Theta^A_{\mathbf{w}})\Big[1 - \frac{3}{2}\langle v^2_{\mathrm{x}} + v^2_{\mathrm{y}}\rangle - \frac{3}{2}\langle \boldsymbol{w}^2_{\mathrm{x}'} + \boldsymbol{w}^2_{\mathrm{y}'}\rangle\Big] + \\ & \sqrt{6}\,[d^{(2)}_{00}(\Theta^A_{\mathbf{v}})d^{(2)}_{02}(\Theta^A_{\mathbf{w}})\langle \boldsymbol{w}^2_{\mathrm{x}'} - \boldsymbol{w}^2_{\mathrm{y}'}\rangle + d^{(2)}_{02}(\Theta^A_{\mathbf{v}})d^{(2)}_{00}(\Theta^A_{\mathbf{w}})\langle v^2_{\mathrm{x}} - v^2_{\mathrm{y}}\rangle] + \\ & 6\, d^{(2)}_{01}(\Theta^A_{\mathbf{v}})d^{(2)}_{01}(\Theta^A_{\mathbf{w}})\langle v_{\mathrm{x}}(t')\boldsymbol{w}_{\mathrm{x}'}(t'+\tau) + v_{\mathrm{x}}(t'+\tau)\boldsymbol{w}_{\mathrm{x}'}(t')\rangle\end{aligned}$$

$$\begin{aligned}\mathscr{K}_1(\tau) = {} & 4\, d^{(2)}_{10}(\Theta^A_{\mathbf{v}})d^{(2)}_{10}(\Theta^A_{\mathbf{w}})\cos(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}}) \times \Big[1 - \frac{3}{2}\langle v^2_{\mathrm{x}} + v^2_{\mathrm{y}}\rangle - \frac{3}{2}\langle \boldsymbol{w}^2_{\mathrm{x}'} + \boldsymbol{w}^2_{\mathrm{y}'}\rangle\Big] + \\ & \sqrt{6}\, d^{(2)}_{10}(\Theta^A_{\mathbf{v}})[\sin(\Theta^A_{\mathbf{w}})\cos(\Theta^A_{\mathbf{w}})\cos(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\langle \boldsymbol{w}^2_{\mathrm{x}'} - \boldsymbol{w}^2_{\mathrm{y}'}\rangle + 2\sin(\Theta^A_{\mathbf{w}})\sin(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\langle \boldsymbol{w}_{\mathrm{x}'}\boldsymbol{w}_{\mathrm{y}'}\rangle] + \\ & \sqrt{6}\, d^{(2)}_{10}(\Theta^A_{\mathbf{w}})[\sin(\Theta^A_{\mathbf{v}})\cos(\Theta^A_{\mathbf{v}})\cos(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\langle v^2_{\mathrm{x}} - v^2_{\mathrm{y}}\rangle - 2\sin(\Theta^A_{\mathbf{v}})\sin(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\langle v_{\mathrm{x}}v_{\mathrm{y}}\rangle] + \\ & 3\cos(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})[(1 - 2\cos^2(\Theta^A_{\mathbf{v}}) - 2\cos^2(\Theta^A_{\mathbf{w}}) - 4\cos^2(\Theta^A_{\mathbf{v}})\cos^2(\Theta^A_{\mathbf{w}})) \times \\ & \langle v_{\mathrm{x}}(t')\boldsymbol{w}_{\mathrm{x}'}(t'+\tau) + v_{\mathrm{x}}(t'+\tau)\boldsymbol{w}_{\mathrm{x}'}(t')\rangle + \cos(\Theta^A_{\mathbf{v}})\cos(\Theta^A_{\mathbf{w}})\langle v_{\mathrm{y}}(t')\boldsymbol{w}_{\mathrm{y}'}(t'+\tau) + \\ & v_{\mathrm{y}}(t'+\tau)\boldsymbol{w}_{\mathrm{y}'}(t')\rangle] + 3\sin(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}}) \times [\cos(\Theta^A_{\mathbf{w}})\cos(2\Theta^A_{\mathbf{v}})\,\langle v_{\mathrm{x}}(t')\boldsymbol{w}_{\mathrm{y}'}(t'+\tau) + \\ & v_{\mathrm{x}}(t'+\tau)\boldsymbol{w}_{\mathrm{y}'}(t')\rangle - \cos(\Theta^A_{\mathbf{v}})\cos(2\Theta^A_{\mathbf{w}})\langle v_{\mathrm{y}}(t')\boldsymbol{w}_{\mathrm{x}'}(t'+\tau) + v_{\mathrm{y}}(t'+\tau)\boldsymbol{w}_{\mathrm{x}'}(t')\rangle]\end{aligned}$$

$$\begin{aligned}\mathscr{K}_2(\tau) = {} & 4\, d^{(2)}_{20}(\Theta^A_{\mathbf{v}})d^{(2)}_{20}(\Theta^A_{\mathbf{w}})\cos 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\Big[1 - \frac{3}{2}\langle v^2_{\mathrm{x}} + v^2_{\mathrm{y}}\rangle - \frac{3}{2}\langle \boldsymbol{w}^2_{\mathrm{x}'} + \boldsymbol{w}^2_{\mathrm{y}'}\rangle\Big] + \sqrt{6}\, d^{(2)}_{20}(\Theta^A_{\mathbf{v}})[(d^{(2)}_{22}(\Theta^A_{\mathbf{w}}) + \\ & d^{(2)}_{2-2}(\Theta^A_{\mathbf{w}}))\cos 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\langle \boldsymbol{w}^2_{\mathrm{x}'} - \boldsymbol{w}^2_{\mathrm{y}'}\rangle + 2\cos(2\Theta^A_{\mathbf{w}})\sin 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\langle \boldsymbol{w}_{\mathrm{x}'}\boldsymbol{w}_{\mathrm{y}'}\rangle] + \\ & \sqrt{6}d^{(2)}_{20}(\Theta^A_{\mathbf{w}})[(d^{(2)}_{22}(\Theta^A_{\mathbf{v}}) + d^{(2)}_{2-2}(\Theta^A_{\mathbf{v}}))\cos 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}}) \times \langle v^2_{\mathrm{x}} - v^2_{\mathrm{y}}\rangle - 2\cos(2\Theta^A_{\mathbf{v}})\sin 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})\langle v_{\mathrm{x}}v_{\mathrm{y}}\rangle] + \\ & 3\sin(\Theta^A_{\mathbf{v}})\sin(\Theta^A_{\mathbf{w}})[\cos 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}}) \times [\langle v_{\mathrm{y}}(t')\boldsymbol{w}_{\mathrm{y}'}(t'+\tau) + v_{\mathrm{y}}(t'+\tau)\boldsymbol{w}_{\mathrm{y}'}(t')\rangle + \\ & \cos(\Theta^A_{\mathbf{v}})\cos(\Theta^A_{\mathbf{w}})\langle v_{\mathrm{x}}(t')\boldsymbol{w}_{\mathrm{x}'}(t'+\tau) + v_{\mathrm{x}}(t'+\tau)\boldsymbol{w}_{\mathrm{x}'}(t')\rangle] + \\ & \sin 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}})[\cos(\Theta^A_{\mathbf{v}})\langle v_{\mathrm{y}}(t')\boldsymbol{w}_{\mathrm{y}'}(t'+\tau) + v_{\mathrm{y}}(t'+\tau)\boldsymbol{w}_{\mathrm{y}'}(t')\rangle - \cos(\Theta^A_{\mathbf{w}})\langle v_{\mathrm{x}}(t')\boldsymbol{w}_{\mathrm{y}'}(t'+\tau) + \\ & v_{\mathrm{x}}(t'+\tau)\boldsymbol{w}_{\mathrm{y}'}(t')\rangle]] \quad (19)\end{aligned}$$

