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# Attenuation of Cross-Peak Intensities in QUIET-BIRD-NOESY Experiments

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The buildup curves in QUIET-BIRD-NOESY experiments, which are designed to isolate two-spin subsystems within macromolecules, are attenuated by transverse relaxation and evolution under homonuclear couplings during the bilinear rotation decoupling (BIRD) pulse sandwich. If the signals of both source and target spins are attenuated equally (uniform damping), this is readily accounted for by normalizing the cross peaks with respect to the diagonal peaks. However, unequal attenuation of source and target spins (differential damping) affects the initial buildup slopes and hence leads to apparent cross-relaxation rates that are significantly distorted from their true values. A simple method for recognizing this situation and extracting accurate cross-relaxation rates is presented. © 1999 Academic Press

*Key Words:* bilinear rotation decoupling (BIRD); cross-relaxation; nuclear Overhauser effect spectroscopy (NOESY); quenching undesirable indirect external trouble in nuclear Overhauser effect spectroscopy (QUIET-NOESY); spin diffusion.

Spin diffusion is a major obstacle to the determination of accurate macromolecular structures by NMR (*1–4*). In the QUIET-BIRD-NOESY experiment (*5*) spin diffusion is eliminated by inserting a [$^1$H, $^{15}$N] BIRD sequence (*6*) in the center of the mixing time of a NOESY sequence. This allows one to invert the longitudinal magnetization of all $H^N$ protons that are scalar-coupled to $^{15}$N, without significantly affecting the magnetization of $H^C$ protons bound to carbons. To first order, this leads to the cancellation of two-step transfer processes $H^N \rightarrow H^C \rightarrow H^N$ (*7*) so that relaxation rates between $H^N$ protons can be determined without significant interference due to spin diffusion via $H^C$ protons.

At the start of the BIRD sequence (see Fig. 1), the relevant components of the proton magnetization are brought into the transverse plane. During the two intervals of total duration $1/J_{HN}$, the transverse magnetization decays and dephases due to a combination of transverse relaxation and homonuclear scalar couplings. Although the resulting attenuation of the magnetization depends in a complex manner on the dynamics and scalar couplings, we need only consider the overall atten-

uation at the end of the BIRD sequence to determine the fraction of longitudinal proton magnetization that has been inverted. From the perspective of the NOE development, the BIRD sequence may be viewed as an instantaneous but incomplete inversion. Thus, the magnetization components of the source and target spins S and T undergo net transformations $M_z^S \rightarrow -d_S M_z^S$ and $M_z^T \rightarrow -d_T M_z^T$, with damping factors $0 < d_S, d_T < 1$. Longitudinal cross-relaxation processes that occur during the BIRD sequence do not affect the outcome of the experiments.

In principle, these damping factors may be determined by performing two complementary three-dimensional experiments such as HSQC-NOESY and HSQC-QUIET-BIRD-NOESY. The damping factor $d_S$ for a particular amide $H^N$ proton would be given by the ratio of the intensities of the corresponding diagonal peaks in the two experiments. To facilitate the comparison, we used one-dimensional experiments with selective cross-polarization (*8*) as shown in Fig. 1. These methods provide information that is comparable to three-dimensional experiments in a much shorter time, albeit for one amino acid at a time.

The decay of the diagonal-peak amplitude $a_{SS}$ in a NOESY spectrum of a selected source spin S in a protein (actually the $H^N$ proton of alanine A46 in ubiquitin) is shown in Fig. 2. Since the decay is linear for short mixing times, extrapolation back to $\tau_m = 0$ allows one to determine the initial amplitude $a_{SS}(0)$. Extrapolation of the diagonal-peak amplitudes $a'_{SS}(\tau_m)$ in a QUIET-BIRD-NOESY spectrum yields an initial amplitude $a'_{SS}(0)$. The attenuation factor $d_S$ of the source spin S is given by the ratio $d_S = a'_{SS}(0)/a_{SS}(0)$. This method was applied to the amide protons of 23 different amino acids in ubiquitin. The resulting damping factors $d_S$ are shown in Fig. 3. Since all cross-relaxation processes of interest in this work are observed between pairs of $H^N$ protons, Fig. 3 also provides information on the damping factors $d_T$ of the target spins T.

