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# Molecular Symmetry as an Aid to Geometry Determination in Ligand Protein Complexes

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**Dipole–dipole couplings between pairs of spin $\frac{1}{2}$ nuclei, which can be measured from NMR spectra in field-ordered media, offer useful constraints on the orientation of various fragments in molecular systems. However, the orientation of fragments relative to a molecule fixed reference frame is often key to complete structure determination. Here, we demonstrate that the symmetry properties of molecular complexes can aid in the definition of a reference frame. It is shown that a threefold rotational symmetry axis dictates the direction and symmetry of the experimentally determined order tensor for $\alpha$-methyl-mannose in fast exchange among the three symmetry-related binding sites of mannose binding protein. This approach facilitates studies of the geometry of the ligand in the protein–ligand complex and also may provide a novel route to structure determination of a homomultimeric protein.** 

*Key Words:* **symmetry; order tensors; homomultimer; ligand bound geometry; mannose binding protein.**

## INTRODUCTION

Long-range orientational constraints derived from measurements of residual dipolar couplings in partially aligned systems are increasingly being used in the structure determination of macromolecules (*1–6*). These can be included as an error function in a simulated annealing approach (*4*) or they can be used to more directly determine the preferred orientation of known molecular fragments that are part of a macromolecule or a complex involving several molecules (*9*). In the latter approach, the orientation and principal elements of an order tensor for each fragment are determined (*7*). Since for a rigid system, the orientation and principal elements of the order tensor must appear the same from the point of view of each fragment, a structure can be assembled by aligning frames (*8, 9*). This cannot be done without some ambiguity because inversion of frame axes does not affect predicted couplings. However, with minimal additional structural constraints, the proper frame can be selected. A potentially more important limitation is that each determination may require measurement of as many as five independent residual dipolar couplings because an order tensor has five independent elements. Measuring a large number of couplings can be a challenging task when some of the fragments are part of a large system whose resonances cannot be readily assigned. Here, we illustrate how symmetry properties of complex systems can define an appropriate reference frame obviating the need for an independent order tensor determination of one or more of the components.

The particular system we choose for illustration involves the interaction of a saccharide ligand, $\alpha$-methyl-mannose (AMM), with mannose binding protein (MBP), a 53-kDa homotrimeric protein having a threefold rotational symmetry axis and three sugar binding sites related by the same symmetry operation as depicted in Fig. 1. We have previously illustrated the general utility of dipolar coupling measurements in determining the geometry of the bound ligand in this system (*10*). Here, we show formally how the presence of the three symmetry-related binding sites in MBP has a unique consequence on the observed order tensor for AMM bound to MBP, and how this leads to a determination of the AMM bound geometry without the need for any dipolar coupling measurements on the protein itself.

## THEORY

While the effects of molecular symmetry on order tensors were recognized as early as the 1960s in the field of liquid crystal NMR, their utility in structural studies of weakly aligned macromolecules has not been fully exploited (*7*). Here, we formalize the effects of rotational symmetry on residual dipolar couplings in terms of irreducible spherical tensors and describe how the threefold symmetry of MBP can be used to define an appropriate reference frame for determining the AMM-bound geometry. Irreducible spherical tensors have recently been used to describe alignment effects on residual dipolar couplings and the reader is referred to this work for additional clarification (*2*).