where some terms have been simplified: for example, $\langle \mathbf{v}^2_x(t') + \mathbf{v}^2_y(t')\rangle = \langle \mathbf{v}^2_x(t'+\tau) + \mathbf{v}^2_y(t'+\tau)\rangle = \langle \mathbf{v}^2_x + \mathbf{v}^2_y\rangle$.

3. Results and Discussion

It may be interesting to calculate these functions in specific cases. First, one can easily verify that if none of the vectors undergo any local motion, the different parts of the correlation function become

$$\mathscr{K}_0(\tau) = 2d^{(2)}_{00}(\Theta^A_{\mathbf{v}})d^{(2)}_{00}(\Theta^A_{\mathrm{w}})$$

$$\mathscr{K}_1(\tau) = 4d^{(2)}_{10}(\Theta^A_{\mathbf{v}})d^{(2)}_{10}(\Theta^A_{\mathbf{w}})\cos(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathrm{w}})$$

$$\mathscr{K}_2(\tau) = 4d^{(2)}_{20}(\Theta^A_{\mathbf{v}})d^{(2)}_{20}(\Theta^A_{\mathbf{w}})\cos 2(\Phi^A_{\mathbf{v}} - \Phi^A_{\mathbf{w}}) \quad (20)$$

One notices that in such a case, one finds the function previously calculated, i.e., $c_{\mathbf{vw}}(\tau) + c_{\mathbf{wv}}(\tau) = \mathcal{C}_{\mathbf{vw}}^{\text{rigid}}(\tau)$. The correlation function $\mathcal{C}_{\mathbf{vw}}^{\text{rigid}}(\tau)$ due to global motion can be separated from the one induced by local motion, provided all terms in angular brackets except $\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle$ and $\langle \mathbf{w}_{x'}^2 + \mathbf{w}_{y'}^2 \rangle$ vanish. In this case, the local motions of $\mathbf{v}$ and $\mathbf{w}$ are not correlated and these vectors satisfy the following relationships: $\langle \mathbf{v}_x^2 - \mathbf{v}_y^2 \rangle = \langle \mathbf{w}_{x'}^2 - \mathbf{w}_{y'}^2 \rangle = 0$ and $\langle \mathbf{v}_x \mathbf{v}_y \rangle = \langle \mathbf{w}_{x'} \mathbf{w}_{y'} \rangle = 0$). One can then obtain:

$$\mathcal{K}_0(\tau) = 2d_{00}^{(2)}(\Theta_{\mathbf{v}}^A)d_{00}^{(2)}(\Theta_{\mathbf{w}}^A)\left[1 - \frac{3}{2}\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle - \frac{3}{2}\langle \mathbf{w}_{x'}^2 + \mathbf{w}_{y'}^2 \rangle\right]$$

$$\mathcal{K}_1(\tau) = 4d_{10}^{(2)}(\Theta_{\mathbf{v}}^A)d_{10}^{(2)}(\Theta_{\mathbf{w}}^A)\cos(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \left[1 - \frac{3}{2}\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle - \frac{3}{2}\langle \mathbf{w}_{x'}^2 + \mathbf{w}_{y'}^2 \rangle\right]$$

$$\mathcal{K}_2(\tau) = 4d_{20}^{(2)}(\Theta_{\mathbf{v}}^A)d_{20}^{(2)}(\Theta_{\mathbf{w}}^A)\cos 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \left[1 - \frac{3}{2}\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle - \frac{3}{2}\langle \mathbf{w}_{x'}^2 + \mathbf{w}_{y'}^2 \rangle\right] \quad (21)$$

The correlation function $c_{\mathbf{vw}}(\tau) + c_{\mathbf{wv}}(\tau)$ can be separated into two factors that correspond to the anisotropic correlation function without local motion $\mathcal{C}_{\mathbf{vw}}^{\text{rigid}}(\tau)$ for the first part and to the correlation function accounting for local motions $1 - 3/2\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle - 3/2\langle \mathbf{w}_{x'}^2 + \mathbf{w}_{y'}^2 \rangle$ for the second part

$$c_{\mathbf{vw}}(\tau) + c_{\mathbf{wv}}(\tau) = \mathcal{C}_{\mathbf{vw}}^{\text{rigid}}(\tau) \times \left[1 - \frac{3}{2}\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle - \frac{3}{2}\langle \mathbf{w}_{x'}^2 + \mathbf{w}_{y'}^2 \rangle\right] \quad (22)$$

With this simplified expression, we have an easy access to the influence of the amplitudes of local motions. Considering a wobbling-in-a-cone motion, where each vector can oscillate in a cone ($-15° \le \theta_{\mathbf{v,w}} \le +15°$), the cross-correlation factor will be attenuated by a factor of 0.9. For two or three times larger angles ($\pm 30°$ or $\pm 45°$), the attenuation factors will be 0.6 or 0.07.

