

Residual dipolar coupling constants: An elementary derivation of key equations

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ABSTRACT

Residual dipolar couplings (RDC) have recently found a wide range of applications in high resolution NMR of biomolecules in the liquid state. A non-isotropic orientational distribution of a molecule of interest results in non-zero average dipolar coupling constants. Here, we present an intuitive introduction to the alignment tensor and an elementary derivation of key equations.

KEYWORDS

alignment tensor, coupling constant, dipolar coupling, dipolar Hamiltonian, order matrix, probability matrix, residual dipolar coupling, Saupe matrix.

INTRODUCTION

While dipolar couplings are the dominant interactions in solid state NMR of spin 1/2 nuclei, they are averaged to zero for isotropically reorienting molecules in the liquid state. This makes it possible to achieve high-resolution spectra with relative ease in liquid state NMR. On the other hand, a wealth of structural information is lost if dipolar couplings vanish. However, even in liquid state NMR, molecules can be partially aligned, e. g. by external fields (magnetic or electric) or by anisotropic solvents (1-8). For example in liquid crystalline solvents, the dissolved molecules are partially aligned through steric and anisotropic interactions with the solvent molecules, and dipolar couplings can be observed (9, 10). The recent success and wide use of such residual dipolar couplings is due to the development and characterization of several new alignment media such as bicelles (5), filamentous phage Pf1 (11), and polyacrylamide gels (12, 13), which make it possible to create a relatively small, tunable degree of alignment. This allows the spectroscopist to adjust the alignment in such a way, that the size of the average dipolar coupling is in the order of the J couplings. In this case, the resulting spectra are still simple, and dipolar coupling constants can be measured relatively easy by comparing line splittings in isotropic and in aligned samples. Techniques to measure residual dipolar couplings and a wide range of applications have been discussed in a number of articles and reviews (2-8).

Here, we revisit the fundamental question of how to calculate the expected residual dipolar coupling constant for a homonuclear (e.g. ^1H - ^1H) or heteronuclear (e.g. ^{15}N - ^1H) spin pair. This turns out to be a surprisingly simple calculation if we know the orientation and the three principal components of the so-called *alignment tensor*. This *alignment tensor* is a key concept, that is crucial to understand residual dipolar couplings. However, in our experience, many students and even seasoned practitioners in the field of NMR have conceptual

difficulties to fully understand the physical meaning of the alignment tensor which sometimes lead to serious misconceptions (*vide infra*). This may result in part from the common practice in literature, to derive the *alignment tensor* using mathematically elegant, but not very intuitive approaches based on spherical harmonics, their addition theorems, Legendre polynomials, Wigner rotation matrices and a confusing number of angles between various axes.

In contrast, we here use a streamlined geometric approach similar to the original derivation by Saupe (9, 10), which is based on the Cartesian representation of vectors. Except for the most basic rules of matrix and vector multiplication, only elementary mathematics is needed to derive the *alignment tensor*. As a didactical aid on the way to understanding the *alignment tensor*, we discuss the related *probability tensor*. Numerical examples and illustrating figures are used to convey the physical meaning of these tensors. In the Appendix, various expressions for the residual dipolar coupling constants commonly found in literature are derived from the presented key results.

STATIC DIPOLAR COUPLING HAMILTONIAN

We consider two spins I and S with an internuclear vector $\vec{R}$ (see Fig. 1). This vector can be expressed in the form

$$\vec{R} = R \vec{r}, \quad [1]$$

where R is the distance between the two nuclei and $\vec{r}$ is a unit vector pointing in the direction of $\vec{R}$. Similarly, the vector representing the external magnetic field $\vec{B}$ can be expressed in the form

$$\vec{B} = B \vec{b}, \quad [2]$$

where B is the magnitude of the static magnetic field, and $\vec{b}$ is a unit vector pointing in the direction of the magnetic field. In the lab frame (x^L, y^L, z^L) , where by convention the magnetic field points along the z^L axis, the (truncated) dipolar coupling Hamiltonian has the form (14)

$$H_D = 2\pi D \left\{ I_{z^L} S_{z^L} - \frac{1}{2} I_{x^L} S_{x^L} - \frac{1}{2} I_{y^L} S_{y^L} \right\}. \quad [3]$$

If the spins I and S are heteronuclear, the second and third term in the bracket can be neglected, resulting in the simpler weak dipolar coupling Hamiltonian

$$H_D = 2\pi D I_{z^L} S_{z^L}. \quad [4]$$

(which has the same form as the weak heteronuclear J coupling Hamiltonian). In both cases, the dipolar coupling constant (which in the weak coupling limit corresponds directly to the experimentally observed line splittings in units of Hz) is given by (14):

$$D = \frac{\kappa}{R^3} \left(\cos^2 \theta - \frac{1}{3} \right), \quad [5]$$

where θ is the angle between the internuclear vector and the magnetic field (see Fig. 1).

The term

$$\kappa = -\frac{3}{8\pi^2} \gamma_I \gamma_S \mu_0 \hbar \quad [6]$$

only depends on physical constants (14): the gyromagnetic ratios γ_I and γ_S of spin I and S respectively, the Planck constant $\hbar = h/2\pi$ and the permeability of vacuum μ_0 . For example, for ^1H - ^1H , ^{13}C - ^1H and ^{15}N - ^1H spin pairs, $\kappa = -360.3 \text{ kHz } \text{\AA}^3$, $-90.6 \text{ kHz } \text{\AA}^3$ and $36.5 \text{ kHz } \text{\AA}^3$, respectively. The maximum possible value of $\cos^2 \theta$ is 1 (for $\theta = 0$ or π), and hence, according to Eq. [5], the maximum possible dipolar coupling constant is

$$D_{\max} = \kappa / R^3 (1 - 1/3) = (2/3) \kappa / R^3, \quad [7]$$

which corresponds e. g. to 21.7 kHz for a ^{15}N - ^1H spin pair with distance $R = 1.04 \text{ \AA}$.

Remember that the scalar product between two unit vectors is identical to the cosine of the angle θ between the two vectors. Hence, the term $\cos \theta$ in Eq. [5] can always be expressed in the form

$$\cos \theta = \bar{\mathbf{b}}^T \bar{\mathbf{r}}. \quad [8]$$

Here, $\bar{\mathbf{b}}^T$ is a row vector (the transpose of the column vector $\bar{\mathbf{b}}$) which allows us to write the scalar product of the two vectors as a usual matrix product between the 1x3 matrix $\bar{\mathbf{b}}^T$ and the 3x1 matrix $\bar{\mathbf{r}}$ (*vide infra*).

TIME-DEPENDENT AND AVERAGE DIPOLAR COUPLING HAMILTONIAN

Now we consider the two spins I and S to be part of a molecule in solution. In the lab frame, the magnetic field vector $\vec{B}$ is constant (pointing along the z^L axis), but the inter-nuclear vector $\vec{R}$ is now time-dependent (see Fig. 2 A). For simplicity, we assume that the molecule is rigid (no internal dynamics and constant distance R), such that the time-dependence of $\vec{R}$ is solely due to the rotational tumbling motion of the molecule. Hence, the term $\cos\theta$ (and as a result also the dipolar coupling constant D and the dipolar coupling Hamiltonian) is time-dependent. For proteins, the rotational correlation time is in the order of nanoseconds and on the time-scale of the NMR experiment, only the time-averaged dipolar Hamiltonian $\overline{H_D}$ gives rise to splittings in the spectrum (relaxation effects caused by the fluctuations of the dipolar Hamiltonian will not be considered here). The time-averaged dipolar coupling constant

$$\overline{D} = \frac{\kappa}{R^3} \left(\overline{\cos^2 \theta} - \frac{1}{3} \right) \quad [9]$$

represents the so-called *residual dipolar coupling constant*, which depends on the average alignment of the molecule.

