



# Full relaxation matrix analysis of apparent cross-correlated relaxation rates in four-spin systems

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## ABSTRACT

Cross-correlated relaxation (CCR) rates are an established tool for the extraction of relative bond orientations in biomolecules in solution. CCR between dipolar interactions in four-spin systems is a particularly well-suited mechanism. In this paper, a simple approach to analyze systematic experimental errors is formulated in a subspace of the complete four-spin Hilbert space. It is shown that, contrary to the common assumption, the secular approximation of the relaxation matrix is marginal for the most prominent spin systems. With the main focus on the model protein GB3 at room temperature, it is shown that the apparent experimental CCR rates have errors between  $-12\%$  and  $+4\%$  for molecules with a molecular tumbling time of 3.5 ns. Although depending on the specific pulse sequence used, the following rule-of-thumb can be established: Judged by absolute values, the errors for  $H^\alpha-C^\alpha/H^\alpha-C^\beta$ ,  $H^N-N/C^\alpha-C^\beta$ ,  $H^N-N/C^\beta-C^\gamma$  and  $H^N-N/H^\beta-C^\beta$  CCR rates can safely be neglected. However, errors for  $H^N-N/H^N-N$  and  $H^N-N/H^\alpha-C^\alpha$  CCR rates are on the order of  $0.1-0.3\text{ s}^{-1}$  and must be considered. Tabulated correction factors may be used for their extraction. If larger systems are studied, in most cases the errors cannot be neglected anymore. On the other hand, well-calibrated pulses can safely be assumed to be perfect.

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## 1. Introduction

Dipolar cross-correlated relaxation (CCR) effects are an excellent tool to analyze relative bond orientations in molecules [1–6]. In particular, multiple-quantum measurements in four-spin systems offer the possibility to correlate two interspin vectors that do not share a common spin. In most cases,  $\varphi$ ,  $\psi$ , or  $\chi_1$  angles in proteins have been assessed [7–14]. As the CCR rate is a time-averaged observable, it has been proposed to inform on dynamics [1,4,15–21]. A prerequisite of such a study is sufficient measuring precision and accuracy.

It has been shown that different mechanisms affect the extraction of angles from CCR rates. Pulse sequences that make use of decoupling sequences to conserve inphase magnetization are prone to the creation of unwanted antiphase magnetization caused by imperfections in decoupling sequences [22]. Angle calculation from a CCR rate is usually based on the assumption of isotropic molecular tumbling. Obviously, an error is introduced depending on the degree of the anisotropy [4,21,23]. For a four-spin  $\frac{1}{2}$  system, effects due to longitudinal relaxation rates of the passively coupled spins have been examined using perturbation theory [24]. In addition, the nuclear Overhauser enhancement between the two passively coupled spins has different effects on the double- and

zero-quantum coherences which are difficult to account for if their scalar couplings to the MQ spins are similar (that is,  $J_{11S1} \approx J_{12S2}$ ) [25]. Apparent measured CCR rates are also affected by scalar couplings, and interference between them and longitudinal relaxation [26,27]. For example, Hu et al. demonstrated the effect on CCR of longitudinal relaxation and  $J$  couplings in a two-spin  $\frac{1}{2}$  system [27].

This work pursues two goals. First, it is geared towards the specific case of CCR rates in the model protein GB3 [28]. In an attempt to characterize the dynamic behavior of GB3 (molecular tumbling time 3.4 ns at 298 K) more holistically, a large body of CCR rates, mainly from the protein backbone, is currently collected. Some of them, namely those between  $H^N-N/H^\alpha-C^\alpha$ ,  $H^N-N/C^\beta-C^\gamma$  and  $H^N-N/C^\alpha-C^\beta$  have previously been published [5,21,29]. Further spin systems of interest in this investigation are  $H^N-N/H^N-N$ ,  $H^\alpha-C^\alpha/H^\alpha-C^\beta$  and  $H^N-N/H^\beta-C^\beta$ . For this purpose, it is crucial to understand all sources of systematic errors at every stage of the procedure. The measurements are described with four-spin  $\frac{1}{2}$  systems. All experiments are based on multiple-quantum coherences scalar coupled to two longitudinal spins. Errors of the apparent CCR rates are calculated which depend specifically on the pulse sequences, pulse imperfections, the method of CCR rate calculation and spin systems used in those studies. The calculations follow exactly the experimental procedures by evolving the magnetization operators numerically in a full evolution- and relaxation-matrix approach. In doing so, the usual assumption of the secular approximation of the

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matrix is circumvented and off-diagonal elements that alter the apparent CCR rates are explicitly included. Ultimately, correction factors to the apparent CCR rates for specific cases are provided. It is shown that, contrary to the common assumption, the secular approximation is marginal. In some cases, it provides false results while in others it may be safely used.

