# Molecular properties determined from the relaxation of long-lived spin states

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The populations of long-lived spin states, in particular, populations of singlet states that are comprised of antisymmetric combinations of product states, $|\alpha_I \beta_S\rangle - |\beta_I \alpha_S\rangle$, are characterized by very long lifetimes because the dipole-dipole interaction between the two "active" spins I and S that are involved in such states is inoperative as a relaxation mechanism. The relaxation rate constants of long-lived (singlet) states are therefore determined by the chemical shift anisotropy (CSA) of the active spins and by dipole-dipole interactions with passive spins. For a pair of coupled spins, the singlet-state relaxation rate constants strongly depend on the magnitudes and orientations of the CSA tensors. The relaxation properties of long-lived states therefore reveal new information about molecular symmetry and structure and about spectral density functions that characterize the dynamic behavior. © *2007 American Institute of Physics.* [DOI: 10.1063/1.2778429]

## INTRODUCTION

Recently, it has been shown that the population of a long-lived state (LLS) can have a very small relaxation rate constant $R_{LLS}$ (and hence a very long lifetime $T_{LLS} = 1/R_{LLS}$) when relaxation due to dipolar interactions is quenched.[1,2] This occurs, for example, when two spins I and S are temporarily made to behave as if they were magnetically equivalent, which can be achieved either by taking the sample out of the static field or by suppressing the effects of the chemical shifts with suitable decoupling methods.[1,3,4] If the molecule contains only two magnetic nuclei with nonvanishing spins I and S, the long-lived state (LLS) corresponds to a so-called singlet state (SS), i.e., a linear combination of product states that is antisymmetric with respect to the permutation of the two spins: $|\alpha_I \beta_S\rangle - |\beta_I \alpha_S\rangle$. In this case, we may speak of a singlet-state relaxation rate constant $R_S = R_{LLS}$ or, alternatively, of a singlet-state relaxation time constant $T_S = 1/R_S = T_{LLS} = 1/R_{LLS}$. In a system with three or more spins,[5] say I, S, and R, it is, however, preferable to speak of a long-lived state $Q_{LLS}$, which is defined as the eigenstate of the Liouvillian with the smallest eigenvalue $\lambda$ (other than the eigenstate $Q_0$ that corresponds to the sum of all populations, which must remain conserved at all times and must therefore be associated with an eigenvalue $\lambda_0 = 0$).[6] In this paper, we use the general notations $R_{LLS}$ and $T_{LLS}$. Two ways have been proposed to preserve the populations of long-lived (singlet) states: (i) the zero-field method, which requires taking the sample out of the magnetic field[1] and (ii) the high-field method, which requires the application of a suitable radio-frequency irradiation to mask the difference in chemical shifts between the two spins, so that they become in effect magnetically equivalent.[2,7] When the zero-field method is used, the remaining dominant relaxation mechanisms of the long-lived spin states are the intra- or intermolecular dipole-dipole (DD) interactions between either of the two active spins I and S and passive spins R, which may be either nuclear or electronic (e.g., Curie spins with rapid electronic relaxation[8]). If the other magnetic nuclei in the molecule (notably the remaining protons) are substituted by isotopes with low gyromagnetic ratios (e.g., by deuterons) and if the samples are carefully degassed, the lifetimes of the long-lived spin states can be greatly extended. In the high-field method, an additional source of relaxation appears, due to fluctuations of the local fields at the sites of the two active spins that arise through the combination of rotational diffusion and chemical shift anisotropy (CSA). The lifetimes of long-lived states will therefore increase with decreasing static field strength. Long lifetimes $T_{LLS}$ in effect provide a "memory" to the spin system that is much longer than its spin-lattice relaxation time constant $T_1$. This property allows one to study very slow dynamic processes, such as translational diffusion[9,10] and conformational rearrangements,[3] that occur on time scales up to tens of seconds.

Although CSA relaxation is obviously detrimental for the lifetimes $T_{LLS}$ of the long-lived (singlet) states (and hence limits the time scales of the slow processes that can be studied), this effect can be put to good use. Indeed, the measurement of the relaxation rate constants $R_{LLS}$ can be used for the characterization of CSA tensors. Except at very high static fields, the CSA relaxation of protons is usually overshadowed by large dipolar interactions, so that the param-

