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Tilt Angle Dependence of Cross-Relaxation in Off-Resonance ROESY

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We present an efficient experimental method to evaluate whether the effective cross-relaxation rate between a pair of spins vanishes when applying an off-resonance spin-lock field. It is shown that the cross-relaxation rate can be made to vanish even when the two spins concerned resonate at different offsets and experience significantly different tilt angles of their respective spin-lock fields. This is verified experimentally using a sample of ^{15}N-labeled human ubiquitin, through selective excitation of chosen amide protons. The results are relevant for the quantitative interpretation of off-resonance ROESY experiments.

Key Words: **off-resonance ROESY; cross-relaxation; protein dynamics.**

Relaxation experiments employing off-resonance spin-lock fields represent an increasingly important class of methods. Applications of these techniques may be found in the determination of spectral density functions (*1*), the investigation of exchange phenomena (*2, 3*), studies of interference effects between different mechanisms of spin relaxation (*4*), and the assessment of internal dynamical parameters (*5*). It is generally assumed that the tilt angles of the spin-lock fields experienced by the two spins of interest are equal, i.e., $\theta_k = \theta_l$, although this can only be true if their offsets are equal in magnitude. Here, we investigate the behavior of the effective cross-relaxation rate between the two spins when this assumption is not fulfilled. The tilt angle θ_k of the spin-lock field for a given spin is

$$\tan(\theta_k) = \omega_1/\Delta\Omega_k, \qquad [1]$$

where ω_1 is the strength of the spin-lock field and $\Delta\Omega_k$ is the resonance offset. In the case of an isolated two-spin system $\{I_k, I_l\}$, the effective cross-relaxation rate is given by (*6, 7*)

$$\sigma_{\text{eff}}^{k,l} = \sigma_{\text{NOE}}^{k,l}\cos(\theta_k)\cos(\theta_l) + \sigma_{\text{ROE}}^{k,l}\sin(\theta_k)\sin(\theta_l), \qquad [2]$$

where σ_{NOE} and σ_{ROE} are the cross-relaxation rates in the laboratory and rotating frames, respectively. In the slow tumbling limit ($\tau_c \gg 1/\omega_0$), $\sigma_{\text{ROE}} = -2\sigma_{\text{NOE}}$ (*8*). In this limit, the effective cross-relaxation rate should vanish if

$$\cos(\theta_k)\cos(\theta_l) = 2\sin(\theta_k)\sin(\theta_l). \qquad [3]$$

The effective cross-relaxation rate should vanish for all values of θ_k and θ_l that fulfill Eq. [3]. If $\theta_k = \theta_l$, as commonly assumed, the null occurs at 35.3°, but this assumption is by no means necessary.

Figure 1 shows the pulse sequence used for the selective off-resonance ROESY experiments. As described elsewhere (*9*), a preparation period with two selective cross-polarization steps was used to create pure in-phase proton magnetization I_{kx}. Following this period the magnetization was converted into I_{kz} and then rotated adiabatically to lie along the tilted spin-lock field. After the relaxation period, the magnetization was rotated adiabatically back to the z axis. This was achieved by using a trapezoidal envelope of the spin-lock field amplitude (*10*). In analogy to selective 1D NOE experiments, the use of two trapezoidal spin-lock fields separated by an inversion pulse in the middle of the relaxation period leads to better baseline behavior because the recovery of the longitudinal magnetization originating from spins other than those of interest is reduced (*11*). Additional ramping of the spin-lock fields would have complicated the analysis due to cross-relaxation processes that may occur during the ramps. Continuous-wave decoupling was applied to the ^{15}N spins during the spin-lock periods. After the second spin-lock period, the remaining magnetization $I_{kz} + I_{lz}$ was converted to $I_{kx} + I_{lx}$ using a $\pi/2$ pulse. The excitation sculpting method for water suppression (*12*) was found to be useful because the longitudinal water magnetization recovers partly during the spin-lock period.

Representative results of the above pulse sequence are shown in Fig. 2. This corresponds to selective excitation of the S20 amide ("source") proton H^N in ubiquitin and monitoring the D21 amide ("target") proton H^N resonance, which arises due to cross-relaxation. Eight spectra are displayed with increasing RF amplitudes and hence increasing contributions of rotating frame cross-relaxation from left to right. Identical phase corrections were applied to all spectra.