The lab frame Hamiltonian for the dipolar interaction be-

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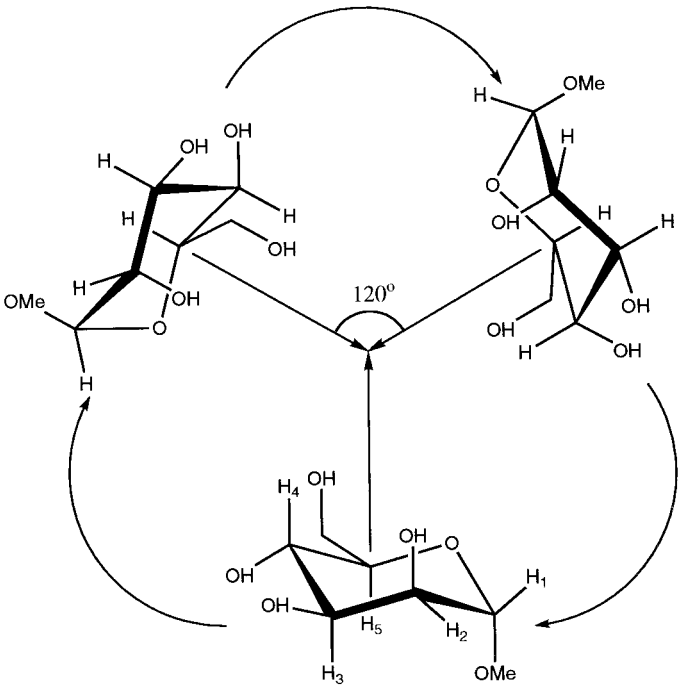


**FIG. 1.** Depiction of the geometrical arrangement of three AMM molecules when bound to three binding sites in MBP related by threefold rotational symmetry. The presence of axial symmetry leads to an axially symmetric tensor with the direction of highest order pointing along the symmetry axis.

tween two nuclei can be given by a scalar contraction of spin and spatial tensor components

$$\frac{H_{DD}}{k} = T_0^2 D_0^2, \qquad [1]$$

where $k$ is a constant that depends on the interacting nuclei and $T_0^2$ and $D_0^2$ are elements of the second rank tensor operators that describe the spin and spatial parts of the interaction respectively; nonsecular terms are not included in Eq. [1] since their time dependence will render them ineffective in determining the energies of the system. For systems in the high field limit, the above equation can be used to give a simple expression for the separation of pairs of lines in a multiplet produced by dipolar couplings,

$$\frac{D_{resid}}{k'} = D_0^2(lab). \qquad [2]$$

Here $k'$ is a constant that has absorbed numerical factors coming from the spin operators as well as the previous constant $k$ and the designation "lab" denotes our choice of the magnetic field direction as the $z$ axis in this frame. The above expression is adequate for a static crystalline system but the presence of molecular motion renders $D_0^2(lab)$ a complex function of orientational averaging. For AMM bound to partially aligned MBP, this averaging will be described by a combination of the overall reorientation of the MBP/AMM complex and exchange of AMM between the three symmetry related binding sites in MBP (Fig. 1). In the case that the two averaging processes are not correlated, i.e., MBP's alignment is not affected by AMM binding, the two averaging processes can be treated independently. To make the effects of averaging clear, we transform $D_0^2(lab)$ through two intermediate reference frames in which the effects of the overall motion of MBP and the averaging among symmetry related sites are easily described. This permits derivation of a closed form expression for residual dipolar couplings for AMM bound to MBP from which the effects of symmetry on dipolar couplings and order tensors can be inferred.

Transformation between frames is easily accomplished using 2nd rank Wigner rotation matrices, with elements $D^2_{k'k}(\alpha, \beta, \gamma)$. The first transformation relates $D_0^2(lab)$ to elements written in the MBP's principal axis system (PAS) for molecular ordering. This transformation is time dependent due to MBP's molecular reorientation. The time dependence is represented by Euler rotations $\Omega(t) = \alpha(t), \beta(t), \gamma(t)$ that relate an instantaneous orientation of MBP's principal order frame to the magnetic field,

$$D_0^2(lab, t) = \sum_{k'=-2}^{2} D^2_{k'}(mbp, t) D^2_{k'0}(\Omega(t)). \qquad [3]$$

The second transformation relates elements written in the principal ordering frame to elements written in MBP's threefold symmetry frame where the symmetry axis is along the $z$ axis. This transformation is time *independent* and is represented by Euler rotations, ($\Phi$):

$$D^2_{k'}(mbp, t) = \sum_{m=-2}^{2} D^2_m(symm, t) D^2_{mk'}(\Phi). \qquad [4]$$