A second aim of this work is to make the approach seizable for a more general use. It can easily be adapted to any spin system and pulse sequence, as only the basic elements of the pulse sequence have to be brought in the correct order. In addition, results for larger molecules with three and six times the tumbling time of GB3 are provided.

## 2. Theory

### 2.1. Full matrix description of cross-correlated relaxation in a four-spin $\frac{1}{2}$ system

In the following, a matrix including spectral frequencies and contributions to relaxation is constructed. In practice, the spin system consists of two spins involved in a multiple-quantum coherence MQ,  $I_1$  and  $S_1$ , and two spins,  $I_2$  and  $S_2$ , which are passively coupled by the scalar couplings  $J_{I_1I_2}$  and  $J_{S_1S_2}$ . This condition restricts the calculation to a subspace of a general four-spin Hilbert space. The MQ subspace is only spanned by transverse operators which are chosen to be the raising and lowering operators.  $I_2$  and  $S_2$  can be expressed by the identity ( $E$ ) and longitudinal operators. These are transformed into the basis formed by  $\alpha$  and  $\beta$  states,  $\{\alpha\alpha, \alpha\beta, \beta\alpha, \beta\beta\}$ . Any magnetization in the subspace under consideration can then be expressed by a 16-dimensional magnetization vector:

$$\vec{M} = (\text{ZQ}_{+-}^{\alpha\alpha}, \text{ZQ}_{+-}^{\alpha\beta}, \text{ZQ}_{+-}^{\beta\alpha}, \text{ZQ}_{+-}^{\beta\beta}, \text{ZQ}_{-+}^{\alpha\alpha}, \text{ZQ}_{-+}^{\alpha\beta}, \text{ZQ}_{-+}^{\beta\alpha}, \text{ZQ}_{-+}^{\beta\beta}, \text{DQ}_{++}^{\alpha\alpha}, \text{DQ}_{++}^{\alpha\beta}, \text{DQ}_{++}^{\beta\alpha}, \text{DQ}_{++}^{\beta\beta}, \text{DQ}_{--}^{\alpha\alpha}, \text{DQ}_{--}^{\alpha\beta}, \text{DQ}_{--}^{\beta\alpha}, \text{DQ}_{--}^{\beta\beta})^T \quad (1)$$

ZQ and DQ are the zero- and double-quantum coherences obtained by the respective combinations of the raising (+) and lowering operators (–) of  $I_1$  and  $S_1$  as indicated in the subscript and the superscripts define the states of  $I_2$  and  $S_2$ .

Double-inphase (IP,IP) and double-antiphase (AP,AP) zero- and double-quantum coherences with respect to the two passively coupled spins are then expressed as:

$$\begin{aligned} \vec{M}_{\text{ZQ}}^{\text{IP,IP}} &= \sqrt{2}(1/4, 1/4, 1/4, 1/4, 1/4, 1/4, 1/4, 1/4, \\ &0, 0, 0, 0, 0, 0, 0, 0)^T \\ \vec{M}_{\text{ZQ}}^{\text{AP,AP}} &= \sqrt{2}(1/4, -1/4, -1/4, 1/4, 1/4, -1/4, -1/4, \\ &1/4, 0, 0, 0, 0, 0, 0, 0)^T \\ \vec{M}_{\text{DQ}}^{\text{IP,IP}} &= \sqrt{2}(0, 0, 0, 0, 0, 0, 0, 0, 1/4, 1/4, 1/4, 1/4, \\ &1/4, 1/4, 1/4)^T \\ \vec{M}_{\text{DQ}}^{\text{AP,AP}} &= \sqrt{2}(0, 0, 0, 0, 0, 0, 0, 0, 1/4, -1/4, -1/4, 1/4, \\ &1/4, -1/4, -1/4, 1/4)^T \end{aligned} \quad (2.1 - 4)$$

The normalization factor is chosen such that the scalar product  $(\vec{M}_{\text{uQ}}^{\text{XP,XP}})^T \cdot \vec{M}_{\text{vQ}}^{\text{YP,YP}} = \delta_{u,v;x,y}$ , where  $\delta_{u,v;x,y}$  is Kronecker's delta function.

