Hindawi
Concepts in Magnetic Resonance Part A, Bridging Education and Research
Volume 2022, Article ID 1617848, 10 pages
https://doi.org/10.1155/2022/1617848

Research Article

Multispin Cross-Correlated Transverse Dipolar NMR Relaxation in Solution

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Received 26 January 2022; Accepted 8 March 2022; Published 12 April 2022

Academic Editor: Rongchun Zhang

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In this paper, we want to consider what would be involved in calculating the R_2 relaxation of amide protons in a protein caused by dipolar interactions with nearby protons, for which there are many. NMR textbooks give analytical equations and sometimes derivations for solution NMR relaxation due to dipolar interactions between two spins. There are also closed equations for dipolar interactions between three spins, which include relaxation interference, also known as cross-correlated cross-relaxation. We here derive an expression for interference between four spins. For larger systems, such as amide protons in a protein, we develop a local-field methodology, from which solution relaxation interference can be computed for a basically limitless number of interacting spins.

1. Introduction

Several researchers have been interested in investigating whether proton-proton dipolar interaction in proteins can be utilized to obtain structural [1, 2] or dynamical information [3]. Here, we want to consider what would be involved in calculating the R_2 relaxation of amide protons in a protein caused by dipolar interactions with other protons and, ultimately, compare those with experiment [4]. NMR textbooks give closed equations and sometimes derivations for solution NMR relaxation due to dipole-dipole relaxation between two spins and for chemical shift anisotropy (CSA) relaxation. There are also closed equations for relaxation due to interactions between three dipoles [5] and two dipoles and the CSA [6]. In these cases, relaxation interference, also known as cross-correlated cross-relaxation, has been taken into account. Dipolar-dipolar relaxation interference has been described very early in the history of NMR [7, 8] and has been exploited to achieve line narrowing [9] and structural information [1, 2]. CSA-dipolar cross-correlated relaxation is at the core of the line narrowing in the TROSY experiment [10].

Here, we derive an analytical expression for dipolar interference between four spins. For larger systems, such as amide protons in a protein, we develop a local-field methodology, from which solution relaxation interference can be computed for a basically limitless number of interacting spins.

2. Theory

Let us start by considering the R_2 relaxation of spin **I** in a rigid molecule with three protons, **S-I-Q**, subject to fluctuating dipolar interactions **IS** and **IQ**. We assume here that **I**, **S,** and **Q** have different chemical shifts and are thus similar, but "unlike." The first step is to add the relaxation rates due to the **IS** and **IQ** dipolar interactions, as given by equation (A.24) of the Appendix.

$$R_2^{I\,\mathrm{total}} = R_2^{\mathrm{IS}} + R_2^{\mathrm{IQ}}, \tag{1}$$

$$R_2^{I\,\mathrm{total}} = \frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2 \left\{ \left(\frac{\gamma_H \gamma_H \hbar}{r_{\mathrm{IS}}^3}\right)^2 + \left(\frac{\gamma_H \gamma_H \hbar}{r_{\mathrm{IQ}}^3}\right)^2 \right\} \cdot \{5J(0) + 9J(\omega_H) + 6J(\omega_{2H})\}. \tag{2}$$

However, in a rigid molecule, the stochastic fluctuations of the **IS** and **IQ** dipolar interactions are 100% correlated and relaxation interference occurs. Relaxation interference is also called cross-correlated cross-relaxation or cross-correlated relaxation.

Interference between two dipolar mechanisms can be qualitatively understood from Figure 1. Here, we consider a linear three-spin system with spin **I** in the center and spin **S** and **Q** equidistant from **I**.

In this case, there are two relaxation rates for spin **I**, one for molecule **A**, where the dipolar fields of **S** and **Q** at the location **I** cancel independent of orientation with respect to the magnetic field, and another for molecule **B**, where the dipoles reinforce each other and fluctuate strongly depending on orientation. This is expressed as

$$R_2^{I\,\mathrm{total}} = R_2^{\mathrm{IS}} + R_2^{\mathrm{IQ}} \pm R_{2\mathrm{CC}}^{\mathrm{IS-IQ}}. \tag{3}$$

Of course, the dipoles flip by R_1 processes. For macromolecules, R_1 rates are much slower than R_2 rates. Thus, for R_2 cross-correlations, one may assume that orientations of the dipoles remain set during the typical R_2 relaxation time.

If the different dipolar permutations also give rise to different resonances, by either scalar or residual static dipolar coupling, one observes four NMR lines for **I**. The two outer lines have equal linewidths, and the two inner lines also have equal, but different, linewidths. Which set will be the broader one depends on the molecular geometry and the sign of the (scalar) coupling. If no such coupling exists, one will observe a superposition of a broader and narrower line for **I**.

In order to obtain quantitative values for the dipolar-dipolar cross-correlation, one starts with expanding the double commutator master equation for R_2 relaxation, as shown in equation (A.16) of the Appendix. We mostly follow a formalism as introduced by [5, 11].

For two R_2 relaxation mechanisms **IS** and **IQ**, modulated by the same motions, the relaxation master equation is

$$\frac{d\langle I_+\rangle}{\mathrm{d}t} = -\frac{1}{2}\sum_{m=-2}^{m=+2}\left\{\left\langle\left[\left[I_+, T_{\mathrm{mIS}} + T_{\mathrm{mIQ}}\right], T_{\mathrm{mIS}}^{\dagger} + T_{\mathrm{mIQ}}^{\dagger}\right]\right\rangle\right\} j_m \omega_m, \tag{4}$$

where T_{mIS} are the tensor operators of the perturbing Hamiltonian, which for dipolar interaction between spins I and S are given by

$$T_0 = 2I_z S_z - \frac{1}{2}\left(I_+ S_- + I_- S_+\right), \tag{5a}$$

$$T_{\pm 1} = \mp\sqrt{\frac{3}{2}}\left(I_\pm S_z + I_z S_\pm\right), \tag{5b}$$

