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Reconstruction of orientations of a moving protein domain from paramagnetic data

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Abstract
We study the inverse problem of determining the position of the moving C-terminal domain in a metalloprotein from measurements of its mean paramagnetic tensor $\bar{\chi}$. The latter can be represented as a finite sum involving the corresponding magnetic susceptibility tensor χ and a finite number of rotations. We obtain an optimal estimate for the maximum probability that the C-terminal domain can assume a given orientation, and we show that only three rotations are required in the representation of $\bar{\chi}$, and that in general two are not enough. We also investigate the situation in which a compatible pair of mean paramagnetic tensors is obtained. Under a mild assumption on the corresponding magnetic susceptibility tensors, justified on physical grounds, we again obtain an optimal estimate for the maximum probability that the C-terminal domain can assume a given orientation. Moreover, we prove that only ten rotations are required in the representation of the compatible pair of mean paramagnetic tensors, and that in general three are not enough. The theoretical investigation is concluded by a study of the coaxial case, when all rotations are assumed to have a common axis. Results are obtained via an interesting connection with another inverse problem, the quadratic complex moment problem. Finally, we describe an application to experimental NMR data.

1. Introduction

The availability of genomic data has created a need for rapid and efficient determination of three-dimensional structures of the corresponding proteins. It is commonly accepted that each protein has a unique fold, but no *a priori* theoretical method is presently available to obtain it from knowledge of the sequence of amino acids which constitute the protein. The primary purpose of this paper is to contribute by addressing a related inverse problem.

There are proteins which exhibit, under particular conditions, a certain degree of freedom. A widely-studied case is that of calmodulin, which consists of two rigid domains, called the N-terminal and C-terminal domains, connected by a short flexible linker. The relative positions of the two domains have been recently studied using numerical methods in [2]. Calmodulin is a metalloprotein, one of the one-third or so of all proteins that contain at least one metal ion. For such proteins, new NMR structural constraints, the paramagnetism-based constraints, can be obtained to study the positions of the domains; see [5]. We focus in this paper on *residual dipolar couplings* (RDC) $\delta_{ab}^{\mathrm{rdc}}$, which are due to the induced partial orientation in high magnetic field caused by anisotropy of the *magnetic susceptibility tensor*, a 3×3 symmetric matrix χ. These are given by the equation (see [14])

$$\delta_{ab}^{\mathrm{rdc}} = \frac{C_{\mathrm{rdc}}}{r_{ab}^5} P_{ab}^* \chi P_{ab}, \tag{1}$$

where C_{rdc} is a constant, $P_{ab} = (x_{ab}, y_{ab}, z_{ab})^*$ is the transposed position vector in the same reference system as the matrix χ, where (x_{ab}, y_{ab}, z_{ab}) are the differences between the coordinates of selected pairs of atoms a and b, and $r_{ab} = \sqrt{x_{ab}^2 + y_{ab}^2 + z_{ab}^2}$.

Since the isotropic part of the magnetic susceptibility tensor χ does not influence the RDC, the trace of χ is usually assumed to be zero, and we will make this assumption throughout the paper.

For many metalloproteins, it is possible to substitute one metal ion with a different one. In these cases more than one set of RDC can be obtained, as different metal ions determine different magnetic susceptibility tensors (see [1, 3]). The removal of the metal ion present in the binding site may cause conformational modifications; however, these can be limited by substituting the metal ion with one having the same charge; see [1].

The binding site of the metal ion in calmodulin belongs to the N-terminal domain. The measured values of RDC for pairs of atoms belonging to the N-terminal domain can be used to determine a good estimate of χ. The measured values of RDC for pairs of atoms belonging to the C-terminal domain can be used to study the relative orientation of the two domains. The rigid structures of both domains are assumed to be known in a reference frame, the lab frame. The orientation of the C-terminal domain with respect to χ may be represented by a rotation matrix R, transforming the lab frame into the orientation of the C-terminal domain. There is an unknown probability measure p in the set of rotations such that the *mean* RDC $\bar{\delta}_{ab}^{\mathrm{rdc}}$ are given by

$$\bar{\delta}_{ab}^{\mathrm{rdc}} = \frac{C_{\mathrm{rdc}}}{r_{ab}^5} \int_{SO_3} (RP_{ab})^* \chi (RP_{ab}) \, \mathrm{d}p(R).$$

(See section 2 for definitions and notation.) Using the 3×3 matrix $\bar{\chi}$ called the *mean paramagnetic tensor*, defined by

$$\bar{\chi}_{ij} = \int_{SO_3} (R^* \chi R)_{ij} \, \mathrm{d}p(R), \tag{2}$$

this formula may be recast in the form

$$\bar{\delta}_{ab}^{\mathrm{rdc}} = \frac{C_{\mathrm{rdc}}}{r_{ab}^5} P_{ab}^* \bar{\chi} P_{ab}. \tag{3}$$

Clearly, the mean RDC of any pair can be calculated from $\bar{\chi}$. On the other hand, $\bar{\chi}$ can be estimated from a number of mean RDC of the C-terminal domain (specifically, five are enough, if the mean RDC are exact, to determine the quadratic form associated with $\bar{\chi}$, while if errors are present a larger number are needed to get a good fit by numerical methods).

In this paper we study the inverse problem of determining the position of the moving C-terminal domain from measurements of $\bar{\chi}$. The fact that $\bar{\chi}$ contains information about the motion of the C-terminal domain is already clear from the following extremal cases.

(i) Suppose the C-terminal domain does not move. Then $p = \delta_{R_0}$ for some rotation R_0 and $\bar{\chi} = R_0^* \chi R_0$ has the same eigenvalues as χ.

(ii) Suppose that all orientations of the C-terminal domain are equally likely, that is, p is the Haar measure (see section 2) in SO_3. Then $\bar{\chi} = 0$, by lemma 3.1.

Note, however, that knowledge of the five distinct entries of the symmetric matrix $\bar{\chi}$ does not allow exact reconstruction of the probability measure p.

We begin our analysis in section 3 by observing that a mean paramagnetic tensor $\bar{\chi}$ can be assumed to be of the special form

$$\bar{\chi} = \sum_j p_j R_j^* \chi R_j, \tag{4}$$

where the sum is finite, $R_j \in SO_3$ for each j, and $\sum_j p_j = 1$ with each $p_j \geqslant 0$. What is of interest to chemists is the maximum probability such that the C-terminal domain can assume a given orientation. They do this by a numerical fit based on an assumption that representation (4) involves a particular number of rotations. This number therefore is also of interest.

In this paper we prove that one can always find a representation (4) of $\bar{\chi}$ involving only three rotations (see theorem 3.5) and that this number is in general the minimum. Moreover, theorem 3.8 provides an optimal estimate of the maximum probability that a given orientation may be assumed by the C-terminal domain.

In practice (see [2, 4]), more than one metal ion can be substituted. We limit the analysis to the two-metal-ion case for simplicity, considering a compatible pair $(\bar{\chi}^1, \bar{\chi}^2)$ of mean paramagnetic tensors, each of form (4), for the *same* rotations R_j and coefficients p_j. The corresponding magnetic susceptibility tensors χ^1 and χ^2 can clearly be assumed to be not proportional; moreover, a slightly stronger assumption (assumption A), that χ^1 and χ^2 do not have a common eigenvector, can be justified on physical grounds. As was noted in [10], the data can now be combined to remove a certain non-uniqueness issue (compare lemma 3.7 and proposition 4.5). In section 4, we use a version of Carathéodory's theorem to prove that a compatible pair of representations can always be found involving at most 10 rotations (see theorem 4.2). It is quite likely that this number may be reduced, but we give an example in section 6 (see also theorem 4.4) to show that under assumption A, at least four rotations are needed in general. Also under assumption A, theorem 4.6, a two-metal-ion counterpart of theorem 3.8, again provides an optimal estimate of the maximum probability that a given orientation may be assumed by the C-terminal domain.

The difficulty of finding the minimum number of rotations involved in representations of mean paramagnetic tensors is highlighted in our discussion in section 5 of an apparently much simpler special case in which all the rotations have a common axis. The minimum number of rotations is then three, but to prove this we resort to a fascinating connection with the famous moment problem, specifically the quadratic complex moment problem. Our result is obtained as an easy corollary of the recent solution of the latter by Curto and Fialkow [6], but this solution is itself by no means simple.

Finally, section 7 describes an application to experimental NMR data.

2. Definitions and notation

As usual, the standard orthonormal basis for n-dimensional Euclidean space $\mathbb{R}^n$ will be $\{e_1, \ldots, e_n\}$, and S^{n-1} is the unit sphere in $\mathbb{R}^n$. The norm in $\mathbb{R}^n$ is denoted by $\|\cdot\|$.

The *dimension* of a set A is the dimension of its affine hull, and written $\dim A$.

The *determinant* and *transpose* of a matrix M are denoted by $\det M$ and M^*, respectively.

We write SO_3 for the special orthogonal group of rotations in $\mathbb{R}^3$. There is a unique rotation-invariant (uniform) probability measure in SO_3, the *Haar measure*; see [13, Satz 1.2.4] for a clever direct construction of this measure. By δ_x we mean the *Dirac measure* of unit mass concentrated at the point x.