OUTLINE AND KEY RESULTS

The goal of this manuscript is to derive a general approach for the calculation of $\overline{D}$ for any pair of spins if the “alignment properties” of the molecule are known. Before we go into the formal derivation, we give a brief outline of the steps and state the final result. First, we move from the lab frame (x^L, y^L, z^L) (c.f. Fig. 2 A) to a frame of reference (x, y, z) that is

fixed to the molecule. In this frame of reference, the term $\overline{\cos^2 \theta}$ can be conveniently expressed with the help of a *probability tensor* $\mathbf{P}$, which is a second order approximation of the orientational probability distribution of the direction of the external magnetic field in the molecular fixed frame of reference (6, 15). This *probability tensor* $\mathbf{P}$ can be represented by an ellipsoid (c.f. Fig. 3 A) with a fixed orientation in the chosen molecular frame (x, y, z). The principal values $P_{\tilde{x}}$, $P_{\tilde{y}}$ and $P_{\tilde{z}}$ of the *probability tensor* (i.e. the lengths of the half axes of the *probability ellipsoid*) are the probabilities to find the magnetic field along the corresponding principal axes of the *probability ellipsoid*, and hence $P_{\tilde{x}} + P_{\tilde{y}} + P_{\tilde{z}} = 1$.

For example, for an isotropically reorienting molecule, $P_{\tilde{x}} = P_{\tilde{y}} = P_{\tilde{z}} = 1/3$, and the *probability ellipsoid* is reduced to a sphere (see Fig. 4 C). On the other hand, if a molecule is fully aligned, $P_{\tilde{x}} = P_{\tilde{y}} = 0$ and $P_{\tilde{z}} = 1$ (by convention, the principal elements are ordered with increasing magnitude), i.e. the probability tensor is reduced to a single line in the direction of the magnetic field.

In general, the principal axes of the *probability ellipsoid* define a special molecular fixed axis system ($\tilde{x}, \tilde{y}, \tilde{z}$), in which the calculation of residual dipolar coupling constants is especially simple (see Fig. 3 B): If we know the three Cartesian components $r_{\tilde{x}}$, $r_{\tilde{y}}$ and $r_{\tilde{z}}$ of any given internuclear unit vector $\bar{r}$ in this principal axis system, the term $\overline{\cos^2 \theta}$ in Eq. [8] is simply given by

$$\overline{\cos^2 \theta} = P_{\tilde{x}} r_{\tilde{x}}^2 + P_{\tilde{y}} r_{\tilde{y}}^2 + P_{\tilde{z}} r_{\tilde{z}}^2. \quad [10]$$

If this simple equation (derived below) is inserted into Eq. [9], the residual coupling constant can be predicted for any arbitrary spin pair in a molecule, as long as the orientation and principal values of the *probability tensor* are known.

With this key result, we can calculate everything, and we could stop here, except that residual dipolar coupling constants are commonly not expressed in terms of the introduced *probability tensor* $\mathbf{P}$ (corresponding in general to a real symmetric 3x3 matrix with trace 1) but in terms of its traceless part (its “resolvent”) $\mathbf{P} - 1/3 \mathbf{1}$, which is called the *alignment tensor* $\mathbf{A}$ (5):

$$\mathbf{A} = \mathbf{P} - \frac{1}{3} \mathbf{1}. \quad [11]$$

The three principal components $A_{\tilde{x}}$, $A_{\tilde{y}}$ and $A_{\tilde{z}}$ of the *alignment tensor* $\mathbf{A}$ are simply given by

$$A_{\tilde{x}} = P_{\tilde{x}} - \frac{1}{3}, \quad A_{\tilde{y}} = P_{\tilde{y}} - \frac{1}{3}, \quad A_{\tilde{z}} = P_{\tilde{z}} - \frac{1}{3}, \quad [12]$$

and the principal axes of $\mathbf{A}$ and $\mathbf{P}$ are identical.

Note that in contrast to the *probability tensor* $\mathbf{P}$ (see Figs. 3 and 4), the *alignment tensor* $\mathbf{A}$ cannot be represented as an ellipsoid, because one or two of the principal components $A_{\tilde{x}}$, $A_{\tilde{y}}$, and $A_{\tilde{z}}$ of the alignment tensor are negative if any of the three components is nonzero due to $A_{\tilde{x}} + A_{\tilde{y}} + A_{\tilde{z}} = 0$. Alternative graphical representations of the effects of the alignment tensor are shown in Figs. 5 and 6 (*vide infra*).

In terms of the principal components of the alignment tensor, the term $(\overline{\cos^2 \theta} - 1/3)$ in the equation for the residual dipolar coupling constant (Eq. [9]) can be expressed as

$$\left(\overline{\cos^2 \theta} - \frac{1}{3} \right) = A_{\bar{x}} r_{\bar{x}}^2 + A_{\bar{y}} r_{\bar{y}}^2 + A_{\bar{z}} r_{\bar{z}}^2. \quad [13]$$

If this equation is inserted into Eq. [9], it is again possible to predict the residual coupling constant for any arbitrary spin pair in a molecule, provided that the orientation and principal values of the *alignment tensor* are known.

Conversely, the *alignment tensor* **A** (or the *probability tensor* **P**) can be determined if a sufficient number of experimental dipolar coupling constants are measured for a given molecule (16). As will be shown below, the *alignment tensor* **A** (and the *probability tensor* **P**) is characterized by five independent parameters. Therefore, at least five dipolar coupling constants need to be measured in order to determine the five unknown parameters (16). In many cases, it is also possible to accurately predict the *alignment tensor* **A** (17) or the *probability tensor* **P** for a given molecule in a given liquid crystalline solvent, and hence to predict the expected dipolar coupling constants for a proposed molecular structure from first principles.

DERIVATION OF THE PROBABILITY AND ALIGNMENT TENSORS

In an arbitrarily chosen molecular frame with axes (x, y, z) (see Fig. 2 B), a given internuclear vector $\bar{\mathbf{R}}$ is constant (still assuming a rigid molecule without internal dynamics):

$$\bar{\mathbf{R}} = R \bar{\mathbf{r}} = R \begin{pmatrix} r_x \\ r_y \\ r_z \end{pmatrix}. \quad [14]$$

However, in this frame of reference, the direction of the magnetic field vector $\bar{\mathbf{B}}$ is time-dependent if the molecule tumbles in solution:

$$\bar{\mathbf{B}} = B \bar{\mathbf{b}} = B \begin{pmatrix} b_x(t) \\ b_y(t) \\ b_z(t) \end{pmatrix}. \quad [15]$$

The definition of $\cos\theta$ via the scalar product of the unit vectors $\bar{\mathbf{b}}$ and $\bar{\mathbf{r}}$ (c.f. Eq. [8]) is valid in any frame of reference. Hence, we can express $\cos\theta$ in the molecular frame as a function of the components of the unit vectors $\bar{\mathbf{b}}$ and $\bar{\mathbf{r}}$, which point in the (varying) direction of the magnetic field $\bar{\mathbf{B}}$ and of the (constant) internuclear vector $\bar{\mathbf{R}}$, respectively:

$$\begin{aligned} \cos\theta &= \bar{\mathbf{b}}^T \cdot \bar{\mathbf{r}} = \begin{pmatrix} b_x(t) & b_y(t) & b_z(t) \end{pmatrix} \begin{pmatrix} r_x \\ r_y \\ r_z \end{pmatrix} \\ &= b_x(t)r_x + b_y(t)r_y + b_z(t)r_z, \end{aligned} \quad [16]$$

and

$$\begin{aligned}
\cos^2 \theta &= (b_x(t)r_x + b_y(t)r_y + b_z(t)r_z)^2 \\
&= b_x^2(t)r_x^2 + b_x(t)b_y(t)r_xr_y + b_x(t)b_z(t)r_xr_z \\
&\quad + b_y(t)b_x(t)r_yr_x + b_y^2(t)r_y^2 + b_y(t)b_z(t)r_yr_z \\
&\quad + b_z(t)b_x(t)r_zr_x + b_z(t)b_y(t)r_zr_y + b_z^2(t)r_z^2.
\end{aligned} \tag{17}$$

