

## COMMUNICATIONS

# Graphical Analysis of the Relative Orientation of Molecular Alignment Tensors for a Protein Dissolved in Two Different Anisotropic Media

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In order to determine precise three-dimensional structures of proteins by residual dipolar coupling constants as the major or even exclusive structural constraints, it is essential to use two anisotropic media. In doing so, a reliable and versatile method for estimating the relative orientation of the alignment tensors for the molecules dissolved in different anisotropic media is required. In this communication, we present a new graphical approach for this purpose, which does not require structural information of the target molecules. The correlation map for the two independent data sets of residual dipolar coupling constants, which can be obtained for the molecules in different anisotropic media, strongly depends on the relative orientation of the alignment tensors. We have simulated the correlation maps for all possible combinations of the Euler angles, which transform one alignment tensor to the other, and compared them to the experimental data sets reported for labeled human ubiquitin. This simple graphical method affords a useful starting point for the structural determinations using residual dipolar couplings. © 2002 Elsevier Science

**Key Words:** residual dipolar coupling; anisotropic media; relative orientation of alignment tensors; correlation map; structural determination by NMR.

### INTRODUCTION

Direct measurements of the relative orientations of internuclear dipolar vectors, internuclear distances, and angles for biomacromolecules through residual dipolar couplings have become possible by developments in the applications of various anisotropic media to partially align the macromolecules in solution (1, 2). These parameters are then used as additional constraints to more precisely determine the three-dimensional structures of proteins and nucleic acids in conjunction with nearly complete sets of NOE, coupling constant, and chemical shift data (3–5). However, the orientations of the dipolar vectors in the alignment tensor cannot be uniquely determined from a single set of residual dipolar coupling measurements, because of the

uncertainty due to the taco-shaped cone nature of the unique axis of the alignment tensor (6). This problem can be alleviated by using an additional orientation of the alignment tensor. However, the relative orientation is not known *a priori*, and so the degrees of freedom are optimized using a least-squares-based search procedure (7). In this paper, we propose a new method for estimating the relative orientation between two different molecular alignment tensors in the absence of any prior structural information. This method is demonstrated using labeled ubiquitin (76 residues) as a model system.

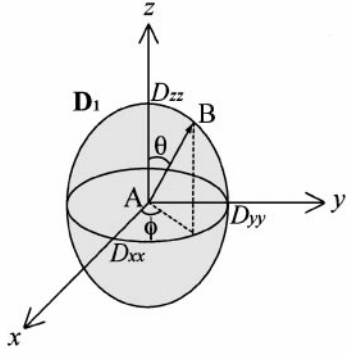
The residual dipolar coupling  $D^{\text{AB}}(\theta, \phi)$  between two directly coupled nuclei A and B can be expressed as (5, 8)

$$\begin{aligned} D^{\text{AB}}(\theta, \phi) &= D_{20}^{\text{PAS}} \sum_{k=-2}^2 D_{0k}(\phi, \theta, 0) \langle D_{k0}(\Omega(t)) \rangle \\ &= D_a^{\text{AB}} \left\{ (3 \cos^2 \theta - 1) + \frac{3}{2} R (\sin^2 \theta \cos 2\phi) \right\}, \end{aligned} \quad [1]$$

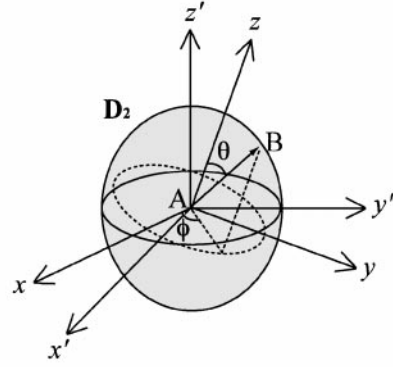
where the transformation from the principal axis frame (PAS) of the dipolar interaction to the molecular alignment tensor and further into the laboratory frame is accomplished using second-rank Wigner rotation matrices with elements  $D_{k'k}(x)$ .  $D_a^{\text{AB}}$  represents the axial component of the molecular alignment tensor for the dipolar pair A–B, and  $R$  is the rhombicity of this tensor.  $\theta$  and  $\phi$  are the polar and azimuthal angles that describe the orientation of the A–B interatomic vector with respect to the molecular alignment frame.  $\Omega(t)$  is the time-dependent Euler angles which transform the molecular alignment tensor from the PAS of the dipolar interaction to the laboratory frame. The time dependence of these angles arises from the molecular reorientation. The angled brackets denote that a time average has been performed to account for this reorientation. Although there is only one value of  $D_a^{\text{AB}}$  and  $R$  for each tensor, there exists one pair of  $\theta$  and  $\phi$  for each dipolar pair. When the molecular alignment tensor is transformed into a second molecular alignment tensor by the Euler angles ( $\alpha, \beta, \gamma$ ) as shown in Fig. 1, the

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Molecular alignment tensor frame 1(x,y,z)



Molecular alignment tensor frame 2(x',y',z')