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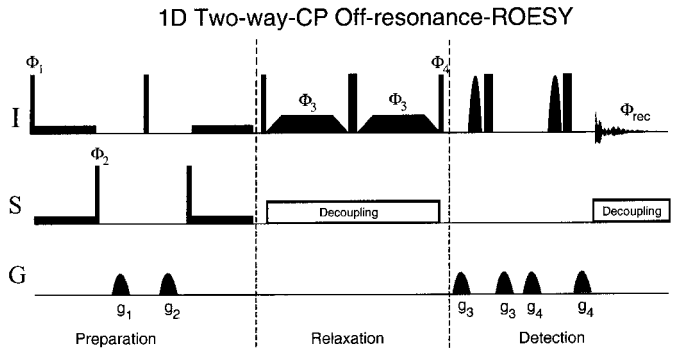

FIG. 1. Pulse sequence used for one-dimensional off-resonance ROESY experiments. Each trapezoidal pulse had a plateau region of 36.8 ms and rise and fall regions of 1.6 ms each. The first $\pi/2$ pulse in the relaxation time converts the selectively excited magnetization I_{kx} into longitudinal magnetization I_{kz}. The first trapezoidal pulse is followed by an inversion pulse to diminish recovery of undesired longitudinal magnetization. After the second trapezoidal pulse, the longitudinal magnetization of both source and target spins is converted to transverse magnetization. The phases ϕ_1, ϕ_2, and ϕ_4 were alternated independently with concomitant alternation of the receiver phase, leading to an 8-step cycle to select the appropriate pathways. In addition, the phase ϕ_3 was cycled through x, y, $-x$, and $-y$, leading to a 32-step cycle. Typical gradient pulses were of 1.0-ms duration with amplitudes $g_1 = 300$ mT/m along the z axis, $g_2 = 250$ mT/m along the z axis, $g_3 = 220$ mT/m along the x axis, and $g_4 = 150$ mT/m along the y axis. Continuous-wave decoupling was applied during the relaxation period, while Garp decoupling was used during acquisition.

The intensities of the target peaks were measured and plotted in Fig. 3 with respect to the RF field strength of the plateau region of the spin-lock field. A fourth-order polynomial was fitted in order to determine the field strength where the cross-relaxation rate vanishes. For the present purpose, only an accurate position of the null was determined. The variation of the peak intensity of the target spin as a function of the strength of the spin-lock field is a function of σ_{ROE} and σ_{NOE}.

Errors in the measurement of the strength of the spin-lock field corresponding to the null of the cross-relaxation rate were evaluated by performing a Monte Carlo analysis using the experimentally measured noise level. The average values and standard deviations of the tilt angles for the source and target spins were obtained for 500 Monte Carlo realizations using Eq. [1]. The results are shown in Fig. 4 for several source and target spins. All experimental points lie near a curve corresponding to Eq. [3]. Some of the deviations can be explained by the fact that the spin pairs studied cannot be considered to be truly isolated two-spin systems. Indeed, the cross-relaxation behavior will be complicated by effects of spin diffusion which can cause a shift in the position of the null of the cross-relaxation rate. However, for the relaxation times considered here these effects are expected to be more pronounced for tilt angles less than 50°.

Figure 4 illustrates that the cross-relaxation rates can be made to vanish even when the tilt angles of the source and target spins are markedly different, provided Eq. [3] is fulfilled. Even in large, slowly tumbling systems, however, the presence of fast local motions may affect the applica-