Manipulation of the initial magnetization vector is simulated by matrices describing pulses, relaxation, scalar coupling and chemical shift evolution. The total time  $T$  is divided up into  $N$  periods of free evolution  $t_i$  and  $(N - 1)$  (possibly multispin-) pulses which are assumed to be executed instantaneously. The pulse operations are given by matrices  $P_i$  and free evolution is given by the matrix  $F$ , which is identical for all periods. In integrated form the magnetization evolution is given as:

$$\vec{M}_{\text{MQ}}^{\text{u,v}}(T) = e^{-Ft_N} \prod_{i=1}^{N-1} P_i e^{Ft_i} \vec{M}_{\text{MQ}}^{\text{u,v}}(0) \quad (3)$$

Free evolution is computed upon diagonalization:

$$e^{Ft_i} = U e^{D t_i} U^{-1} \quad (4)$$

$D$  is the diagonal matrix containing the eigenvalues of  $F$  and  $U$  is the transformation matrix containing the eigenvectors.

The intensity of an observed signal selected by the observation operator  $\vec{M}_{\text{obs}}$  is given by the projection

$$I_{\text{MQ}}^{\text{u,v}} = |(\vec{M}_{\text{obs}})^T \cdot \vec{M}_{\text{MQ}}^{\text{u,v}}(T)| \quad (5)$$

### 2.2. Construction of pulse matrices

The  $P$  matrices executing pulses on  $I_2$ ,  $S_2$  or both have the following form since transitions occur only within the ZQ and DQ subspaces:

$$P_{I_2} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \otimes \tilde{P}_{I_2} \quad (6)$$

$$P_{S_2} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \otimes \tilde{P}_{S_2}$$

The tensor product (direct product) of matrices is used.  $P$  is composed of four identical  $4 \times 4$  submatrices  $\tilde{P}$ . Inversion pulses only applied to  $I_2$  or  $S_2$  are given as:

$$\tilde{P}_{I_2}^{180} = \begin{pmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{pmatrix}; \quad \tilde{P}_{S_2}^{180} = \begin{pmatrix} 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 \end{pmatrix} \quad (7.1 - 2)$$

If the pulses are imperfect, three- or four-spin coherences are generated which are assumed to be undetectable (this can be shown to be true for all pulse sequences analyzed in this work). Therefore,  $\tilde{P}$  is replaced by linear combinations of  $\tilde{P}$  and the identity matrix:

$$\tilde{P}_{I_2} = \begin{pmatrix} \lambda & 0 & \kappa & 0 \\ 0 & \lambda & 0 & \kappa \\ \kappa & 0 & \lambda & 0 \\ 0 & \kappa & 0 & \lambda \end{pmatrix}; \quad \tilde{P}_{S_2} = \begin{pmatrix} \lambda & \kappa & 0 & 0 \\ \kappa & \lambda & 0 & 0 \\ 0 & 0 & \lambda & \kappa \\ 0 & 0 & \kappa & \lambda \end{pmatrix} \quad (8.1-2)$$

$$\lambda = \frac{1 + \text{Cos}(\phi)}{2}; \quad \kappa = \frac{1 - \text{Cos}(\phi)}{2}$$

$\phi$  is the effective angle rotation caused by the pulse. Simultaneous execution of pulses on both  $I_2$  and  $S_2$  is described by:

$$\tilde{P}_{I_2 S_2} = \tilde{P}_{I_2} \tilde{P}_{S_2} \quad (9)$$

Inversion pulses on  $I_1$  and  $S_1$  induce transitions between ZQ and DQ coherences:

$$P_{I_1}^{180} = \begin{pmatrix} 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix} \otimes \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}; \quad (10.1 - 2)$$

$$P_{S_1}^{180} = \begin{pmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{pmatrix} \otimes \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

Here no modification for imperfect pulses is used since it is assumed that they lead to dephasing of the MQ magnetization by

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