**FIG. 1.** A schematic representation of the two molecular alignment frames in which the tensors  $\mathbf{D}_1(x, y, z)$  and  $\mathbf{D}_2(x', y', z')$  for a dipolar pair A–B are defined.  $\theta$  and  $\phi$  are the polar and azimuthal angles, respectively.  $\mathbf{D}_1$  is transformed into alignment tensor frame 2 using the Euler rotation matrix  $R(\alpha, \beta, \gamma)$ . The transformed  $\mathbf{D}_1$  in frame 2 is denoted by the dashed hemisphere (i.e., for clarity only the  $D_{xx}$ ,  $D_{yy}$  plane is shown). The axial ( $D_a$ ) and rhombic ( $D_r$ ) components of the alignment tensor,  $\mathbf{D}$ , are defined as  $\frac{1}{3}[D_{zz} - (D_{xx} + D_{yy})/2]$  and  $\frac{1}{3}(D_{xx} - D_{yy})$ , respectively. The rhombicity ( $R$ ) is defined as  $D_r/D_a$ . The principal axis values (i.e.,  $D_{xx}$ ,  $D_{yy}$ , and  $D_{zz}$ ) of alignment tensor 1 (i.e.,  $\mathbf{D}_1$ ) are indicated in alignment frame 1.

dipolar coupling can be rewritten as

$$\begin{aligned}
 D^{AB}(\theta, \phi, \alpha, \beta, \gamma) &= D_{20}^{\text{PAS}} \sum_{k=-2}^2 D_{0k}(\phi, \theta, 0) D_{kk'}(\alpha, \beta, \gamma) \langle D_{k'0}(\Omega(t)) \rangle \\
 &= D_a^{AB} \left[ \frac{1}{2}(3 \cos^2 \theta - 1)(3 \cos^2 \beta - 1) - \frac{3}{2} \sin 2\theta \sin 2\beta \right. \\
 &\quad \times \cos(\alpha + \phi) + \frac{3}{2} \sin^2 \theta \sin^2 \beta \cos(2\alpha + 2\phi) \\
 &\quad + \frac{3}{2} R \left\{ \frac{1}{2}(3 \cos^2 \theta - 1) \sin^2 \beta \cos 2\gamma + \frac{1}{2} \sin 2\theta \right. \\
 &\quad \times \{ \sin \beta (\cos \beta + 1) \cos(\alpha + 2\gamma + \phi) + \sin \beta (\cos \beta - 1) \\
 &\quad \times \cos(\alpha - 2\gamma + \phi) \} + \sin^2 \theta \left\{ \cos^4 \left( \frac{\beta}{2} \right) \cos(2\alpha + 2\gamma + 2\phi) \right. \\
 &\quad \left. \left. + \sin^4 \left( \frac{\beta}{2} \right) \cos(2\alpha - 2\gamma + 2\phi) \right\} \right]. \quad [2]
 \end{aligned}$$

Hence, by comparing the correlation pattern of two sets of experimentally determined residual dipolar couplings from two alignment tensors (i.e.,  $\mathbf{D}_1$  and  $\mathbf{D}_2$ ) with theoretical simulations, the possibilities for the relative orientation between the two tensors can be reduced because the correlation pattern is strongly dependent on the alignment. From the symmetric character and trigonometric relationships in Eq. [2], it can be shown that the residual dipolar coupling has same value for any of the eight following orientations:  $(\theta, \phi, \alpha, \beta, \gamma)$ ,  $(\theta, \phi, \alpha, \beta, \gamma + \pi)$ ,  $(\theta, \pi - \phi, \pi - \alpha, \beta, \pi - \gamma)$ ,  $(\theta, \pi - \phi, \pi - \alpha, \beta, -\gamma)$ ,  $(\theta, \phi, \pi + \alpha, \pi - \beta, \pi - \gamma)$ ,  $(\theta, \phi, \pi + \alpha, \pi - \beta, -\gamma)$ ,  $(\theta, \pi - \phi, -\alpha, \pi - \beta, \gamma)$ , and  $(\theta, \pi - \phi, -\alpha, \pi - \beta, \pi + \gamma)$ . Hence, due to this symmetry, we can reduce the “volume” of Euler

space that must be searched from  $0^\circ \leq \alpha < 360^\circ$ ,  $0^\circ \leq \beta < 180^\circ$ , and  $0^\circ \leq \gamma < 360^\circ$  to  $0^\circ \leq \alpha < 180^\circ$ ,  $0^\circ \leq \beta < 180^\circ$ , and  $0^\circ \leq \gamma < 90^\circ$  so that only the orientation  $(\alpha, \beta, \gamma)$  is found. If the A–B vectors are distributed isotropically, this correlation will have a similar shape to a two-dimensional (2D) separated local field (SLF) spectrum of a polycrystalline powder (9). In practice, however, the number of dipolar couplings that can be measured in anisotropic solution compared to the polycrystalline state is limited, and thus the sensitivity of this method is inferior to the 2D SLF spectrum method. After determining the Euler angles linking the two alignment tensors,  $\theta$  and  $\phi$  or each dipolar pair can then be determined from Eqs. [1] and [2].