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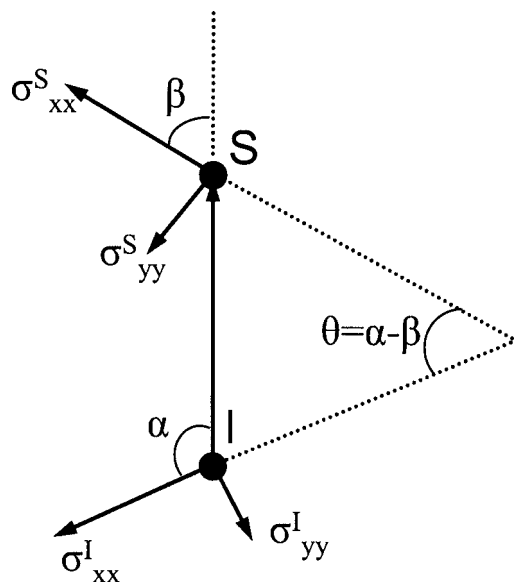
FIG. 1. Principal values and relative orientations of CSA tensors in a system of two spins I and S such as two aromatic protons. The $\sigma_{zz}$ components (not shown) of the two CSA tensors are assumed to be both perpendicular to the plane of the figure and hence to the internuclear vector $r_{IS}$. The $\sigma^I_{xx}$ and $\sigma^S_{xx}$ components subtend an angle $\theta$.

eters of proton CSA tensors are often difficult to determine with reasonable accuracy, and it is often necessary to exploit the field dependence of CSA autorelaxation rate constants and/or CSA/DD cross-correlation effects. It will be shown in this paper that the CSA relaxation of long-lived (singlet) states critically depends on molecular symmetry properties. In two-spin systems, both the principal components of the two CSA tensors and their relative orientation affect the relaxation rate constants of long-lived states. The CSA contribution to relaxation vanishes if the two tensors have identical principal components and parallel principal axes. This relaxation mechanism is similar to that due to random field fluctuations in the absence of a static magnetic field.[11] The relaxation rate constant $R_{LLS}$ of a long-lived state involving two active spins I and S is affected by DD interactions I-$R_i$ and S-$R_i$ with passive spins $R_i$ (with $i=1,2,\ldots,N$.) The resulting DD contributions $R_{LLS}^{DD}$ to the relaxation rate constants of long-lived states depend on the molecular symmetry. If $N=1$, one can extract valuable information on the (average) position of the third spin R relative to the first two. If spin R is mobile, averaging effects may allow one to gain novel insight into internal dynamics. This paper focuses on the interpretation of the experimentally determined relaxation rate constants $R_{LLS}$ of long-lived states in terms of CSA ten-

sors and DD interactions, which in turn may provide insight into molecular symmetry, structure, and dynamics.

## THEORY AND EXPERIMENTS

First, we shall address the effects of CSA interactions on the relaxation of a long-lived (singlet) state in a system with only two spins I and S. The principal axes and orientations of the CSA tensors characteristic for a planar molecule are defined in Fig. 1. All the calculations assume isotropic rotational diffusion. The tensor operators for the dipolar I-S interaction and for the CSA interactions of spins I and S, along with the relevant frequencies, are given in Table I.

The autorelaxation time constants of the normalized operators Q that span the basis of the density operator may comprise contributions from DD and CSA mechanisms:

$$\Gamma_{Q,Q} = \Gamma_{Q,Q}^{DD} + \Gamma_{Q,Q}^{CSA}. \tag{1}$$

The contribution from the dipolar interactions is:[12]

$$R_{Q,Q}^{DD} = \Gamma_{Q,Q}^{DD} = \frac{1}{2}\sum_q \langle Q|[A_2^{-q},[A_2^q,Q]]\rangle J(\omega_q). \tag{2}$$

In the extreme narrowing regime ($\tau_c \ll 1/\omega_0$), the spectral density function is almost white,[13] i.e., $J(0) \approx J(\omega_0) \approx J(2\omega_0)$, while for macromolecules ($\tau_c \gg 1/\omega_0$), one has $J(0) \gg J(\omega_0) \approx J(2\omega_0) \approx 0$. Numerical simulations were run for two typical correlation times. To mimic the behavior of small molecules, we chose $\tau_c=0.05$ ns, which yields the ratio $J(0):J(\omega_0):J(2\omega_0)=1.25:1.18:1$ at $\omega_0/(2\pi)=800$ MHz. For macromolecules such as proteins, we considered $\tau_c=5$ ns, which gives the ratio $J(0):J(\omega_0):J(2\omega_0)=2500:4:1$ at 800 MHz. If decoupling is efficient,[4] a two-spin system has four eigenstates: the singlet state $|S_0\rangle=N\{|\alpha_I\beta_S\rangle-|\beta_I\alpha_S\rangle\}$ (with a norm $N=2^{-1/2}$) and the three triplet states ($|T_{+1}\rangle=|\alpha_I\alpha_S\rangle$, $|T_0\rangle=N\{|\alpha_I\beta_S\rangle+|\beta_I\alpha_S\rangle\}$, and $|T_{-1}\rangle=|\beta_I\beta_S\rangle$).[2] The expectation values $p(Q)$ of the populations of these four eigenstates can be expressed in terms of Cartesian operators in Liouville space,

$$p(S_0) = |S_0\rangle\langle S_0| = \frac{E}{4} - I_xS_x - I_yS_y - I_zS_z = \frac{E}{4} - \boldsymbol{I}\cdot\boldsymbol{S}, \tag{3a}$$

$$p(T_{+1}) = |T_{+1}\rangle\langle T_{+1}| = \frac{E}{4} + \frac{I_z}{2} + \frac{S_z}{2} + I_zS_z, \tag{3b}$$

$$p(T_0) = |T_0\rangle\langle T_0| = \frac{E}{4} + I_xS_x + I_yS_y - I_zS_z, \tag{3c}$$