Finally, we can relate elements written in MBP's symmetry frame to elements written in the PAS of the dipolar interaction using the Euler transformation denoted by ($\Theta$). In the latter case, only one element, $D_0^2(pas)$, is finite due to the properties of the dipolar interaction (*2*):

$$D^2_m(symm, t) = \sum_{l=-2}^{2} D^2_l(pas) D^2_{lm}(\Theta(t)) = D_0^2(pas) D^2_{0m}(\Theta(t)). \qquad [5]$$

The final time dependence is now in the angles $\Theta$ that describe motion among symmetry related sites in MBP in a frame in which the symmetry axis is the $z$ axis. Since we are interested

in averaging the dipolar interaction, we can replace explicit time dependent terms with averages. Denoting averaging with brackets, the $p$-fold rotational symmetry averaging of terms in Eq. [5] is given by

$$\langle D^2_{0m}(\Theta(t))\rangle = \frac{1}{p}\sum_{q=1}^{p} D^2_{0m}\left(\alpha + q\,\frac{2\pi}{p},\,\beta,\,\gamma\right). \qquad [6]$$

It can be shown that for $p \geq 3$, all Wigner matrix elements vanish except for $D^2_{00}$ (*12*). Furthermore, as $D^2_{00}$ depends only on the angles $\beta$,

$$\langle D^2_{00}\rangle = D^2_{00}(\beta)$$

and

$$\langle D^2_m(symm)\rangle = D^2_0(pas)\,D^2_{00}(\beta). \qquad [7]$$

Substituting $\langle D^2_0(symm)\rangle$ from Eq. [7] for $D^2_{\mathrm{m}}(symm, t)$ in Eq. [4], and substituting the resulting expression for $D^2_{k'}(mbp, t)$ into Eq. [3] yields an expression for $D^2_k(lab, t)$,

$$D^2_0(lab, t) = \sum_{k'=-2}^{2} D^2_0(pas)\,D^2_{00}(\beta)\,D^2_{0k'}(\Phi)\,D^2_{k'0}(\Omega(t)). \qquad [8]$$

Substitution into Eq. [1] leads to

$$\frac{D_{resid}}{k'} = \sum_{k'=-2}^{2} D^2_0(pas)\,D^2_{00}(\beta)\,D^2_{0k'}(\Phi)\langle D^2_{k'0}(\Omega(t))\rangle, \qquad [9]$$

where the angle brackets again indicate the time average over MBP's molecular reorientation. The averaged elements in Eq. [9] can be related to the axial and rhombic components of MBPs alignment tensor ($\hat{\mathrm{A}}$) (*2*),

$$\langle D^2_{00}(\Omega(t))\rangle = \Delta\mathrm{A}$$
$$\langle D^2_{10}(\Omega(t))\rangle = \langle D^2_{-10}(\Omega(t))\rangle = 0$$
$$\langle D^2_{20}(\Omega(t))\rangle = \langle D^2_{-20}(\Omega(t))\rangle = \sqrt{\frac{3}{8}}\,\delta\mathrm{A}$$

and

$$\frac{D_{resid}}{k'} = \left\{\Delta\mathrm{A}D^2_{00}(\Phi) + \sqrt{\frac{3}{8}}\,\delta\mathrm{A}[D^2_{02}(\Phi) + D^2_{0-2}(\Phi)]\right\}D^2_{00}(\beta). \qquad [10]$$

Note that the first term in Eq. [10] is a constant. It is a measure of AMM's order in the system as a result of a combination of MBP's ordering tendency and its own averaging among symmetry related states. The only dependence on the geometry of a spin–spin interaction vector in the molecular symmetry frame is in

$$D^2_{00}(\beta) \propto \left(\frac{3\cos^2\beta - 1}{2}\right).$$

The dependence is only on $\beta$, the angle relative to the $z$ axis (the symmetry axis), and not on rotation about the $z$ axis. Hence the system from the point of view of AMM has axial symmetry.