The *coordinate axis reflections* in $\mathbb{R}^3$ will be denoted by $S_i, i = 1, 2, 3$, where S_i is the diagonal matrix with 1 in place ii, and -1 in the remaining diagonal places. It will be convenient to write S_0 for the identity matrix. The matrix with all entries zero will be denoted by 0.

3. The one-metal-ion case

In this section we study the information that can be gathered from measurements arising from one metal ion.

Let $\mu_{\min}, \mu_2$, and $\mu_{\max}$ be the eigenvalues of the magnetic susceptibility tensor χ, in increasing order, and let

$$\chi_d = \begin{pmatrix} \mu_{\min} & 0 & 0 \\ 0 & \mu_2 & 0 \\ 0 & 0 & \mu_{\max} \end{pmatrix} \tag{5}$$

be the standard diagonalized form of χ. The calculated values (see [3]) show that in physical situations no eigenvalue can be zero, and all eigenvalues are distinct. We shall therefore make this assumption in this paper. Then, using the fact that trace $\chi = 0$, we have

$$\mu_{\min} < 0 < \mu_{\max} \qquad \text{and} \qquad -2\mu_{\max} < \mu_{\min} < -\mu_{\max}/2. \tag{6}$$

Lemma 3.1. *Let the mean paramagnetic tensor $\bar{\chi}$ be given by (2). If p is the Haar measure in SO_3, then $\bar{\chi} = 0$.*

Proof. We may assume that $\chi = \chi_d$ is given by (5). The matrix for the rotation R around an axis parallel to the unit vector (x, y, z) by angle $0 \leqslant \alpha < 2\pi$ is given by

$$R = \begin{pmatrix} \cos\alpha + (1-\cos\alpha)x^2 & (1-\cos\alpha)xy - z\sin\alpha & (1-\cos\alpha)xz + y\sin\alpha \\ (1-\cos\alpha)xy + z\sin\alpha & \cos\alpha + (1-\cos\alpha)y^2 & (1-\cos\alpha)yz - x\sin\alpha \\ (1-\cos\alpha)xz - y\sin\alpha & (1-\cos\alpha)yz + x\sin\alpha & \cos\alpha + (1-\cos\alpha)z^2 \end{pmatrix}. \tag{7}$$

(Compare [15, p 582].) In terms of spherical polar coordinates (ρ, θ, ϕ), where $\rho \geqslant 0$, $0 \leqslant \theta < 2\pi$ and $0 \leqslant \phi \leqslant \pi$, we have $x = \rho\cos\theta\sin\phi$, $y = \rho\sin\theta\sin\phi$, and $z = \rho\cos\phi$. Substituting these values into (7), and using the formula (see [9, (4.77)])

$$\mathrm{d}p(R) = \frac{1}{4\pi^2}\sin^2(\alpha/2)\sin\phi\,\mathrm{d}\alpha\,\mathrm{d}\theta\,\mathrm{d}\phi,$$

the right-hand side of (2) can be shown to be equal to zero for all i and j by routine calculation. We omit the details. □

We assume henceforth that the mean paramagnetic tensor is given by

$$\bar{\chi} = \sum_j p_j R_j^* \chi R_j, \tag{8}$$

where the sum is finite, $R_j \in SO_3$ for each j, and $\sum_j p_j = 1$ with each $p_j \geqslant 0$. (It is convenient to allow $p_j = 0$, though of course in this case the corresponding term in (8) can

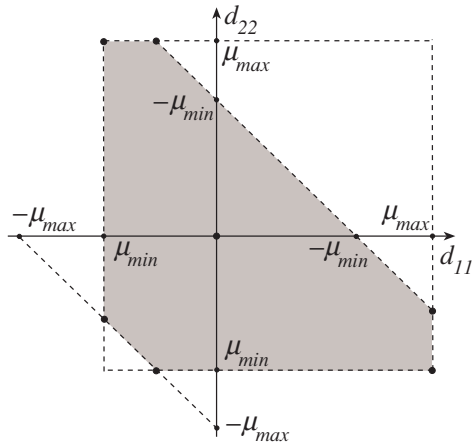

Figure 1. Hexagon (shaded) representing the set H of diagonal matrices.

be dropped.) This assumption, that the general representation (2) of $\bar{\chi}$ can be replaced by (8), is equivalent to assuming that the measure p in (2) is concentrated on a finite set of rotations. Theorem 3.5 below justifies the assumption.

Let $V = V(\chi)$ be the set of all mean paramagnetic tensors of form (8). Each matrix in V is symmetric and so can be represented as a point in $\mathbb{R}^5$, for example by taking, in some particular order, the entries of the upper triangular part of the matrix excepting the lower right diagonal entry. In this way V can be regarded as a subset of $\mathbb{R}^5$. The following lemma collects some basic properties of V.

Lemma 3.2. *Let $\bar{\chi} \in V$. Then*

(i) *$\bar{\chi}$ is symmetric,*
(ii) $\operatorname{trace}\bar{\chi} = 0$,
(iii) *V is a compact convex subset of $\mathbb{R}^5$, and*
(iv) *$R^*\bar{\chi}R \in V$, for each $R \in SO_3$.*

Proof. Property (i) is trivial and (ii) follows from the linearity of the trace and its invariance for similar matrices. As for (iii), representation (8) shows that V is precisely the convex hull in $\mathbb{R}^5$ of the compact set $\{R^*\chi R : R \in SO_3\}$. Property (iv) follows from the fact that the product of rotations is a rotation. □

Let H be the set of all diagonal matrices $D = (d_{ij})$ with zero trace and with eigenvalues in the interval $[\mu_{\min}, \mu_{\max}]$. Since $\mu_{\min} \leqslant d_{33} = -d_{11} - d_{22} \leqslant \mu_{\max}$, each element of H is represented by the point (d_{11}, d_{22}) in the shaded hexagon shown in figure 1. Each vertex of the hexagon corresponds to a diagonal matrix whose diagonal entries are the eigenvalues $\mu_{\min}$, μ_2 and $\mu_{\max}$ of χ, ordered in one of the six possible ways. Therefore each such vertex corresponds to a matrix $Q_i^*\chi_d Q_i$, where Q_i is one of the six rotation matrices that permute the axes of the reference system (possibly reversing the direction of an axis). The vertices are all distinct because the strict inequalities (6) hold.

Theorem 3.3. *The set V is the set of symmetric 3×3 matrices with zero trace and with eigenvalues in the interval $[\mu_{\min}, \mu_{\max}]$.*

Proof. Let $\bar{\chi} \in V$. Then $\bar{\chi}$ is symmetric and has zero trace, by lemma 3.2(i) and (ii). Moreover, for each unit vector x, using (8), we obtain

$$x^*\bar{\chi}x = \sum_j p_j (R_j x)^* \chi (R_j x) \leqslant \sum_j p_j \mu_{\max} = \mu_{\max}.$$

Similarly, $x^*\bar{\chi}x \geqslant \mu_{\min}$. It follows that the eigenvalues of $\bar{\chi}$ lie in the interval $[\mu_{\min}, \mu_{\max}]$.

Now suppose that M is symmetric with zero trace and with eigenvalues in the interval $[\mu_{\min}, \mu_{\max}]$. Then M is similar to a diagonal matrix R^*MR in H, which is represented by a point in the shaded hexagon in figure 1. This point is a convex combination of three (not necessarily distinct) vertices of the hexagon; let the corresponding rotations be Q_{i_j}, $j = 1, 2, 3$. Then $R^*MR = \sum_{j=1}^{3} p_j Q_{i_j}^* \chi_d Q_{i_j}$, yielding

$$M = \sum_{j=1}^{3} p_j \left(Q_{i_j} R^*\right)^* \chi_d \left(Q_{i_j} R^*\right).$$

Comparing (8), we see that $M \in V$ and the theorem is proved. □

The previous theorem and its proof give the following corollary.

Corollary 3.4. *The set V has the following properties.*

(i) The zero matrix $0 \in V$,
(ii) $\dim V = 5$, *and*
(iii) each $\bar{\chi} \in V$ has a representation (8) involving at most three rotations R_j.

