



Structural constraints from residual tensorial couplings in high resolution NMR without an explicit term for the alignment tensor

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Abstract

Structural restraints from residual tensorial couplings in high resolution NMR are usually incorporated into molecular structure calculation programs by an energy penalty function which depends on the knowledge of the alignment tensor. Here, we show that the alignment tensor enters in linear form into such a function. Therefore, the explicit appearance of the alignment tensor can be eliminated from the penalty function. This avoids the necessity of a determination of magnitude and rhombicity of the alignment tensor in the absence of structural information. The price for this procedure is a slightly shallower energy landscape. Simulations in the vicinity of the energy minimum for the backbone of human ubiquitin show that the reduction in curvature is on the order of a few percent.

A new type of structural information has become available in high resolution NMR by the introduction of an anisotropic orientation of solute molecules (Saupe and Englert, 1963; Bothner-By et al., 1981; Tolman et al., 1995; Vold and Prosser, 1996; Tjandra and Bax, 1997; Tjandra et al., 1997). This information results from the anisotropy of certain second rank tensor interactions which vanish to first order under isotropic conditions. A weakly anisotropic distribution of the molecular orientation reintroduces these interactions to such an extent that they are easily detected under the conditions of solution NMR applied to biomacromolecules. Relevant interactions are the dipolar couplings between the magnetic nuclei, the anisotropy of the chemical shift, and the quadrupolar couplings. The effect of the anisotropic distribution of molecular orientations is conveniently described by a set of order parameters which are known as the alignment tensor or the Saupe order matrix (Saupe, 1964; Emsley, 1996). The size of the residual tensorial interactions is a function of both the alignment tensor and of the orientation of the individual chemical groups with respect

to the alignment tensor's principal axes system; hence, the content of intramolecular structural information.

In order to extract this information from the measured interaction energies, some knowledge about the alignment tensor is a prerequisite. This tensor is characterized by five independent parameters, e.g. its largest principal value, the rhombicity, and the three Euler angles describing the orientation of the principal axes with respect to the molecular coordinates. For an unknown molecular structure, the largest principal value and rhombicity of the alignment tensor can be determined in principle from a histogram of measured interaction energies under the assumption that the distribution of individual chemical group orientations is isotropic (Clare et al., 1998a). An analogous technique has been described for the determination of the magnitude and rhombicity of the diffusion tensor from relaxation data (Clare et al., 1998b). As an extension to this method, it has also been proposed to refine the value of the rhombicity of the tensor by a grid search during the structure calculations (Clare et al., 1998c). In the absence of any further knowledge on the molecular structure, the orientation of the tensor relative to the molecule is unknown. Therefore, in the energy minimization routines used in molecular struc-

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ture calculations, the Euler angles are usually left as free floating parameters (Tjandra et al., 1997).

In this communication, we use the fact that the residual tensorial interactions can be described by a function which is linear in the five parameters corresponding to the irreducible representation of the alignment tensor. Therefore, the problem of finding an alignment tensor and a molecular structure that best fit the experimentally measured tensorial interactions can be separated into a linear least squares minimization for the alignment tensor and a non-linear least squares minimization for the internal degrees of freedom of the molecule. As a consequence, the alignment tensor can be eliminated completely from the fitting problem (Golub and Pereyra, 1973). This approach amounts to implicitly adjusting the linear parameters for each given set of the non-linear parameters. Such a minimization is, of course, less sensitive to a variation in the non-linear parameters than it would be if the orientation tensor were completely known. However, the results of simulations using a known protein structure indicate that the loss in the discriminative power of the fit is not much reduced compared to the more complicated approach where experimentally determined values for the largest principal value and the rhombicity are used.