By setting $\mathbf{v} = \mathbf{w}$, one can find the autocorrelation case discussed by Daragan et al.[16,32] In the autocorrelation case, the function $1-3\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle$ corresponds to the so-called model-free order parameter $S_{\mathbf{v}}^2$ related to the vector $\mathbf{v}$[16]

$$\mathcal{C}_{\mathbf{vv}}(\tau) = \mathcal{C}_{\mathbf{vv}}^{\text{rigid}}(\tau)(1 - 3\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle) \quad (23)$$

We notice that the parameter we calculated is the average $1/2(S_{\mathbf{v}}^2 + S_{\mathbf{w}}^2) = 1 - 3/2\langle \mathbf{v}_x^2 + \mathbf{v}_y^2 \rangle - 3/2\langle \mathbf{w}_{x'}^2 + \mathbf{w}_{y'}^2 \rangle$. Thus, if one assumes that the auto-correlation model-free order parameter is typically between 0.8 and 1, local motions have approximately the same effect in the cross-correlation case. The anisotropy of rotational diffusion must be considered as the dominant factor if its effect on the cross-correlation rate is higher than the effect of the order parameter, i.e., 20%. As a consequence of eq 22, the effect of local motions can be represented by a scaling factor. Thus, the anisotropy of rotational diffusion can be taken into account separately, as described in ref 37 For $\theta_{\mathbf{vw}} = 0$, if $D_{\|}/D_{\perp} \ge 1.45$ or $D_{\|}/D_{\perp} \le 0.65$ (in a symmetrical diffusion tensor), the error due to anisotropy is 20%. In these cases, it seems obvious to take anisotropy of rotational diffusion into account. However, for human ubiquitin, a 17% anisotropy will lead to an error of 9%. Associated with a maximum error of 20% due to internal dynamics, the overall error is equal to 27%. This tends to show that even if internal motion is supposed to be a decisive factor, one should be aware of the pernicious effects of anisotropy.

It will be shown that the model-free analysis cannot really be applied to calculate the intervals of cross-correlation rates. A more elaborate motional model should be used to derive such intervals. For correlated motions, one has to assume some model to calculate average values such as $\langle \mathbf{v}_x^2 \pm \mathbf{v}_y^2 \rangle$, $\langle \mathbf{w}_{x'}^2 \pm \mathbf{w}_{y'}^2 \rangle$ and $\langle \mathbf{v}_x \mathbf{v}_y \rangle$, $\langle \mathbf{w}_{x'} \mathbf{w}_{y'} \rangle$. For the terms that are functions of τ, we used the approach followed by Daragan et al.[16] These functions may be characterized by appropriate average values and a correlation time τ_l characteristic of local motions

$$\langle \mathbf{v}_x(t')\mathbf{w}_{x'}(t' + \tau) + \mathbf{w}_{x'}(t')\mathbf{v}_x(t' + \tau) \rangle = \langle \mathbf{v}_x \mathbf{w}_{x'} \rangle e^{-\tau/\tau_l}$$

$$\langle \mathbf{v}_x(t')\mathbf{w}_{y'}(t' + \tau) + \mathbf{w}_{y'}(t')\mathbf{v}_x(t' + \tau) \rangle = \langle \mathbf{v}_x \mathbf{w}_{y'} \rangle e^{-\tau/\tau_l}$$

$$\langle \mathbf{v}_y(t')\mathbf{w}_{y'}(t' + \tau) + \mathbf{w}_{y'}(t')\mathbf{v}_y(t' + \tau) \rangle = \langle \mathbf{v}_y \mathbf{w}_{y'} \rangle e^{-\tau/\tau_l}$$

$$\langle \mathbf{v}_y(t')\mathbf{w}_x{}'(t' + \tau) + \mathbf{w}_x{}'(t')\mathbf{v}_y(t' + \tau) \rangle = \langle \mathbf{v}_y \mathbf{w}_{x'} \rangle e^{-\tau/\tau_l} \quad (24)$$

To simplify these expressions, we assumed that the motions of $\mathbf{v}$ and $\mathbf{w}$ are not correlated. Then, we have

$$\langle \mathbf{v}_x \mathbf{w}_{x'} \rangle = \langle \mathbf{v}_x \rangle \langle \mathbf{w}_{x'} \rangle = 0 \quad (25)$$

because of the symmetry of the fluctuations (i.e., $\langle \mathbf{v}_x \rangle = \langle \mathbf{w}_{x'} \rangle = 0$). Thus, the parameter τ_l does not play any role, because the correlation between $\mathbf{v}$ and $\mathbf{w}$ is not taken into account (Terms such as $\langle \mathbf{v}_x(t')\mathbf{w}_{x'}(t' + \tau) + \mathbf{w}_{x'}(t')\mathbf{v}_x(t' + \tau) \rangle$ will also vanish, and only the terms $\langle \mathbf{v}_x^2 \pm \mathbf{v}_y^2 \rangle$, $\langle \mathbf{w}_{x'}^2 \pm \mathbf{w}_{y'}^2 \rangle$ and $\langle \mathbf{v}_x \mathbf{v}_y \rangle$, $\langle \mathbf{w}_{x'} \mathbf{w}_{y'} \rangle$, where τ_l does not play any role- will remain). To calculate the remaining terms, we made use of the method developped by Daragan et al.[16] We introduced two random rotations, $\vec{\omega}(t')$ and $\vec{\omega}'(t')$ acting on the vectors $\mathbf{v}$ and $\mathbf{w}$. As we said, if the motions of $\mathbf{v}$ and $\mathbf{w}$ are not correlated, the random rotations $\vec{\omega}(t')$ and $\vec{\omega}'(t')$ are not correlated. The average values of these rotations vanish $\langle \omega(t') \rangle = \langle \omega'(t') \rangle = 0$. First, we will consider the cross-correlation between dipolar $^{13}C^{\alpha} - {}^{1}H^{\alpha}$ interactions in proteins. To calculate the random rotations $\vec{\omega}(t')$ and $\vec{\omega}'(t')$, we supposed that the main sources of motion are the rotations about the two neighboring bonds. For each vector, one has to consider two rotations of angles ϕ and ψ around the $^{13}C_{\alpha}{}^{15}N$ and $^{13}C_{\alpha}{}^{13}C'$ bonds. The resulting transformation may be approximated by the sum of the two rotations[15]

$$\vec{\omega} = \vec{\phi} + \vec{\psi} \quad (26)$$

According to Daragan et al.[16]

$$\omega^2 = \phi^2 + \psi^2 + 2\vec{\phi}.\vec{\psi} \quad (27)$$

and

$$\langle \vec{\phi}.\vec{\psi} \rangle = c_{\phi\psi}\sqrt{\langle \phi^2 \rangle \langle \psi^2 \rangle} \quad (28)$$

where $-1 \le c_{\phi\psi} \le 1$ and $\langle \phi^2 \rangle = 2||\vec{\phi}^{\max}||^3/3$ with $-\phi^{\max} \le \phi \le \phi^{\max}$. We will consider that the rotations $\vec{\phi}$ and $\vec{\psi}$ are not correlated, i.e., $c_{\phi\psi} = 0$ to simplify the numerical calculations.