For an isolated two-spin subsystem, the buildup curves are described by solving suitably adapted Solomon equations. If we neglect the fact that the inversion causes all components to be negative, we obtain
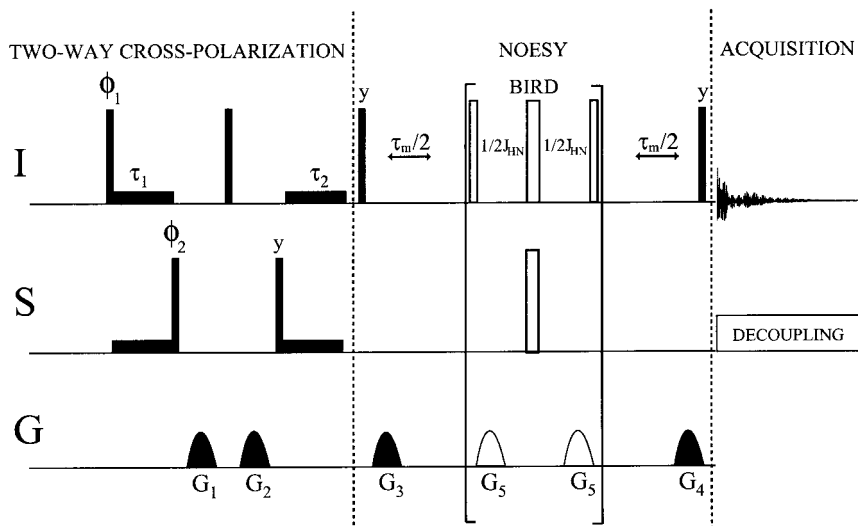
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**FIG. 1.** Pulse sequence used for 1D NOE measurements using two-way selective cross-polarization combined with quenching of undesirable indirect external trouble (QUIET) by bilinear rotation decoupling (BIRD) in nuclear Overhauser effect spectroscopy (NOESY). For optimal cross-polarization efficiency, $\tau_1 = \tau_2 = {}^1J_{NH}^{-1}$ (i.e., 10.9 ms for ${}^1$H and ${}^{15}$N in amides). The carrier frequencies of the I and S channels must be set to the resonance frequencies of the selected ${}^1$H and ${}^{15}$N spins, which can be taken from an HSQC spectrum. The RF fields used for cross-polarization typically have amplitudes of approximately ${}^1J_{NH}^{-1}/2$ (i.e., 45 Hz in amides). The phases $\Phi_1$ and $\Phi_2$ must be alternated between $+y$ and $-y$ with concomitant alternation of the receiver phase, resulting in a four-step cycle to select the appropriate pathways. Pulses without phase labels are applied along the $x$ axis. The open symbols represent pulses and gradients used for the (optional) QUIET-BIRD sequence.

$$\Delta \mathbf{M}_z(\tau_m) = \exp\{-\mathbf{R}\tau_m/2\}\mathbf{D}\exp\{-\mathbf{R}\tau_m/2\}\Delta \mathbf{M}_z(\tau_m = 0),$$

$$[1]$$

where the matrix $\mathbf{D}$ contains the damping factors $d_S$ and $d_T$,

$$\mathbf{D} = \begin{bmatrix} d_S & 0 \\ 0 & d_T \end{bmatrix} = \begin{bmatrix} d - \delta & 0 \\ 0 & d + \delta \end{bmatrix}, \qquad [2]$$

with the average damping $d = (d_T + d_S)/2$ and the differential damping $\delta = (d_T - d_S)/2$. For uniform damping ($d_S = d_T = d$, hence $\delta = 0$), Eq. [1] can be simplified:

$$\Delta \mathbf{M}_z(\tau_m, \delta = 0) = d \exp\{-\mathbf{R}\tau_m\}\Delta \mathbf{M}_z(\tau_m = 0). \qquad [3]$$

If the cross-peak amplitudes $a'_{ST}(\tau_m)$ are normalized with respect to the diagonal-peak amplitudes $a'_{SS}(0)$, the common damping factor $d$ is eliminated. The buildup behavior will be identical for all damping constants $d$, and the initial slopes will not be affected by the absence or presence of a BIRD sequence if the signals are normalized.