The notation follows closely the one given by Emsley (1996). We consider a molecule which is fixed at a certain position in space. For the interactions of the magnetic moments of its nuclei, which are of interest with respect to residual orientation in high resolution NMR, the general form of the nuclear Hamiltonian H in irreducible tensorial form can be given as

$$H = \sum_{\lambda} \sum_{m=-2}^2 (-1)^m T_{\lambda}^{2m} A_{\lambda}^{2-m} g_{\lambda} \quad (1)$$

where T_{λ}^{2m} is a second rank, irreducible tensor representing the spatial degrees of freedom of the λ th interaction, A_{λ}^{2m} the nuclear spin operator associated with it, and g_{λ} a numerical interaction constant. In the high field case, only diagonal elements of this Hamiltonian with respect to the Zeeman Hamiltonian are considered. This means, for a magnetic field in the direction of the z-axis, only terms of H with $m = 0$ are relevant:

$$H \approx \sum_{\lambda} T_{\lambda}^{20} A_{\lambda}^{20} g_{\lambda} \quad (2)$$

The translational motion of a molecule in the sample tube leaves this Hamiltonian invariant if the static

magnetic field is homogeneous. Rotations of the molecule will affect the spatial part (T_{λ}^{2m}) but not the spin part (A_{λ}^{2m}) of the Hamiltonian. For the sake of simplicity, we assume that the molecule does not undergo significant internal motions. In such a case, the instantaneous $T_{\lambda}^{20}(t)$ tensor element can be represented by an overall rotation of the molecule:

$$T_{\lambda}^{20}(t) = \sum_{m=-2}^2 T_{\lambda,0}^{2m} D_{m0}^2(\vec{\Omega}(t)) \quad (3)$$

where $T_{\lambda,0}^{2m}$ represents T_{λ}^{2m} in the reference frame of the laboratory at time point $t = 0$, and $D_{m0}^2(\vec{\Omega}(t))$ a Wigner matrix corresponding to the instantaneous Euler angles $\vec{\Omega}(t) = (\alpha(t), \beta(t), \gamma(t))$ of the molecule at time point t . Observables in high resolution NMR are associated with the time (and ensemble) average of H . This average can be expressed as an average of T_{λ}^{20} over all rotations of the molecule:

$$\langle T_{\lambda}^{20} \rangle = \sum_{m=-2}^2 T_{\lambda,0}^{2m} \langle D_{m0}^2 \rangle \quad (4)$$

The average $\langle D_{m0}^2 \rangle$ is called order parameter. Its knowledge determines the average of H and all observables completely:

$$\begin{aligned} \langle H \rangle &= \sum_{m=-2}^2 \langle D_{m0}^2 \rangle \sum_{\lambda} T_{\lambda,0}^{2m} A_{\lambda}^{20} g_{\lambda} \\ &\equiv \sum_{m=-2}^2 S_m^* \sum_{\lambda} T_{\lambda,0}^{2m} A_{\lambda}^{20} g_{\lambda} \end{aligned} \quad (5)$$

where the definition $\langle D_{m0}^2 \rangle \equiv S_m^*$ has been used. Note that the complex conjugate in the definition gives the right side of Equation 5 the form of a canonical scalar product. The tensor elements for the dipolar, CSA, and quadrupolar interactions in a form suitable for Equation 5 are summarized in Table 1. It is clear from this table that, once a reference frame is chosen in the laboratory, the elements of $T_{\lambda,0}^{2m}$ only depend on the internal geometry of the molecule, i.e. on its internal degrees of freedom, e.g. its dihedral angles. Equation 5 separates these internal degrees of freedom from the rotational order of the molecule. As the Wigner matrices form a complete set of functions acting on the Euler angles (Brink and Satchler, 1993), any kind of orientational order can be described completely by specifying the ensemble averages $\langle D_{mm'}^l \rangle$

Table 1. Elements of the irreducible representation of the Hamiltonian for orientation dependent interactions relevant to high resolution NMR