TABLE I. Tensor operators $A_2^{\pm q}$ that are relevant for dipolar IS interactions, with $a=1/2$, $b=\sqrt{3/2}$, and $D=\hbar(\mu_0/4\pi)(\gamma_I\gamma_S/r_{IS}^3)$. The other symbols have their usual meaning. For two protons separated by 2 Å, $D$ is 94.3 kHz. The tensor operators $T_j^{(K)\pm q}$ for the CSA interactions of spins K=I or S are defined using $\Delta\sigma_{jj}^K=\sigma_{jj}^K-\sigma_{zz}^K$, $j=x,y$, and K=I,S.

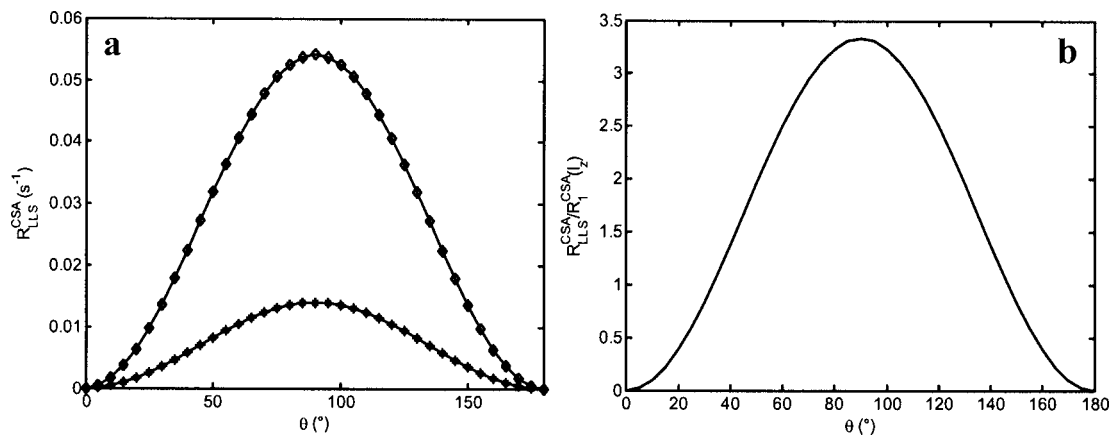| $q$ | $A_2^q$ | $A_2^{-q}$ | $T_j^{(K)q}$ | $T_j^{(K)-q}$ | $\omega_q$ |
|---|---|---|---|---|---|
| 0 | $aD(4I_zS_z-I^+S^--I^-S^+)$ | $aD(4I_zS_z-I^+S^--I^-S^+)$ | $4a(\Delta\sigma_{jj}^K\omega_0/3)K_z$ | $4a(\Delta\sigma_{jj}^K\omega_0/3)K_z$ | 0 |
| 1 | $bD(I_zS^++I^+S_z)$ | $bD(I_zS^-+I^-S_z)$ | $b(\Delta\sigma_{jj}^K\omega_0/3)K^+$ | $b(\Delta\sigma_{jj}^K\omega_0/3)K^-$ | $\omega$ |
| 2 | $bDI^+S^+$ | $bDI^-S^-$ | $\cdots$ | $\cdots$ | $2\omega$ |

FIG. 2. (a) Theoretical CSA contributions $R_{\mathrm{LLS}}^{\mathrm{CSA}}$ to the relaxation rate constants of long-lived (singlet) states in the system of Fig. 1 at 400 MHz (lower curve) and 800 MHz (upper curve), considering two CSA tensors with cylindrical symmetry ($\sigma_{xx} \neq \sigma_{yy} = \sigma_{zz}$) and with the same anisotropy $\Delta\sigma_{xx}^I = \Delta\sigma_{xx}^S = 10$ ppm for both spins I and S, as a function of the angle $\theta = \alpha - \beta$ between the orientations of the $\sigma_{xx}^I$ and $\sigma_{xx}^S$ components. The rate constants were calculated using Eq. (5), with a correlation time $\tau_c = 0.05$ ns. (b) Theoretical ratio of $R_{\mathrm{LLS}}^{\mathrm{CSA}}$ to the CSA contribution to the longitudinal relaxation rate constants $R_1^{\mathrm{CSA}}(I_z) = R_1^{\mathrm{CSA}}(S_z)$ of the individual spins as a function of the angle $\theta$. This ratio does not depend on the static field $B_0$.