If there is a differential damping effect, i.e., if $\delta = (d_T - d_S)/2 \neq 0$, this has a more pernicious effect on the buildup behavior. Figure 4 demonstrates how the initial slopes depend on the extent of differential damping. If the initial slopes are analyzed in a naive manner, the apparent cross-relaxation rates $\sigma_{ST}^{app}$ will differ from their true value $\sigma_{ST}^{true}$ by an amount dependent upon $\delta$. Although Fig. 3 shows that the deviation $\delta$ is small for most pairs of N$^H$ protons, care must be used for residues which have significantly different damping factors $d_S$ and $d_T$.

Since the damping parameters $d_S$ and $d_T$ can be determined independently by the method shown in Fig. 2, it is possible to
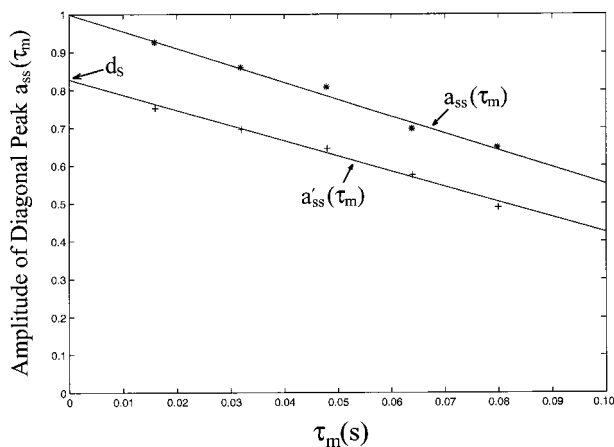


**FIG. 2.** Representative example of the determination of the damping parameter $d_S$ for a source proton S. The NOESY diagonal-peak intensity $a_{SS}(\tau_m)$ of the amide proton $S = H_{A46}^N$ in ubiquitin, uniformly enriched in ${}^{15}$N, was measured without the BIRD sequence for five mixing times $\tau_m$. Linear regression was used to extrapolate back to $\tau_m = 0$. This was repeated for the source peak $a'_{SS}(\tau_m)$ obtained by QUIET-BIRD-NOESY. The ratio of the intercepts of the two regressions $d_S = a'_{SS}(0)/a_{SS}(0)$ provides the damping factor $d_S$ for $S = H_{A46}^N$. The initial slopes give an estimate of the self-relaxation rate $\rho_S$. Each 1D experiment required approximately 3 min.
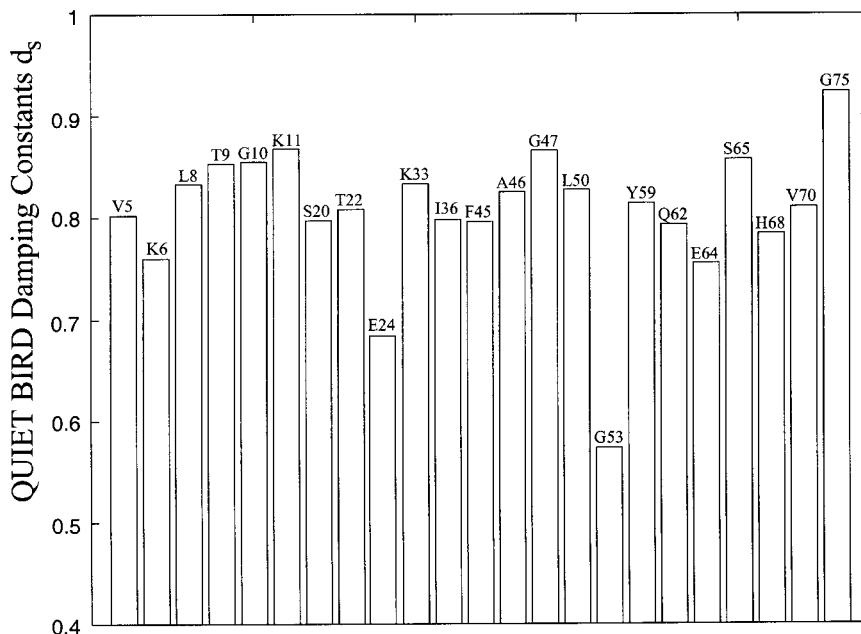
**FIG. 3.** Damping parameters $d_S$ determined as in Fig. 2 for the $H^N$ protons of 23 of the 76 amino acids of ubiquitin, identified by their residue numbers, in $H_2O/D_2O$ = 9/1, pH 4.5, $T$ = 303 K, and $B_0$ = 9.4 T (400 MHz). The average value of the damping constants $d_S$ was 0.805, ranging from $d_S$ = 0.925 for the highly mobile penultimate residue $H^N_{G75}$ to $d_S$ = 0.573 for $H^N_{G53}$, which is located in a tight turn between a $\beta$-sheet and an $\alpha$-helix. The $T_2$ is short probably due to conformational exchange.