Note that Eq. [17] can also be expressed in the form

$$\cos^2 \theta = \begin{pmatrix} r_x & r_y & r_z \end{pmatrix} \begin{pmatrix} b_x^2(t) & b_x(t)b_y(t) & b_x(t)b_z(t) \\ b_x(t)b_y(t) & b_y^2(t) & b_y(t)b_z(t) \\ b_x(t)b_z(t) & b_y(t)b_z(t) & b_z^2(t) \end{pmatrix} \begin{pmatrix} r_x \\ r_y \\ r_z \end{pmatrix}. \tag{18}$$

Hence, the time average of $\cos^2 \theta$ is given by

$$\overline{\cos^2 \theta} = \begin{pmatrix} r_x & r_y & r_z \end{pmatrix} \begin{pmatrix} \overline{b_x^2(t)} & \overline{b_x(t)b_y(t)} & \overline{b_x(t)b_z(t)} \\ \overline{b_x(t)b_y(t)} & \overline{b_y^2(t)} & \overline{b_y(t)b_z(t)} \\ \overline{b_x(t)b_z(t)} & \overline{b_y(t)b_z(t)} & \overline{b_z^2(t)} \end{pmatrix} \begin{pmatrix} r_x \\ r_y \\ r_z \end{pmatrix} = \vec{r}^T \mathbf{P} \vec{r}. \tag{19}$$

We call the matrix

$$\mathbf{P} = \begin{pmatrix} \overline{b_x^2(t)} & \overline{b_x(t)b_y(t)} & \overline{b_x(t)b_z(t)} \\ \overline{b_x(t)b_y(t)} & \overline{b_y^2(t)} & \overline{b_y(t)b_z(t)} \\ \overline{b_x(t)b_z(t)} & \overline{b_y(t)b_z(t)} & \overline{b_z^2(t)} \end{pmatrix} \tag{20}$$

the *probability matrix*. For a known *probability matrix* $\mathbf{P}$, the residual dipolar coupling constant (Eq. [9]) is given by

$$\bar{D} = \frac{\kappa}{R^3} \left(\bar{\mathbf{r}}^T \mathbf{P} \bar{\mathbf{r}} - \frac{1}{3} \right). \quad [21]$$

The matrix $\mathbf{P}$ is real, symmetric, and has a trace of 1 because

$$\text{tr}\{\mathbf{P}\} = P_{xx} + P_{yy} + P_{zz} = \overline{b_x^2(t)} + \overline{b_y^2(t)} + \overline{b_z^2(t)} = \overline{(b_x^2(t) + b_y^2(t) + b_z^2(t))} = 1 \quad [22]$$

since by definition, $\bar{\mathbf{b}}$ is a unit vector, and hence, $b_x^2(t) + b_y^2(t) + b_z^2(t) = 1$ for all times t .

Therefore, $\mathbf{P}$ is fully specified by only five independent parameters. The matrix $\mathbf{P}$ can be represented graphically as an ellipsoid (see Fig. 3 and 4). The three principal axes $\tilde{x}$, $\tilde{y}$ and $\tilde{z}$ of this ellipsoid are defined by the three eigenvectors of the matrix $\mathbf{P}$ and the lengths of the three half axes are defined by the eigenvalues $P_{\tilde{x}}$, $P_{\tilde{y}}$ and $P_{\tilde{z}}$ (see Fig. 3 A).

In the special frame of reference defined by this principal axis system (see Fig. 3 B), the matrix $\mathbf{P}$ is diagonal:

$$\mathbf{P} = \begin{pmatrix} P_{\tilde{x}} & 0 & 0 \\ 0 & P_{\tilde{y}} & 0 \\ 0 & 0 & P_{\tilde{z}} \end{pmatrix}. \quad [23]$$

In this case the eigenvalues (principal values) $P_{\tilde{x}} = \overline{b_{\tilde{x}}^2}$, $P_{\tilde{y}} = \overline{b_{\tilde{y}}^2}$ and $P_{\tilde{z}} = \overline{b_{\tilde{z}}^2}$ are the probabilities to find the magnetic field along the principal axes $\tilde{x}$, $\tilde{y}$ and $\tilde{z}$, respectively.

Therefore we call $\mathbf{P}$ simply the *probability tensor*. (Rigorously, $\mathbf{P}$ corresponds to the sum of the zero and second order term of a spherical harmonics expansion of the probability

distribution function describing the orientation of a reference vector relative to a rigid body (4, 15)).

In the principal axis system, Eq. [21] for the calculation of the residual dipolar coupling reduces simply to

$$\overline{D} = \frac{\kappa}{R^3} \left(P_{\tilde{x}} r_{\tilde{x}}^2 + P_{\tilde{y}} r_{\tilde{y}}^2 + P_{\tilde{z}} r_{\tilde{z}}^2 - \frac{1}{3} \right). \quad [24]$$

For example, in the static case, $\bar{\mathbf{b}} = \begin{pmatrix} b_x \\ b_y \\ b_z \end{pmatrix}$ is constant, and hence,

$$\mathbf{P} = \begin{pmatrix} b_x^2 & b_x b_y & b_x b_z \\ b_x b_y & b_y^2 & b_y b_z \\ b_x b_z & b_y b_z & b_z^2 \end{pmatrix}. \quad [25]$$

The matrix has a much simpler form in the principal axis frame $(\tilde{x}, \tilde{y}, \tilde{z})$ where the $\tilde{z}$ axis is parallel to the vector $\bar{\mathbf{b}}$. In this reference frame,

$$\bar{\mathbf{b}} = \begin{pmatrix} b_{\tilde{x}} \\ b_{\tilde{y}} \\ b_{\tilde{z}} \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \text{ and } \mathbf{P} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 1 \end{pmatrix}. \quad [26]$$

In this case, the probability ellipsoid is reduced to a line along the $\tilde{z}$ axis and the dipolar coupling constant is

$$\overline{D} = D = \frac{\kappa}{R^3} \left(r_z^2 - \frac{1}{3} \right). \quad [27]$$

For a completely isotropically reorienting molecule, the averages $\overline{b_x(t)b_y(t)}$, $\overline{b_x(t)b_z(t)}$, $\overline{b_y(t)b_z(t)}$ are zero, and $P_{\tilde{x}} = P_{\tilde{y}} = P_{\tilde{z}} = 1/3$, i. e. the *probability matrix*

$$\mathbf{P} = \begin{pmatrix} \frac{1}{3} & 0 & 0 \\ 0 & \frac{1}{3} & 0 \\ 0 & 0 & \frac{1}{3} \end{pmatrix} \quad [28]$$

is diagonal in any molecular fixed frame of reference. Hence, there is an equal probability of 1/3 for the magnetic field direction to point along all three axes of reference. The corresponding probability ellipsoid is a sphere with radius 1/3 (see Fig. 4 C), and the residual dipolar coupling constant is

$$\overline{D} = \frac{\kappa}{R^3} \left(\frac{1}{3} (r_{\tilde{x}}^2 + r_{\tilde{y}}^2 + r_{\tilde{z}}^2) - \frac{1}{3} \right) = 0. \quad [29]$$

Fig. 4 A shows an example of an axially symmetric probability ellipsoid with the principal values $P_{\tilde{x}} = P_{\tilde{y}} = 0.25$ and $P_{\tilde{z}} = 0.5$. Fig. 4 B shows an example without axial symmetry where $P_{\tilde{x}} = 0.2$, $P_{\tilde{y}} = 0.3$ and $P_{\tilde{z}} = 0.5$. Note that the lack of axial symmetry simply means that there are two different probabilities $P_{\tilde{x}} \neq P_{\tilde{y}}$ for the magnetic field to point along the principal axes $\tilde{x}$ and $\tilde{y}$ of the molecular-fixed probability tensor. However, this does by no means imply that in the lab frame there are different probabilities for the molecule to be

aligned along the x^L or y^L direction. For example in the case shown in Fig. 4 B, $P_{\tilde{x}} = 0.2$, $P_{\tilde{y}} = 0.3$ and $P_{\tilde{z}} = 0.5$ are the probabilities that the $\tilde{x}$, $\tilde{y}$ and $\tilde{z}$ axes are aligned parallel to B_0 .