## RESULTS AND DISCUSSION

Simulations of the dependence of the correlation pattern of two sets of residual  $^{15}\text{N}$ - $^1\text{H}$  dipolar couplings on the two Euler angles  $\alpha$  and  $\beta$  ( $\gamma$  set to  $0^\circ$ ) are shown in Fig. 2 and on  $\alpha$  and  $\gamma$  ( $\beta$  set to  $60^\circ$ ) in Fig. 3. In the simulations the values of  $D_a^{AB}$  and  $R$  for the two tensors were obtained from previous measurements on human ubiquitin ( $D_a^{\text{NH}} = 15.85$  Hz and  $R = 0.48$  for  $\mathbf{D}_1$  (measured in charged bicelle solution) and  $D_a^{\text{NH}} = 9.42$  Hz and  $R = 0.163$  for  $\mathbf{D}_2$  (measured in uncharged bicelle solution)) (10). The interpretation of these correlation plots is straightforward. For the first set of residual dipolar couplings contained in  $\mathbf{D}_1$ , the dipolar couplings in the vertical axis lead to a maximum value of  $D^{\text{NH}}$  when  $r^{\text{NH}}$  is parallel to the  $z$  axis ( $\theta = 0^\circ$ ) of  $\mathbf{D}_1$ . If  $r^{\text{NH}}$  is parallel to the  $y$  axis ( $\theta = 90^\circ$ ;  $\phi = 90^\circ$ ) of  $\mathbf{D}_1$ , there are pronounced ridges. Similar relationships occur for the horizontal axis which corresponds to the second set of residual dipolar couplings contained in  $\mathbf{D}_2$ . By comparing Figs. 2 and 3, it can be seen that this correlation is particularly dependent on  $\beta$ . This is because the rotation of the  $\beta$  angle corresponds to rotation of the  $z$  axis of the molecular alignment tensor.