We considered the effects of these dynamical processes on the $^{13}C_i^{\alpha}\ {}^{1}H_i^{\alpha} - {}^{13}C_{i+1}^{\alpha}\ {}^{1}H_{i+1}^{\alpha}$ cross-correlation rate. For the generation of the map of the Figure 2, we used $\tau_c = 0.14$ ns, and $D_{//}/D_{\perp} = 1.27$. Then, 180 values for the angle $\theta_{\mathbf{vw}}$ have been used. Ten different values between 0° and 180° for each orientation angle of the pair of vector (as defined by Deschamps et al.[37]) have been taken. The axes of the small rotations were taken to be the bond vectors $N_i^H{-}C_i^{\alpha}$, $N_{i+1}^H{-}C_{i+1}^{\alpha}$ and $C_i^{\alpha}{-}C'_i$, $C_{i+1}^{\alpha}{-}C'_{i+1}$, and two relative orientations of these bond vectors

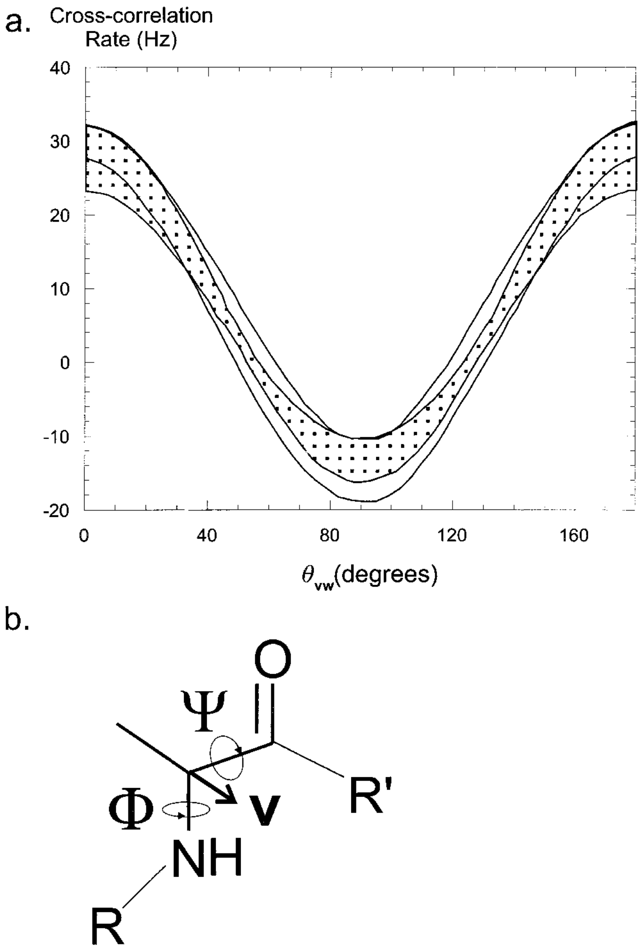

Figure 2. (a) Areas covered by the cross-correlation rates $R(\theta_{vw})$ for $\tau_c = 4.14$ ns and $D_{//}/D_{\perp} = 1.24$:[34] The gray area represents the dispersion of the rates arising from overall rotational anisotropy and local motions due to small rotations about the neighboring bond vectors (b) with maximal amplitudes equal to 10°. The dotted area corresponds to the area generated by rotational anisotropy[37] multiplied by a local order parameter $0.8 \leq S^2 \leq 1.0$. b. The vector $\boldsymbol{v} = \boldsymbol{r}(^{13}C_i^{\alpha}\ ^1H_i^{\alpha})$ in an amino acid may undergo small rotations about the two neighboring bond vectors.

with respect to **v** or **w** were used. Three different maximum amplitudes were used for each rotation $\vec{\phi}$ and $\vec{\psi}$: 0°, $\phi^{max}/2$ and ϕ^{max} or 0°, $\psi^{max}/2$ and ψ^{max}. For each value of θ_{vw}, 12 960 000 pairs {orientation,dynamics} were explored and the corresponding cross-correlation rates were recorded. The upper and lower bound of these rates were represented in Figure 2, for a maximum amplitude of all rotations about bonds set to 10°. This area was compared to the rates obtained by describing the local motion by a local order parameter $0.8 \leq S^2_{vw} \leq 1$. The shapes of these two maps are rather different. One can notice that the S^2 method tends to minimize errors for θ_{vw} values that are near to 54.7° and maximize them near 0° or 180°. Thus, it is shown that cross-correlation may be studied more efficiently by considering real local motions.