The above expression can be related to the more typical expression for couplings in the presence of axial symmetry ordering using a principal order parameter $S_{zz}$,

$$\frac{D_{resid}}{k'} = S_{zz}\left(\frac{3\cos^2\theta - 1}{2}\right)$$
$$S_{zz} = \left\{\Delta\mathrm{A}\left(\frac{3\cos^2\theta' - 1}{2}\right) + \frac{3}{4}\,\delta\mathrm{A}(\sin^2\theta'\cos 2\phi')\right\}, \qquad [11]$$

where $\theta'$ and $\phi'$ are spherical angles which define the position of the MBP symmetry axis in MBP's PAS. The above expression holds for molecules possessing a threefold or greater symmetry axis, including MBP itself, regardless of the nature of the alignment force. We can therefore conclude that the experimentally determined order tensor for AMM bound to trimeric MBP will be axially symmetric and the principal axis will point along MBP's three fold symmetry axis. This provides a reference frame that can be used to constrain the bound geometry of AMM, without the need to independently determine an order tensor for MBP. This is valuable information for structure determination.

## RESULTS

It is possible to experimentally verify some aspects of this predicated behavior by examining the symmetry properties of the order tensor determined using residual dipolar coupling measurements from AMM bound to trimeric MBP. In the pyranose ring of AMM, there are five directly bonded $^1$H–$^{13}$C pairs, two of which point in unique directions (C1–H1, C2–H2), and three of which depart by small amounts from a third direction (C3–H3, C4–H4, C5–H5). While these interaction vectors can be confidently used to determine the three independent elements of an axially symmetric tensor, a more general order tensor determination requires that the small orientational differences between internuclear vectors C3–H3, C4–H4, and C5–H5 be known to a high level of confidence. The dependence on this requirement can be reduced by including

**TABLE 1**
**Residual Dipolar Coupling Measurements for Free and Bound AMM**

| Internuclear vectors in AMM | Free AMM[a] | AMM bound to MBP[b] |
|---|---|---|
| C1–H1 (Hz) | −3.5 ± 0.7 | −22.6 ± 2.2 |
| C2–H2 (Hz) | −7.3 ± 0.7 | −28.7 ± 2.2 |
| C3–H3 (Hz) | 9.2 ± 0.9 | 20 ± 3.5 |
| C4–H4 (Hz) | 12.1 ± 0.7 | 23.9 ± 2.9 |
| C5–H5 (Hz) | 7.8 ± 0.5 | 30.5 ± 3 |
| C6–H6(S) (Hz) | 3.0 ± 0.9 | 25.9 ± 6.1 |
| C6–H6′(R) (Hz) | −5.0 ± 0.9 | −21.3 ± 6.3 |

[a] Residual dipolar couplings were calculated from the differences between couplings measured at 25 and 39°C for AMM dissolved in a 5% bicelle. The program Xrambo was used in data analysis to extract coupling values and associated errors (*17, 10*). For the C6 data, only a single slice through the center of the doublet was used to generate frequency domain data as input for Xrambo analysis.

[b] Residual dipolar couplings were calculated using the residual dipolar coupling data from AMM both in the presence and absence of MBP and using a binding constant to calculate the residual dipolar contributions from the AMM bound state. Coupling values and associated errors were extracted using the program Xrambo (*17, 10*). For the C6 data, only a single slice through the center of the doublet was used to generate frequency domain data as input for Xrambo analysis.

two additional independent measurements from the internuclear vectors C6–H6 and C6–H6′ in the hydroxymethyl group. These measurements have their own complications because these internuclear vectors can vary through rotations about the C5–C6 linkage. However, reasonable models for these variations exist. The rotations generally sample three energetically favorable staggered conformations, denoted by "gg," "gt," and "tg" (*13*). Moreover, it is well documented, from interpretation of high resolution NMR data using empirical Karplus equations, that the gg conformation is the dominant conformation for AMM, with approximate populations given by (gg:gt:tg, 6:4:0) (*13*). This information can be used to include measurements from the hydroxymethyl group in the order tensor determination for AMM.

We have previously reported measurements of five $^{1}H–^{13}C$ residual dipolar couplings between internuclear vectors in the pyranose ring of $^{13}C$-enriched AMM, dissolved in a dilute bicelle medium both in the presence and absence of MBP. Details of these experiments have previously been described (*10*) and residual dipolar coupling measurements and corresponding errors are summarized in Table 1. In addition, we include measurements from the hydroxymethyl group (C6–H6 and C6–H6′).