In the NMR literature, it is not customary to consider the *probability tensor* $\mathbf{P}$ (which can be nicely depicted as an ellipsoid), but to use its traceless part which is called the *alignment tensor*

$$\mathbf{A} = \mathbf{P} - \frac{1}{3}\mathbf{1}. \quad [30]$$

If we multiply $\mathbf{A}$ from the left with the unit row vector $\bar{\mathbf{r}}^T$ and from the right with the column vector $\bar{\mathbf{r}}$ and using Eq. [19] and Eq. [30], we get

$$\begin{aligned} \bar{\mathbf{r}}^T \mathbf{A} \bar{\mathbf{r}} &= \bar{\mathbf{r}}^T \left(\mathbf{P} - \frac{1}{3}\mathbf{1} \right) \bar{\mathbf{r}} \\ &= \bar{\mathbf{r}}^T \mathbf{P} \bar{\mathbf{r}} - \frac{1}{3} \bar{\mathbf{r}}^T \bar{\mathbf{r}} \\ &= \overline{\cos^2 \theta} - \frac{1}{3}, \end{aligned} \quad [31]$$

which can also be used to calculate the residual dipolar coupling constant in Eq. [21]:

$$\overline{D} = \frac{\kappa}{R^3} (\bar{\mathbf{r}}^T \mathbf{A} \bar{\mathbf{r}}). \quad [32]$$

$\mathbf{P}$ and $\mathbf{A}$ have the same principal axis system $(\tilde{x}, \tilde{y}, \tilde{z})$ (except for a possible reordering of the axis labels if the convention is used that $|P_{\tilde{x}}| \leq |P_{\tilde{y}}| \leq |P_{\tilde{z}}|$ and $|A_{\tilde{x}}| \leq |A_{\tilde{y}}| \leq |A_{\tilde{z}}|$), and the principal values are related by

$$A_{\tilde{x}} = P_{\tilde{x}} - \frac{1}{3},$$

$$A_{\tilde{y}} = P_{\tilde{y}} - \frac{1}{3},$$

and

$$A_{\tilde{z}} = P_{\tilde{z}} - \frac{1}{3}, \tag{33}$$

with $A_{\tilde{x}} + A_{\tilde{y}} + A_{\tilde{z}} = 0$.

In the principal axis system

$$\left(\overline{\cos^2 \theta} - \frac{1}{3} \right) = A_{\tilde{x}} r_{\tilde{x}}^2 + A_{\tilde{y}} r_{\tilde{y}}^2 + A_{\tilde{z}} r_{\tilde{z}}^2, \tag{34}$$

and hence, the residual dipolar coupling constant is given by

$$\overline{D} = \frac{\kappa}{R^3} (A_{\tilde{x}} r_{\tilde{x}}^2 + A_{\tilde{y}} r_{\tilde{y}}^2 + A_{\tilde{z}} r_{\tilde{z}}^2). \tag{35}$$

The *alignment tensor* cannot be represented as an ellipsoid, because at least one of the principal values is always negative if $\mathbf{A} \neq 0$.

In Fig. 5, we show a graphical representation of the $\mathbf{A}$ tensors which correspond to the $\mathbf{P}$ tensors shown in Fig. 4. The plots show the surfaces where the term $\left| \frac{\bar{\mathbf{r}}^T \mathbf{A} \bar{\mathbf{r}}}{R^3} \right|$ is constant. Hence, if spin I is assumed to be located in the origin, the plots show the possible locations of spin S for which the residual dipolar coupling constant has the same magnitude. For the case of an isotropically reorienting molecule (spherical *probability tensor*), the residual dipolar coupling is always zero, and no such surface exists.

The dependence of the scaling factor $(\overline{\cos^2 \theta} - 1/3)$ on the orientation of the internuclear vector is sometimes shown by the color of a unit sphere. For the three cases shown in Fig. 4 and 5, the corresponding grayscale coded surface representations of the alignment tensors are shown in Fig. 6. The grayscale intensity represents the scaling factor of a residual dipolar coupling constant if spin I is located at the origin and spin S is moved over the surface, i. e. assuming a constant internuclear distance.

For example, in the axially symmetric case shown in Fig. 6 A with $A_{\tilde{x}} = A_{\tilde{y}} = -1/12$ and $A_{\tilde{z}} = 1/6$, the scaling factor $(\overline{\cos^2 \theta} - 1/3)$ is zero if the $\tilde{z}$ -component of the internuclear vector is $r_{\tilde{z}} = \sqrt{1/3}$, which is straightforward to see if Eq. [34] is set to zero and using $r_{\tilde{x}}^2 + r_{\tilde{y}}^2 = 1 - r_{\tilde{z}}^2$. This corresponds to an angle of $\vartheta = \arccos \sqrt{1/3} = 54.74^\circ$ (the *magic angle*) between the internuclear vector and the $\tilde{z}$ -axis. For the case shown in Fig. 6 B with $A_{\tilde{x}} = -2/15$, $A_{\tilde{y}} = -1/30$ and $A_{\tilde{z}} = 1/6$, the polar angle ϑ , when the scaling factor is zero, depends also on the azimuthal angle φ between the $\tilde{x}$ -axis and the projection of $\bar{\mathbf{r}}$ on the $\tilde{x}/\tilde{y}$ plane. For example, in the $\tilde{x}/\tilde{z}$ plane, the scaling factor is zero if $r_{\tilde{z}} = 2/3$

(corresponding to $\vartheta = \arccos(2/3) = 48.19^\circ$), and in the $\tilde{y}/\tilde{z}$ plane, the scaling factor is zero if $r_{\tilde{z}} = \sqrt{1/6}$ ($\vartheta = \arccos\sqrt{1/6} = 65.91^\circ$). In the isotropic case shown in Fig. 6 C, the scaling factor $(\overline{\cos^2 \theta} - 1/3)$ is zero for all orientations of the internuclear vector $\bar{\mathbf{R}}$.

APPENDIX

In the appendix, the key equations (Eq. [24] and Eq. [35]) for the calculation of the residual dipolar coupling constant $\bar{D}$ are reexpressed in various forms found in literature. If the unit vector $\bar{\mathbf{r}}$ is defined in terms of the polar coordinates ϑ and φ in the principal axis system of the alignment tensor $\mathbf{A}$, then

$$\bar{\mathbf{r}} = \begin{pmatrix} r_{\tilde{x}} \\ r_{\tilde{y}} \\ r_{\tilde{z}} \end{pmatrix} = \begin{pmatrix} \sin \vartheta \cos \varphi \\ \sin \vartheta \sin \varphi \\ \cos \vartheta \end{pmatrix} \quad [36]$$

and hence (according to Eq. [34]):

$$\left(\overline{\cos^2 \theta} - \frac{1}{3} \right) = A_{\tilde{x}} \sin^2 \vartheta \cos^2 \varphi + A_{\tilde{y}} \sin^2 \vartheta \sin^2 \varphi + A_{\tilde{z}} \cos^2 \vartheta. \quad [37]$$

This can be simplified by noting that $\cos^2 \varphi = (1 + \cos 2\varphi)/2$ and $\sin^2 \varphi = (1 - \cos 2\varphi)/2$:

$$\begin{aligned} \left(\overline{\cos^2 \theta} - \frac{1}{3} \right) &= \frac{A_{\tilde{x}}}{2} \sin^2 \vartheta + \frac{A_{\tilde{x}}}{2} \sin^2 \vartheta \cos 2\varphi + \frac{A_{\tilde{y}}}{2} \sin^2 \vartheta - \frac{A_{\tilde{y}}}{2} \sin^2 \vartheta \cos 2\varphi + A_{\tilde{z}} \cos^2 \vartheta \\ &= \frac{A_{\tilde{x}} + A_{\tilde{y}}}{2} \sin^2 \vartheta + \frac{A_{\tilde{x}} - A_{\tilde{y}}}{2} \sin^2 \vartheta \cos 2\varphi + A_{\tilde{z}} \cos^2 \vartheta \end{aligned} \quad [38]$$