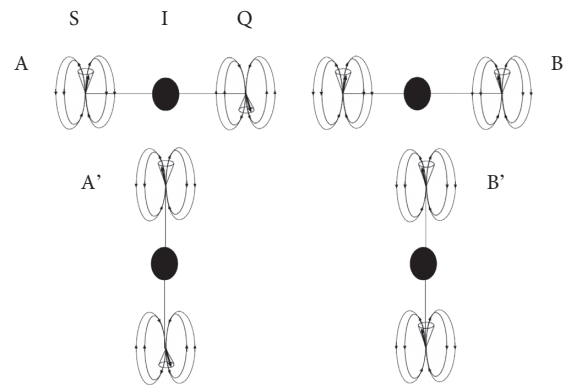

Figure 1: Pictorial representation of dipolar cross-correlation in a linear S-I-Q system. The dipolar fields of **S** and **Q** at the location of I for A, independent of orientation with respect to the magnetic field (vertical). In B, the dipolar fields of **S** and **Q** reinforce each other at the location of **I** and modulate strongly as a function of molecular orientation.

$$T_{\pm 2} = \sqrt{\frac{3}{2}}\left(I_\pm S_\pm\right), \tag{5c}$$

with similar equations for T_{mIQ}.

$j_m \omega_m$ are the spectral densities of the molecular motion at the frequencies of the bilinear spin operators including magnitude terms.

In the expansion, the "diagonal" terms such as

$$\sum_{m=-2}^{m=+2}\left\{\left\langle\left[\left[I_+, T_{\mathrm{mIS}}\right], T_{\mathrm{mIS}}^{\dagger}\right]\right\rangle\right\} j_m \omega_m, \tag{6a}$$

$$\sum_{m=-2}^{m=+2}\left\{\left\langle\left[\left[I_+, T_{\mathrm{mIQ}}\right], T_{\mathrm{mIQ}}^{\dagger}\right]\right\rangle\right\} j_m \omega_m, \tag{6b}$$

and their complex conjugates will give rise to the autocorrelation rates R_2^{IS} and R_2^{IQ}, as shown in equation (A.22) of the Appendix.

In the cross terms,

$$\sum_{m=-2}^{m=+2}\left\{\left\langle\left[\left[I_+, T_{\mathrm{mIS}}\right], T_{\mathrm{mIQ}}^{\dagger}\right]\right\rangle\right\} j_m^{\mathrm{cc}} \omega_m, \tag{7a}$$

$$\sum_{m=-2}^{m=+2}\left\{\left\langle\left[\left[I_+, T_{\mathrm{mIQ}}\right], T_{\mathrm{mIS}}^{\dagger}\right]\right\rangle\right\} j_m^{\mathrm{cc}} \omega_m, \tag{7b}$$

and their complex conjugates, only spin operators with exactly the same precession frequency will be relevant, i.e., only the pair ($I_z S_z$, $I_z Q_z$) and the four terms $I_+ S_z$, $I_- S_z$, $I_+ Q_z$, and $I_- Q_z$.

We obtain the following equation from equations (6a) and (6b):

$$-\frac{d\langle I_+\rangle}{\mathrm{d}t} = \langle 4[[I_+, I_z S_z], I_z Q_z]\rangle j_0^{\mathrm{cc}}(0) + \frac{3}{2}\langle[[I_+, I_- S_z], I_+ Q_z]\rangle j_1^{\mathrm{cc}}(\omega_I), \tag{8}$$

where the ½ term is dropped because there are two equivalent cross terms, as given in equations (7a) and (7b).

The cross-correlation spectral densities are

$$j_m^{cc}\omega_m = -\frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{\gamma_I\gamma_S\hbar}{r_{IS}^3}\right)\left(\frac{\gamma_I\gamma_Q\hbar}{r_{IQ}^3}\right)P_2\cos\left(\theta_{IS-IQ}\right)J\left(\omega_m\right), \tag{9}$$

with, for isotropic motion,

$$J\left(\omega_m\right) = \frac{2}{5}\frac{\tau_c}{1+\omega_m^2\tau_c^2}, \tag{10}$$

where the $P_2\cos\left(\theta_{IS-IQ}\right) = 1/2\left(3\cos^2\left(\theta_{IS-IQ}\right) - 1\right)$ term originates from transforming the **IQ** dipolar vector into the **IS** frame or vice versa and using the addition theorem of spherical harmonics. Here, θ_{IS-IQ} is the angle between vectors **IS** and **IQ** and τ_c is the (rotational) correlation time.

In contrast to the R_2 autocorrelation, the double commutators drive cross-relaxation

$$-\frac{d\langle I_+\rangle}{dt} = \langle 4I_+S_zQ_z\rangle j_0^{cc}(0) + \frac{3}{4}\langle 4I_+S_zQ_z\rangle j_1^{cc}(\omega_I), \tag{11a}$$

and back

$$-\frac{d\langle 4I_+S_zQ_z\rangle}{dt} = \langle I_+\rangle j_0^{cc}(0) + \langle I_+\rangle j_1^{cc}(\omega_I). \tag{11b}$$

One ends up with a set of coupled differential equations:

$$\frac{d}{dt}\begin{pmatrix} I_+ \\ 4I_+S_zQ_z \end{pmatrix} = -\begin{bmatrix} R_{2I}^{IP} & R_{2I}^{CC} \\ R_{2I}^{CC} & R_{2I}^{AP} \end{bmatrix}\begin{pmatrix} I_+ \\ 4I_+S_zQ_z \end{pmatrix}. \tag{12}$$