We applied this method to human ubiquitin. Knowing 10 solution structures determined by Cornilescu et al.,[33] and the dipolar-dipolar $^{13}C_i^{\alpha}\ ^1H_i^{\alpha} - {}^{13}C_{i+1}^{\alpha}\ ^1H_{i+1}^{\alpha}$ cross-correlation rates measured by Chiarparin et al.[28] we plotted in Figure 3 the cross-correlation rate against the average angle θ_{vw} in the 10 structures. The vertical error bars correspond to the experimental errors and the horizontal error bars, to the intervals covered by the angles θ_{vw} in the 10 solution-state structures. We represented two areas containing the allowed pairs {θ_{vw},cross-correlation

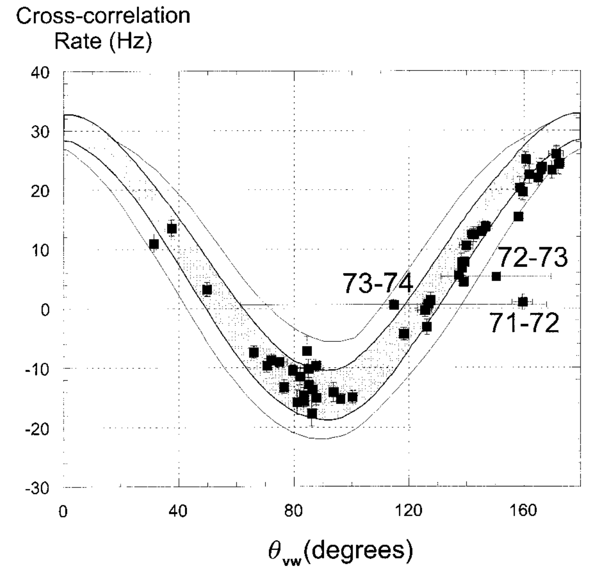

Figure 3. Plots of the areas covered by cross-correlation rates for the rotational diffusion tensor calculated by Tjandra et al.[34] with $D_{//}/D_{\perp} = 1.24$ and $\tau_c = 4.14$ ns, for small rotations about the neighboring bond vectors of maximal amplitudes equal to 10° in dark gray or 15° in light gray. The squares represent 54 experimental cross-correlation rates measured by Chiarparin et al.[28] for dipolar $^{13}C_i^{\alpha}\ ^1H_i^{\alpha}$ and $^{13}C_{i+1}^{\alpha}\ ^1H_{i+1}^{\alpha}$ interactions. The vertical bars correspond to the experimental errors[28] and the horizontal bars to the intervals covered by the angles θ_{vw} in the 10 solution-state structures from Cornilescu et al.[33]

rate} for two maximum amplitudes of small rotations about the neighboring bond vectors, respectively equal to 10° and 15°, using $\tau_c = 4.14$ ns and $D_{//}/D_{\perp} = 1.27$ (obtained from Tjandra et al.[34]). With rotations of maximal amplitudes equal to 10°, one could explain most of the experimental results. Only three pairs of residues, 71−72, 72−73, and 73−74, feature behaviors that cannot be explained by 15° rotations. One could use these areas to determine structural constraints from cross-correlation rates. Knowing approximatively the rotational anisotropy of a protein from X-ray studies or relaxation rates analysis,[38] and assuming 15° rotations, one can use the calculated map to determine the intervals in which each angle θ_{vw} must lie from the knowledge of the cross-correlation rates and of the associated experimental errors.

Another question can be addressed with this method. One may be interested in knowing when anisotropy has to be taken into account in the interpretation of cross-correlation rates. With this aim in view, we plotted in Figure 4, on the same graph, the areas covered by allowed cross-correlation rates for different values of the rotational anisotropy, looking for the limit above which it is impossible to neglect it. For 5° rotations, the limit is $D_{//}/D_{\perp} = 1.5$. For 10° and 15° rotations, the anisotropy should be considered if $D_{//}/D_{\perp} \geq 2.0$.

A simple example can be interesting. Let us consider the motion of two relatively fixed internuclear vectors. For example, two neighboring CH vectors belonging to an aromatic base of an RNA molecule. The motions of the two vectors are totally concerted provided they belong to the same aromaric ring. In a first approach, we can describe this motion as a single rotation $\vec{\omega}$ of the base in the plane perpendicular to the axis of the RNA helix (see Figure 5). For the vector **v**, the first three Euler angles that define the average orientation are: $\alpha = 0°$, $\beta = 90°$ and $\gamma = 0°$. For vector **w**, the three Euler angles are: $\alpha = \theta_{vw}$, $\beta = 90°$ and $\gamma = 0°$. Note that $\gamma = 0°$ because the dipolar Hamiltonian is symmetric, and it is used as a reference for the choice of the (*x*,*y*,*z*) or (*x*′,*y*′,*z*′) coordinates. The rotation