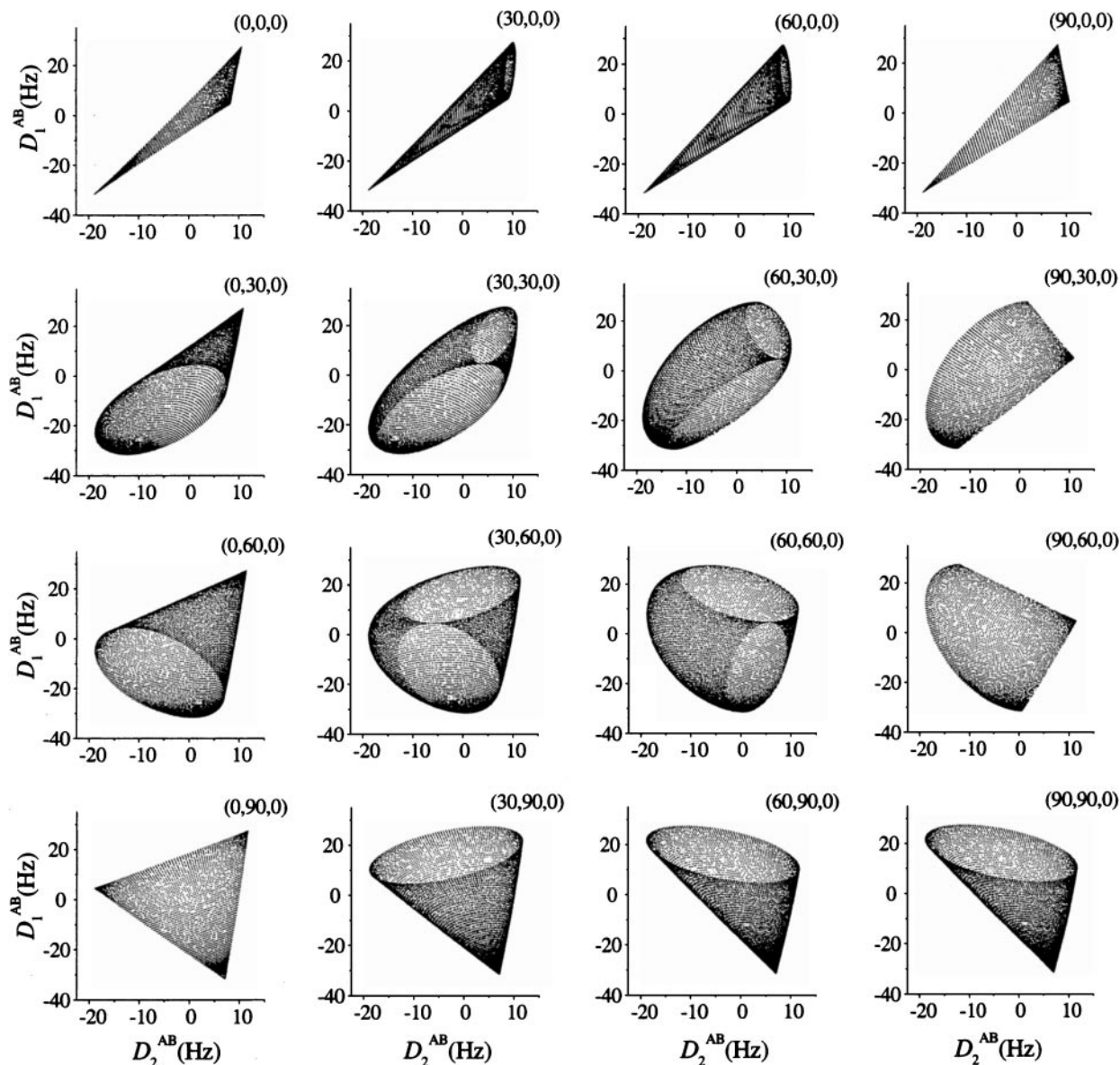


FIG. 2. The  $\alpha$  and  $\beta$  angle dependence of the correlation plots of simulated dipolar couplings. The  $\gamma$  angle is fixed to zero.  $D_1^{\text{AB}}$  and  $D_2^{\text{AB}}$  are the residual dipolar couplings calculated for  $D_a = 15.85$ ,  $R = 0.48$ , and  $D_a = 9.42$ ,  $R = 0.16$ , respectively. These correlations are calculated at  $1^\circ$  intervals for  $\theta$  and  $\phi$ .

A series of dipolar coupling correlation plots obtained from the individual normalized (with respect to NH dipolar coupling)  $^1D^{\text{NH}}$ ,  $^1D^{C_\alpha\text{H}_\alpha}(\text{NH})$ ,  $^1D^{C^{\text{N}}(\text{NH})}$ ,  $^1D^{C_\alpha C'}(\text{NH})$ , and  $^2D^{C^{\text{HN}}(\text{NH})}$  couplings and the ensemble of all five couplings for human ubiquitin from the data sets reported in Ref. (10) are shown in Fig. 4. The normalization of the dipolar couplings was performed as described in Ref. (10) (i.e.,  $D^{\text{AB}}(\text{NH}) = D^{\text{AB}}(\gamma_{\text{N}}\gamma_{\text{H}}\langle r_{\text{NH}}^{-3} \rangle / (\gamma_{\text{A}}\gamma_{\text{B}}\langle r_{\text{AB}}^{-3} \rangle))$ ), and taking the internuclear distances to be 1.02, 1.08, 1.34, 1.52, and 2.07 Å for the N–H,  $C_\alpha$ – $H_\alpha$ , N– $C'$ ,  $C_\alpha$ – $C'$ , and  $C'$ – $H^{\text{N}}$  bond lengths, respectively.

The most probable Euler angles are estimated in the following way. Correlation plots are initially calculated for all

combinations of Euler angles at  $10^\circ$  intervals. Then each correlation plot is divided into a mesh with 5-Hz intervals along both residual dipolar coupling axes. Then, the number of the points per mesh square for the simulated and experimental plots are counted and normalized to the total number of points in each case. Matching of the simulated to the experimental data then proceeds by defining a merit function as follows: if both the normalized experimental and the simulated data have more than one point per square or both have less than or equal to one point per square, the square takes the value of 1 merit point (0 otherwise). Finally, the values of all the squares are summed. This total provides a measure of the fit between the