Here, R_{2I}^{IP} and R_{2I}^{AP} are the in-phase (IP) R_2 rate for the $<I_+>$ coherence and antiphase (AP) R_2 rate for the $4<I_+S_zI_z>$ coherence. The antiphase terms relax faster because of the extra $\mathbf{S_z}$ and $\mathbf{Q_z}$ R_1 relaxation. For macromolecules, the difference can be neglected because R_1 is small as compared to R_2, and one can easily diagonalize the rate matrix and obtain relaxation "eigenvectors"

$$\frac{d}{dt}\begin{pmatrix} (I_+ + 4I_+S_zQ_z) \\ (I_+ - 4I_+S_zQ_z) \end{pmatrix} = -\begin{bmatrix} R_2^{IS} + R_2^{IQ} + R_{2I}^{CC} & 0 \\ 0 & R_2^{IS} + R_2^{IQ} - R_{2I}^{CC} \end{bmatrix}\begin{pmatrix} (I_+ + 4I_+S_zQ_z) \\ (I_+ - 4I_+S_zQ_z) \end{pmatrix}, \tag{13}$$

with

$$R_{2I}^{CC} = \frac{1}{4}\left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{\gamma_I\gamma_S\hbar}{r_{IS}^3}\right)\left(\frac{\gamma_I\gamma_Q\hbar}{r_{IQ}^3}\right)P_2\cos\left(\theta_{IS-IQ}\right)\{4J(0) + 3J(\omega_I)\}. \tag{14}$$

The total $\mathbf{R_2}$ relaxation for proton **i**, as stated before in equation (3), is then given by

$$R_2^{I\,\text{total}} = R_2^{IS} + R_2^{IQ} \pm R_{2CC}^{IS-IQ}. \tag{15}$$

One notices that ^{1}H **IS**-autorelaxation contains a $5J(0)$ term (equation (2)), while the dipolar cross-correlation has a $4J(0)$ term (equation (14)). The extra $J(0)$ in autorelaxation is due to the fact that $J(0)$ and $J(\omega_I - \omega_S)$ are for two protons similar enough for the spectral densities to add (see Appendix), but $(\omega_I - \omega_S)$ and $(\omega_I - \omega_Q)$ are different enough for the spin operators to diphase and not cross-correlate. Thus, there will not be a complete cancelation of relaxation, as suggested in the situation of Figure 1. It can still happen, if the differences in distances IS and IQ would make up for the 4/5 term.

If one cannot make the approximation that R_{2I}^{IP} and R_{2I}^{AP} are equal, the rates become

$$R_2^{I\,\text{total}} = \frac{1}{2}R_{2I}^{IP} + \frac{1}{2}R_{2I}^{AP} \pm \sqrt{\frac{R_{2I}^{IP}xR_{2I}^{IP}}{4} - \frac{R_{2I}^{IP}xR_{2I}^{AP}}{2} + \frac{R_{2I}^{AP}xR_{2I}^{AP}}{4} + R_{2CC}^{IS-IQ}xR_{2CC}^{IS-IQ}}. \tag{16}$$

Let us now extend this formalism to a 4-spin system, with a central spin **I** and 3 other spins **S**, **Q,** and **P** in its vicinity.

From the master equation, we arrive at three cross terms:

$$-\frac{d\langle I_+\rangle}{dt} = \langle 4[[I_+, I_zS_z], I_zQ_z]\rangle j_0^{cc}(0) + \frac{3}{2}\langle [[I_+, I_-S_z], I_+Q_z]\rangle j_1^{cc}(\omega_I)$$
$$+\langle 4[[I_+, I_zS_z], I_zP_z]\rangle j_0^{cc}(0) + \frac{3}{2}\langle [[I_+, I_-S_z], I_+P_z]\rangle j_1^{cc}(\omega_I) \quad (17)$$
$$+\langle 4[[I_+, I_zQ_z], I_zP_z]\rangle j_0^{cc}(0) + \frac{3}{2}\langle [[I_+, I_-Q_z], I_+P_z]\rangle j_1^{cc}(\omega_I),$$

leading to terms such as $4I_+S_zQ_z$, $4I_+S_zP_z$, and $4I_+P_zQ_z$, driven by the rates

$$R_{2I}^{\mathrm{IS-IQ}} = \frac{1}{4}\left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{\gamma_I\gamma_S\hbar}{r_{IS}^3}\right)\left(\frac{\gamma_I\gamma_Q\hbar}{r_{IQ}^3}\right) \cdot P_2\cos\left(\theta_{\mathrm{IS-IQ}}\right)\{4J(0) + 3J(\omega_I)\}, \quad (18a)$$

$$R_{2I}^{\mathrm{IS-IP}} = \frac{1}{4}\left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{\gamma_I\gamma_S\hbar}{r_{\mathrm{IS}}^3}\right)\left(\frac{\gamma_I\gamma_P\hbar}{r_{\mathrm{IP}}^3}\right) \cdot P_2\cos\left(\theta_{\mathrm{IS-IP}}\right)\{4J(0) + 3J(\omega_I)\}, \quad (18b)$$

$$R_{2I}^{\mathrm{IP-IQ}} = \frac{1}{4}\left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{\gamma_I\gamma_Q\hbar}{r_{IQ}^3}\right)\left(\frac{\gamma_I\gamma_P\hbar}{r_{IP}^3}\right) \cdot P_2\cos\left(\theta_{\mathrm{IQ-IP}}\right)\{4J(0) + 3J(\omega_I)\}. \quad (18c)$$

This appears to lead to eight different relaxation rates ($R_2^{\mathrm{IS}} + R_2^{\mathrm{IQ}} + R_2^{\mathrm{QS}} \pm R_{2\mathrm{CC}}^{\mathrm{IS-IQ}} \pm R_{2\mathrm{CC}}^{\mathrm{IS-IP}} \pm R_{2\mathrm{CC}}^{\mathrm{IP-IQ}}$). However, that cannot be right; adding one more dipole **P** to the **S-I-Q** situation should just give rise to two more rates, four in total. So, one should not stop here and construct the full relaxation matrix.