Interaction	$T_{\lambda,0}^{2m}$	A^{20}	g_λ
Dipolar	$D_{0m}^2(\vartheta_D, \vartheta_D, 0) = \sqrt{\frac{4\pi}{5}} Y_{2m}(\vartheta_D, \varphi_D)$	$\vec{I}^1 \cdot \vec{I}^2 - 3I_z^1 I_z^2$	$\frac{\mu_0 \gamma_1 \gamma_2 \hbar}{4\pi r_{12}^3}$
CSA	$\sum_{m'=-2}^2 D_{m'm}^2(\tilde{\Omega}_{CSA}) \eta_{CSA}^{m'}$	I_z	$\frac{2}{3} \gamma B_0 \Delta\sigma$
Quadrupolar	$\sum_{m'=-2}^2 D_{m'm}^2(\tilde{\Omega}_{Quad}) \eta_{Quad}^{m'}$	$\vec{I} \cdot \vec{I} - 3I_z I_z$	$\frac{-eQ}{4\hbar I(2I-1)} \left(\frac{\partial^2 V}{\partial Z^2} \right)$

The size of the Hamiltonians is measured in angular frequency units. r_{12} is the internuclear distance associated with the dipolar interaction between nuclei 1 and 2, $\Delta\sigma = \sigma_{zz} - (\sigma_{xx} + \sigma_{yy})/2$ is the anisotropy of the chemical shielding tensor, $\partial^2 V / \partial Z^2$ is the expectation value of the electric field gradient at the position of the nucleus, eQ has its usual definition as the nuclear quadrupole moment, $\eta^m = (\eta/\sqrt{6}, 0, 1, 0, \eta/\sqrt{6})$ describes the asymmetry for the CSA or quadrupolar interaction with the convention $\eta = (T_{xx} - T_{yy})/[T_{zz} - (T_{xx} + T_{yy} + T_{zz})/3]$, where $T_{\alpha\alpha}$ are the principal values of the interaction tensor. The Wigner rotation matrices $D_{m'm}^2(\tilde{\Omega})$ transform the principal axis system of the interaction tensor into the reference frame of the molecule.

for all $l = 0, 1, 2, \dots$; $m = -l, -l+1, \dots, l$; $m' = -l, -l+1, \dots, l$ (Emsley, 1996). However, one realizes from Equation 5 that only the five elements $\langle D_{m0}^2(\alpha, \beta, \gamma) \rangle = (-1)^m \sqrt{4\pi/5} \langle Y_{2m}(\beta, \gamma) \rangle \equiv S_m^*$ are relevant for describing ensemble averages of high resolution NMR. This five-dimensional vector S_m transforms under rotations as any other second rank tensor. It corresponds to the irreducible representation of the Saupe order matrix $S_{\alpha\beta} = \langle 3 \cos \theta_\alpha \cos \theta_\beta - \delta_{\alpha\beta} \rangle / 2$; $\alpha, \beta = x, y, z$ (Saupe, 1964). The relation between both representations is given by Clebsch-Gordan coefficients:

$$\begin{aligned} S_0 &= S_{zz} \\ S_1 &= \sqrt{2/3}(S_{xz} - iS_{yz}) \\ S_2 &= \sqrt{1/6}S_{xx} - \sqrt{1/6}S_{yy} - i\sqrt{2/3}S_{xy} \\ S_m &= (-1)^m S_{-m}^* \end{aligned} \quad (6a)$$

$$\begin{aligned} S_{zz} &= S_0 \\ S_{xx} &= \sqrt{3/8}(S_2 + S_{-2}) - S_0/2 \\ S_{yy} &= -\sqrt{3/8}(S_2 + S_{-2}) - S_0/2 \\ S_{xy} &= S_{yx} = i\sqrt{3/8}(S_2 - S_{-2}) \\ S_{xz} &= S_{zx} = \sqrt{3/8}(S_1 - S_{-1}) \\ S_{yz} &= S_{zy} = i\sqrt{3/8}(S_1 + S_{-1}) \end{aligned} \quad (6b)$$

Once S_m is specified, it is thus possible to find the Saupe order matrix $S_{\alpha\beta}$ by Equation 6b and to derive the principal axis system and eigenvalues of the orientation tensor by standard procedures of linear algebra.