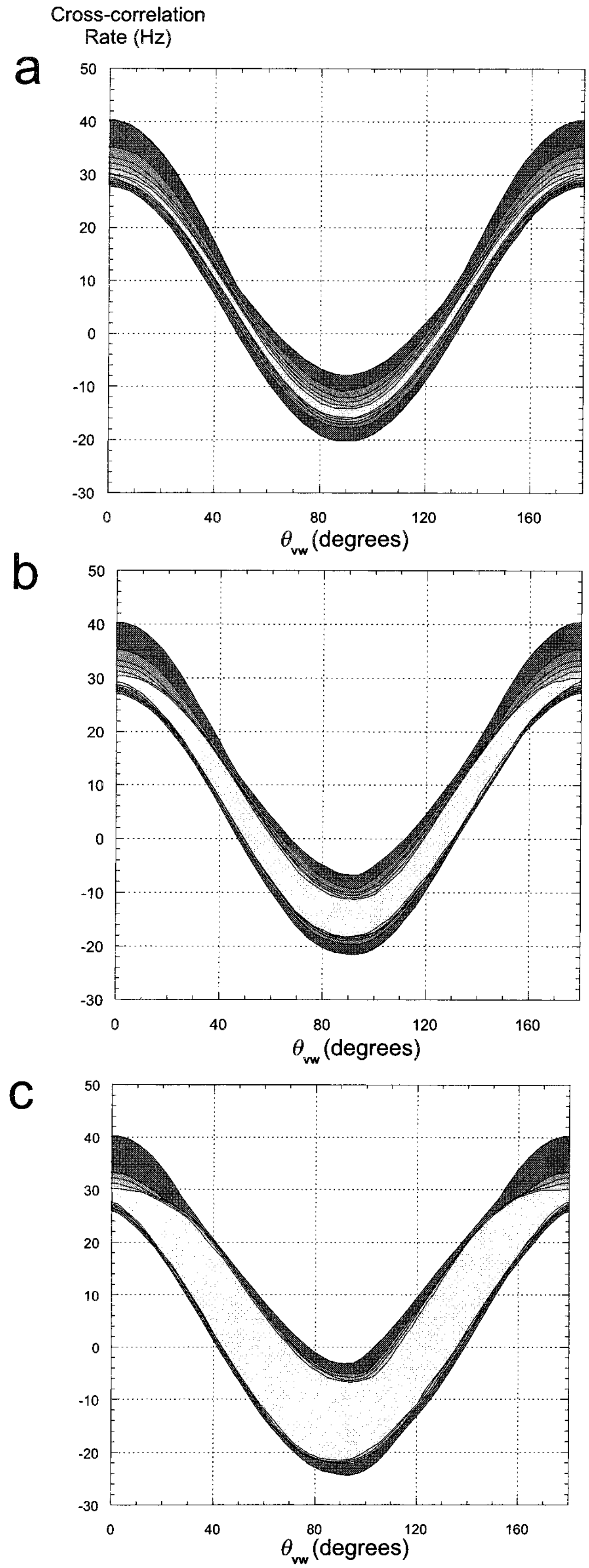

Figure 4. Plots of the areas obtained for different rotational anisotropy ratios $D_{//}/D_{\perp}$ equal to 1.0, 1.1, 1.2, 1.3, 1.5, and 2.0 (from light to dark gray), considering small rotations about the neighboring bond vectors of amplitudes equal to the following: a. 5°, b. 10° and c. 15°.

involved in the motional process is about the x or x' axis, which leads to $\langle \mathbf{v}_x \rangle = \langle \mathbf{w}_{x'} \rangle = 0$. It is now easy to calculate the value

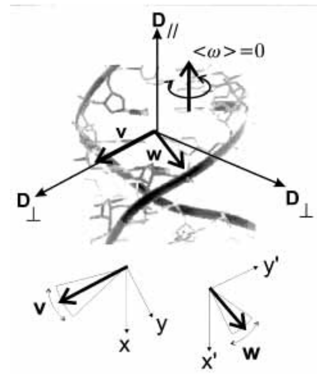

Figure 5. Scheme showing two internuclear vectors attached to a base in an RNA double helix, which are both perpendicular to $D_{//}$. The two vectors undergo the same motional processes that may be described by a random rotation $\vec{\omega}$.

of the cross-correlation rate as a function of $\theta_{\mathbf{vw}}$ and $\langle \mathbf{v}_{y}^2 \rangle = \langle \mathbf{w}_{y'}^2 \rangle = \langle y^2 \rangle$. The result is given by

$$K_0(\tau) = \frac{1}{2}$$

$$K_1(\tau) = 0$$

$$K_2(\tau) = \frac{3}{2}\cos(2\theta_{\mathbf{vw}})[1 - 4(\langle y^2 \rangle - \langle y(t')y(t' + \tau)\rangle)] \quad (29)$$

One can assume that $\langle y(t')y(t' + \tau)\rangle = \langle y^2 \rangle e^{-\tau/\tau_l}$ where τ_l is the local correlation time. Then

$$c_{\mathbf{vw}}(\tau) + c_{\mathbf{wv}}(\tau) = \frac{1}{10}[e^{-6D_{\perp}\tau} + 3\cos 2\theta_{\mathbf{vw}}[1 - 4\langle y^2 \rangle(1 - e^{-\tau/\tau_l})]e^{-(2D_{\perp}+4D_{||})\tau}] \quad (30)$$

The value of $j^{q}_{\mathbf{vw}}(0)$ gives an idea of the dipole−dipole cross-correlation and can be written, according to eq 7

$$j^{q}_{\mathbf{vw}}(0) = \frac{1}{10}\left[\frac{1}{6D_{\perp}} + 3\cos 2\theta_{\mathbf{vw}}\left(\frac{1 - 4\langle y^2 \rangle}{2D_{\perp} + 4D_{||}} + \frac{4\langle y^2 \rangle \tau_l}{1 + \tau_l(2D_{\perp} + 4D_{||})}\right)\right] \quad (31)$$

Note that, for a value of $\theta_{\mathbf{vw}} = 45°$, no effect of the local motion may be observed. The only term that depends on τ_l, is equal to $4\langle y^2 \rangle \tau_l/1+\tau_l(2D_{\perp}+4D_{||})$. If $\tau_l \ll 1/(2D_{\perp} + 4D_{||})$, this term is equivalent to τ_l and the result is given by

$$j^{q}_{\mathbf{vw}}(0) \approx \frac{1}{10}\left[\frac{1}{6D_{\perp}} + 3\cos 2\theta_{\mathrm{vw}}\frac{1 - 4\langle y^2 \rangle}{2D_{\perp} + 4D_{||}}\right] \quad (32)$$

The effect of the motion is approximately proportional to $-12\cos 2\theta_{\mathbf{vw}}\langle \mathbf{y}^2 \rangle/(2D_{\perp} + 4D_{||})$. The motion will lower the cross-correlation rate if $\theta \in [0°, 45°] \cup [135°, 180°]$. The cross-correlation rate will be greater if $\theta \in [45°, 135°]$. Moreover, if $\tau_l \gg 1/(2D_{\perp} + 4D_{||})$, this term is equivalent to $1/2D_{\perp}+4D_{||}$ and the result is

$$j^{q}_{\mathbf{vw}}(0) \approx \frac{1}{10}\left[\frac{1}{6D_{\perp}} + 3\cos 2\theta_{\mathbf{vw}}\frac{1}{2D_{\perp} + 4D_{||}}\right] \quad (33)$$

As expected, this result does not depend on the amplitude and is exactly equal to the result obtained without any motion. Indeed, if such a motion is sufficiently slow and of sufficiently small amplitude, it has no effect on the cross-correlation rate.

Conclusions

We were able to derive a rather complicated expression of the cross-correlation function that can be simplified in many cases, or implemented in a computer program to choose a projection in the multidimensional space of the parameters. We used it to determine the possible cross-correlation rates for a given angle $\theta_{\mathbf{vw}}$ between two internuclear vectors, considering a range of small amplitude motions in a protein. We compared the results with rates determined in human ubiquitin and saw that two random rotations about two neighboring bond vectors with maximum amplitudes $\omega^{\max} = 15°$ are sufficient to explain the experimental results. We determined the limit above which anisotropy cannot be neglected for different amplitudes of local motion. An RNA molecule has been taken as an example to explain the simplification of the cross-correlation function in special cases.

Acknowledgment. I am indebted to Prof. G. Bodenhausen for his assistance and careful reading of the manuscript. This work has been supported by funds of the CNRS and of the Ministère de l'Education Nationale et de la Recherche.