Proof. Only (ii) requires comment. For this, note that if $\bar{\chi} \in V$ has eigenvalues in $(\mu_{\min}, \mu_{\max})$, then by continuity of the eigenvalues with respect to the elements of the matrix, a neighbourhood of $\bar{\chi}$ in $\mathbb{R}^5$ also belongs to V. □

Theorem 3.5. *Suppose that $\bar{\chi}$ is a mean paramagnetic tensor of the general form (2). Then $\bar{\chi}$ also has a representation of form (8) involving at most three rotations.*

Proof. Note that representation (2) implies that $\bar{\chi}$ is symmetric and trace $\bar{\chi} = 0$. For each unit vector x, we have

$$x^*\bar{\chi}x = \int_{SO_3} (Rx)^* \chi (Rx)\, \mathrm{d}p(R) \leqslant \int_{SO_3} \mu_{\max}\, \mathrm{d}p(R) = \mu_{\max},$$

and similarly $\mu_{\min} \leqslant x^*\bar{\chi}x$. It follows that the eigenvalues of $\bar{\chi}$ lie in the interval $[\mu_{\min}, \mu_{\max}]$. By theorem 3.3, $\bar{\chi} \in V$, and the proof is completed by corollary 3.4 (iii). □

Lemma 3.6. *The zero matrix $0 \in V$ has no representation of form (8) involving only two rotations. However, 0 can be represented in the form*

$$0 = \tfrac{1}{3}\chi_d + \tfrac{1}{3}P_1^*\chi_d P_1 + \tfrac{1}{3}P_2^*\chi_d P_2, \tag{9}$$

where P_1 and P_2 are the two rotations that cyclicly permute the axes.

Proof. Suppose that $0 = p_1 R_1^* \chi R_1 + p_2 R_2^* \chi R_2$, for some $R_1, R_2 \in SO_3$, where $p_1 + p_2 = 1$. Then $p_1^3 \det(R_1^*\chi R_1) = -p_2^3 \det(R_2^*\chi R_2)$, so $\left(p_1^3 + p_2^3\right) \det \chi = 0$. This implies $\det \chi = 0$, contradicting the fact that the eigenvalues of χ are nonzero.

A direct computation with

$$P_1 = \begin{pmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \end{pmatrix} \qquad \text{and} \qquad P_2 = \begin{pmatrix} 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{pmatrix}$$

establishes representation (9). □

Note that (9) is not the only way to represent the zero matrix. For instance, if $-\mu_{\min} > \mu_{\max}$, then direct calculation yields

$$0 = \frac{\mu_{\max}}{\mu_{\max} - \mu_{\min}}\chi_d + \frac{\mu_{\min} + \mu_{\max}}{2\mu_{\min} + \mu_{\max}} P_1^* \chi_d P_1 + \frac{-\left(\mu_{\min}^2 + \mu_{\min}\mu_{\max} + \mu_{\max}^2\right)}{(\mu_{\max} - \mu_{\min})(2\mu_{\min} + \mu_{\max})} Q_2^* \chi_d Q_2, \quad (10)$$

where

$$Q_2 = \begin{pmatrix} 0 & 0 & -1 \\ 0 & 1 & 0 \\ 1 & 0 & 0 \end{pmatrix}.$$

Similarly, if $-\mu_{\min} < \mu_{\max}$, we have

$$0 = \frac{\mu_{\min}}{\mu_{\min} - \mu_{\max}}\chi_d + \frac{\mu_{\min} + \mu_{\max}}{2\mu_{\max} + \mu_{\min}} P_2^* \chi_d P_2 + \frac{-\left(\mu_{\min}^2 + \mu_{\min}\mu_{\max} + \mu_{\max}^2\right)}{(\mu_{\min} - \mu_{\max})(2\mu_{\max} + \mu_{\min})} Q_2^* \chi_d Q_2. \quad (11)$$

Note that the denominators of the coefficients in (10) and (11) are nonzero, by (6); however, these coefficients now depend on the eigenvalues.

The only rotations R such that $R^*\chi_d R = \chi_d$ are the identity S_0 and the coordinate axis reflections $S_i, i = 1, 2, 3$ (see section 2). There is a consequent non-uniqueness in the representation (8) of certain $\bar{\chi}$, as the following lemma demonstrates.

Lemma 3.7. *Let $\bar{\chi} = R_0^* \chi_d R_0$ for some $R_0 \in SO_3$. Then every representation of $\bar{\chi}$ as in (8) can also be written as*

$$\bar{\chi} = \sum_{i=0}^{3} p_i (S_i R_0)^* \chi_d (S_i R_0), \quad (12)$$

where $p_i, i = 0, \ldots, 3$ are arbitrary nonnegative coefficients with $\sum_i p_i = 1$.

Proof. Note that for each i we have $(S_i R_0)^* \chi_d (S_i R_0) = R_0^* \chi_d R_0$. Therefore if $p_i, i = 0, \ldots, 3$ are arbitrary nonnegative coefficients with $\sum_i p_i = 1$, then (12) holds.

On the other hand, the eigenvalues of χ_d and $\bar{\chi}$ are the same. If $\bar{\chi} = \sum_j p_j R_j^* \chi_d R_j$, then $\chi_d = R_0 \bar{\chi} R_0^* = \sum_j p_j (R_j R_0^*)^* \chi_d R_j R_0^*$. Therefore

$$\mu_{\max} = e_3^* \chi_d e_3 = \sum_j p_j (R_j R_0^* e_3)^* \chi_d R_j R_0^* e_3 \leqslant \sum_j p_j \mu_{\max} = \mu_{\max}.$$

It follows that for each j, $(R_j R_0^* e_3)^* \chi_d R_j R_0^* e_3 = \mu_{\max}$, and hence, since the eigenvalues of χ_d are simple, that $R_j R_0^* e_3 = \pm e_3$. Analogously, using $\mu_{\min}$, we obtain $R_j R_0^* e_1 = \pm e_1$ for each j. This shows that for each j, there is an i such that $R_j R_0^* = S_i$, i.e., $R_j = S_i R_0$. Thus (12) holds. □

Let $\bar{\chi}$ be a mean paramagnetic tensor, and let R_0 be a given rotation. From the physical point of view, it is important to obtain an estimate of the maximum probability $p_{\max}$ for which the C-terminal domain can assume the relative orientation R_0. In practice (see [2]) it is reasonable to suppose that the orientations for which small values of $p_{\max}$ are obtained cannot be assumed by the domain. The following result is an optimal estimate.

Theorem 3.8. *Let $\bar{\chi}$ be of form (8), let $R_0 \in SO_3$, and let $p_{\max}$ be the maximum probability such that the C-terminal domain can assume the relative orientation R_0. Then*

$$p_{\max} = \sup\left\{0 \leqslant t < 1 : \frac{\bar{\chi} - t R_0^* \chi_d R_0}{1 - t} \in V\right\}. \quad (13)$$

Proof. Let

$$t_{\max} = \sup\left\{0 \leqslant t < 1 : \frac{\bar{\chi} - tR_0^*\chi_d R_0}{1-t} \in V\right\}. \tag{14}$$

We consider various cases.

Suppose first that $\bar{\chi} = R^*\chi_d R$ for some $R \in SO_3$. If $R = S_i R_0$ for some $0 \leqslant i \leqslant 3$, then $p_{\max} = 1$ because $\bar{\chi} = R_0^* S_i^* \chi_d S_i R_0 = R_0^*\chi_d R_0$, and $t_{\max} = 1$ because $(\bar{\chi} - tR_0^*\chi_d R_0)/(1-t) = \bar{\chi} \in V$ for each $t < 1$. On the other hand, if $R \neq S_i R_0$ for $i = 0, \ldots, 3$, then $p_{\max} = 0$ by lemma 3.7. Moreover, if $(R^*\chi_d R - tR_0^*\chi_d R_0)/(1-t) \in V$ for some $0 < t < 1$, then by (8) we have

$$R^*\chi_d R = tR_0^*\chi_d R_0 + (1-t)\sum_{j>0} p_j R_j^*\chi_d R_j,$$

and this is impossible by lemma 3.7 because $R \neq S_i R_0$. Hence $t_{\max} = 0$.

Now suppose that $\bar{\chi} \notin \{R^*\chi_d R : R \in SO_3\}$, and suppose the C-terminal domain has relative orientation R_0 with probability p_0. Then $\bar{\chi} = p_0 R_0^*\chi_d R_0 + \sum_{j>0} p_j R_j^*\chi_d R_j$, and so

$$\frac{\bar{\chi} - p_0 R_0^*\chi_d R_0}{1-p_0} = \sum_{j>0} \frac{p_j}{1-p_0} R_j^*\chi_d R_j \in V,$$

since $\sum_{j>0} p_j/(1-p_0) = 1$. This shows that $p_{\max} \leqslant t_{\max}$. To prove the reverse inequality, note first that since $\bar{\chi} \notin \{R^*\chi_d R : R \in SO_3\}$, the numerator of $(\bar{\chi} - tR_0^*\chi_d R_0)/(1-t)$ is different from 0 for t close to 1 and the denominator tends to 0 as $t \to 1$. Since V is compact, this implies that $t_{\max} < 1$. Now by the definition of $t_{\max}$, we have $(\bar{\chi} - t_{\max} R_0^*\chi_d R_0)/(1-t_{\max}) \in V$, so

$$\frac{\bar{\chi} - t_{\max} R_0^*\chi_d R_0}{1-t_{\max}} = p_0 R_0^*\chi_d R_0 + \sum_{j>0} p_j R_j^*\chi_d R_j, \tag{15}$$

where $\sum_j p_j = 1$ and $p_j \geqslant 0$ for $j \geqslant 0$. Setting $t_0 = t_{\max} + (1-t_{\max})p_0$, (15) implies that

$$\frac{\bar{\chi} - t_0 R_0^*\chi_d R_0}{1-t_0} = \sum_{j>0} \frac{p_j}{1-p_0} R_j^*\chi_d R_j \in V.$$