Also, one must take cross-relaxation between the different four-spin terms into account (here only showing some $J(0)$ terms),

$$-\frac{d\langle 4I_+S_zQ_z\rangle}{dt} = \langle 4[[4I_+S_zQ_z, I_zS_z], I_zP_z]\rangle = \langle 4I^+Q_zP_z\rangle, \quad (19a)$$

$$-\frac{d\langle 4I_+S_zQ_z\rangle}{dt} = \langle 4[[4I_+S_zQ_z, I_zQ_z], I_zP_z]\rangle = \langle 4I_+S_zP_z\rangle, \quad (19b)$$

$$-\frac{d\langle 4I_+S_zP_z\rangle}{dt} = \langle 4[[4I_+S_zP_z, I_zP_z], I_zQ_z]\rangle = \langle 4I_+S_zQ_z\rangle, \quad (19c)$$

and three reverse processes.

The differential equations in matrix form will now become

$$\frac{d}{dt}\begin{pmatrix} I_+ \\ 4I_+S_zQ_z \\ 4I_+S_zP_z \\ 4I_+Q_zP_z \end{pmatrix} = -\begin{vmatrix} R_{2I} & R_{2\mathrm{cc}}^{\mathrm{SQ}} & R_{2\mathrm{cc}}^{\mathrm{SP}} & R_{2\mathrm{cc}}^{\mathrm{QP}} \\ R_{2\mathrm{cc}}^{\mathrm{SQ}} & R_{2I} & R_{2\mathrm{cc}}^{\mathrm{QP}} & R_{2\mathrm{cc}}^{\mathrm{SP}} \\ R_{2\mathrm{cc}}^{\mathrm{SP}} & R_{2\mathrm{cc}}^{\mathrm{QP}} & R_{2I} & R_{2\mathrm{cc}}^{\mathrm{SQ}} \\ R_{2\mathrm{cc}}^{\mathrm{QP}} & R_{2\mathrm{cc}}^{\mathrm{SP}} & R_{2\mathrm{cc}}^{\mathrm{SQ}} & R_{2I} \end{vmatrix}\begin{pmatrix} I_+ \\ 4I_+S_zQ_z \\ 4I_+S_zP_z \\ 4I_+Q_zP_z \end{pmatrix}. \quad (20)$$

Here, we are neglecting the differences between R_2 in-phase and antiphase relaxation. This matrix has four eigenvalues, which correspond to what must be physically correct. While the exact eigenvalues of this matrix could potentially be analytically obtained, it becomes harder when differences between R_2 in-phase and antiphase relaxation have to be considered as well. However, because matters will quickly become more complicated when even more spins interact, we will not attempt to derive an analytical solution for the four-spin case here. For instance, in a protein, there are typically at least 20 protons around every amide proton in a 6 Å sphere which would all have to be taken into account. In such a case, one would need to diagonalize a $262144 * 262144$ matrix (2^{20-2}).

We must take another approach. To arrive at an estimation for the effects in a multiproton spin system, we will start from a "solid-state NMR" point of view. We calculate $B_{\mathrm{loc}(i)}^{\Omega}$, the net local magnetic field at center proton **i** due to the **M** surrounding protons **j** [12] for a certain orientation of the molecule in the external magnetic field:

$$B_{\mathrm{loc}(i)}^{\Omega} = \frac{\mu_0}{4\pi}\sum_{j\neq i}^{j=M}\mathbb{D}\left(\frac{\gamma_i\gamma_j}{r_{ij}^3}\right)P_2\left(\cos\theta_{ij}\right). \quad (21)$$

Here, θ_{ij} is the angle between the internuclear vector **ij** and the magnetic field direction Ω in the molecular frame. $\mathbb{D}$ represents a particular distribution of the signs of the z-components of the dipoles **j**. If one varies the magnetic

field direction according to a sphere distribution and adds the results, one obtains the powder pattern for that particular distribution $\mathbb{D}$. Subsequently, one coadds all powder patterns for different values of $\mathbb{D}$ and normalizes to arrive at the "cross-correlated" dipolar powder pattern for the ^{1}HN under consideration.

It is the time dependence of $\mathbf{B_{loc}}$, as caused by molecular motion, that drives the solution NMR dipolar relaxation. The R_2 relaxation is then obtained as the second moment of the (cross-correlated) powder pattern [12]:

$$R_{2i}^{\text{solution}} = 4\tau_c \sum_{\Omega} \left(B_{\text{loc}(i)} - B_{\text{loc}(i)}^{\Omega} \right)^2, \tag{22}$$

where the brackets indicate average overall orientations. In practice, we permute only the signs of the eight closest protons (256 different distributions $\mathbb{D}$) and treat the spins further out with a single random distribution where spins up and down are on average equal. So, each distribution then gives rise to an individual resonance for **i** with its own R_2 rate. The sum of all of those creates the inhomogeneous sum-line for resonance **i**.

3. Verification

We verified the algorithm with a three-atom arrangement (see Table 1) with results in Figure 2. In this figure, the green points were calculated from equations (14) and (15) for dipolar-dipolar cross-correlation, while the drawn black line was calculated using the "solid-state" approach. The results are identical. In red is a relaxation curve calculated from the straight addition of R_2 (IS) and R_2 (IQ) (see equation (1)). It is clear that the cross-correlation cannot be neglected, except when one considers the first part of the curve. Fitting a single exponential against a complete cross-correlated relaxation curve (dashed line) yields an erroneous rate.

In Figure 3(a), we show calculated R_2 relaxation rates for the HN of Asp45 of the protein GB1. This amide has 21 proton neighbors within a sphere of 6 Å. Here, the difference between relaxation curves with and without cross-correlations is smaller than the example case in Figure 2. From this, one would tend to conclude that many protons cancel each other's cross-correlations. However, that is not necessarily true; in Figure 3(b), we show calculated R_2 relaxation rates for the HN of Asn35 of the protein GB1. This amide has more (36) proton neighbors within a sphere of 6 Å, yet the difference between relaxation curve with and without cross-correlations is larger than for Asp45. This happens because the R_2 relaxation of Asn35 is dominated by two close-by protons.