Appendix

More explicitly

$$\begin{aligned}
\mathcal{G}_0(\tau) = {} & d_{00}^{(2)}(\Theta_{\mathbf{v}}^A)d_{00}^{(2)}(\Theta_{\mathbf{w}}^A)\Big[1 - \frac{3}{2}(\mathbf{v}_{x}^2(t') + \mathbf{v}_{y}^2(t')) - \frac{3}{2}(\mathbf{w}_{x'}^2(t'+\tau) + \mathbf{w}_{y'}^2(t'+\tau))\Big] + \\
& \sqrt{\frac{3}{2}}[d_{00}^{(2)}(\Theta_{\mathbf{v}}^A)d_{02}^{(2)}(\Theta_{\mathbf{w}}^A)[\mathbf{w}_{x'}^2(t'+\tau) - \mathbf{w}_{y'}^2(t'+\tau)] + \\
& d_{02}^{(2)}(\Theta_{\mathbf{v}}^A)d_{00}^{(2)}(\Theta_{\mathbf{w}}^A)[\mathbf{v}_{x}^2(t') - \mathbf{v}_{y}^2(t')]] + \\
& 3d_{01}^{(2)}(\Theta_{\mathbf{v}}^A)d_{01}^{(2)}(\Theta_{\mathbf{w}}^A)[(\mathbf{v}_x(t')\mathbf{w}_{x'}(t'+\tau) + \mathbf{v}_y(t')\mathbf{w}_{y'}(t'+\tau)) + \\
& (\mathbf{v}_x(t')\mathbf{w}_{x'}(t'+\tau) - \mathbf{v}_y(t')\mathbf{w}_{y'}(t'+\tau))]
\end{aligned}$$

$$\begin{aligned}
\mathcal{G}_1(\tau) = {} & 2d_{10}^{(2)}(\Theta_{\mathbf{v}}^A)d_{10}^{(2)}(\Theta_{\mathbf{w}}^A)\cos(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A)\Big[1 - \frac{3}{2}(\mathbf{v}_{x}^2(t') + \\
& \mathbf{v}_{y}^2(t')) - \frac{3}{2}(\mathbf{w}_{x'}^2(t'+\tau) + \mathbf{w}_{y'}^2(t'+\tau))\Big] + \\
& \sqrt{\frac{3}{2}}d_{10}^{(2)}(\Theta_{\mathbf{v}}^A)\,[\sin(\Theta_{\mathbf{w}}^A)\cos(\Theta_{\mathbf{w}}^A)\cos(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& (\mathbf{w}_{x'}^2(t'+\tau) - \mathbf{w}_{y'}^2(t'+\tau)) + \\
& 2\sin(\Theta_{\mathbf{w}}^A)\sin(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A)(\mathbf{w}_{x'}(t'+\tau)\mathbf{w}_{y'}(t'+\tau))] + \\
& \sqrt{\frac{3}{2}}d_{10}^{(2)}(\Theta_{\mathbf{w}}^A)\,[\sin(\Theta_{\mathbf{v}}^A)\cos(\Theta_{\mathbf{v}}^A)\cos(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& (\mathbf{v}_{x}^2(t') - \mathbf{v}_{y}^2(t')) - 2\sin(\Theta_{\mathbf{v}}^A)\sin(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A)(\mathbf{v}_x(t')\mathbf{v}_y(t'))] + \\
& 3\cos(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A)[(1 - 2\cos^2(\Theta_{\mathbf{v}}^A) - 2\cos^2(\Theta_{\mathbf{w}}^A) - \\
& 4\cos^2(\Theta_{\mathbf{v}}^A)\cos^2(\Theta_{\mathbf{w}}^A)) \times \\
& (\mathrm{v}_x(t')\mathrm{w}_{x'}(t'+\tau)) + \cos(\Theta_{\mathrm{v}}^A)\cos(\Theta_{\mathrm{w}}^A)(\mathrm{v}_y(t')\mathrm{w}_{y'}(t'+\tau))] + \\
& 3\sin(\Phi_{\mathrm{v}}^A - \Phi_{\mathrm{w}}^A)[\cos(\Theta_{\mathbf{w}}^A)\cos(2\Theta_{\mathbf{v}}^A)(\mathbf{v}_x(t')\mathbf{w}_{y'}(t'+\tau)) - \\
& \cos(\Theta_{\mathbf{v}}^A)\cos(2\Theta_{\mathbf{w}}^A)(\mathbf{v}_y(t')\mathbf{w}_{x'}(t'+\tau))]
\end{aligned}$$

$$\begin{aligned}
\mathcal{G}_2(\tau) = {} & 2d_{20}^{(2)}(\Theta_{\mathbf{v}}^A)d_{20}^{(2)}(\Theta_{\mathbf{w}}^A)\cos 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& \Big[1 - \frac{3}{2}(\mathbf{v}_{x}^2(t') + \mathbf{v}_{y}^2(t')) - \frac{3}{2}(\mathbf{w}_{x'}^2(t'+\tau) + \mathbf{w}_{y'}^2(t'+\tau))\Big] + \\
& \sqrt{\frac{3}{2}}d_{20}^{(2)}(\Theta_{\mathbf{v}}^A)[(d_{22}^{(2)}(\Theta_{\mathbf{w}}^A) + d_{2-2}^{(2)}(\Theta_{\mathbf{w}}^A))\cos 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& (\mathbf{w}_{x'}^2(t'+\tau) - \mathbf{w}_{y'}^2(t'+\tau)) + \\
& 2\cos(2\Theta_{\mathbf{w}}^A)\sin 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A)(\mathbf{w}_{x'}(t'+\tau)\mathbf{w}_{y'}(t'+\tau))] + \\
& \sqrt{\frac{3}{2}}d_{20}^{(2)}(\Theta_{\mathbf{w}}^A)[(d_{22}^{(2)}(\Theta_{\mathbf{v}}^A) + d_{2-2}^{(2)}(\Theta_{\mathbf{v}}^A))\cos 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& (\mathbf{v}_{x}^2(t') - \mathbf{v}_{y}^2(t')) - 2\cos(2\Theta_{\mathbf{v}}^A)\sin 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& (\mathbf{v}_x(t')\mathbf{v}_y(t'))] + \frac{3}{2}\sin(\Theta_{\mathbf{v}}^A)\sin(\Theta_{\mathbf{w}}^A) \times \\
& [[1 + \cos(\Theta_{\mathbf{v}}^A)\cos(\Theta_{\mathbf{w}}^A)]\cos 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& (\mathbf{v}_x(t')\mathbf{w}_{x'}(t'+\tau) + \mathbf{v}_y(t')\mathbf{w}_{y'}(t'+\tau)) + \\
& [\cos(\Theta_{\mathbf{v}}^A) + \cos(\Theta_{\mathbf{w}}^A)] \\
& \sin 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A)(\mathbf{v}_y(t')\mathbf{w}_{x'}(t'+\tau) - \mathbf{v}_x(t')\mathbf{w}_{y'}(t'+\tau)) - \\
& [1 - \cos(\Theta_{\mathbf{v}}^A)\cos(\Theta_{\mathbf{w}}^A)]\cos 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A) \times \\
& (\mathbf{v}_x(t')\mathbf{w}_{x'}(t'+\tau) - \mathbf{v}_y(t')\mathbf{w}_{y'}(t'+\tau)) - \\
& [\cos(\Theta_{\mathbf{v}}^A) - \cos(\Theta_{\mathbf{w}}^A)] \\
& \sin 2(\Phi_{\mathbf{v}}^A - \Phi_{\mathbf{w}}^A)(\mathbf{v}_y(t')\mathbf{w}_{x'}(t'+\tau) + \mathbf{v}_x(t')\mathbf{w}_{y'}(t'+\tau))]
\end{aligned} \tag{34}$$