Since $\mathbf{A}$ is a traceless matrix, $A_{\tilde{x}} + A_{\tilde{y}} = -A_{\tilde{z}}$, and we can rewrite Eq. [38] as

$$\left(\overline{\cos^2 \theta} - \frac{1}{3}\right) = A_{\tilde{z}} \left(\cos^2 \vartheta - \frac{\sin^2 \vartheta}{2}\right) + \frac{A_{\tilde{x}} - A_{\tilde{y}}}{2} \sin^2 \vartheta \cos 2\varphi. \quad [39]$$

The pre-factor of $A_{\tilde{z}}$ can be further simplified by using the relation $\sin^2 \vartheta = 1 - \cos^2 \vartheta$:

$$\begin{aligned} \cos^2 \vartheta - \frac{\sin^2 \vartheta}{2} &= \cos^2 \vartheta - \frac{(1 - \cos^2 \vartheta)}{2} \\ &= \frac{1}{2} (3 \cos^2 \vartheta - 1). \end{aligned} \quad [40]$$

Thus, we arrive at

$$\left(\overline{\cos^2 \theta} - \frac{1}{3}\right) = \frac{A_{\tilde{z}}}{2} (3 \cos^2 \vartheta - 1) + \frac{A_{\tilde{x}} - A_{\tilde{y}}}{2} \sin^2 \vartheta \cos 2\varphi. \quad [41]$$

Eq. [41] can alternatively be expressed in terms of the principal values $S_{\tilde{x}}$, $S_{\tilde{y}}$ and $S_{\tilde{z}}$ of the *Saupe matrix* (or *order matrix*) $\mathbf{S}$, which is simply the *alignment matrix* $\mathbf{A}$ scaled by a factor of 3/2, if the optical axis of the liquid crystal is collinear with the direction of the magnetic field (9, 10):

$$\mathbf{S} = 3/2 \mathbf{A}. \quad [42]$$

Hence,

$$\left(\overline{\cos^2 \theta} - \frac{1}{3}\right) = \frac{1}{3} \left\{ S_z (3 \cos^2 \vartheta - 1) + (S_{\tilde{x}} - S_{\tilde{y}}) \sin^2 \vartheta \cos 2\varphi \right\} \quad [43]$$

Often, the *axial component* A_a of the alignment tensor is defined as (5)

$$A_a = \frac{3}{2} A_z = S_z, \quad [44]$$

and the *rhombic component* A_r of the alignment tensor is defined as

$$A_r = A_{\tilde{x}} - A_{\tilde{y}} = \frac{2}{3} (S_{\tilde{x}} - S_{\tilde{y}}) \quad [45]$$

With these definitions, we can express Eqs. [41] and [43] as

$$\left(\overline{\cos^2 \theta} - \frac{1}{3}\right) = \frac{1}{3} \left\{ A_a (3 \cos^2 \vartheta - 1) + \frac{3}{2} A_r \sin^2 \vartheta \cos 2\varphi \right\}, \quad [46]$$

which in turn is often written as

$$\begin{aligned} \left(\overline{\cos^2 \theta} - \frac{1}{3}\right) &= \frac{A_a}{3} \left\{ (3 \cos^2 \vartheta - 1) + \frac{3}{2} R \sin^2 \vartheta \cos 2\varphi \right\} \\ &= \frac{A_a}{3} \left\{ (3 \cos^2 \vartheta - 1) + \eta \sin^2 \vartheta \cos 2\varphi \right\} \end{aligned} \quad [47]$$

where

$$R = \frac{A_r}{A_a} \quad [48]$$

is called the *rhombicity* of the *alignment tensor* and

$$\eta = \frac{A_{\bar{x}} - A_{\bar{y}}}{A_{\bar{z}}} = \frac{S_{\bar{x}} - S_{\bar{y}}}{S_{\bar{z}}} = \frac{3}{2} R \quad [49]$$

is called the *asymmetry parameter* which describes the deviation from axially symmetric ordering (6).

So far, we have assumed a rigid molecule that tumbles in solution. In the presence of internal motions the derivation of residual dipolar couplings becomes more complicated (6, 18, 19). Provided the alignment process is not affected by intramolecular motion, the analysis is relatively straightforward. If the internal motion of the internuclear vector $\bar{r}$ is axially symmetric with respect to the average orientation $\bar{r}_{av}$, the dipolar coupling expected for this average orientation is scaled by a factor λ , which is identical to a generalized order parameter S ($0 \leq S \leq 1$) (18). The latter corresponds mathematically to the spin relaxation order parameter (19, 20), but exhibits a sensitivity to motions extending to the millisecond time scale (6, 18). This leads to the following equation of the residual dipolar coupling constant:

$$\bar{D} = S \frac{\kappa}{3} \frac{A_a}{R^3} \left\{ 3 \cos^2 \vartheta - 1 + \eta \sin^2 \vartheta \cos 2\varphi \right\} \quad [50]$$

This expression is often rewritten using the *maximum dipolar coupling* $D_{\max} = (2/3)\kappa / R^3$ (c. f. Eq. [7]) or the so called *magnitude of the residual dipolar coupling tensor* $D_a = D_{\max} A_a / 2$ (7):

$$\begin{aligned}\bar{D} &= S D_a \left\{ \frac{3}{2} \cos^2 \vartheta - 1 \right\} + \eta \sin^2 \vartheta \cos 2\varphi \\ &= S \frac{D_{\max}}{2} A_a \left\{ \frac{3}{2} \cos^2 \vartheta - 1 \right\} + \eta \sin^2 \vartheta \cos 2\varphi \\ &= S D_{\max} A_a \left\{ P_2(\cos \vartheta) + \frac{\eta}{2} \sin^2 \vartheta \cos 2\varphi \right\},\end{aligned}\tag{51}$$

where $P_2(x) = (3 \cos^2 x - 1)/2$ is the second-order Legendre polynomial.

Finally, we use the results derived in this manuscript to introduce the concepts of the *generalized degree of order* (GDO) of a given alignment tensor $\mathbf{A}$ (22) and the *generalized angle* between two different alignment tensors $\mathbf{A}^{(1)}$ and $\mathbf{A}^{(2)}$ (23).

In complete analogy to the scalar product between two real vectors, the scalar product between two real matrices (e. g. two alignment matrices $\mathbf{A}^{(1)}$ and $\mathbf{A}^{(2)}$) is defined as

$$\langle \mathbf{A}^{(1)} | \mathbf{A}^{(2)} \rangle = \sum_{i,j} A_{ij}^{(1)} A_{ij}^{(2)}\tag{52}$$

and the norm $|\mathbf{A}|$ of the real matrix $\mathbf{A}$ is given by

$$|\mathbf{A}| = \sqrt{\langle \mathbf{A} | \mathbf{A} \rangle} = \sqrt{\sum_{i,j} A_{ij}^2}.\tag{53}$$

The maximum order is found for the static case, where the probability tensor $\mathbf{P}_{\max}$ is given by Eq. [26] in the principal axis system. The corresponding maximum alignment tensor $\mathbf{A}_{\max} = \mathbf{P}_{\max} - 1/3 \mathbf{1}$ has the form

$$\mathbf{A}_{\max} = \begin{pmatrix} -1/3 & 0 & 0 \\ 0 & -1/3 & 0 \\ 0 & 0 & 2/3 \end{pmatrix}. \quad [54]$$

The norm of $\mathbf{A}_{\max}$ is given by

$$|\mathbf{A}_{\max}| = \sqrt{\frac{1}{9} + \frac{1}{9} + \frac{4}{9}} = \sqrt{\frac{2}{3}}. \quad [55]$$

The *generalized degree of order* (GDO) of a given order matrix $\mathbf{A}$ can be defined as

$$\text{GDO} = \frac{|\mathbf{A}|}{|\mathbf{A}_{\max}|} = \sqrt{\frac{3}{2}} |\mathbf{A}|. \quad [56]$$

In terms of the *Saupe matrix* $\mathbf{S} = 3/2 \mathbf{A}$ (c. f. Eq. [42]), this can be written as (6, 22)

$$\text{GDO} = \sqrt{\frac{2}{3}} |\mathbf{S}|. \quad [57]$$

In literature, the symbol “ ϑ ” is often used for the GDO but we do not use the symbol here in order to avoid confusion with the polar angle ϑ defined in Eq. [36].