This manuscript was written in anticipation of the interpretation of experimental $T_{1\text{rho}}$ data for protein amide protons, which we hope may contribute to more complete understanding of protein molecular dynamics (E.R.P. Zuiderweg and D.A. Case, in preparation). We conclude from the current analysis that one can still fit a single exponential to the beginning of the $T_{1\text{rho}}$ relaxation curves, even when many protons interact. This result is the same as for a 3-spin network, but that was not previously demonstrated.

Table 1: Coordinates of an arbitrary three-spin arrangement (Å). $d_{\text{IS}} = 2$ Å, $d_{\text{IQ}} = 2.236$ Å, and $P_2\ \cos(\theta_{\text{IS-IQ}}) = 0.70$.

	x	y	z
Center spin (I)	0	0	0
Neighbor (S)	0	2	0
Neighbor (Q)	0	−2	1

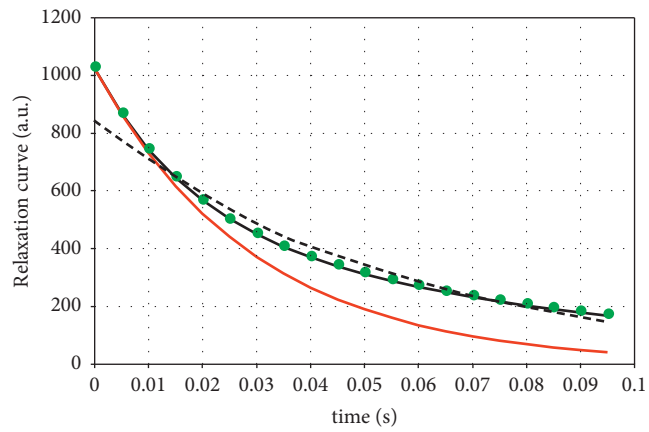

Figure 2: The green points were calculated from equations (14) and (15) for dipolar-dipolar cross-correlation, while the solid black line was calculated using the "solid-state" approach (equations (21) and (22)). Fitting a single exponential against a complete cross-correlated relaxation curve (dashed line) yields an erroneous rate. In red is a relaxation curve calculated from the straight addition of R_2 (IS) and R_2 (IQ) (equation (1)). A (isotropic) rotational correlation time τ_c of 10 ns was used in all calculations.

However, how far down a relaxation curve can go varies from proton to proton environment. Judging from Figure 3(b), one should limit the recording of $T_{1\text{rho}}$ relaxation curves to values larger than $0.8\text{x}I_0$, where I_0 is the initial value of the decay curve.

4. Description of the Codes

The computer program requires as input a "protonated" PDB file, the radius of the sphere of protons around the amide protons one is interested in, the rotational correlation time, and the spectrometer frequency. Basically, the program consists of four nested loops: amides, protons around amides, permutation of dipole signs of these surrounding protons, and rotation of the magnetic field vector in the molecular frame.

A set of 10 nested loops permutes the dipolar signs of the closest 10 hydrogens (1024 distributions). The more remote hydrogens in the sphere (if any) have their dipolar signs assigned according to a 50% random chance. The local dipolar field at a certain ^{1}HN due to the surrounding protons in a certain permutation $\mathbb{D}$ of surrounding dipoles is calculated according to equation (20). Here, the program takes the differences between "like" and "unlike" spins (see Appendix) into account. Then, the program calculates, according to equation (22), the R_2 relaxation rate due to that permutation, by rotating the external field

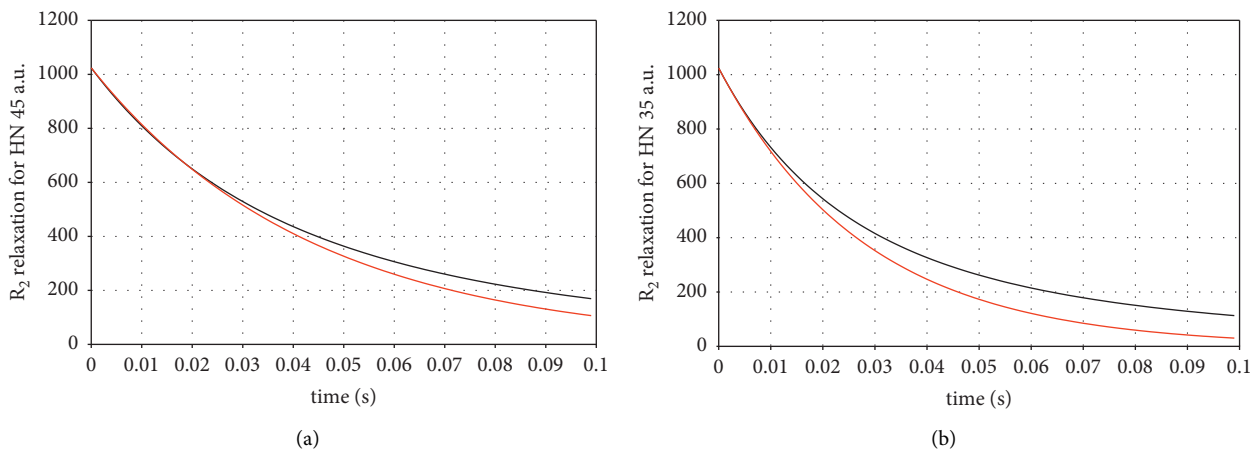

FIGURE 3: The calculated R_2 relaxation rates (a) for the HN of Asp45 of the protein GB1 which has 21 proton neighbors within a sphere of 6 Å and (b) for the HN of Asn35 with 37 proton neighbors within a sphere of 6 Å. The black curves are with cross-correlations (equations (21) and (22)), while the red curves are computed as the sum of the R_2 rates (equation (1)). A (isotropic) rotational correlation time τ_c of 10 ns was used in all calculations.

direction (the z-axis) through the molecular frame using an isotropic spherical distribution (5000 orientations) (http://corysimon.github.io/articles/uniformdistn-on-sphere/).