Using these residual dipolar couplings, assuming the dominant gg conformation for the hydroxymethyl group geometry, and using coordinates from an AMBER minimized AMM structure, order tensor elements were determined for AMM using a singular value decomposition approach (*14*). These determinations were repeated 10,000 times, sampling dipolar coupling values from a normal distribution about measured values using the estimated errors. A satisfactory description of the principal order tensor elements is presented in Fig. 2.

A discussion of the symmetry property of the order tensor is most conveniently done in terms of an asymmetry parameter $\eta$ defined in terms of the principal elements of the order tensor as $(S_{xx} - S_{yy})/S_{zz}$, using the convention $|S_{zz}| \geq |S_{yy}| \geq |S_{xx}|$. In Fig. 2, we show possible asymmetry parameter values determined for AMM in the absence of MBP (light bars) and in the presence of MBP (dark bars). In the absence of MBP, the order tensor is moderately asymmetric with the most probable asymmetry parameter $\eta_{max} = 0.54$. No solutions were obtained when using either the gt or tg conformations for the hydroxymethyl group, consistent with the presence of a dominant gg conformation. However, mixtures of the gt and gg conformation with ranges of populations (0:6–3:6) do produce solutions. The departure from literature values of gt:gg = 4:6 might be due to the oversimplification in using a two state model to describe what is more likely a continuos distribution of conformations, or there may be some small media dependent perturbations.

Residual dipolar couplings for AMM bound to MBP can be extracted using the known binding constant for MBP and the measured couplings, in the presence and absence of MBP (*10*). These derived values are also included in Table 1. The resulting asymmetry parameters for AMM bound to MBP, shown in Fig. 2 as black bars, make it clear that the determined order tensor is nearly axially symmetric. The most probable asymmetry parameter $\eta_{max} = 0.12$ is near the theoretical value of $\eta_{theo} = 0.0$. While the asymmetry solutions for free and bound AMM slightly overlap, the principal order parameter and orientation of the determined order tensors (not shown here) are very different, confirming a unique MBP effect on residual dipolar couplings measurements in AMM. Again, no solutions were found when using either the gt or tg conformation for the hydroxymethyl group, suggesting that the gg conformation remains the dominant conformation in the AMM bound state.

## DISCUSSION

The determined order tensor for AMM bound to MBP is nearly as we anticipated from our analysis of averaging about a threefold symmetry axis; it is axially symmetric. The averaging also predicts that the determined direction of highest order will point along the symmetry axis of MBP. This alignment has been used to facilitate determination of the binding geometry of AMM in MBP (*10*).

The symmetry effects described here for AMM bound to MBP can also be used to great advantage in studying other protein ligand interactions. Many proteins involved in cell surface recognition exist and function in homomultimeric states (*15, 16*). The cooperative interactions of these mulltim-