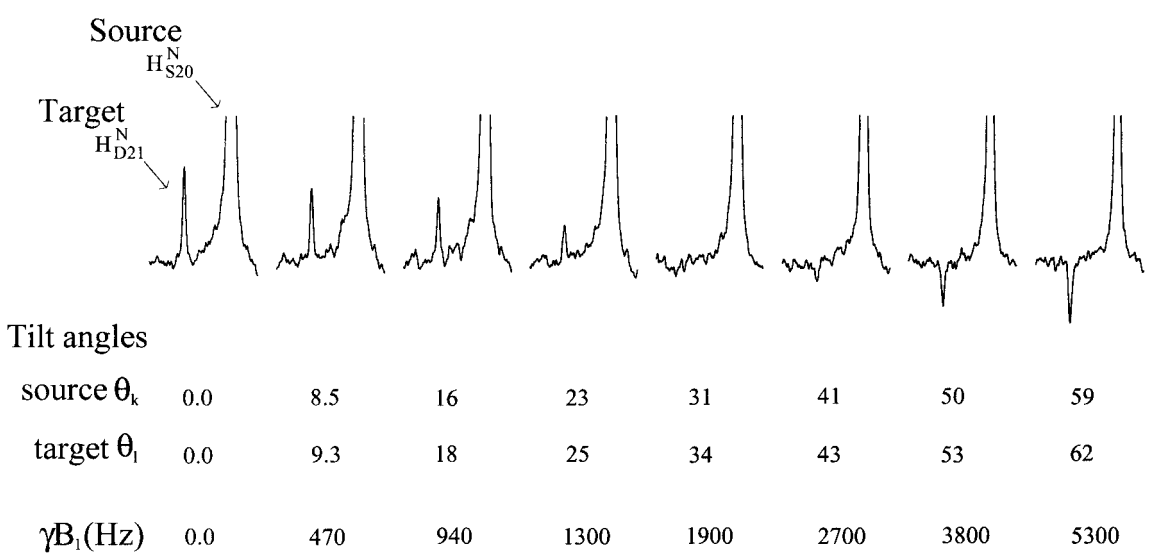

FIG. 2. Series of spectra of ^{15}N-labeled human ubiquitin obtained with the sequence of Fig. 1, acquired with a Bruker Avance 300-MHz spectrometer equipped with Acustar triple axes gradient amplifiers and a multinuclear 5-mm TXI probe with three self-shielded gradient coils. The carrier frequency of the trapezoidial pulses remained constant for each of the eight measurements, while the spin-lock amplitude was increased from left to right to increase the tilt angles. The amide proton H^N of serine S20 (clipped signal) acts as the source spin and the amide proton H^N of aspartic acid D21 as the target. The resonance offsets of the source and target signals were separated by 291 Hz. The spin-lock frequency was maintained at 3435 Hz downfield from the source resonance and 3145 Hz downfield from the target resonance. The change of sign of the target peak from left to right demonstrates the increasing role of rotating frame cross-relaxation.

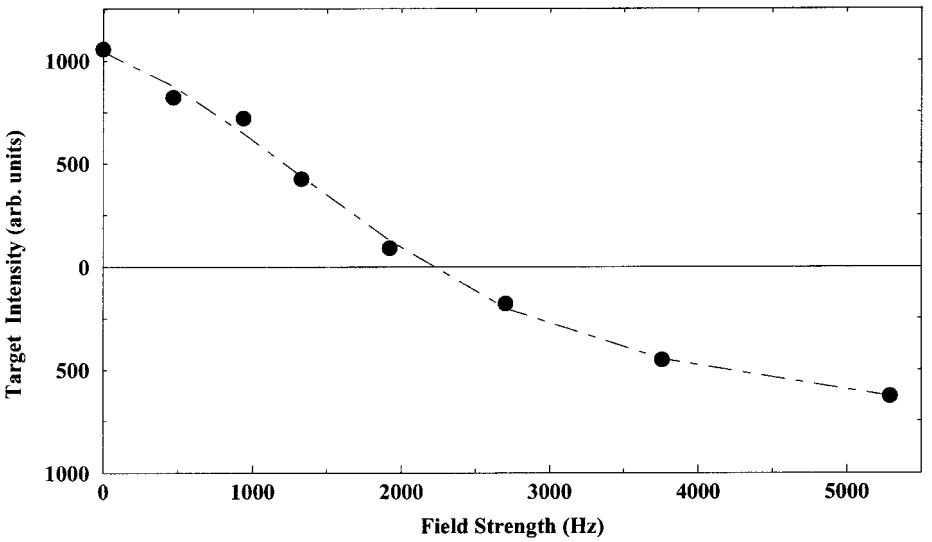

FIG. 3. Plot of the measured intensity of the D21 amide H^N target peak of Fig. 2 with a fourth-order polynomial fit.

bility of Eq. [3], so that it is important to verify its validity experimentally. To some extent, alternating the position of the carrier frequency of the spin-lock field symmetrically about the center of the spectrum on successive scans can reduce the effect of the spread of tilt angles, provided that the distribution of resonance frequencies is moderate (*13, 14*). However, this method is not sufficient at higher fields and for paramagnetic systems where it is essential to consider explicitly that the tilt angles of the effective fields depend on the offset.

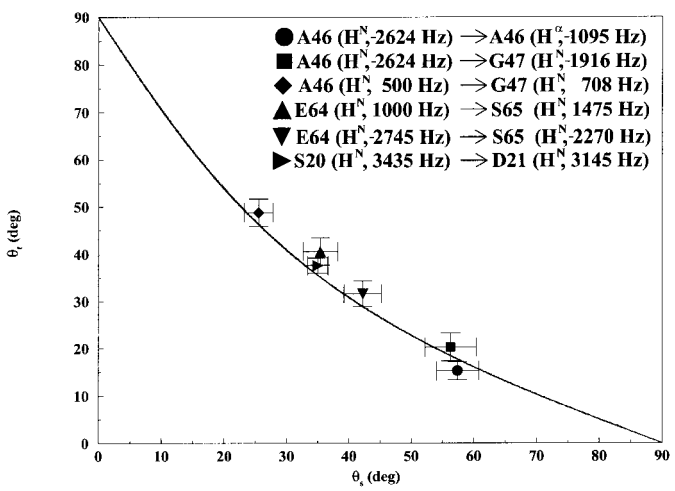

FIG. 4. Experimentally determined tilt angles (θ_l, θ_k) corresponding to the null of the cross-relaxation rates for six pairs of source and target spins. The solid line corresponds to Eq. [3], which is applicable to the slow motion limit. Local dynamics may lead to slight deviations from Eq. [3]. The offset frequencies of the source and target with respect to the RF carrier are given in parentheses. The RF field amplitude γB_1 has been varied between 5290 and 0 Hz for all pairs of source and target spins.

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