$$p(T_{-1}) = |T_{-1}\rangle\langle T_{-1}| = \frac{E}{4} - \frac{I_z}{2} - \frac{S_z}{2} + I_z S_z. \tag{3d}$$

The long-lived state eigenoperator $Q_{\mathrm{LLS}}$, which decays with a relaxation rate constant $R_{\mathrm{LLS}}$, corresponds to the difference between the population of the singlet state and the mean of the populations of the three triplet states.[6,10] With Eq. (3), one obtains the following expression for the rotation-invariant normalized operator of the long-lived state:

$$Q_{\mathrm{LLS}} = -\frac{2}{\sqrt{3}}(I_x S_x + I_y S_y + I_z S_z) = -\frac{2}{\sqrt{3}} \boldsymbol{I} \cdot \boldsymbol{S}. \tag{4}$$

Since $Q_{\mathrm{LLS}}$ commutes with all tensor operators that represent the I-S DD interaction, it is not affected by dipolar relaxation between the two active spins, i.e., $\Gamma_{Q_{\mathrm{LLS}}}^{\mathrm{DD}} = 0$ if there are no further spins. On the other hand, the contribution of the CSA interactions of spins I and S to the relaxation of $Q_{\mathrm{LLS}}$ is

$$
\begin{aligned}
R_{\mathrm{LLS}}^{\mathrm{CSA}} &= \Gamma_{\mathrm{LLS}}^{\mathrm{CSA}} \\
&= \frac{1}{2}\sum_q \sum_{K,L=I,S} \sum_{i,j=x,y} \langle Q_{\mathrm{LLS}}|[T_i^{(K)-q},[T_j^{(L)q},Q_{\mathrm{LLS}}]]\rangle \\
&\quad \times J(\omega_q) P_2(\cos\theta(\sigma_{ii}^K,\sigma_{jj}^L)),
\end{aligned} \tag{5}
$$

where $P_2$ is the Legendre polynomial and $\theta(\sigma_{ii}^I,\sigma_{jj}^S)$ is the angle between the principal axes $\sigma_{ii}^I$ and $\sigma_{jj}^S$ of the CSA tensors of the two spins, and the tensor operators $T_j^{(K)}$ with $K=I$ or $S$ are given in Table I. If both CSA tensors have cylindrical symmetry (e.g., if $\sigma_{xx}^I \neq \sigma_{yy}^I = \sigma_{zz}^I$ and $\sigma_{xx}^S \neq \sigma_{yy}^S = \sigma_{zz}^S$), using the notation $\Delta\sigma^{I,S} = \Delta\sigma_{xx}^{I,S} - \Delta\sigma_{zz}^{I,S}$, the CSA contributions to the autorelaxation rate constant of long-lived (singlet) state involving spins I and S are given by

$$
\begin{aligned}
R_{\mathrm{LLS}}^{\mathrm{CSA}} &= \frac{2}{27}[(\Delta\sigma^I)^2 + (\Delta\sigma^S)^2]\omega_0^2[2J(0) + 3J(\omega_0)] \\
&\quad - \frac{4}{27}\Delta\sigma^I \Delta\sigma^S \omega_0^2[2J(0) + 3J(\omega_0)]\frac{3\cos^2\theta - 1}{2}, 
\end{aligned} \tag{6}
$$

where $\theta = \theta(\sigma_{xx}^I, \sigma_{xx}^S)$ is the angle between the principal axes $\sigma_{xx}^I$ and $\sigma_{xx}^S$.

If the fluctuations of the DD and CSA interactions are correlated, there can be a partial conversion (cross relaxation) of IS singlet-state populations into a difference of Zeeman polarizations ($I_z - S_z$). This type of cross relaxation is effectively suppressed by the coherent terms in the Hamiltonian.[13]

## Similar CSA tensors with nonparallel principal axes

In some cases, it can be inferred from molecular symmetry that the principal values of the CSA tensors of spins I and S must be similar. In this case, it may be possible to determine the relative orientations of the two tensors from the relaxation rate constants of long-lived states. If the two CSA tensors are similar and axially symmetric, we can further simplify the notation by using $\Delta\sigma = \Delta\sigma^I = \Delta\sigma^S$, and Eq. (6) becomes

$$R_{\mathrm{LLS}}^{\mathrm{CSA}} = \frac{2}{9}(\Delta\sigma\omega_0)^2(1 - \cos^2\theta)[2J(0) + 3J(\omega_0)], \tag{7}$$

where $\theta = \theta(\sigma^I, \sigma^S)$ is again the angle between the principal axes $\sigma_{xx}^I$ and $\sigma_{xx}^S$. The autorelaxation rate constant of Eq. (7) depends only on the chemical shift anisotropy $\Delta\sigma$ and on the relative orientation $\theta$ of the two tensors [Fig. 2(a)]. In the extreme narrowing limit, we have

$$R_{\mathrm{LLS}}^{\mathrm{CSA}} = \frac{10}{9}(\Delta\sigma\omega_0)^2 J(0)(1 - \cos^2\theta). \tag{8}$$