The GDO is independent of the molecular-fixed frame, in which the *alignment tensor* $\mathbf{A}$ is expressed. In the principal axis system only the diagonal elements of $\mathbf{A}$ are nonzero and Eq. [56] simplifies to

$$\text{GDO} = \sqrt{\frac{3}{2}} \sqrt{A_x^2 + A_y^2 + A_z^2}. \quad [58]$$

For axially symmetric *alignment tensors* ($A_x = A_y = -A_z/2$) this simplifies further to (22):

$$\begin{aligned} \text{GDO} &= \sqrt{\frac{3}{2} \left(\frac{1}{4} A_z^2 + \frac{1}{4} A_z^2 + A_z^2 \right)} = \frac{3}{2} \sqrt{A_z^2} \\ &= \frac{3}{2} |A_z| = |S_z|. \end{aligned} \quad [59]$$

With the help of the scalar product, we can also define the *generalized angle* β between two *alignment tensors* $\mathbf{A}^{(1)}$ and $\mathbf{A}^{(2)}$ which correspond e. g. to two different alignment media.

If the matrix representations of $\mathbf{A}^{(1)}$ and $\mathbf{A}^{(2)}$ are given in a common molecular frame of reference, the cosine of the *generalized angle* β between these *alignment tensors* can be defined as the normalized scalar product between them (23):

$$\cos \beta = \frac{\langle \mathbf{A}^{(1)} | \mathbf{A}^{(2)} \rangle}{|\mathbf{A}^{(1)}| |\mathbf{A}^{(2)}|}. \quad [60]$$

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FIGURE CAPTIONS

Figure 1:

Definition of the angle θ between the internuclear vector $\vec{R}$ (connecting spins I and S) and the magnetic field vector $\vec{B}$. The unit vectors $\vec{r}$ and $\vec{b}$ point in the direction of $\vec{R}$ and $\vec{B}$, respectively.

Figure 2:

Effect of molecular tumbling of a rigid molecule as seen (Panel A) from the lab frame of reference (with axes x^L, y^L, z^L) and (Panel B) from an arbitrary molecular frame of reference (with axes x, y, z). In the lab frame (Panel A), the magnetic field $\vec{B}$ is constant and points by definition along the z^L axis, whereas the internuclear vector $\vec{R}$ keeps changing its direction. In a molecular frame (Panel B), the situation is reversed: here, any given internuclear vector is constant, whereas the orientation of the magnetic field is time-dependent.

Figure 3:

The molecule, a given internuclear vector $\vec{R}$ and the *probability ellipsoid* (a graphical representation of the *probability tensor* $\mathbf{P}$, c.f. Eq. [25]) are shown (Panel A) in an arbitrarily chosen molecular frame (c.f. Fig. 2 B) and (Panel B) in the special coordinate system (with axes $\tilde{x}, \tilde{y}, \tilde{z}$) defined by the principal axes of the *probability ellipsoid*.

Figure 4:

Examples of three characteristic *probability ellipsoids* (graphical representations of the *probability tensor* $\mathbf{P}$, c.f. Eq. [25]) as seen from the principal axis system with axes $\tilde{x}, \tilde{y}, \tilde{z}$ (c.f. Fig. 3 B). Panel A shows an axially symmetric *probability ellipsoid* with $P_{\tilde{x}} = P_{\tilde{y}} = 0.25$ and $P_{\tilde{z}} = 0.5$ (Panel A). Panel B depicts a rhombic *prob-*

ability ellipsoid with $P_{\tilde{x}} = 0.2$, $P_{\tilde{y}} = 0.3$ and $P_{\tilde{z}} = 0.5$. Panel C shows an isotropic *probability ellipsoid* with $P_{\tilde{x}} = P_{\tilde{y}} = P_{\tilde{z}} = 1/3$.

Figure 5:

Graphical representations of the *alignment tensors* (Panel A) which correspond to the three *probability tensors* shown in Fig. 4 A-C. The principal components of the *alignment tensor* are (A) $A_{\tilde{x}} = A_{\tilde{y}} = 0.25 - 1/3 = -1/12$, $A_{\tilde{z}} = 0.5 - 1/3 = -1/6$, (B) $A_{\tilde{x}} = 0.2 - 1/3 = -2/15$, $A_{\tilde{y}} = 0.3 - 1/3 = -1/30$, $A_{\tilde{z}} = 0.5 - 1/3 = 1/6$ and (C) $A_{\tilde{x}} = A_{\tilde{y}} = A_{\tilde{z}} = 1/3 - 1/3 = 0$. The plots show the surfaces where $(\tilde{\mathbf{r}}^T \mathbf{A} \tilde{\mathbf{r}}) R^3 = 1 \text{ \AA}^{-3}$ (light gray) or $-1 \text{ \AA}^{-3}$ (dark gray) if the $\tilde{x}$, $\tilde{y}$ and $\tilde{z}$ axes are labelled in units of $\text{\AA}$.

Figure 6:

For the three cases shown in Fig. 4 and 5 with (A) $A_{\tilde{x}} = A_{\tilde{y}} = -1/12$, $A_{\tilde{z}} = -1/6$, (B) $A_{\tilde{x}} = -2/15$, $A_{\tilde{y}} = -1/30$, $A_{\tilde{z}} = 1/6$ and (C) $A_{\tilde{x}} = A_{\tilde{y}} = A_{\tilde{z}} = 0$ the magnitude of the scaling factor $(\overline{\cos^2 \theta} - 1/3)$ is coded on a unit sphere as a function of the orientation of the internuclear vector $\tilde{\mathbf{R}}$ (white: vanishing scaling factor). Positive and negative scaling factors are denoted by the respective sign.