Also note the following simple relations between the values of the order matrix in an arbitrary coordinated system $(S_m, S_{\alpha\beta})$ and these values in the principal axis system $(S'_m, S'_{\alpha\beta})$:

$$\begin{aligned} \sum_{m=-2}^2 S_m S_m^* &= \frac{2}{3} \sum_{\alpha, \beta=x, y, z} S_{\alpha\beta}^2 \\ &= S_{zz}'^2 \left(1 + \frac{\eta^2}{3} \right) \end{aligned} \quad (7a)$$

$$\begin{aligned} \frac{S_0^3}{4} - \frac{3}{2}S_0(S_2 S_{-2} + \frac{1}{2}S_1 S_{-1}) + \frac{3}{4}\sqrt{\frac{3}{2}}(S_{-2} S_1^2 \\ + S_{-1}^2 S_2) \\ = \text{Det}(S_{\alpha\beta}) \\ = S_{zz}'^3 (1 - \eta^2)/4 \end{aligned} \quad (7b)$$

where the asymmetry parameter η is usually defined in the principal axis system as $0 \leq \eta = (S'_{xx} - S'_{yy})/S'_{zz} \leq 1$.

In order to reveal the structural information about the molecule which is contained in Equation 5, resonance frequencies are measured which correspond to changes in the spin states $|1\rangle \rightarrow |2\rangle$ for the interaction λ . If we denote such a frequency by ω_λ , it follows from Equation 5 that

$$\begin{aligned} \omega_\lambda &= \sum_{m=-2}^2 S_m^* T_{\lambda,0}^{2m} g'_\lambda \\ &\equiv \sum_{m=-2}^2 S_m^* T_{\lambda,0}^{2m} g_\lambda (\langle 2|A_\lambda^{20}|2\rangle - \langle 1|A_\lambda^{20}|1\rangle) \end{aligned} \quad (8)$$

It is clear from Table 1 that for the relevant transitions in a spin 1/2 system, both in the case of dipolar and CSA interactions, $g'_\lambda = \pm g_\lambda$.

Under the assumption of a rigid molecule with n internal degrees of freedom represented by the vector $\vec{\alpha} = (\alpha_1, \dots, \alpha_n)$, the problem of finding a best structure of the molecule compatible with a set of N

measured frequencies ω_λ^{obs} can be stated as finding a set of parameters $\hat{\vec{\alpha}}$ and \hat{S}_m that minimizes the function

$$\chi^2(\vec{\alpha}, \vec{S}) \equiv r(\vec{\alpha}, \vec{S}) = \sum_{\lambda=1}^N \left[\omega_\lambda^{obs} - \sum_{m=-2}^2 S_m^* T_{\lambda,0}^{2m}(\vec{\alpha}) g'_\lambda \right]^2 \quad (9)$$

Whereas Equation 9 presents a non-linear least squares problem with respect to $\vec{\alpha}$, it is a linear least squares problem with respect to the order parameter S_m . For such a class of problems, it is well known (Golub and Pereyra, 1973) that one can define a modified non-linear least squares problem $r_2(\vec{\alpha})$ which does not depend on S_m . Thus, it is possible to optimize $r_2(\vec{\alpha})$ first with respect to $\vec{\alpha}$ and then to obtain, a posteriori, the optimal parameters \hat{S}_m . In order to follow as closely as possible the notation of Golub and Pereyra (1973), we introduce the matrix $\{\Phi(\vec{\alpha})\}_{\lambda,m} = T_{\lambda,0}^{2m}(\vec{\alpha}) g'_\lambda$ and the vector $\vec{\omega}^{obs} = \omega_\lambda^{obs}$, $r_2(\vec{\alpha})$ is then given by:

$$r_2(\vec{\alpha}) = \|\vec{\omega}^{obs} - \Phi(\vec{\alpha})\Phi^+(\vec{\alpha})\vec{\omega}^{obs}\|^2 \quad (10)$$

where $\|\cdot\|$ is the Euclidean norm and $\Phi^+(\vec{\alpha})$ is the Moore–Penrose generalized inverse of $\Phi(\vec{\alpha})$. For any given $\vec{\alpha}$, the optimal set of order parameters $\hat{S}(\vec{\alpha}) = \hat{S}_m(\vec{\alpha})$ is given by the minimal least squares solution