This expression can be compared with the CSA contributions to the longitudinal relaxation rate constants $R_I = 1/T_I$ of the individual spins,

$$R_1^{\mathrm{CSA}}(I_z) = \Gamma_{I_z,I_z}^{\mathrm{CSA}} = R_1^{\mathrm{CSA}}(S_z) = \Gamma_{S_z,S_z}^{\mathrm{CSA}} = \frac{1}{3}(\Delta\sigma\omega_0)^2 J(0). \tag{9}$$

Therefore, the ratio between the CSA contributions $R_{\mathrm{LLS}}^{\mathrm{CSA}}$ to the autorelaxation rate constant of a long-lived state and the CSA contributions $R_1^{\mathrm{CSA}}$ to the longitudinal relaxation rate constants of the individual spins yields
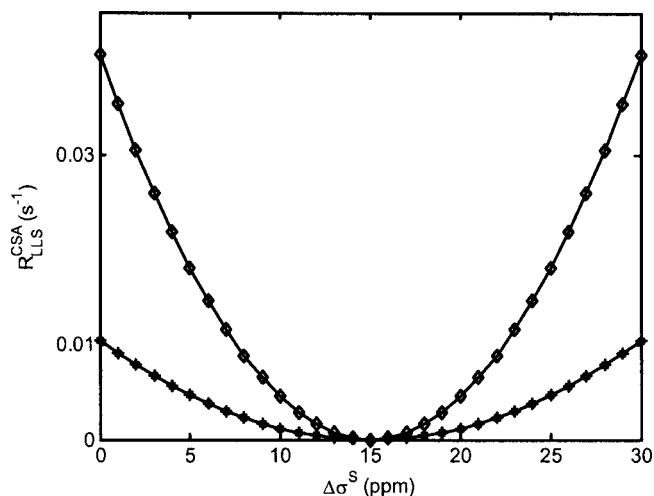
FIG. 3. Theoretical CSA contributions $R_{LLS}^{CSA}$ to the relaxation rate constants of long-lived (singlet) states in the spin system of Fig. 1 at 400 MHz (lower curve) and 800 MHz (upper curve), considering two CSA tensors with cylindrical symmetry ($\sigma_{xx} \neq \sigma_{yy} = \sigma_{zz}$) and parallel principal components ($\theta = 0$) as a function of the anisotropy $\Delta\sigma^S = \Delta\sigma_{xx}^S$, which is varied in the range of $0 < \Delta\sigma^S < 2\Delta\sigma^I$, with $\Delta\sigma^I = \Delta\sigma_{xx}^I = 15$ ppm. The rate constants were calculated using Eq. (5) for a correlation time $\tau_c$ of 0.05 ns.

$$R_{LLS}^{CSA}/R_1^{CSA} = 10(1 - \cos^2\theta)/3, \tag{10}$$

thus providing direct information about the relative orientation of the two CSA tensors, i.e., about the angle subtended between their principal axes [Fig. 2(b)]. Note that one must exclude the (field-independent) dipolar contributions $R_{LLS}^{DD}$, $R_1^{DD}(I_z)$, and $R_1^{DD}(S_z)$ to the relaxation rate constants. Once the angle $\theta$ and the spectral density $J(0)$ are known, the anisotropy $\Delta\sigma$ can in principle be determined from Eq. (8).

## Different CSA tensors with parallel principal components

If the principal components of the CSA tensors are parallel and the CSA tensors have cylindrical symmetry (e.g., if $\sigma_{xx}^I \neq \sigma_{yy}^I = \sigma_{zz}^I$ and $\sigma_{xx}^S \neq \sigma_{yy}^S = \sigma_{zz}^S$), the rate constant given in Eq. (6) only depends on the difference $\Delta\sigma^I - \Delta\sigma^S = [(\sigma_{xx}^I - \sigma_{zz}^I) - (\sigma_{xx}^S - \sigma_{zz}^S)]$,

$$R_{LLS}^{CSA} = \frac{2}{27}(\Delta\sigma^I - \Delta\sigma^S)^2 \omega_0^2 [2J(0) + 3J(\omega_0)]. \tag{11}$$

Thus, for two protons with $\Delta\sigma^I - \Delta\sigma^S = 5$ ppm in the extreme narrowing limit ($\tau_c = 0.05$ ns), the relaxation rate constants calculated from Eq. (11) are $R_{LLS}^{CSA} = 0.0012$ s$^{-1}$ at 400 MHz and 0.0045 s$^{-1}$ at 800 MHz. These values increase to $R_{LLS}^{CSA} = 0.0472$ and 0.1876 s$^{-1}$ for $\tau_c = 5$ ns. The rate constants in Eq. (11) vanish regardless of the static field if the two CSA tensors, besides having parallel orientations, are also equal in magnitude. The calculations using Eq. (11) were confirmed numerically using the GAMMA program,[14] as described in the Appendix below.