The relaxation rate for that permutation $\mathbb{D}$ is then used to compute a R_2 relaxation curve. The computed R_2 relaxation curves for all different permutations $\mathbb{D}$ are then added to obtain the complete R_2 relaxation curve, as shown in Figures 2 and 3.

The program is written in Fortran 90 and contains no references to outside libraries. The source code is available from the author.

Appendix

This appendix presents a refresher on dipolar R_2 relaxation, mostly following the formalism, as developed by Goldman [11] and further extended by [13], to help follow the algebra in the main body of the paper.

When the density operator σ is subject to both a time-independent (eigen) Hamiltonian $\mathscr{H}_0$ and a time-dependent perturbing dipolar Hamiltonian $\mathscr{H}_1(t)$, its evolution, in units of $\hbar$, is given by

$$\frac{d}{dt}\sigma(t) = -i\left[\mathscr{H}_0 + \mathscr{H}_1(t), \sigma(t)\right]. \tag{A.1}$$

Transforming to the rotating frame of the time-independent Hamiltonian, one obtains

$$\frac{d}{dt}\tilde{\sigma}(t) = -i\left[\widetilde{\mathscr{H}}_1(t), \tilde{\sigma}(t)\right], \tag{A.2}$$

with the time-dependent Hamiltonian also transformed to the rotating frame. One keeps in mind that this equation describes an ensemble average. First, one imposes on equation (A.2) that the density operator cannot evolve ("relax") when it was not perturbed in the first place (e.g., by a r.f. pulse). Thus, one makes the substitution

$$\frac{d}{dt}\tilde{\sigma}(t) = -i\left[\widetilde{\mathscr{H}}_1(t), \tilde{\sigma}(0) - \tilde{\sigma}_{eq}\right], \tag{A.3}$$

where $\tilde{\sigma}_{eq}$ is the density operator at equilibrium, which is not evolving under $\mathscr{H}_0$ (i.e., the usual "high temperature approximation with $\tilde{\sigma}_{eq} \sim I_z$).

One uses the Hausdorff expansion to integrate equation (A.3):

$$\begin{aligned}\tilde{\sigma}(t) = \tilde{\sigma}(0) &- i\int_0^t \left[\widetilde{\mathscr{H}}_1(t\prime), \tilde{\sigma}(0) - \tilde{\sigma}_{eq}\right] dt' \\ &- \int_0^t \int_0^{t'} \left[\widetilde{\mathscr{H}}_1(t'), \left[\widetilde{\mathscr{H}}_1(t''), \tilde{\sigma}(0) - \tilde{\sigma}_{eq}\right]\right] dt'\,dt'' + \ldots.\end{aligned} \tag{A.4}$$

Since the dipolar Hamiltonian has zero average in solution, the second term in equation (A.4) vanishes. The fourth and higher-order terms also vanish because the perturbing Hamiltonian causes only small changes of the density operator, allowing the expansion to converge rapidly. One thus obtains the formal relaxation equation:

$$\tilde{\sigma}(t) = \tilde{\sigma}(0) - \int_0^t \int_t^{t'} \left[\widetilde{\mathscr{H}}_1(t'), \left[\widetilde{\mathscr{H}}_1(t''), \tilde{\sigma}(0) - \tilde{\sigma}_{eq}\right]\right] dt'\,dt''. \tag{A.5}$$

The perturbing Hamiltonian fluctuates not only by the fluctuating dipole-dipole interactions between spins I caused by random molecular motion captured in the terms $F_m^{DD}(t)$ but also by frequencies of the spin operators I, S, and I_+S_- (ωI, ωS, ωI–ωS, etc.).

It can be abbreviated as

$$\widetilde{\mathscr{H}_{IS}^{DD}}(t) = \sum_{m=-2}^{m=2} F_m^{DD}(t)\widetilde{T_{mIS}}. \tag{A.6}$$

T_{mIS} are the tensor operators of the perturbing Hamiltonian, which for dipolar interaction between spins I and S are given by

$$T_0 = 2I_zS_z - \frac{1}{2}\left(I_+S_- + I_-S_+\right), \tag{A.7a}$$

$$T_{\pm 1} = \mp\sqrt{\frac{3}{2}}\left(I_\pm S_z + I_z S_\pm\right), \tag{A.7b}$$

$$T_{\pm 2} = \sqrt{\frac{3}{2}}\left(I_\pm S_\pm\right). \tag{A.7c}$$

On expanding, this becomes

$$\begin{aligned}\widetilde{\mathscr{H}}_{\mathrm{IS}}^{\mathrm{DD}}(t) = &+2F_0^{\mathrm{IS}}(t)I_zS_z - \frac{1}{2}F_0^{\mathrm{IS}}(t)\left(I_+S_- + I_-S_+\right)e^{i\left(\omega_I-\omega_S\right)t}\\ &+\sqrt{\frac{3}{2}}F_1^{\mathrm{IS}}(t)\left(I_+S_z\right)e^{i\left(\omega_I\right)t} - \sqrt{\frac{3}{2}}F_1^{\mathrm{IS}}(t)\left(I_-S_z\right)e^{i\left(\omega_I\right)t}\\ &+\sqrt{\frac{3}{2}}F_1^{\mathrm{IS}}(t)\left(I_zS_+\right)e^{i\left(\omega_S\right)t} - \sqrt{\frac{3}{2}}F_1^{\mathrm{IS}}(t)\left(I_zS_-\right)e^{i\left(\omega_S\right)t}\\ &+\sqrt{\frac{3}{2}}F_2^{\mathrm{IS}}(t)\left(I_+S_+\right)e^{i\left(\omega_I+\omega_S\right)t} + \sqrt{\frac{3}{2}}F_2^{\mathrm{IS}}(t)\left(I_-S_-\right)e^{i\left(\omega_I+\omega_S\right)t},\end{aligned} \tag{A.8}$$