correct for the distortions of the buildup curves illustrated in Fig. 4. The matrix **D** of Eq. [1] may be factorized as

$$\mathbf{D} = d\left\{ \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} + \frac{\delta}{d} \begin{bmatrix} -1 & 0 \\ 0 & 1 \end{bmatrix} \right\} \qquad [4]$$
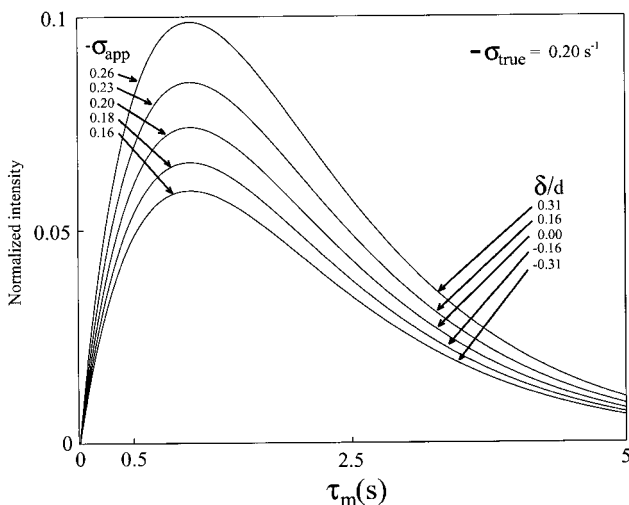


**FIG. 4.** Distortion of buildup curves due to differential damping. The simulated curves represent the transfer of longitudinal magnetization from a source spin S to a target spin T according to Eq. [5]. For all curves, the self-relaxation rates were $\rho_S = \rho_T = 1 \text{ s}^{-1}$ and the true cross-relaxation rate was $\sigma^{true}_{ST} = -0.2 \text{ s}^{-1}$. The initial slopes (apparent cross-relaxation rates $\sigma^{app}_{ST}$) were determined for $d = (d_T + d_S)/2 = 0.8$ and various differential damping rates $\delta = (d_T - d_S)/2$ between 0.25 and −0.25.

so that Eq. [3] may be reformulated,

$$\Delta\mathbf{M}_z(\tau_m, \delta \neq 0)$$
$$= \Delta\mathbf{M}^{uniform}_z(\tau_m, \delta = 0) + \Delta\mathbf{M}^{differential}_z(\tau_m), \quad [5]$$

where

$$\Delta\mathbf{M}^{differential}_z(\tau_m) = \delta \, \exp\{-\mathbf{R}\tau_m/2\} \begin{bmatrix} -1 & 0 \\ 0 & 1 \end{bmatrix}$$
$$\times \exp\{-\mathbf{R}\tau_m/2\}\Delta\mathbf{M}_z(\tau_m = 0).$$