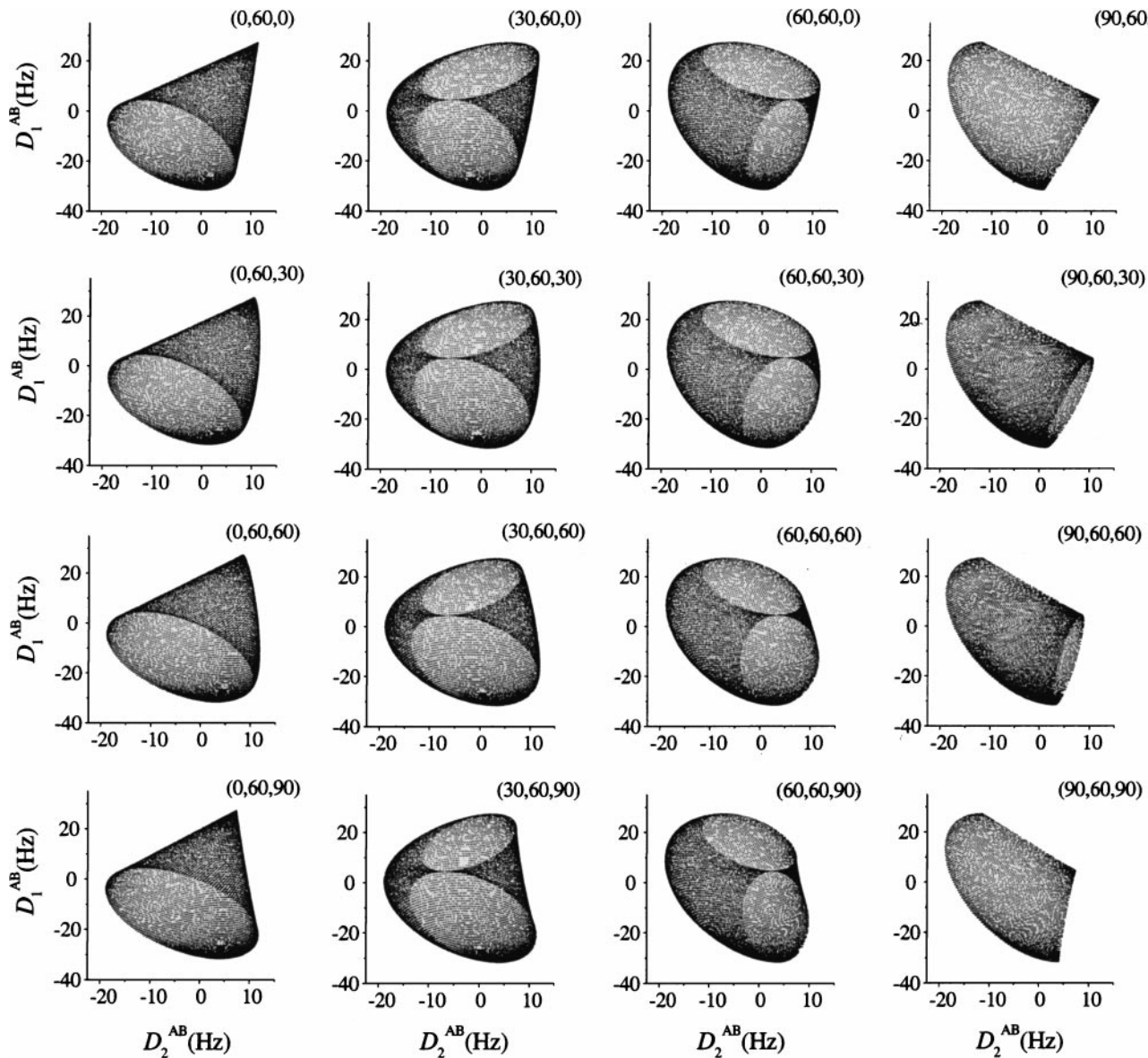
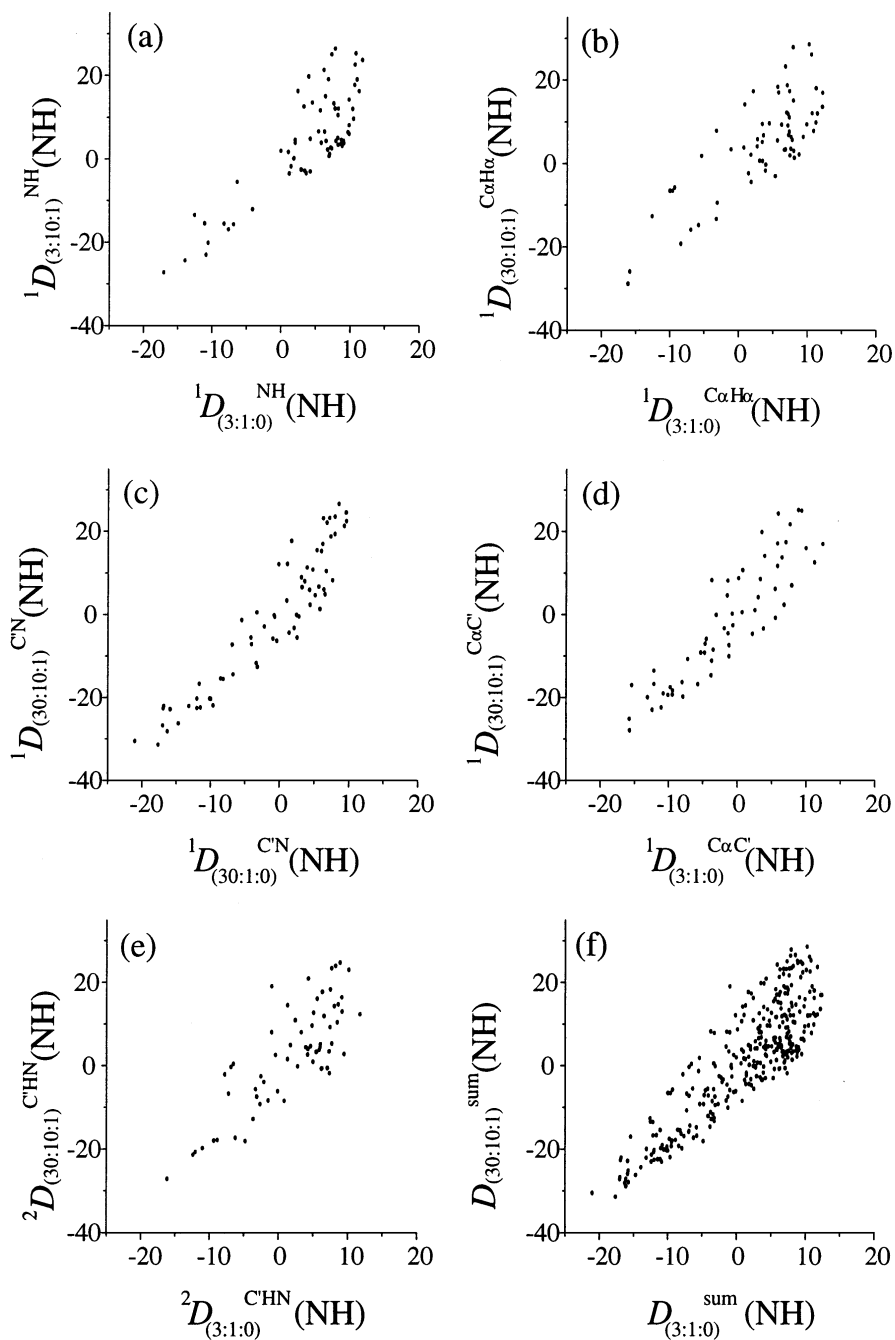