with

$$F_0^{\mathrm{IS}}(t) = -\left(\frac{\mu_0}{4\pi}\right)\left(\frac{\gamma_I\gamma_S\hbar}{r_{\mathrm{IS}}^3}\right)\frac{3\cos^2\theta(t) - 1}{2} = F_0^{\mathrm{IS}*}(t), \tag{A.9a}$$

$$F_{\pm 1}^{\mathrm{IS}}(t) = \pm\left(\frac{\mu_0}{4\pi}\right)\left(\frac{\gamma_I\gamma_S\hbar}{r_{\mathrm{IS}}^3}\right)\sqrt{\frac{3}{2}}\sin\theta(t)\cos\theta(t)e^{\pm i\varphi(t)} = -F_{\mp 1}^{\mathrm{IS}*}(t), \tag{A.9b}$$

$$F_{\pm 2}^{\mathrm{IS}}(t) = -\left(\frac{\mu_0}{4\pi}\right)\left(\frac{\gamma_I\gamma_S\hbar}{r_{\mathrm{IS}}^3}\right)\sqrt{\frac{3}{8}}\sin^2\theta(t)e^{\pm 2i\varphi(t)} = F_{\mp 2}^{\mathrm{IS}*}(t). \tag{A.9c}$$

Here, μ_0 is the permittivity of vacuum, $\hbar$ is the Planck's constant divided by 2π, γ's are the gyromagnetic ratios, and r_{IS} is the distance between the dipoles. The angles $\theta(t)$ and $\varphi(t)$ are fluctuating polar angles between the IS vector and the direction of the external magnetic field.

In terms of equations (A.6), (A.7a), (A.7b), and (A.7c), equation (A.5) now becomes

$$\tilde{\sigma}(t) - \tilde{\sigma}(0) = \sum_{m,n=-2}^{m,n=2}\left[T_m\left[T_n^\dagger, \left(\tilde{\sigma}(0) - \tilde{\sigma}_{\mathrm{EQ}}\right)\right]\right] \times \int_0^t\int_0^{t'} F_m^{\mathrm{IS}}(t')F_n^{\mathrm{IS}*}(t'')e^{i\left(\omega_m t_I - \omega_n t''\right)t}\mathrm{d}t''\mathrm{d}t'. \tag{A.10}$$

Here, one recognizes $\int_0^t F_m^{\mathrm{IS}}(t')F_n^{\mathrm{IS}*}(t'')\,\mathrm{d}t'$ as a time correlation function (AC(τ)) of $F_m^{\mathrm{IS}}(t')$ (make the substitution $t'' = t' + \tau$). One makes the common assumption that time correlation functions decay exponentially:

$$\mathrm{AC}(\tau) = \mathrm{AC}(0)e^{-(\tau)/\tau_c}, \tag{A.11}$$

with a single rotational correlation time τ_c in case of isotropic motion

The second integral in equation (A.10) is the Fourier transform of that time correlation function, which takes the shape of a Lorentzian:

$$J(\omega) = \frac{2}{5}\frac{\tau_c}{1 + \omega^2\tau_c^2}. \tag{A.12}$$

From the 25 terms in equation (A.10), only the ones with equal frequencies $e^{i(\omega_m t_I - \omega_m t'')t}$ contribute. This is called the Redfield kite, named after the author of one of the earliest papers on NMR relaxation [14]. It suffices to just take the five diagonal terms into account and drop the second summation.

One arrives at the "master equation" of relaxation [11]

$$\frac{d\langle\tilde{\sigma}\rangle}{\mathrm{d}t} = -\frac{1}{2}\sum_{m=-2}^{m=+2}\left[T_{\mathrm{mIS}},\left[T_{\mathrm{mIS}}^{\dagger},\left(\tilde{\sigma}(0)-\tilde{\sigma}_{\mathrm{EQ}}\right)\right]\right]j_m, \tag{A.13}$$

with

$$\begin{aligned} j_m &= \frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{\gamma_I\gamma_S\hbar}{r_{\mathrm{IS}}^3}\right)^2 J(\omega_m) \\ &= \frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2\left(\frac{\gamma_I\gamma_S\hbar}{r_{\mathrm{IS}}^3}\right)^2\frac{2}{5}\frac{\tau_c}{1+\omega_m^2\tau_c^2}, \end{aligned} \tag{A.14}$$

assuming isotropic motion.

It is interesting to consider the time development of an observable Q:

$$\frac{d\langle Q\rangle}{\mathrm{d}t} = \mathrm{Trace}\left\{Q\frac{d\langle\tilde{\sigma}\rangle}{\mathrm{d}t}\right\}. \tag{A.15}$$

The master equation for the time development of an observable Q is derived from (A.12) using commutation algebra and no new assumptions:

$$\frac{d\langle Q\rangle}{\mathrm{d}t} = -\frac{1}{2}\sum_{m=-2}^{m=+2}\left\{\left\langle\left[\left[Q,T_{\mathrm{mIS}}\right],T_{\mathrm{mIS}}^{\dagger}\right]\right\rangle - \left\langle\left[\left[Q,T_{\mathrm{mIS}}\right],T_{\mathrm{mIS}}^{\dagger}\right]\right\rangle_{\mathrm{eq}}\right\}j_{mm}. \tag{A.16}$$

Now, we are finally ready to derive the R_2 relaxation rate for $<I_+>$. We start by setting the equilibrium term to zero:

$$\frac{d\langle I_+\rangle}{\mathrm{d}t} = -\frac{1}{2}\sum_{m=-2}^{m=+2}\left\langle\left[\left[I_+,T_{\mathrm{mIS}}\right],T_{\mathrm{mIS}}^{\dagger}\right]\right\rangle j_{mm}. \tag{A.17}$$