The second term in Eq. [5] describes how differential damping affects the buildup behavior. This is described pictorially in Fig. 5. With the knowledge of the differential damping $\delta = (d_T - d_S)/2$, the correction term may be obtained. It is then possible to obtain the "undistorted" buildup curve corresponding to $\delta = 0$ by subtracting a correction curve from the experimentally obtained buildup curve. This allows one to determine the true cross-relaxation rate $\sigma^{true}_{ST}$. The correction term can be derived without prior knowledge of the entire relaxation matrix, provided that the two damping factors $d_S$ and $d_T$ have been determined independently as described above.

Although the analysis was presented for two-spin systems only, the principle may be extended to multiple-spin networks. With a sample labeled with $^{15}N$ and $^{13}C$, it is also possible to determine the damping coefficients of protons that are scalar-
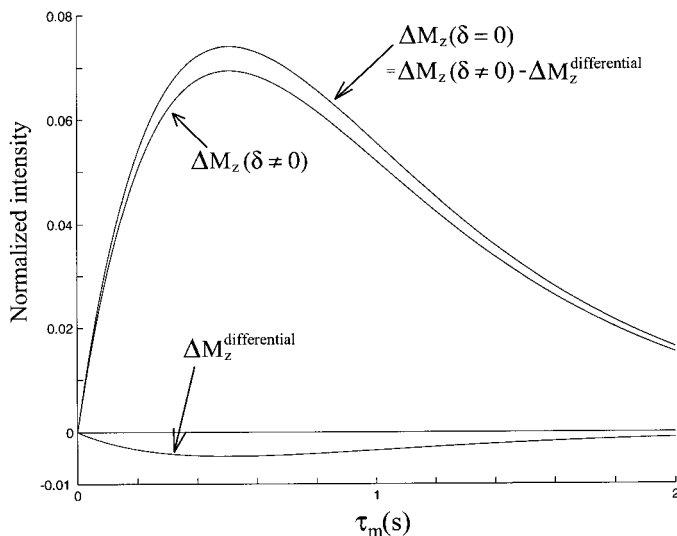
**FIG. 5.** Normalized buildup curves $a'_{ST}(\tau_m)/a'_{SS}(\tau_m = 0)$ describing the transfer of longitudinal magnetization from a source spin S to a target spin T, simulated using Eq. [5]. Self-relaxation rates $\rho_S = \rho_T = 1$ s$^{-1}$, cross-relaxation rate $\sigma_{ST}^{true} = -0.2$ s$^{-1}$ with a uniform damping factor $d = 0.75$, and differential damping factor $\delta = 0.05$. The initial slope of the curve $\Delta M_z^{uniform}$ can be obtained by subtracting from the initial slope of $\Delta M_z$ ($\delta \neq 0$) (which can be observed experimentally) the correction term $\Delta M_z^{differential}$. The correction term can be calculated from the knowledge of $d_S$ and $d_T$ (or, equivalently, $d$ and $\delta$) and an estimate of the cross-relaxation rate. In this figure, this is evaluated as $\sigma_{ST}^{true} = [(-0.187) - (0.013)]$ s$^{-1} = -0.200$ s$^{-1}$. $\sigma_{ST}^{true}$ may be determined unambiguously through iterative adjustment, recognizing that Eq. [5] is valid only if the correct $\sigma_{ST}^{true}$ is used for the simulations.

coupled to $^{13}$C, using QUIET-NOESY experiments that incorporate a [$^1$H, $^{13}$CH] BIRD sequence. Knowledge of damping coefficients for protons bound to different heteronuclei would allow one to extract various cross-relaxation rates, while distortions due to spin diffusion are eliminated. Experiments designed to edit relaxation pathways that use selective pulses of finite duration may be subject to similar complications if there is significant differential attenuation of the magnetization during the pulses.

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