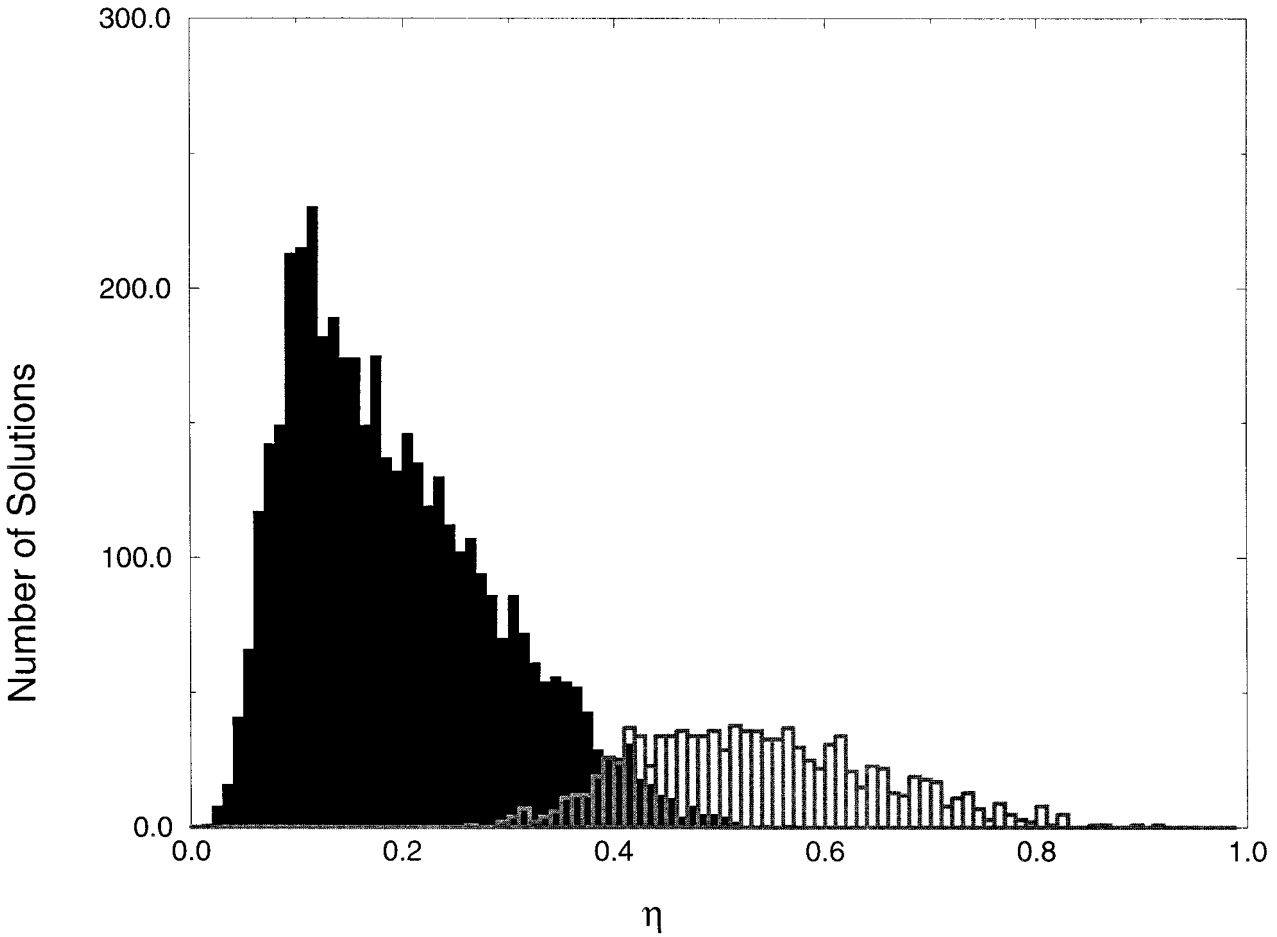


**FIG. 2.** Histogram plot of Determined Asymmetry parameters $|\eta|$ for Free (open bars) and Bound (filled bars) AMM. Ten thousand sets of order tensor elements were determined that reproduce residual dipolar coupling measurements within experimental certainty. The distribution of derived asymmetry parameters are shown.

ers amplify binding affinities and specificities (*16*). Thus, there should be a large number of systems for which the use of symmetry in defining molecular geometry is useful. Furthermore, this approach overcomes size limitations often imposed by conventional assignment strategies. The MBP trimer is large (53 kDa) and assignment of the NMR resonances needed for an independent determination of its order tensor will not be easy.

In cases where the protein assignments are available, however, it may be that structural aspects of homomultimeric proteins themselves can be obtained by taking advantage of these symmetry effects. Measurement of $^{15}N$–$^{1}H$ residual dipolar couplings in MBP can be used in the determination of the orientation of the symmetry axis from the point of view of a domain and a hence a structural model for the multimer can be generated (*9*). This may overcome a long standing obstacle in structure determination of homooligomers from NOE data, namely, the difficulty in distinguishing intra- from interresidue NOEs (*11*).

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