FIG. 3. The  $\alpha$  and  $\gamma$  angle dependence of the correlation plots of simulated dipolar couplings. The  $\beta$  angle is fixed to  $60^\circ$ .

simulated and experimental data for each particular Euler angle combination. The resulting three-dimensional data matrix (each Euler angle constitutes one dimension) is then searched to find the global maximum, which corresponds to the best fit between the simulated and experimental data. This Euler angle searching procedure is then repeated with smaller angular increments to provide a more accurate estimate of the Euler angles that define the relative orientation. As an example, a two-dimensional slice (i.e., contour plot) of the three-dimensional data matrix with  $\gamma = 160^\circ$  is shown in Fig. 5. The sensitivity of the total merit function to the  $\alpha$  and especially the  $\beta$  angle is clearly shown ( $\gamma$  is the least sensitive). The results of the search procedure gave eight global maxima (i.e., orientations of equal likelihood) to be

$(\alpha, \beta, \gamma) = (47^\circ, 6^\circ, 177^\circ)$  with the other seven maxima being as listed after Eq. [2].

To confirm that the orientations determined using our pattern matching procedure were valid, we compared our findings with those determined from X-ray data. From the X-ray structure (pdb code 1ubq) (12) for the 3D structure of human ubiquitin, four possible relative orientations of the principal axis frame of alignment tensor to the X-ray coordinate frame were obtained according to the best-fit method, using order matrix analysis of residual dipolar couplings via singular value decomposition (13), for each of the two sets of residual dipolar couplings. This gave 16 (i.e., the product of the four possible orientations for  $\mathbf{D}_1$  to the X-ray coordinate frame times the



**FIG. 4.** A series of correlation plots obtained from the individual normalized (a)  ${}^1D^{\text{NH}}$ , (b)  ${}^1D^{\text{C}\alpha\text{H}\alpha}(\text{NH})$ , (c)  ${}^1D^{\text{C}^{\text{N}}}(\text{NH})$ , (d)  ${}^1D^{\text{C}\alpha\text{C}' }(\text{NH})$ , and (e)  ${}^2D^{\text{C}^{\text{H}}\text{N}}(\text{NH})$  couplings and (f) the sum of all five couplings for human ubiquitin.

four possible orientations for  $\mathbf{D}_2$  to the X-ray coordinate frame) candidates for the relative orientation  $(\alpha, \beta, \gamma)$  between the two alignment tensors. From the symmetric character of Eq. [2], the residual dipolar coupling has same value for any of the following orientations:  $(\alpha, \beta, \gamma)$ ,  $(\alpha, \beta, \gamma + \pi)$ ,  $(\pi - \alpha, \beta, \pi - \gamma)$ , and  $(\pi - \alpha, \beta, -\gamma)$ . Hence, the correlation plots of these 16 candidates can be represented by four orientations as  $(56.4^\circ,$

$7.2^\circ, 160.2^\circ)$ ,  $(303.6^\circ, 7.2^\circ, 19.8^\circ)$ ,  $(56.4^\circ, 172.8^\circ, 19.8^\circ)$ , and  $(303.6^\circ, 172.8^\circ, 160.2^\circ)$ . Figure 6 shows the correlation plots of the simulated dipolar couplings of these four orientations. By comparing Figs. 6a and 6d with Fig. 4f, it can be seen that there is good agreement between the experimental and simulated results. It is emphasized that the Euler angles used in calculating Fig. 6a are very close to those determined by our fitting