Since $t_{\max} < 1$, if $p_0 > 0$ we have $t_0 > t_{\max}$, contradicting the definition of $t_{\max}$. Therefore $p_0 = 0$, and substituting this in (15), we see that

$$\bar{\chi} = t_{\max} R_0^*\chi_d R_0 + (1-t_{\max})\sum_{j>0} p_j R_j^*\chi_d R_j$$

and hence that $t_{\max} \leqslant p_{\max}$, completing the proof. □

4. The two-metal-ion case

Let χ^k, $k = 1, 2$ be magnetic susceptibility tensors relative to two different metal ions, and for $k = 1, 2$, let

$$V^k = \left\{\bar{\chi}^k : \bar{\chi}^k = \sum_j p_j R_j^*\chi^k R_j, \sum_j p_j = 1, p_j \geqslant 0\right\},$$

be the corresponding sets of mean paramagnetic tensors. Let

$$V^{1,2} = \left\{\bar{\chi}^{1,2} = (\bar{\chi}^1, \bar{\chi}^2) : \bar{\chi}^k = \sum_j p_j R_j^*\chi^k R_j, k = 1, 2\right\} \tag{16}$$

be the set of compatible pairs of mean paramagnetic tensors. Each $\bar{\chi}^{1,2} \in V^{1,2}$ has a representation

$$\bar{\chi}^{1,2} = \left(\sum_j p_j R_j^* \chi^1 R_j, \sum_j p_j R_j^* \chi^2 R_j \right), \tag{17}$$

where $\sum_j p_j = 1$ and each $p_j \geqslant 0$. Note that both these coefficients p_j and the rotations R_j are the same for each component of $\bar{\chi}^{1,2}$ in (17). Just as we were able to regard V as a subset of $\mathbb{R}^5$ in section 3, so we can regard $V^{1,2}$ as a subset of $\mathbb{R}^5 \times \mathbb{R}^5 = \mathbb{R}^{10}$.

We summarize some properties of $V^{1,2}$ in the following lemma.

Lemma 4.1. *The set $V^{1,2}$ has the following properties.*

(i) $V^{1,2}$ is a compact convex subset of $V^1 \times V^2$.

Moreover, if χ^1 and χ^2 are not proportional, then

(ii) the origin in $\mathbb{R}^{10}$ belongs to the interior of $V^{1,2}$, and hence
(iii) $\dim V^{1,2} = 10$.

Proof. By definition, $V^{1,2} \subset V^1 \times V^2$, and (16) shows that $V^{1,2}$ is precisely the convex hull of the compact set $\{(R^*\chi^1 R, R^*\chi^2 R) : R \in SO_3\}$ in $\mathbb{R}^{10}$.

Though (iii) follows from (ii), our proof will actually supply both statements at once. Suppose for simplicity that $\chi^1 = \chi_d^1$ is diagonal. Consider the section $\Pi^2 = \{\bar{\chi}^2 : (0, \bar{\chi}^2) \in V^{1,2}\}$. Using lemma 3.6, we can represent the zero matrix $0 \in V^1$ in form (9). Then

$$\bar{\chi}^2 = \tfrac{1}{3}\chi^2 + \tfrac{1}{3}P_1^*\chi^2 P_1 + \tfrac{1}{3}P_2^*\chi^2 P_2 \in \Pi^2.$$

A straightforward computation shows that $\bar{\chi}^2$ has zero diagonal entries and all other entries equal to $x = (1/3)\chi_{12}^2 + (1/3)\chi_{13}^2 + (1/3)\chi_{23}^2$, and hence has eigenvalues $2x$, $-x$ and $-x$. By lemma 3.2(iv), every matrix of the form $R^*\bar{\chi}^2 R$ belongs to Π^2, because $R^*0R = 0$. Then Π^2 includes the three diagonal matrices having eigenvalues $2x$, $-x$ and $-x$, and hence, by convexity, a triangle T^2 containing the origin in $\mathbb{R}^5$. If $x \neq 0$, the origin in $\mathbb{R}^5$ belongs to the relative interior of T^2.

If $x = 0$, then we have

$$\chi_{23}^2 = -\chi_{13}^2 - \chi_{12}^2. \tag{18}$$

Suppose first that the eigenvalues $\mu_{\min}^1$ and $\mu_{\max}^1$ of χ^1 satisfy $-\mu_{\min}^1 > \mu_{\max}^1$. Then we can repeat the above argument, using instead of (9) the alternative representation (10) of the zero matrix. Let $\bar{M}^2$ be the corresponding matrix in Π^2, given by (10) with χ_d replaced by χ^2, noting that the coefficients in this representation of $\bar{M}^2$ depend on $\mu_{\min}^1$ and $\mu_{\max}^1$.

We claim that $\bar{M}^2 \neq 0$. Indeed, if the non-diagonal entries of $\bar{M}^2$ are all zero, then in particular $\bar{M}_{12}^2 = 0$ and $\bar{M}_{13}^2 = 0$. Using (18), these two equations form a system of two homogeneous linear equations in the variables χ_{12}^2 and χ_{13}^2, whose determinant may be calculated to be

$$2\frac{\left(2\mu_{\max}^1 + \mu_{\min}^1\right)\left(\mu_{\max}^1 + \mu_{\min}^1\right)\left(\left(\mu_{\min}^1\right)^2 + \mu_{\max}^1\mu_{\min}^1 + \left(\mu_{\min}^1\right)^2\right)}{\left(\mu_{\max}^1 - \mu_{\min}^1\right)^2\left(2\mu_{\min}^1 + \mu_{\max}^1\right)^2}.$$

Since this determinant is nonzero, we have $\chi_{12}^2 = \chi_{13}^2 = 0$ and hence that χ^2 is diagonal. In this case a straightforward calculation shows that $\bar{M}_{11}^2 = \bar{M}_{22}^2 = 0$, and hence $\bar{M}^2 = 0$, if and only χ_d^1 and χ^2 are proportional. Our hypothesis therefore implies that $\bar{M}^2 \neq 0$ and proves the claim.

Since $\bar{M}^2 \neq 0$, it has at least one nonzero eigenvalue and hence at least two nonzero eigenvalues, because trace $\bar{M}^2 = 0$. Then, as above, there is again a triangle T^2 containing the origin in $\mathbb{R}^5$ in its relative interior.

If $-\mu^1_{\min} < \mu^1_{\max}$, we repeat the above argument, using instead of (10) the representation (11) of the zero matrix. In this case the same conclusion can be reached similarly.

Every matrix similar to a diagonal matrix with its eigenvalues in T^2 belongs to Π^2, so $\dim \Pi^2 = 5$.

The previous argument can be applied also to the section $\Pi^1 = \{M^1 : (M^1, 0) \in V^{1,2}\}$ (using the lab frame for the second metal ion as the reference frame), yielding a triangle T^1 in Π^1 containing the origin in $\mathbb{R}^5$ in its relative interior and allowing us to conclude that $\dim \Pi^1 = 5$. Then, by convexity, $\dim V^{1,2} = 10$, and the origin is an interior point of $V^{1,2}$. □

A few remarks are appropriate in connection with the previous lemma. Firstly, an alternative to our proof of part (iii) would be to find an explicit set of 11 points in $V^{1,2}$ whose convex hull has positive volume in $\mathbb{R}^{10}$, and the fact that the origin in $\mathbb{R}^{10}$ belongs to $V^{1,2}$ can easily be proved using lemma 3.1. However, our proof of these facts via part (ii) provides more information.

Secondly, the proof shows that if $x = 0$, we have found a representation of the origin in $V^{1,2}$ involving only three rotations.

Thirdly, we see from lemma 4.1 that $\dim V^{1,2} = 5$ if χ^1 and χ^2 are proportional, and $\dim V^{1,2} = 10$ otherwise. Thus dimensionally speaking, we can gather more information in the two-metal-ion case if and only if χ^1 and χ^2 are not proportional.

Theorem 4.2. *Each $\bar{\chi}^{1,2} \in V^{1,2}$ has a representation of form (17) involving at most 10 rotations.*

Proof. As in the proof of lemma 4.1(i), $V^{1,2}$ is the convex hull of the connected set

$$C = \{(R^*\chi^1 R, R^*\chi^2 R) : R \in SO_3\}$$

in $\mathbb{R}^{10}$. Suppose that $\bar{\chi}^{1,2} \in V^{1,2}$. By a version of Carathéodory's theorem (see, e.g., [12, theorem 1.1.4]) due to Fenchel [7] (see also [11, theorem 1.4]), there is a subset D of C consisting of at most 10 points such that $\bar{\chi}^{1,2}$ belongs to the convex hull of D. This proves the theorem. □

For our next results, we require a slightly stronger assumption than that χ^1 and χ^2 are not proportional, which however is justified on physical grounds (see [3]):

Assumption A. The matrices χ^1 and χ^2 do not have a common eigenvector.