References and Notes

(1) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(2) Tolman, J. R.; Flanagan, J. M.; Kennedy, M. A.; Prestegard, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9297.
(3) Bax, A.; Tjandra, N. *J. Biomol. NMR* **1997**, *10*, 289.
(4) Tjandra, N; Bax, A. *Science* **1997**, *278*, 1111.
(5) Favro, L. D. *Phys. Rev.* **1960**, *119*, 53.
(6) Woessner, D. E. *J. Chem. Phys.* **1962**, *37*, 647.
(7) Woessner, D. E. *J. Chem. Phys.* **1965**, *42*, 1855.
(8) Huntress, W. T. *J. Chem. Phys.* **1968**, *48*, 3524.
(9) Bopp, T. T. *J. Chem. Phys.* **1967**, *47*, 3621.
(10) Wallach, D. *J. Chem. Phys.* **1967**, *47*, 5258.
(11) Woessner, D. E.; Snowden, B. S.; Meyer, G. H. *J. Chem. Phys.* **1969**, *50*, 719.
(12) Wittebort, R. J.; Szabo, A. *J. Chem. Phys.* **1978**, *69*, 1722.
(13) Lipari, G.; Szabo, A. *J. Chem. Phys.* **1982**, *104*, 4546.
(14) Clore, G. M.; Szabo, A.; Bax, A.; Kay, L. E.; Driscoll, P. C.; Gronenborn, A. M. *J. Am. Chem. Soc.* **1990**, *112*, 4989.
(15) Daragan, V. A.; Mayo, K. H. *J. Phys. Chem.* **1996**, *100*, 8378.
(16) Daragan, V. A.; Mayo, K. H. *J. Phys. Chem.* **1999**, *103*, 6829.
(17) Brutscher, B.; Skrynnikov, N. R.; Bremi, T.; Brüschweiler, R.; Ernst, R. R. *J. Magn. Reson.* **1998**, *130*, 346.
(18) Fischer, M. W. F.; Zeng, L.; Pang, Y.; Hu, W.; Majumdar, A.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **1997**, *119*, 12 629.
(19) Hus, J.-C.; Marion, D.; Blackledge, M. *J. Am. Chem. Soc.* **1999**, *121*, 2311.
(20) Richter, C.; Griesinger, C.; Felli, I.; Cole, P. T.; Varani, G.; Schwalbe, H. *J. Biomol. NMR* **1999**, *15*, 241.
(21) Reif, B.; Hennig, M.; Griesinger, C. *Science* **1997**, *276*, 1230.
(22) Yang, D.; Konrat, R.; Kay, L. E. *J. Am. Chem. Soc.* **1997**, *119*, 11 938.
(23) Yang, D.; Gardner, K.; Kay, L. E. *J. Biomol. NMR* **1998**, *11*, 213.
(24) Yang, D.; Kay, L. E. *J. Am. Chem. Soc.* **1998**, *120*, 9880.
(25) Pelupessy, P.; Chiarparin, E.; Ghose, R.; Bodenhausen, G. *J. Biomol. NMR* **1999**, *13*, 375.
(26) Chiarparin, E.; Pelupessy, P.; Ghose, R.; Bodenhausen *J. Am. Chem. Soc.* **1999**, *121*, 6876.
(27) Pelupessy, P.; Chiarparin, E.; Ghose, R.; Bodenhausen, G. *J. Biomol. NMR* **1999**, *14*, 277.
(28) Chiarparin, E.; Pelupessy, P.; Ghose, R.; Bodenhausen, G. *J. Am. Chem. Soc.* **2000**, *122*, 1758.
(29) Reif, B.; Diener, A.; Hennig, M.; Maurer, M. and Griesinger, C. *J. Magn. Reson.* **2000**, *143*, 45.

(30) Sprangers, R.; Bottomley, M. J.; Linge, J. P.; Schultz, J.; Nilges, M.; Sattler, M. *J. Biomol. NMR* **2000**, *16*, 47.
(31) Lee, K. L.; Rance, M.; Chazin, W. J.; Palmer, A. G. *J. Biomol. NMR* **1997**, *9*, 287.
(32) Daragan, V. A.; Mayo, K. H. *J. Magn. Reson.* **1998**, *130*, 329.
(33) Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 6836.
(34) Tjandra, N.; Feller, S. E.; Pastor, R. W.; Bax, A. *J. Am. Chem. Soc.* **1995**, *117*, 12 562.
(35) Abragam, A. *Les Principes du Magnétisme Nucleaire*; PUF; Paris 1961.
(36) Szabo, A. *J. Chem. Phys.* **1984**, *81*, 150.
(37) Deschamps, M.; Bodenhausen, G. *ChemPhysChem* **2001**, *2*, 543.
(38) Millet, O.; Pons, M. *J. Biomol. NMR* **2001**, *19*, 181.