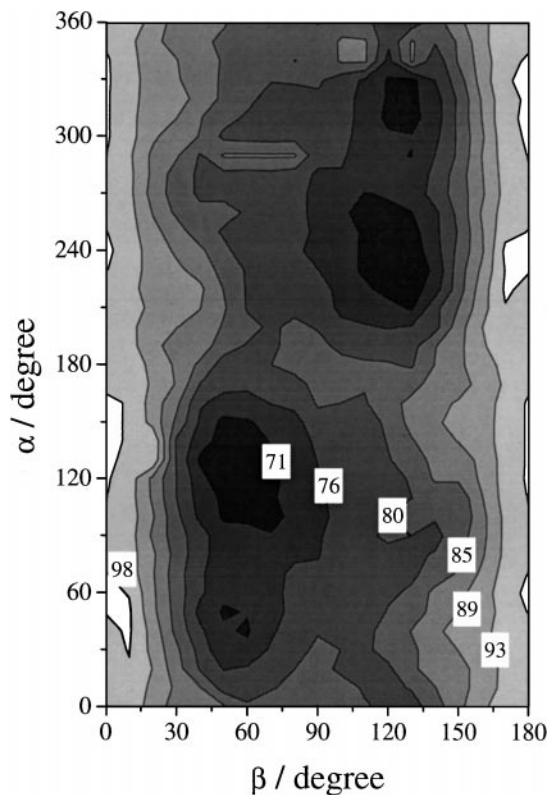


FIG. 5. A two-dimensional slice at  $\gamma = 160^\circ$  of the three-dimensional data matrix which shows the degree of matching of the simulated data set to the experimental data set for different combinations of the Euler angles. The contours reflect the degree of matching as a percentage of the best match (i.e., each of the eight most likely orientations scores 100%).

procedure (i.e.,  $(47^\circ, 6^\circ, 177^\circ)$ ). This close agreement clearly indicates the usefulness and validity of the present approach.

The theoretical dependence of the correlation plot of the residual dipolar coupling on the rhombicity  $R$  is shown in Fig. 7. Although the plots were dependent on  $R$ , the axial components,  $D_a^{AB}$ , of the molecular alignment tensor served only to scale the correlation patterns. The scaling by the axial component can be removed by normalizing the magnitudes of the measured dipolar couplings. The scaling factor is the ratio of the square root of the sum of the squares of all dipolar couplings in alignment tensor 1 divided by the square root of the same sum for alignment tensor 2. For the two data sets of human ubiquitin, this ratio is 1.63 and it is almost the same as  $D_{a1}/D_{a2} = 1.68$ . Hence, it is better to use this ratio for  $D_a^{AB}$  and use a histogram (11) to determine  $R$ . Thus, this method only needs two sets of residual dipolar coupling data and does not require the three-dimensional structure of the molecule.

In conclusion, we have shown that the relative orientations of the molecular alignment tensors determined in different anisotropic media can be obtained from the correlation of two sets of residual dipolar couplings in the absence of any structural information. It can be realized that this method is a “two-

dimensional” extension of the histogram method for determining the axial and rhombic components from the normalized residual dipolar couplings (11). Although the resolution of  $\gamma$  determined by this new method is low, the resolution is better for  $\alpha$  and the more important  $\beta$  angle. Besides, it is important to note that the precision of the Euler angles determined depend on the difference in orientation between  $\mathbf{D}_1$  and  $\mathbf{D}_2$ , and in the respective rhombicities.

Though other approaches can be used to determine the relative orientation of the molecular alignment tensors in the absence of any structural information (7), our method does not require sophisticated software and allows the solution to be visually verified. Further, although preferable, the new method does not require all four types of residual dipolar coupling (i.e.,  $^1D^{NH}$ ,  $^1D^{CN}$ ,  $^1D^{CaC'}$ , and  $^2D^{CHN}$ ) data. Thus, by using this new approach the relative orientation of the molecular alignment tensors can be easily estimated and consequently the information content of measured residual dipolar coupling data sets can be checked before starting (lengthy) structure calculations.

*Supplementary material.* A complete set of the simulated data in the Euler space,  $0^\circ \leq \alpha < 360^\circ$ ,  $0^\circ \leq \beta < 180^\circ$ , and  $0^\circ \leq \gamma < 360^\circ$  to  $0^\circ \leq \alpha < 180^\circ$ ,  $0^\circ \leq \beta < 180^\circ$ , and  $0^\circ \leq \gamma < 90^\circ$ , at  $30^\circ$  intervals, and all programs used in this study are available, together with instructions, upon request to kainosho@nmr.chem.metro-u.ac.jp.