As a consequence, χ^1 and χ^2 cannot both be diagonal. It is always possible to suppose that χ^1 is diagonal, by appropriately choosing the reference frame.

Lemma 4.3. *Suppose that assumption A holds. If $\chi^1 = \chi^1_d$ is diagonal, then there are at most three zero entries in χ^2.*

Proof. Suppose there are at least four zero entries in χ^2. In view of the symmetry of χ^2, it is not possible that all the diagonal entries and at least one non-diagonal entry are zero, since this would imply that $\det \chi^2 = 0$. Then, since χ^2 has zero trace, it can have at most one zero diagonal entry, and since it is symmetric, there must be at least four non-diagonal zero entries. It follows that there is an index i such that the only nonzero entry in row i or in column i of

χ^2 is the diagonal entry χ^2_{ii}. But then e_i is an eigenvector of both χ^1_d and χ^2, contradicting assumption A. □

Theorem 4.4. *Suppose that assumption A holds and suppose that in the reference frame where χ^1 is diagonal, each non-diagonal entry of χ^2 is nonzero. Then there exists a $\bar{\chi}^{1,2} \in V^{1,2}$ that has no representation of form (17) involving less than four rotations.*

Proof. We may assume that $\chi^1 = \chi^1_d$ is diagonal. Let $p_j > 0, j = 0, \ldots, 3$ satisfy $\sum_j p_j = 1$, and define

$$\bar{\chi}^{1,2} = \left(\chi^1_d, \bar{\chi}^2\right) = \left(\sum_{j=0}^{3} p_j S_j \chi^1_d S_j, \sum_{j=0}^{3} p_j S_j \chi^2 S_j\right), \tag{19}$$

where the rotations S_j are as defined in section 2. By lemma 3.7, every representation of χ^1_d, and hence every representation of $\bar{\chi}^{1,2}$, involves only the rotations S_j. Suppose that

$$\bar{\chi}^{1,2} = \left(\chi^1_d, \bar{\chi}^2\right) = \left(\sum_{j=0}^{3} q_j S_j \chi^1_d S_j, \sum_{j=0}^{3} q_j S_j \chi^2 S_j\right), \tag{20}$$

where $q_j \geqslant 0$ for $j = 0, \ldots, 3$ and $\sum_j q_j = 1$, is another such representation. Let $c_j = p_j - q_j$, $j = 0, \ldots, 3$, so that

$$c_0 + c_1 + c_2 + c_3 = 0. \tag{21}$$

By (19) and (20), we have

$$\sum_{j=0}^{3} c_j S_j \chi^2 S_j = 0,$$

and this implies that

$$\left.\begin{aligned} \chi^2_{12}(c_0 - c_1 - c_2 + c_3) &= 0 \\ \chi^2_{13}(c_0 - c_1 + c_2 - c_3) &= 0 \\ \chi^2_{23}(c_0 + c_1 - c_2 - c_3) &= 0 \end{aligned}\right\}.$$

Each entry of χ^2 appearing in this system may be cancelled, by our hypothesis. The resulting system of three equations, together with (21), forms a linear system whose determinant is nonzero. Therefore $c_j = 0$ and so $q_j = p_j > 0$ for all j, completing the proof. □

Note that the hypothesis in theorem 4.4 that each non-diagonal entry of χ^2 is nonzero holds quite generally, in fact for all but a set of χ^2's of zero measure.

The next result, proved in [10, theorem 1], shows that the non-uniqueness in lemma 3.7 can be avoided in the two-metal-ion case.

Proposition 4.5. *Suppose that assumption A holds. Let $\bar{\chi}^{1,2} = (R_0^* \chi^1 R_0, R_0^* \chi^2 R_0)$ for some $R_0 \in SO_3$. Then there is no other representation of $\bar{\chi}^{1,2}$ of form (17) in which the coefficients p_j are all positive.*

The following theorem is an optimal estimate for the maximum probability for which the C-terminal domain can assume a given relative orientation.

Theorem 4.6. *Suppose that assumption A holds. Let* $\bar{\chi}^{1,2} \in V^{1,2}$, *let* $R_0 \in SO_3$, *and let* $p_{\max}$ *be the maximum probability for which the C-terminal domain can assume the relative orientation* R_0. *Then*

$$p_{\max} = \sup\left\{0 \leqslant t < 1 : \frac{\bar{\chi}^{1,2} - t(R_0^*\chi^1 R_0, R_0^*\chi^2 R_0)}{1-t} \in V^{1,2}\right\}. \tag{22}$$

Proof. The proof is very similar to that of theorem 3.8 for the one-metal-ion case. Indeed, the latter can be followed step by step with only very minor changes, using proposition 4.5 instead of lemma 3.7, and we therefore omit the details. □

Corollary 4.7. *Suppose that assumption A holds. We have*

$$p_{\max} \leqslant t^{1,2} = \min_{k=1,2} \sup\left\{0 \leqslant t < 1 : \frac{\bar{\chi}^k - tR_0^*\chi^k R_0}{1-t} \in V^k\right\}.$$

Proof. Note that

$$t^{1,2} = \sup\left\{0 \leqslant t < 1 : \frac{\bar{\chi}^{1,2} - t(R_0^*\chi^1 R_0, R_0^*\chi^2 R_0)}{1-t} \in V^1 \times V^2\right\},$$

so the result follows from lemma 4.1(i). □

The value $p_{\max}$ is very hard to compute, because is not easy to check whether a given pair (M^1, M^2) of symmetric matrices with zero trace belongs to $V^{1,2}$. On the other hand, theorem 3.3 provides a straightforward method of checking whether $M^k \in V^k$. In this way, $t^{1,2}$ can easily be computed.

A numerical way to estimate the probability for which the C-terminal domain can assume a given relative orientation R_0 is to scan the possible representations involving R_0. This has been done in practice in [2].

5. The coaxial case

In this section we consider the particular case when the linker between the two domains is confined to a single dihedral angle. This may happen in the determination of the fold of tertiary or residual protein structures.

We begin with the one-metal case. Note that while it is always possible to apply the general theory of section 4, obtaining from corollary 3.4 a representation of the mean paramagnetic tensor $\bar{\chi}$ involving only three rotations, these are not coaxial rotations, so a separate analysis is required.

In the coaxial case, we are restricting the rotations to a subset

$$O_u = \{R \in SO_3 : R(u) = u\}$$

of SO_3 consisting of rotations preserving a common oriented axis in a fixed direction $u \in S^2$. By choosing a suitable reference frame, we may assume that $u = e_3$. (In this frame, however, the matrix χ is not necessarily diagonal.) Let V_{e_3} be the set of all mean paramagnetic tensors of the form

$$\bar{\chi} = \sum_j p_j R_{\theta_j}^* \chi R_{\theta_j}, \tag{23}$$

where for each j,

$$R_{\theta_j} = \begin{pmatrix} \cos\theta_j & \sin\theta_j & 0 \\ -\sin\theta_j & \cos\theta_j & 0 \\ 0 & 0 & 1 \end{pmatrix}, \tag{24}$$

and as usual, $\sum_j p_j = 1$ and $p_j \geqslant 0$ for each j.

A straightforward computation shows that (23) and (24) imply that the entries of $\bar{\chi}$ are given by

$$\begin{aligned}
\bar{\chi}_{11} &= \frac{\chi_{11}+\chi_{22}}{2} + \left(\frac{\chi_{11}-\chi_{22}}{2}\right) B_1 - \chi_{12} B_2, \\
\bar{\chi}_{12} &= \left(\frac{\chi_{11}-\chi_{22}}{2}\right) B_2 + \chi_{12} B_1, \\
\bar{\chi}_{13} &= \chi_{13} A_1 - \chi_{23} A_2, \\
\bar{\chi}_{22} &= \frac{\chi_{11}+\chi_{22}}{2} - \left(\frac{\chi_{11}-\chi_{22}}{2}\right) B_1 + \chi_{12} B_2, \\
\bar{\chi}_{23} &= \chi_{13} A_2 + \chi_{23} A_1, \\
\bar{\chi}_{33} &= \chi_{33},
\end{aligned} \tag{25}$$

where

$$\begin{aligned}
A_1 &= \sum_j p_j \cos\theta_j, \qquad & A_2 &= \sum_j p_j \sin\theta_j. \\
B_1 &= \sum_j p_j \cos 2\theta_j, \qquad & B_2 &= \sum_j p_j \sin 2\theta_j.
\end{aligned} \tag{26}$$

Lemma 5.1. *Let $\bar{\chi} \in V$ be of form (8), where (i) e_3 is not an eigenvector of χ, (ii) $\chi_{11} - \chi_{22}$ and χ_{12} are not both zero and (iii) $\bar{\chi}_{33} = \chi_{33}$. Then it is possible to determine unique constants A_1, A_2, B_1 and B_2 such that $\bar{\chi}$ is given by (25).*

Proof. Using (25), the constants A_1 and A_2 can be found by solving the system

$$\left.\begin{aligned} \chi_{13} A_1 - \chi_{23} A_2 &= \bar{\chi}_{13} \\ \chi_{23} A_1 + \chi_{13} A_2 &= \bar{\chi}_{23} \end{aligned}\right\};$$

the determinant vanishes if and only if $\chi_{13} = 0$ and $\chi_{23} = 0$, and this happens if and only if e_3 is an eigenvector of χ, excluded by (i).