Expanding equation (A.15), one obtains

$$-\frac{d\langle I_+\rangle}{\mathrm{d}t} = \frac{1}{2}\left\{A+B+B'+C+C'+D+D'+E+E'\right\}, \tag{A18}$$

with the terms

$$\begin{aligned}
A &= 4\left\langle\left[\left[I_+,I_zS_z\right],I_zS_z\right]\right\rangle j_0(0) = \langle I_+\rangle j_0(0), \\
B &= +\frac{1}{4}\left\langle\left[\left[I_+,I_+S_-\right],I_-S_+\right]\right\rangle j_0(\omega_I-\omega_S) = 0, \\
B\prime &= +\frac{1}{4}\left\langle\left[\left[I_+,I_-S_+\right],I_+S_-\right]\right\rangle j_0(\omega_I-\omega_S) = \frac{1}{4}\langle I_+\rangle j_0(\omega_I-\omega_S), \\
C &= +\frac{3}{2}\left\langle\left[\left[I_+,I_+S_z\right],I_-S_z\right]\right\rangle j_1(\omega_I) = 0, \\
C\prime &= +\frac{3}{2}\left\langle\left[\left[I_+,I_-S_z\right],I_+S_z\right]\right\rangle j_1(\omega_I) = \frac{3}{4}\langle I_+\rangle j_1(\omega_I), \\
D &= +\frac{3}{2}\left\langle\left[\left[I_+,I_zS_+\right],I_zS_-\right]\right\rangle j_1(\omega_S) = \frac{3}{4}\langle I_+\rangle j_1(\omega_S), \\
D\prime &= +\frac{3}{2}\left\langle\left[\left[I_+,I_zS_-\right],I_zS_+\right]\right\rangle j_1(\omega_S) = \frac{3}{4}\langle I_+\rangle j_1(\omega_S), \\
E &= +\frac{3}{2}\left\langle\left[\left[I_+,I_+S_+\right],I_-S_-\right]\right\rangle j_2(\omega_I+\omega_S) = 0, \\
E\prime &= +\frac{3}{2}\left\langle\left[\left[I_+,I_-S_-\right],I_+S_+\right]\right\rangle j_2(\omega_I+\omega_S) = \frac{3}{2}\langle I_+\rangle j_2(\omega_I+\omega_S).
\end{aligned} \tag{A.19}$$

For the expansion of the commutators, one uses

$$[I_+, I_z] = -I_+; [I_-, I_z] = I_-; [I_+, I_-] = 2I_z, \quad \text{(A.20a)}$$

and the product rules

$$I_z I_z = \frac{1}{4}E; I_+ I_- = \frac{1}{2}E + I_z; I_- I_+ = \frac{1}{2}E - I_z, \quad \text{(A.20b)}$$

$$I_z I_+ = -I_+ I_z = \frac{1}{2}I_+; I_z I_- = -I_- I_z = -\frac{1}{2}I_-, \quad \text{(A.20c)}$$

with E being the unit matrix.

One finds that all double commutators in equation (A.19) result in terms linear in I_+, yielding an uncoupled differential equation

$$-\frac{d\langle I_+\rangle}{dt} = R_2^I \langle I_+\rangle, \quad \text{(A.21)}$$

with

$$R_2^I = \frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_I \gamma_S \hbar}{r_{IS}^3}\right)^2 \{4J(0) + 3J(\omega_I) + 6(\omega_S) + J(\omega_I - \omega_S) + 6J(\omega_I + \omega_S)\}. \quad \text{(A.22)}$$

For two protons with different chemical shifts, one makes the substitutions

$$J(\omega_I - \omega_S) \cong J(0); J(\omega_S) \cong J(\omega_I) \cong J(\omega_H), \quad \text{(A.23)}$$

which yields

$$R_2^I = \frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_H \gamma_H \hbar}{r_{IS}^3}\right)^2 \{\{5J(0) + 9J(\omega_H) + 6J(2\omega_H)\}. \quad \text{(A.24)}$$

This is called the "unlike" proton-proton dipolar relaxation equation.

In the case of two protons **I** and **I′** with identical chemical shifts, or when the chemical shifts are forced to be equal during a spin lock, the cross terms between different coherence orders in the dipolar Hamiltonian cannot be neglected.

In particular, two cross terms between the single quantum terms C and D of equation (A.19) enter in the relaxation master equation, giving rise to cross-relaxation to the other spin:

$$[[I_+, I_- I_z'], I_z I_+'] = 2[I_z I_z', I_z I_+'] = \frac{1}{2}[I_z', I_+'] = \frac{1}{2}I_+'. \quad \text{(A.25)}$$

Similarly, the frequencies of $J(0)$ and zero-quantum terms in this case are precisely equal, so that two cross terms between terms A and B of equation (A.17) will occur as well:

$$-[[I_+, I_z I_z'], I_- I_+'] = [I_+ I_z', I_{z-} I_+'] = \frac{1}{2}I_+'. \quad \text{(A.26)}$$

Capturing both transfers into one equation yields

$$-\frac{d\langle I_+\rangle}{dt} = \frac{1}{2}\left\{j_0(0) + \frac{3}{2}j_1(\omega_H)\right\}\langle I_+'\rangle \equiv \sigma_{\mathrm{ROE}}\langle I_+'\rangle, \quad \text{(A.27)}$$

$$\sigma_{\mathrm{ROE}} = \frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_I \gamma_S \hbar}{r_{IS}^3}\right)^2 \{4J(0) + 6(\omega_I)\}. \quad \text{(A.28)}$$

The cross-relaxation rate in equation (A.28) is called the rotating frame Overhauser effect and was first discovered and quantified by [15] using the name "camel-spin."

Since the I and I' chemical shifts are identical, one may add equation (A.28) to equation (A.24) to obtain

$$R_2^I = \frac{1}{8}\left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_H \gamma_H \hbar}{r_{IS}^3}\right)^2 \{\{9J(0) + 15J(\omega_H) + 6J(2\omega_H)\}. \quad \text{(A29)}$$

This equation holds two protons with identical chemical shifts, or spinlocked. It is called the "like" spin relaxation equation. This has led to confusion. A better nomenclature would be to call this the identical spin relaxation equation.

Data Availability

No experimental data were used in this work.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors thank Professor Arno Kentgens at the University of Nijmegen for discussions and encouragement.

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