In a two-spin system where the CSA tensors are known to have cylindrical symmetry ($\sigma_{xx} \neq \sigma_{yy} = \sigma_{zz}$) and a known relative orientation $\theta$, the variation of the relaxation rate constant $R_{LLS}^{CSA}$ of the long-lived (singlet) state as a function of the $B_0$ field can provide a measure of the difference between the anisotropies of the two spins, $\Delta\sigma^I - \Delta\sigma^S$ (Fig. 3). The

profiles of $R_{LLS}^{CSA}$ in the slow motion limit are very similar to those for extreme narrowing in Fig. 3.

## Dipolar interactions with an external passive spin R

The symmetry properties of a long-lived state also determine the effects of dipolar interactions I-R and S-R between the two active spins I and S that are involved in the long-lived state and an external passive spin R. If the position of spin R deviates from the bisecting plane, the dipolar contributions to $R_{LLS}$ increase parabolically [trajectory A in Fig. 4], provided the distance between spin R and the center of the line connecting spins I and S is maintained constant. For this circular trajectory, the sum of the dipolar interactions I-R and S-R is *not* constant since it depends on the sum of the inverse distances to the sixth power ($1/r_{IR}^6 + 1/r_{SR}^6$). On the other hand, if we move spin R along a trajectory so as to keep the sum of these dipolar interactions constant [see trajectory B in Fig. 4], the rate constant $R_{LLS}^{DD}$ increases with the angle $\Omega$.

This behavior is similar to that observed for two CSA interactions as a function of the relative orientation $\theta$ in Eq. (6). It can be seen that in both cases, the symmetry has similar effects on the relaxation of the two-spin order $2I_zS_z$ and of the long-lived (singlet) states. The latter have, in the fast tumbling limit, lower relaxation rate constants, i.e., $R_{zz} > R_{LLS}$ (Fig. 4). The dependence of the relaxation properties of a long-lived state on the geometry of a three-spin system should allow one to extract information regarding the position of spin R with respect to spins I and S. The long intrinsic lifetimes of long-lived states may afford an experimental determination of structural information in cases when the third spin is relatively remote from the first two (i.e., when the interactions to be determined are weak).

## Experimental evidence

In order to obtain information about molecular symmetry and structure using Eqs. (8) and (9), the CSA contributions $R_1^{CSA}(I_z)$ and $R_1^{CSA}(S_z)$ to the longitudinal relaxation rate constants of the two active spins need to be known. In practice, this may be difficult for protons since dipolar interactions tend to dominate CSA effects, often by more than an order of magnitude, even in high magnetic fields.[13] However, the longitudinal relaxation rate constants $R_1^{CSA}(I_z)$ and $R_1^{CSA}(S_z)$ may be measured in a similar molecule where the spin under investigation (say, spin I) has a similar environment, while the dipolar interaction with the other proton spin (S) is removed by substitution of the latter, for instance, by a chlorine atom.