In the linear system (25), the condition trace $\bar{\chi} = 0$ may be used to eliminate one equation involving B_1 and B_2. The constants B_1 and B_2 can then be found by solving the system

$$\left.\begin{aligned} \tfrac{\chi_{11}-\chi_{22}}{2} B_1 - \chi_{12} B_2 &= -\tfrac{\chi_{11}+\chi_{22}}{2} + \bar{\chi}_{11} \\ \chi_{12} B_1 + \tfrac{\chi_{11}-\chi_{22}}{2} B_2 &= \bar{\chi}_{12} \end{aligned}\right\};$$

the determinant vanishes if and only if $\chi_{11} = \chi_{22}$ and $\chi_{12} = 0$, impossible by (ii). Of course, (iii) must hold for $\bar{\chi}$ to be given by (25). □

If hypothesis (i) of lemma 5.1 does not hold, there are two cases: if $\bar{\chi}_{13} = 0$ and $\bar{\chi}_{23} = 0$, then any A_1 and A_2 is a solution, while if $\bar{\chi}_{13}$ or $\bar{\chi}_{23}$ are not zero, there is no solution. (Note that $\bar{\chi} \in V_{e_3}$ would guarantee that $\bar{\chi}_{13} = \bar{\chi}_{23} = 0$.) If (ii) does not hold, there are also two cases: if $\bar{\chi}_{11} - (\chi_{11} + \chi_{22})/2 = 0$ and $\bar{\chi}_{12} = 0$, then any B_1 and B_2 is a solution, while otherwise there is no solution.

Lemma 5.1 shows that, under certain conditions, starting with any $\bar{\chi} \in V$ it is possible to find constants A_1, A_2, B_1 and B_2 such that (25) holds. However, to find a coaxial representation (23), coefficients p_j and angles θ_j satisfying (26) also need to be determined. This is not possible in general. An obvious necessary condition is that $A_1^2 + A_2^2 \leqslant 1$ and $B_1^2 + B_2^2 \leqslant 1$. This condition is not sufficient, however. To determine such a condition, we let

$$A = A_1 + A_2\mathrm{i} \qquad \text{and} \qquad B = B_1 + B_2\mathrm{i} \tag{27}$$

be complex constants, and define

$$M(1) = \begin{pmatrix} 1 & A & \bar{A} \\ \bar{A} & 1 & \bar{B} \\ A & B & 1 \end{pmatrix}, \tag{28}$$

where $\bar{A}$ and $\bar{B}$ are the complex conjugates of A and B, respectively.

Theorem 5.2. *Let $\bar{\chi} \in V$ be of form (8), where the hypotheses of lemma 5.1 are satisfied. Let A_1, A_2, B_1 and B_2 be determined from (25), where $A_1^2 + A_2^2 \leqslant 1$ and $B_1^2 + B_2^2 \leqslant 1$ and suppose that* $\det M(1) \geqslant 0$*, where $M(1)$ is as in (28). Then $\bar{\chi} \in V_{e_3}$, with the coaxial representation (23). Moreover, the minimum number of rotations in this representation is equal to the rank of $M(1)$.*

Proof. Consider the problem of finding, given complex numbers $\gamma_{00}, \gamma_{01}, \gamma_{10}, \gamma_{02}, \gamma_{11}$ and γ_{20}, a non-negative Borel measure μ in the complex plane such that

$$\gamma_{kl} = \int \bar{z}^k z^l \, \mathrm{d}\mu,$$

for $0 \leqslant k + l \leqslant 2$. This is the *quadratic complex moment problem*; see, for example, [6]. This problem contains our existence problem. To see this, note first that (26) and (27) are equivalent to

$$A = \sum_j p_j \, \mathrm{e}^{\mathrm{i}\theta_j} = \int \mathrm{e}^{\mathrm{i}\theta} \, \mathrm{d}\mu \qquad \text{and} \qquad B = \sum_j p_j (\mathrm{e}^{\mathrm{i}\theta_j})^2 = \int (\mathrm{e}^{\mathrm{i}\theta})^2 \, \mathrm{d}\mu, \tag{29}$$

the first and second moments of the complex variable $z = \exp(\mathrm{i}\theta)$ with respect to the discrete measure $\mu = \sum_j p_j \delta_{\theta_j}$. Then it is easy to check that solving (29) is equivalent to solving the quadratic complex moment problem with $\gamma_{00} = \gamma_{11} = \gamma_{22} = 1$, $\gamma_{01} = A$, $\gamma_{10} = \bar{A}$, $\gamma_{02} = B$ and $\gamma_{20} = \bar{B}$.

Curto and Fialkow [6, theorem 1.3] solve the quadratic complex moment problem, proving that a measure μ exists if and only if the matrix

$$\Gamma = \begin{pmatrix} \gamma_{00} & \gamma_{01} & \gamma_{10} \\ \gamma_{10} & \gamma_{11} & \gamma_{20} \\ \gamma_{01} & \gamma_{02} & \gamma_{11} \end{pmatrix}$$

has non-negative eigenvalues, and proving also that μ may be taken to be supported on a set of points whose cardinality equals the rank of Γ. Using the above values for γ_{kl} and (28), we see that $\Gamma = M(1)$. The condition that $M(1)$ has non-negative eigenvalues is equivalent to the condition that the principal minors of $M(1)$ are non-negative. Since $M(1)$ is given by (28) and since $|A| \leqslant 1$ and $|B| \leqslant 1$ by hypothesis, the latter condition holds and the theorem follows. □

Theorem 5.2 can be used to check if there exists a coaxial representation of a general pair $\bar{\chi}^{1,2}$ of mean paramagnetic tensors, given by (17). A candidate oriented rotation axis in the

direction $u \in S^2$ may be determined from the system

$$\left.\begin{aligned} u^*\bar{\chi}^1 u &= u^*\chi^1 u \\ u^*\bar{\chi}^2 u &= u^*\chi^2 u \\ \|u\| &= 1 \end{aligned}\right\}.$$

It is then possible to use the reference frame where $u = e_3$. In this frame, if the constants A_1, A_2, B_1 and B_2 of lemma 5.1 are the same for both $\bar{\chi}^1$ and $\bar{\chi}^2$, and if they satisfy the conditions of theorem 5.2, there is a coaxial representation of $\bar{\chi}^{1,2}$.

6. A representation requiring four rotations

Theorem 4.4 provides a relatively easy example in which at least four rotations are needed to represent a pair of mean paramagnetic tensors, but only under extra assumptions. In this section we present a more complicated example showing that three rotations are in general not enough, without the assumptions of theorem 4.4. More precisely, starting with any pair (χ^1, χ^2) satisfying assumption A, we shall construct a pair $(\bar{\chi}^1, \bar{\chi}^2)$ of mean paramagnetic tensors obtained from (χ^1, χ^2) with a combination of four rotations. We then show that $(\bar{\chi}^1, \bar{\chi}^2)$ has a unique representation and hence cannot be represented with fewer than four rotations.

We use a slight generalization of the coaxial representation. Let

$$O_{u^-} = \{R \in SO_3 : R(u) = -u\}$$

be the set of rotations reversing the orientation of a common fixed axis parallel to $u \in S^2$. Then $R \in O_{e_3^-}$ if and only if $R = S_1 R_{\theta_j}$, where R_{θ_j} is given by (24). Let $O_{e_3^\pm} = O_{e_3} \cup O_{e_3^-}$ and let

$$V_{e_3^\pm} = \left\{ \bar{\chi} = \sum_j R_j^* \chi R_j, \quad R_j \in O_{e_3^\pm} \right\}.$$

Then $\bar{\chi} \in V_{e_3^\pm}$ if and only if

$$\bar{\chi} = \sum_{j:R_{\theta_j} \in O_{e_3}} p_j R_{\theta_j}^* \chi R_{\theta_j} + \sum_{j:R_{\theta_j} \in O_{e_3^-}} p_j R_{\theta_j}^* S_1 \chi S_1 R_{\theta_j}. \tag{30}$$