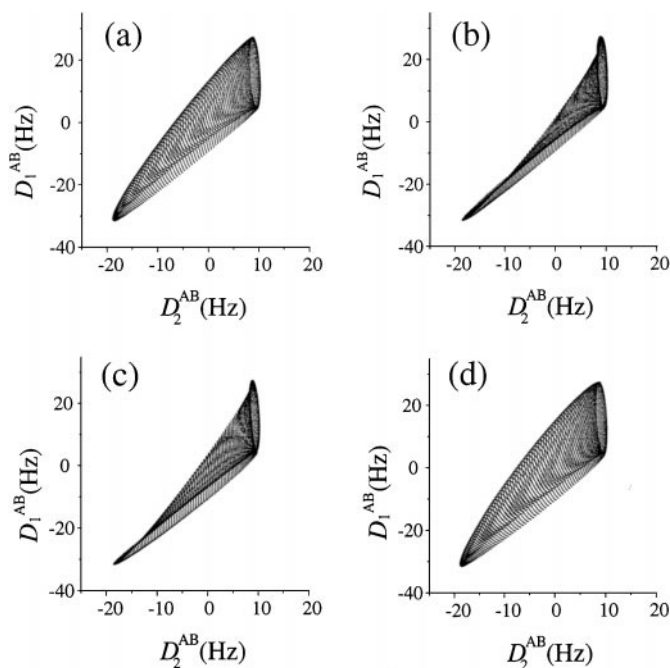


FIG. 6. Correlation plots of simulated dipolar couplings using the relative orientation determined using the order tensor approach for four different orientations: (a)  $(56.4^\circ, 7.2^\circ, 160.2^\circ)$ , (b)  $(303.6^\circ, 7.2^\circ, 19.8^\circ)$ , (c)  $(56.4^\circ, 172.8^\circ, 19.8^\circ)$ , and (d)  $(303.6^\circ, 172.8^\circ, 160.2^\circ)$ .

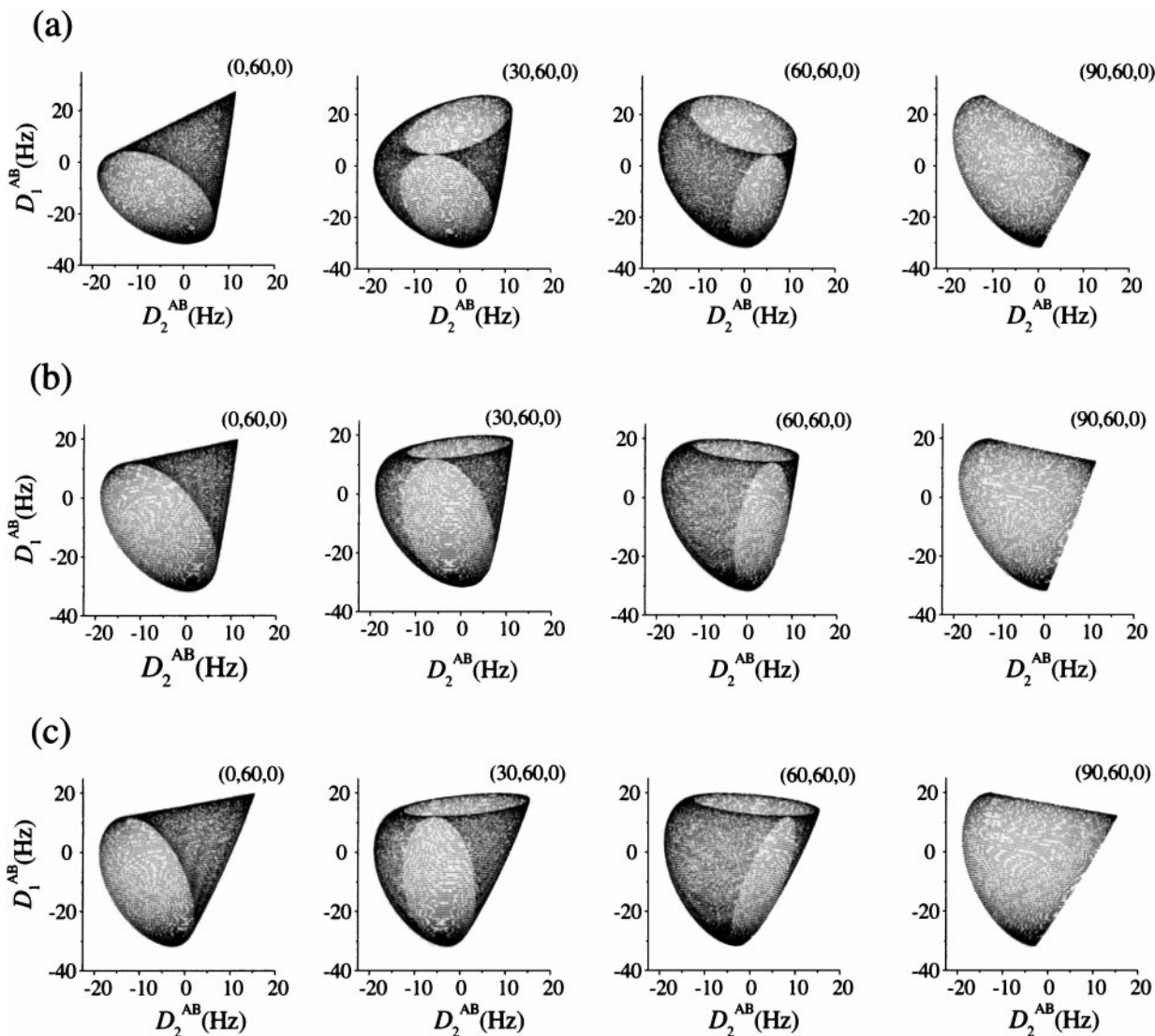


FIG. 7. The rhombicity dependence of the correlation plots of the simulated dipolar couplings. (a)  $R_1 = 0.48$ ,  $R_2 = 0.16$ ; (b)  $R_1 = 0.16$ ,  $R_2 = 0.16$ ; and (c)  $R_1 = 0.163$ ,  $R_2 = 0.48$ .

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