The longitudinal and long-lived (singlet) state relaxation rate constants have been measured in two related molecules (Fig. 5): pentachlorobenzene (which contains an isolated $I = 1/2$ proton that is predominantly relaxed through CSA) and trichlorophenol (with two coupled protons that experience both dipolar and CSA interactions). We shall assume for simplicity that the CSA tensors of the protons are the same in the two molecules. The measurements were carried out at static fields of 9.4 and 14.1 T (400 and 600 MHz for protons), yielding the rate constants given in Table II.
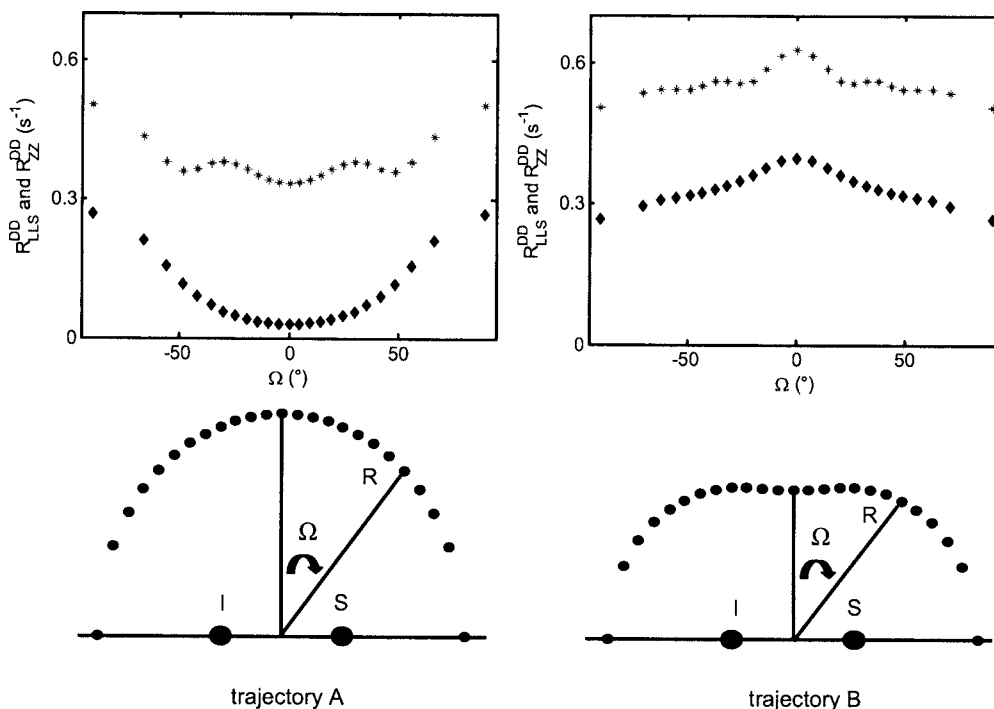
FIG. 4. Calculated DD contributions to the relaxation rate constants of long-lived states ($R_{\mathrm{LLS}}^{\mathrm{DD}}$, filled diamonds) and of longitudinal two-spin order $2I_zS_z$ ($R_{\mathrm{ZZ}}^{\mathrm{DD}}$, stars), due to dipolar interactions I-R and S-R with a third passive spin R, at 400 MHz, for a correlation time $\tau_c=0.05$ ns. In trajectory A (left panel), the distance of spin R from the midpoint between spins I and S was kept constant (3 Å), while the angle $\Omega$ was varied between $-90°$ and $90°$. In trajectory B (right panel), the third spin R was displaced on a trajectory so as to keep $(1/r_{IR}^6+1/r_{SR}^6)=0.016$ Å$^{-6}$. The rate constants were calculated as explained in the Appendix.

As has been shown in the previous sections for long-lived states created on pairs of spins I and S, while the dipolar interaction between the I and S nuclei is effectively silenced, these states can still be relaxed via dipolar interactions with external spins. In the case of the trichlorophenol molecule in water solution, some contributions to the relaxation of the intramolecular long-lived state are expected to arise from interactions with spins in the solvent and from intermolecular dipole-dipole interactions. The characteristic correlation times of these interactions are extremely short, and their spectral density functions are therefore expected not to show any dependence on the magnetic field.
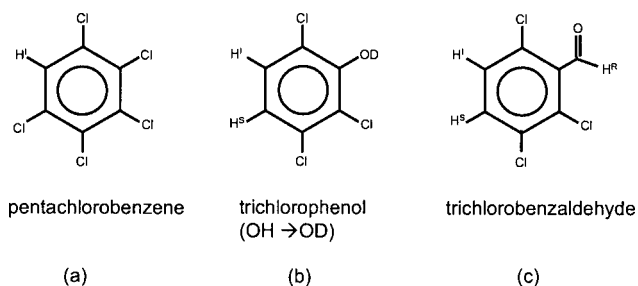


FIG. 5. (a) Pentachlorobenzene, containing an isolated proton I predominantly relaxed by chemical shift anisotropy, with a CSA tensor assumed to be axially symmetric. (b) Trichlorophenol, with a deuterated hydroxyl group, containing two protons I and S with CSA tensors that have similar anisotropies $(\sigma_{xx}^S-\sigma_{zz}^S)\approx(\sigma_{xx}^I-\sigma_{zz}^I)$. (c) Trichlorobenzaldehyde, with a similar pair of aromatic protons with active spins I and S with $(\sigma_{xx}^S-\sigma_{zz}^S)\approx(\sigma_{xx}^I-\sigma_{zz}^I)$ and an aldehyde proton $H^R$ with a passive spin R giving rise to dipolar I-R and S-R interactions, leading to contributions $R_{\mathrm{LLS}}^{\mathrm{DD}}$ to the relaxation rate constant of the long-lived (singlet) state involving the active spins I and S.