A computation similar to that in section 5 shows that (24) and (30) imply that the entries of $\bar{\chi}$ are given by

$$\begin{aligned} \bar{\chi}_{11} &= \frac{\chi_{11} + \chi_{22}}{2} + \left(\frac{\chi_{11} - \chi_{22}}{2}\right)\left(B_1^+ + B_1^-\right) - \chi_{12}\left(B_2^+ - B_2^-\right), \\ \bar{\chi}_{12} &= \left(\frac{\chi_{11} - \chi_{22}}{2}\right)\left(B_2^+ + B_2^-\right) + \chi_{12}\left(B_1^+ - B_1^-\right), \\ \bar{\chi}_{13} &= \chi_{13}\left(A_1^+ - A_1^-\right) - \chi_{23}\left(A_2^+ + A_2^-\right), \\ \bar{\chi}_{22} &= \frac{\chi_{11} + \chi_{22}}{2} - \left(\frac{\chi_{11} - \chi_{22}}{2}\right)\left(B_1^+ + B_1^-\right) + \chi_{12}\left(B_2^+ - B_2^-\right), \\ \bar{\chi}_{23} &= \chi_{13}\left(A_2^+ - A_2^-\right) + \chi_{23}\left(A_1^+ + A_1^-\right), \\ \bar{\chi}_{33} &= \chi_{33}, \end{aligned} \tag{31}$$

where

$$\begin{aligned} A_1^+ &= \sum_{j:R_j\in O_{e_3}} p_j\cos\theta_j, & A_2^+ &= \sum_{j:R_j\in O_{e_3}} p_j\sin\theta_j,\\ A_1^- &= \sum_{j:R_j\in O_{e_3^-}} p_j\cos\theta_j, & A_2^- &= \sum_{j:R_j\in O_{e_3^-}} p_j\sin\theta_j,\\ B_1^+ &= \sum_{j:R_j\in O_{e_3}} p_j\cos 2\theta_j, & B_2^+ &= \sum_{j:R_j\in O_{e_3}} p_j\sin 2\theta_j,\\ B_1^- &= \sum_{j:R_j\in O_{e_3^-}} p_j\cos 2\theta_j, & B_2^- &= \sum_{j:R_j\in O_{e_3^-}} p_j\sin 2\theta_j. \end{aligned} \tag{32}$$

Define

$$\begin{aligned} A^+ &= A_1^+ + \mathrm{i}A_2^+, & A^- &= A_1^- + \mathrm{i}A_2^-,\\ B^+ &= B_1^+ + \mathrm{i}B_2^+, & B^- &= B_1^- + \mathrm{i}B_2^-. \end{aligned} \tag{33}$$

Lemma 6.1. *Let $\chi^1 = \chi_d^1$ and let χ^2 satisfy $\chi_{12}^2 \neq 0$. Then*

(i) $\bar\chi_{13}^2 = \chi_{13}^2\big(A_1^+ - A_1^-\big) - \chi_{23}^2\big(A_2^+ + A_2^-\big)$, $\bar\chi_{23}^2 = \chi_{13}^2\big(A_2^+ - A_2^-\big) + \chi_{23}^2\big(A_1^+ + A_1^-\big)$, and
(ii) B^+ and B^- can uniquely be determined from χ^1 and χ^2.

Proof. Using (31) with χ and $\bar\chi$ replaced by χ^2 and $\bar\chi^2$, respectively, we obtain (i).

Since χ_d^1 is diagonal, (31) with χ and $\bar\chi$ replaced by χ_d^1 and $\bar\chi_d^1$, respectively, yields

$$\left.\begin{aligned} \left(\tfrac{\chi_{11}^1-\chi_{22}^1}{2}\right)\big(B_1^+ + B_1^-\big) &= \bar\chi_{11}^1 - \tfrac{\chi_{11}^1+\chi_{22}^1}{2}\\ \left(\tfrac{\chi_{11}^1-\chi_{22}^1}{2}\right)\big(B_2^+ + B_2^-\big) &= \bar\chi_{12}^1. \end{aligned}\right\} \tag{34}$$

Similarly, (31) with χ and $\bar\chi$ replaced by χ^2 and $\bar\chi^2$, respectively, gives

$$\left.\begin{aligned} \left(\tfrac{\chi_{11}^2-\chi_{22}^2}{2}\right)\big(B_1^+ + B_1^-\big) - \chi_{12}^2\big(B_2^+ - B_2^-\big) &= \bar\chi_{11}^2 - \tfrac{\chi_{11}^2+\chi_{22}^2}{2}\\ \left(\tfrac{\chi_{11}^2-\chi_{22}^2}{2}\right)\big(B_2^+ + B_2^-\big) + \chi_{12}^2\big(B_1^+ - B_1^-\big) &= \bar\chi_{12}^2. \end{aligned}\right\} \tag{35}$$

Since the eigenvalues of χ_d^1 are simple, $\chi_{11}^1 \neq \chi_{22}^1$, so $\big(B_1^+ + B_1^-\big)$ and $\big(B_2^+ + B_2^-\big)$ can be uniquely determined from (34) and substituted into (35). Then, since $\chi_{12}^2 \neq 0$, $\big(B_1^+ - B_1^-\big)$ and $\big(B_2^+ - B_2^-\big)$ can be uniquely determined from (35). Finally, (ii) follows since these values can be added and subtracted to obtain B_k^+ and B_k^-, $k = 1, 2$ and hence B^+ and B^-. □

Lemma 6.2. *Let $\bar\chi \in V_{e_3^\pm}$ be as in (30). If $|B^+| + |B^-| = 1$, then there are θ^+ and θ^- such that $\theta_j = \theta^+ (\mathrm{mod}\,\pi)$ for all j such that $R_{\theta_j} \in O_{e_3}$ and $\theta_j = \theta^- (\mathrm{mod}\,\pi)$ for all j such that $R_{\theta_j} \in O_{e_3^-}$. Also,*

$$B^+ = \gamma\exp(2\theta^+\mathrm{i}) \qquad \text{and} \qquad B^- = (1-\gamma)\exp(2\theta^-\mathrm{i}), \tag{36}$$

where $\gamma = \sum_{j:R_j\in O_{e_3}} p_j$.

Proof. Equations (32) and (33) imply that

$$|B^+| = \left|\sum_{j:R_j\in O_{e_3}} p_j\exp(2\theta_j\mathrm{i})\right| \leqslant \sum_{j:R_j\in O_{e_3}} p_j = \gamma. \tag{37}$$

Similarly,

$$|B^-| \leqslant \sum_{j:R_j \in O_{e_3^-}} p_j = 1 - \gamma. \tag{38}$$

Then

$$1 = |B^+| + |B^-| \leqslant \gamma + (1 - \gamma) = 1,$$

so equality holds in both (37) and (38). Equality holds in (37) if and only if the vectors $\exp(2\theta_j \mathrm{i})$ have the same direction, and this occurs if and only if there exists θ^+ such that $2\theta_j = 2\theta^+ (\mathrm{mod}\, 2\pi)$. Then $B^+ = \gamma \exp(2\theta^+ \mathrm{i})$, and similarly we obtain $B^- = (1 - \gamma) \exp(2\theta^- \mathrm{i})$. □

Theorem 6.3. *Suppose that χ^1 and χ^2 satisfy assumption A. Then there exists $\bar{\chi}^{1,2} = (\bar{\chi}^1, \bar{\chi}^2) \in V^{1,2}$ with a unique representation (17) involving exactly four rotations with positive coefficients p_j.*

Proof. We may assume that $\chi^1 = \chi^1_d$ is diagonal. Suppose first that $\chi^2_{12} \neq 0$. By lemma 4.3, χ^2_{13} and χ^2_{23} cannot both be zero, so $\chi^2_{13} + \mathrm{i}\chi^2_{23} = r \exp(\theta \mathrm{i})$ for some $0 \leqslant \theta < 2\pi$ and $r > 0$. Choose θ_1 and θ_2 so that

$$\theta_1 + \theta \neq \theta_2 - \theta, \tag{39}$$

and define

$$\bar{\chi}^k = \tfrac{1}{4} R^*_{\theta_1} \chi^k R_{\theta_1} + \tfrac{1}{4} R^*_{\theta_1+\pi} \chi^k R_{\theta_1+\pi} + \tfrac{1}{4} R^*_{\theta_2} S_1 \chi^k S_1 R_{\theta_2} + \tfrac{1}{4} R^*_{\theta_2+\pi} S_1 \chi^k S_1 R_{\theta_2+\pi}, \tag{40}$$

for $k = 1, 2$. From this definition and (32), we obtain

$$\begin{aligned} A^+ &= \tfrac{1}{4} (\exp(\theta_1 \mathrm{i}) + \exp((\theta_1 + \pi)\mathrm{i})) = 0, \\ A^- &= \tfrac{1}{4} (\exp(\theta_2 \mathrm{i}) + \exp((\theta_2 + \pi)\mathrm{i})) = 0, \end{aligned} \tag{41}$$

$$B^+ = \tfrac{1}{2} \exp(2\theta_1 \mathrm{i}) \qquad \text{and} \qquad B^- = \tfrac{1}{2} \exp(2\theta_2 \mathrm{i}). \tag{42}$$

By lemma 6.1(i) and (41), we also obtain

$$\bar{\chi}^2_{13} + \mathrm{i}\bar{\chi}^2_{23} = \left(\chi^2_{13} + \mathrm{i}\chi^2_{23}\right) A^+ - \left(\chi^2_{13} - \mathrm{i}\chi^2_{23}\right) A^- = 0. \tag{43}$$