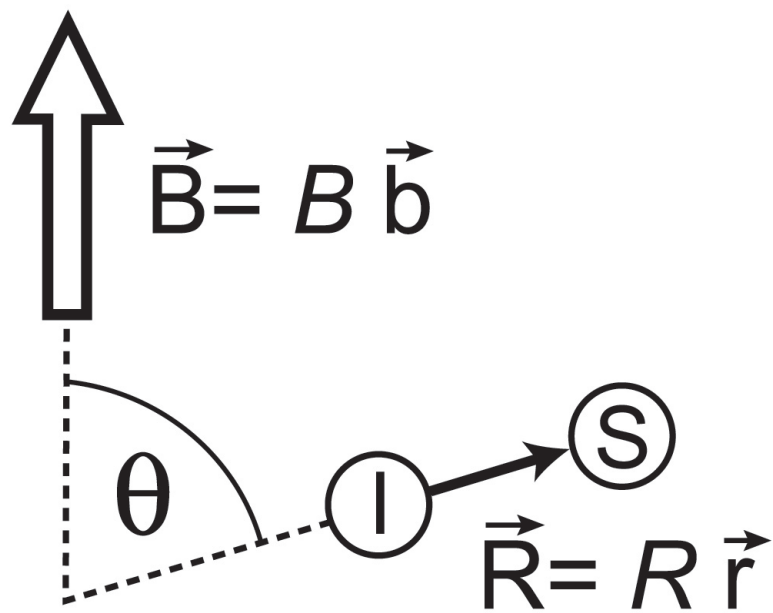


Figure 1

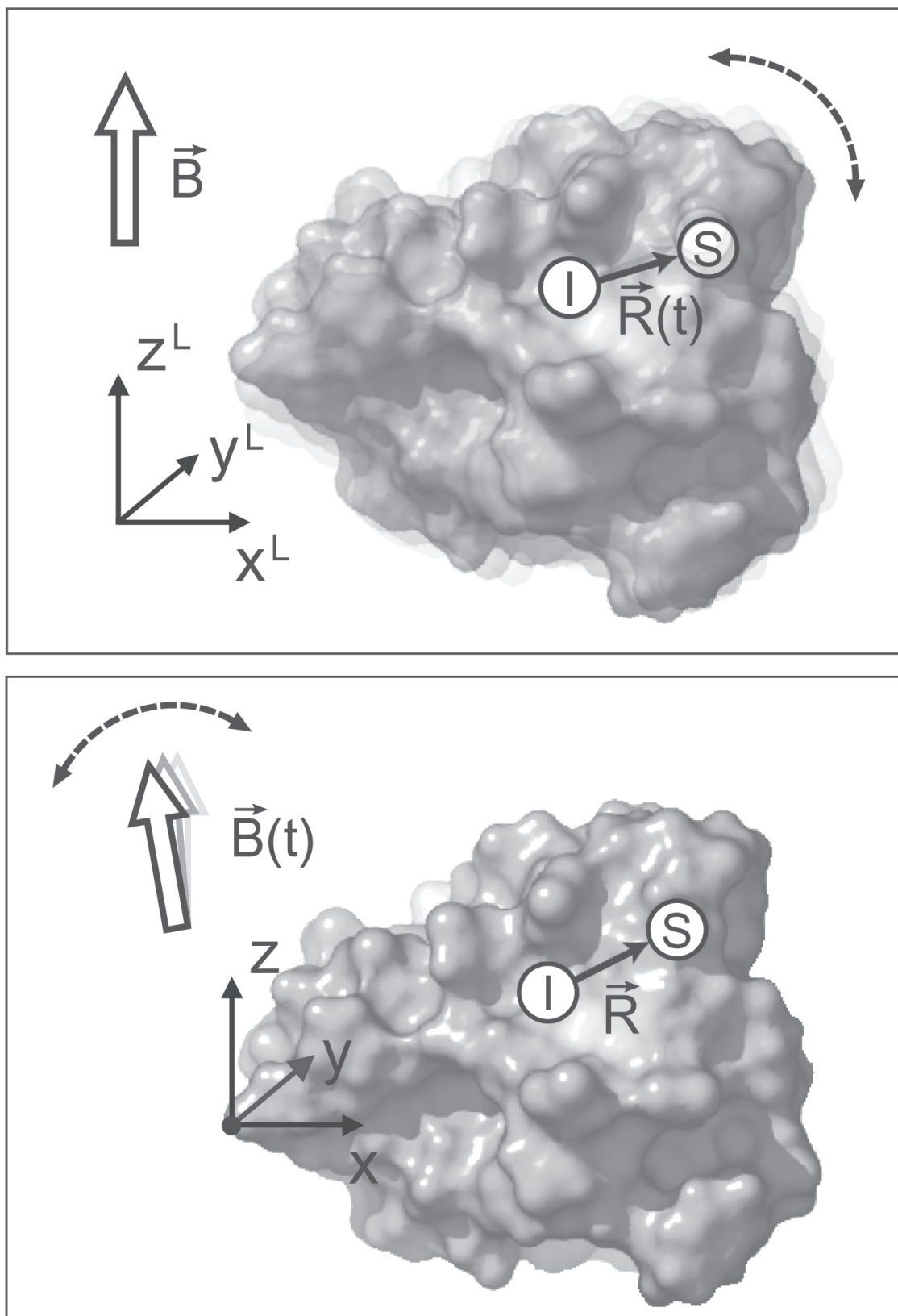


Figure 2

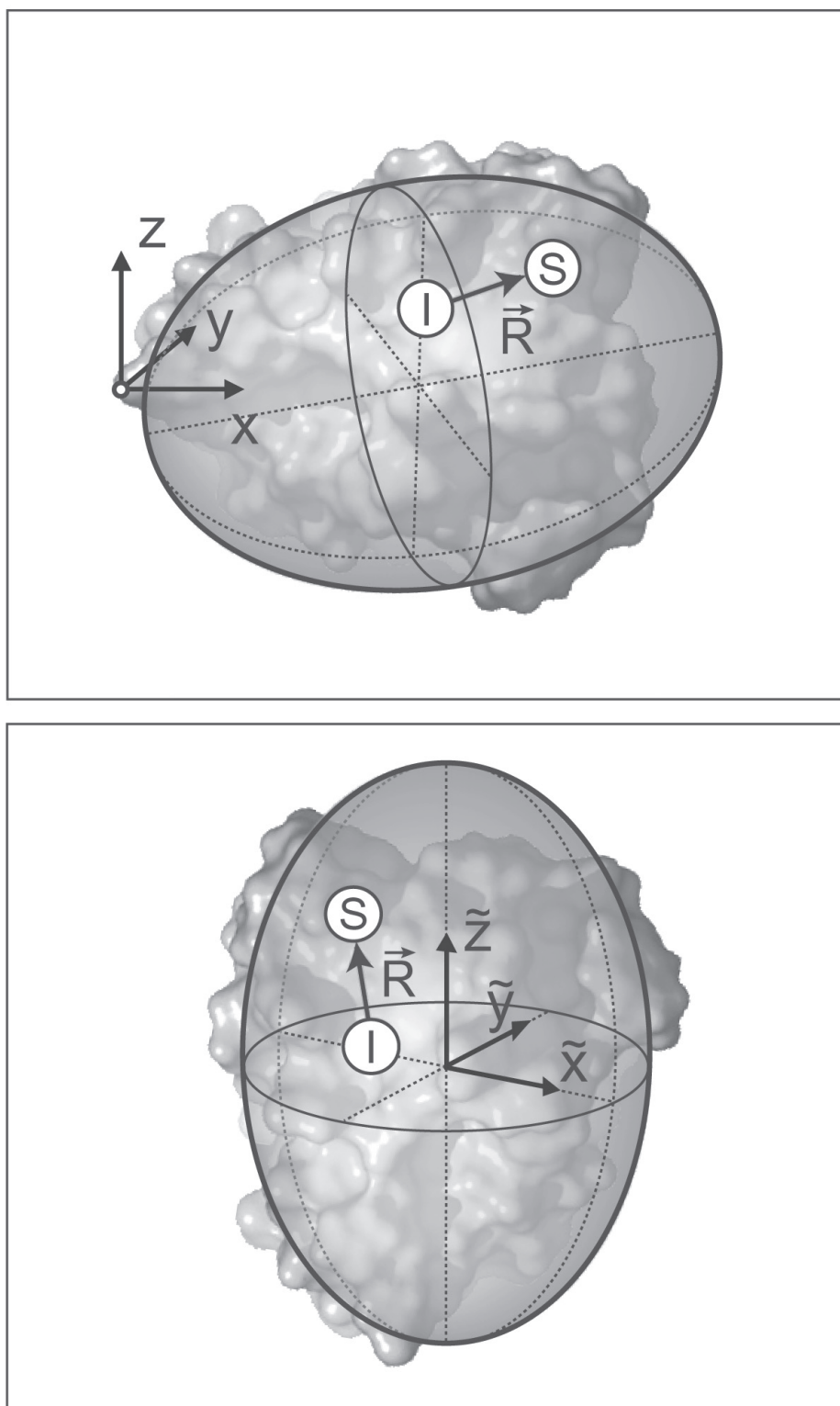


Figure 3

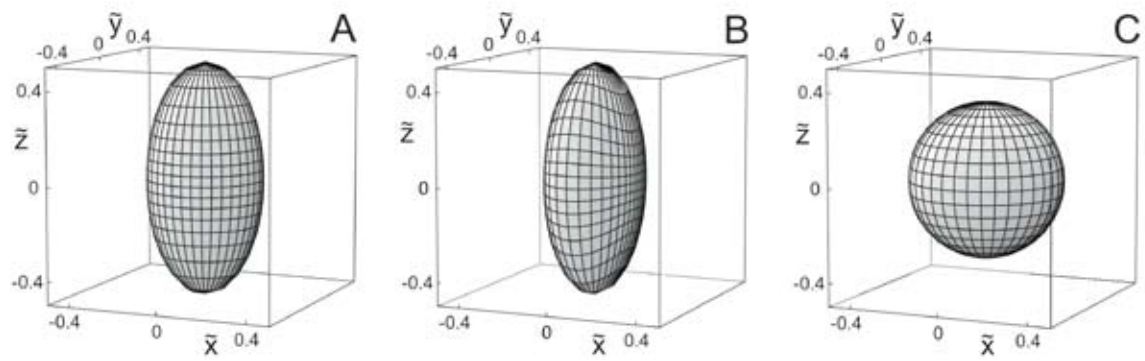


Figure 4

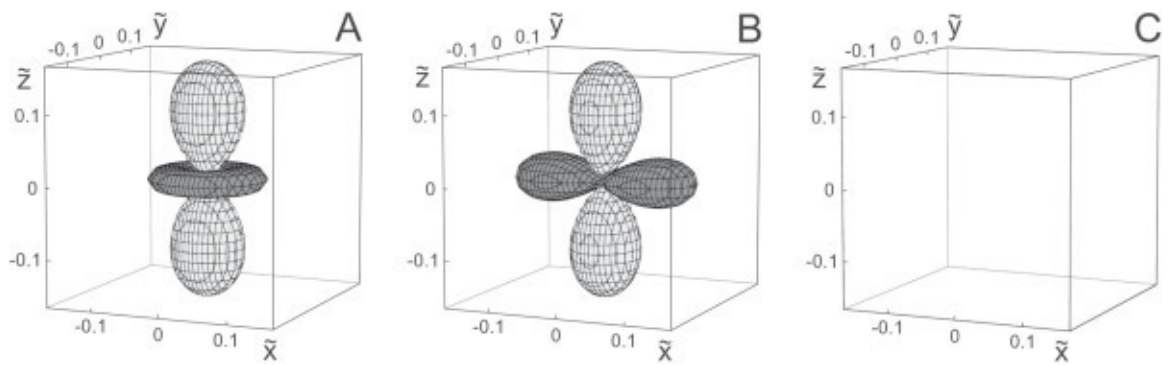