The only field-dependent contribution to relaxation is therefore related to the magnitudes of the CSA tensor operators of the two spins (Table I), which increase linearly with the field. The same holds for the contributions to the longitudinal relaxation rate constant of the hydrogen nucleus in pentachlorobenzene. Consequently, in order to eliminate possible contributions from interactions other than the CSA's, we have considered the ratio of differences between rate constants measured at different fields,

$$F = \frac{R_{\mathrm{LLS}}^{\mathrm{CSA}}(600\ \mathrm{MHz}) - R_{\mathrm{LLS}}^{\mathrm{CSA}}(400\ \mathrm{MHz})}{R^{\mathrm{CSA}}(I_z)(600\ \mathrm{MHz}) - R^{\mathrm{CSA}}(I_z)(400\ \mathrm{MHz})}. \quad (12)$$

The experimental ratio was found to be $F=3.7\pm1.1$. With reference to Fig. 2(b), this ratio implies that the relative orientation $\theta$ of the most shielded components of the two CSA tensors $\sigma^I$ and $\sigma^S$ must fulfill the inequality $\theta>60°$. The errors of 30% in the experimental ratio $F$ and therefore in $\theta$

TABLE II. Experimental relaxation rate constants of longitudinal magnetization $R_1(I_z)$ in pentachlorobenzene (containing a single isolated proton) and experimental relaxation rate constants of long-lived (singlet) states $R_{\mathrm{LLS}}$ in trichlorophenol (with two coupled protons) at 400 and 600 MHz. To measure $R_{\mathrm{LLS}}$ at two fields, the rf field amplitude used for continuous-wave irradiation in the mixing time was $\omega_1/(2\pi)=1.2$ kHz at 400 MHz and 1.7 kHz at 600 MHz, so that the ratio $\Delta\Omega/\omega_1$ between the isotropic chemical shift difference $\Delta\Omega=\Omega_I-\Omega_S$ and the amplitude $\omega_1$ was the same at both fields ($\Delta\Omega/\omega_1=0.0814$).

| [1]H frequency (MHz) | $R_1(I_z)$ (s$^{-1}$) | $R_{\mathrm{LLS}}$ (s$^{-1}$) |
|---|---|---|
| 400 | 0.017 10±0.000 05 | 0.0150±0.0007 |
| 600 | 0.018 18±0.000 01 | 0.0190±0.0003 |

are relatively large, due to the propagation of errors on the ratio of differences between experimental values.

Using the same procedure, the relaxation rate constants $R_{LLS}$ of the long-lived states in trichlorobenzaldehyde (three-spin system) and trichlorophenol (two-spin system) were also determined (Fig. 5) in order to investigate the effects of DD interactions between the active spins I and S and a third passive spin R on the relaxation rate constant of a long-lived state.

## CONCLUSIONS

It has been shown that CSA contributions to relaxation properties of long-lived (singlet) states depend on the symmetry of the molecules and on the relative orientations of the principal components of the two CSA tensors. Dipolar interactions between the active spins I and S and a third spin R critically depend on molecular symmetry. Understanding these relationships can be valuable for dynamic and structural studies by NMR.

## ACKNOWLEDGMENTS

## APPENDIX: SIMULATIONS

To calculate contributions of various relaxation mechanisms to the Liouvillian, we considered two protons separated by a distance $|\mathbf{r}_{IS}| = 2$ Å with two axially symmetric CSA tensors ($\sigma_{xx}^I \neq \sigma_{yy}^I = \sigma_{zz}^I$ and $\sigma_{xx}^S \neq \sigma_{yy}^S = \sigma_{zz}^S$) with different anisotropies $\sigma_{xx}^I - \sigma_{zz}^I = 15$ ppm and $\sigma_{xx}^S - \sigma_{zz}^S = 10$ ppm, with correlation times $\tau_c = 0.05$ and 5 ns. The theoretical relaxation rate constants of Eq. (11) were compared with

GAMMA simulations incorporating coherent evolution under the full spin Hamiltonian (expressed in units of $\hbar$ as usual), in addition to dipolar and CSA relaxation mechanisms,

$$H = \omega_I I_z + \omega_S S_z + 2\pi J_{IS}\mathbf{I} \cdot \mathbf{S} + \omega_1(I_x + S_x). \quad (A1)$$

The simulations were performed considering a difference between the chemical shifts of 0.19 ppm, a scalar coupling $J_{IS} = -12$ Hz, and a radio-frequency amplitude $\omega_1/(2\pi) = 5$ kHz, applied half-way between the chemical shifts. The decay of the populations of the long-lived (singlet) states was monitored for 1000 different relaxation intervals. The decays were then fitted to exponential functions using three fitting parameters (initial amplitude, decay rate constant, and dc offset). When the effect of a third spin R was calculated, this spin was considered at an offset of 4 Hz without any scalar couplings, i.e., $J_{IR} = J_{SR} = 0$.

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