To show that representation (40) is unique, consider an arbitrary representation of $\bar{\chi}^{1,2}$ of form (17). Since χ^1_d is given by (5), (40) implies that $\bar{\chi}^1_{13} = \bar{\chi}^1_{23} = 0$ and $\bar{\chi}^1_{33} = \mu^1_{\max}$, so e_3 is an eigenvector of $\bar{\chi}^1$ corresponding to $\mu^1_{\max}$. Moreover

$$\mu^1_{\max} = e_3^* \bar{\chi}^1 e_3 = \sum_j p_j (R_j e_3)^* \chi^1_d R_j e_3 \leqslant \sum_j p_j \mu^1_{\max} = \mu^1_{\max},$$

so that $R_j e_3 = \pm e_3$ for any j. It follows that $R_j \in O_{e_3^\pm}$ for any j. This shows that in our arbitrary representation $\bar{\chi}^1$ and $\bar{\chi}^2$ are actually both of form (30). Then (32) holds, so by lemma 6.1(ii), B^+ and B^- are uniquely determined. Therefore their values are given by (42), so $|B^+| = |B^-| = 1/2$ and we can apply lemma 6.2 and determine angles θ^+ and θ^- such that $\theta_j = \theta^+ (\mathrm{mod}\, \pi)$ for all j such that $R_{\theta_j} \in O_{e_3}$ and $\theta_j = \theta^- (\mathrm{mod}\, \pi)$ for all j such that $R_{\theta_j} \in O_{e_3^-}$. Comparing (42) with (36), we see that without loss of generality, $\theta^+ = \theta_1$ and $\theta^- = \theta_2$. It follows that the arbitrary representation must be of the form

$$\bar{\chi}^k = p_1 R^*_{\theta_1} \chi^k R_{\theta_1} + p_2 R^*_{\theta_1+\pi} \chi^k R_{\theta_1+\pi} + p_3 R^*_{\theta_2} S_1 \chi^k S_1 R_{\theta_2} + p_4 R^*_{\theta_2+\pi} S_1 \chi^k S_1 R_{\theta_2+\pi}, \tag{44}$$

where $p_j \geqslant 0$, $j = 1, \ldots, 4$ and $\sum_j p_j = 1$, for $k = 1, 2$.

Comparing (36) and (42), we find that $\gamma = p_1 + p_2 = p_3 + p_4 = 1/2$, so we may write

$$p_1 = \frac{s}{2}, \qquad p_2 = \frac{1-s}{2}, \qquad p_3 = \frac{t}{2} \qquad \text{and} \qquad p_4 = \frac{1-t}{2},$$

where $0 \leqslant s, t \leqslant 1$. Using (32), we find that for representation (44),

$$A^+ = (s - 1/2)\exp(\theta_1 \mathrm{i}) \qquad \text{and} \qquad A^- = (t - 1/2)\exp(\theta_2 \mathrm{i}). \tag{45}$$

By lemma 6.1(i) and (43),

$$\begin{aligned} 0 = \bar{\chi}^2_{13} + \mathrm{i}\bar{\chi}^2_{23} &= \left(\chi^2_{13} + \mathrm{i}\chi^2_{23}\right)A^+ - \left(\chi^2_{13} - \mathrm{i}\chi^2_{23}\right)A^- \\ &= (s - 1/2)\exp((\theta_1 + \theta)\mathrm{i}) - (t - 1/2)\exp((\theta_2 - \theta)\mathrm{i}). \end{aligned}$$

Our choice of θ implies that $s = t = 1/2$ and hence $p_j = 1/4$ for $j = 1, \ldots, 4$, proving the theorem under the assumption $\chi^2_{12} \neq 0$.

Finally, if $\chi^2_{12} = 0$, then $\chi^2_{23} \neq 0$ by lemma 4.3. Then we can repeat the whole construction, replacing e_3 with e_1 and $\mu^1_{\min}$ with $\mu^1_{\max}$, to obtain the required example. Of course, all the results of this section must be reformulated accordingly. □

7. Application to experimental NMR data

In the previous sections we studied, from the mathematical point of view, the problem of reconstructing the position of a moving protein terminal domain using RDC spectroscopy data. Any sufficient number of measurements involving two different metal ions is equivalent to knowledge of the ten coefficients defining the mean paramagnetic tensors. It is evident that there is no hope of reconstructing the position of the terminal with ten numbers, apart from special situations such as the extreme case, mentioned in the introduction, when the terminal domain does not move. In the case of calmodulin the C-terminal domain is effectively moving, because the absolute value of the eigenvalues of the mean paramagnetic tensors is about 10% of those of the corresponding magnetic susceptibility tensors (see [2]).

The information we can gather from the mean paramagnetic tensors is of a probabilistic nature. For any particular relative orientation of the moving C-terminal domain, there is a maximum probability $0 \leqslant p_{\max} \leqslant 1$ that the domain assumes that orientation. The conformational space can then be sampled to look for the most favoured orientations, that is, those having a large maximum probability.

If data from a single metal ion are available, the maximum probability is given by (13), and can easily be calculated using the bound on the eigenvalues given in theorem 3.3. A simple way to combine data from two different metal ions would be to use the minimum of the two values found, that is, the quantity $t^{1,2}$ of corollary 4.7. While this is perfectly feasible, one would only end up with a rough estimate (from above) of $p_{\max}$.

The standard way to calculate $p_{\max}$ using real data is via the following fitting procedure. For each of two metal ions indexed by $j = 1$ or 2, we are given a set $\left\{\delta^j_{a_i b_i}\right\}$ of RDC experimental measurements for couples of atoms a_i and b_i. The collections of atoms a_i and b_i need not be the same for the two metal ions. Given a suitable finite set of rotations R_j and probabilities p_j summing to one, the mean paramagnetic tensors can be obtained from (17). For $j = 1, 2$, we can replace $\bar{\chi}$ by $\bar{\chi}^j$ in (3) to calculate the corresponding mean RDC values $\left\{\bar{\delta}^j_{a_i b_i}\right\}$. A target function TF can then be defined to measure the difference between the observed measurements and the corresponding calculated values. Roughly speaking, this is given by

$$\mathrm{TF} = \sum_i \left\|\bar{\delta}^1_{a_i b_i} - \delta^1_{a_i b_i}\right\| + \sum_i \left\|\bar{\delta}^2_{a_i b_i} - \delta^2_{a_i b_i}\right\|,$$

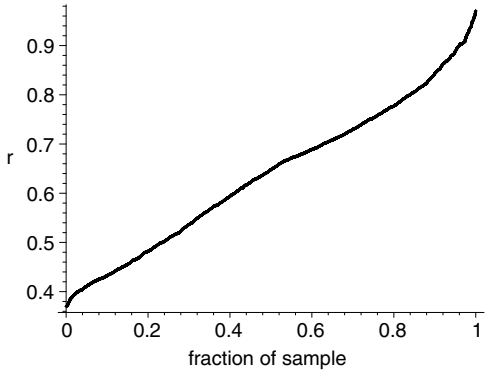

Figure 2. Comparison of $p_{\max}$ and $t^{1,2}$.

for a suitable norm $\|\cdot\|$. In practice, TF is more complicated, involving error filtering and multi-stage normalization.

With the target function in hand, the first step is to determine an optimal noise level, that is, the smallest value of TF using ten relative orientations, each represented by a rotation, with variable probabilities, as suggested by theorem 4.2. Next, the conformational space of relative orientations, again represented by rotations, must be sampled. For each rotation R_0 of the sample, we look for the corresponding maximum probability $p_{\max}$. A tentative probability p_0 is such that $p_0 \leqslant p_{\max}$ if and only if there exist other rotations with probabilities summing to $1 - p_0$ which, when combined with the fixed rotation R_0 with probability p_0, give a value of TF not significantly larger than the optimal noise level. The largest p_0 for which this can be done is a reasonable estimate (from below) of $p_{\max}$ for R_0. The search for $p_{\max}$ involves a bisection method with respect to the variable p_0.

The use of ten rotations in the fitting procedure can bring numerical problems. We seek the absolute minimum of a very bumpy function of 39 real variables, three for each rotation and nine for the probabilities. This is not an easy task, even when methods such as simulated annealing are used. It is advisable to use as much precision as possible in the single minimization required for the determination of the optimal noise level, because it is a reference value employed throughout the procedure. This is not feasible when sampling the conformational space, due to the very large number of minimizations required. A practical way of reducing the number of rotations is to start the minimizations using four variable rotations, according to the results of section 6. It can then be checked whether, repeating the procedure with five rotations, the value of $p_{\max}$ remains the same. It can be shown that if this is the case, the estimate found is a good one.

We made some numerical experiments to measure the improvement obtained by using $p_{\max}$ instead of $t^{1,2}$. We used the RDC of NH couples of the C-terminal domain of calmodulin. Data arising from Tb^{3+} and Tm^{3+} metal ions were obtained by the Center for Magnetic Resonance of the University of Florence.

Figure 2 shows the values of $r = p_{\max}/t^{1,2}$ for a sample of 8000 rotations. The graph should be read in the following way. A point (x, y) of the graph means that a value of r smaller (i.e., better) than y is obtained for a fraction x of the rotations considered in the sample. For example, half of the rotations in the sample showed a maximum probability reduced by a factor 0.65 or better. This reduction should aid the localization of the zones where the C-terminal domain has the largest probability of being found.

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