Figure 5

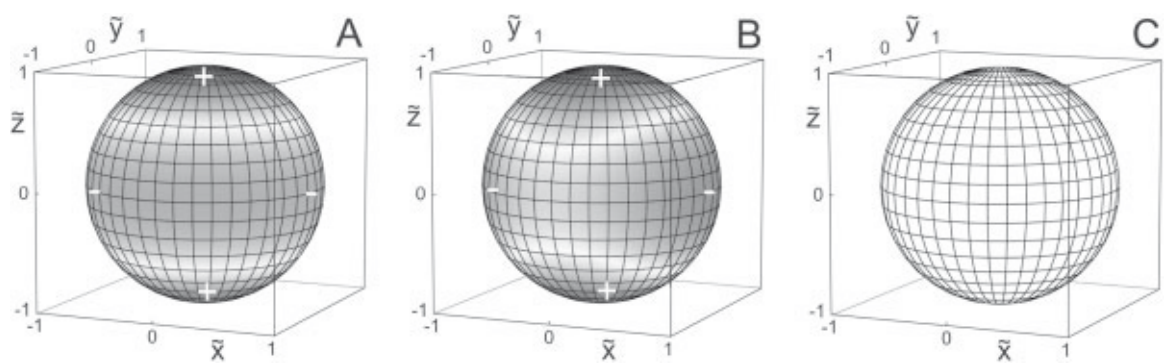


Figure 6

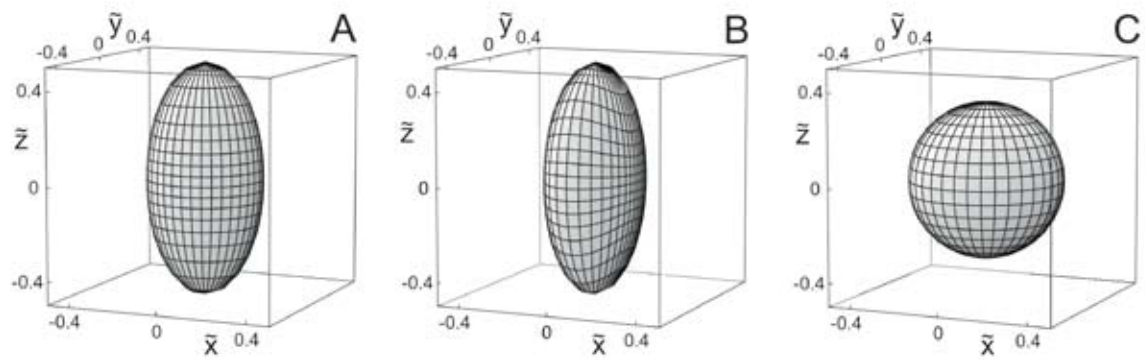


Figure 4

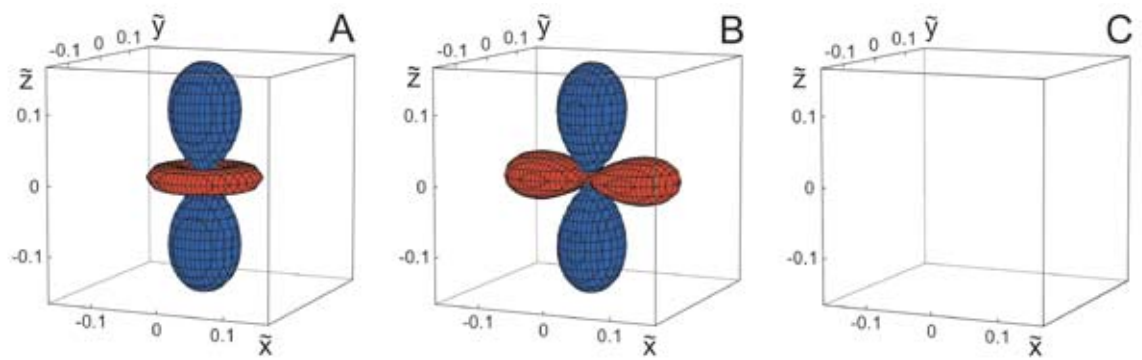


Figure 5 (color)

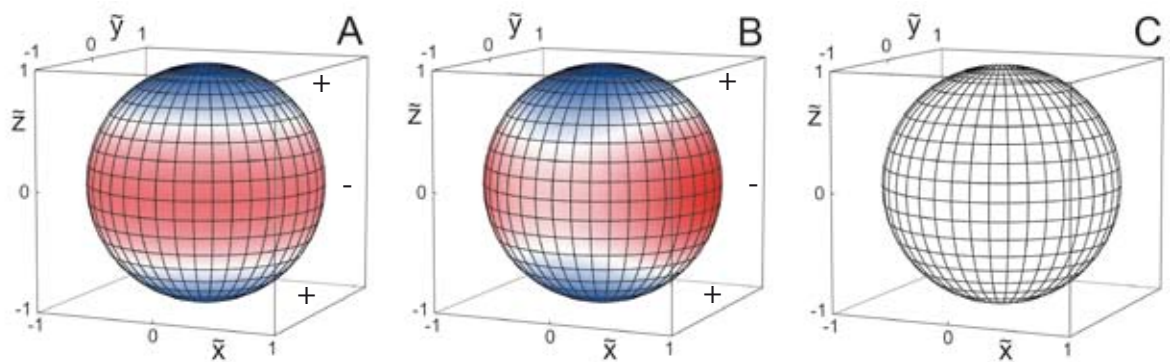


Figure 6 (color)