$$\hat{S}^*(\vec{\alpha}) = \Phi^+(\vec{\alpha})\vec{\omega}^{obs} \quad (11)$$

This optimal set of parameters $\hat{S}(\vec{\alpha})$ minimizes $r(\vec{\alpha}, \vec{S})$ at the point $\vec{\alpha}$ and the value of $r(\vec{\alpha}, \hat{S}(\vec{\alpha}))$ is given by $r_2(\vec{\alpha})$. The problem of minimizing Equation 9 is therefore reduced to minimizing Equation 10 with respect to the non-linear parameters $\vec{\alpha}$ alone. Once an optimal set of parameters $\hat{\vec{\alpha}}$ is found, the optimal set of order parameters \hat{S}_m for this choice of $\hat{\vec{\alpha}}$ can be derived from Equation 11.

The Moore–Penrose generalized inverse $\Phi^+(\vec{\alpha})$ can be calculated from a singular value decomposition of $\Phi(\vec{\alpha})$ (Campbell and Meyer, 1979). As shown by Golub and Pereyra (1973), it is, however, not even necessary to compute $\Phi^+(\vec{\alpha})$ during the minimization of $r_2(\vec{\alpha})$. It is sufficient to reduce $\Phi(\vec{\alpha})$ to trapezoidal form. The value of $r_2(\vec{\alpha})$ can then be calculated from the reducing matrix and $\vec{\omega}^{obs}$ directly.

We have tested the validity of this approach for the fitting of backbone torsion angles from dipolar coupling data in the structure of human ubiquitin (Figure 1). All of the following simulations were carried

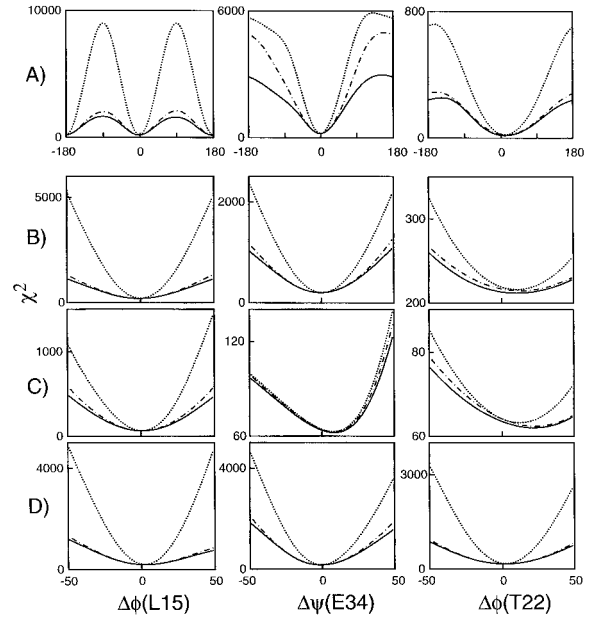


Figure 1. χ^2 deviations of dipolar couplings as a function of the change in one backbone torsion angle in the ubiquitin crystal structure (Vijay-Kumar et al., 1987) assuming different levels of knowledge about the orientation tensor. Simulated experimental dipolar couplings with a random error of 10% were derived from the crystal structure for a symmetric and an asymmetric orientation tensor (see text). Single backbone torsion angles, i.e. either ϕ of L15 (left column), ψ of T34 (middle column) or ϕ of T22 (right column), were varied in steps of 3° around their crystal structure values. For each of these configurations, the dipolar couplings were recalculated for the various orientation tensor models and χ^2 was determined as the sum of squared differences between these and the simulated experimental couplings. Dotted lines (model 1): complete knowledge of the orientation tensor was assumed. Dashed lines (model 2): knowledge of the largest principal value and the asymmetry parameter of the orientation tensor was assumed and Euler angles were derived from a simplex minimization. Solid lines (model 3): no knowledge about the orientation tensor was assumed and χ^2 was derived by the pseudo-inverse approach according to Equation 10. Panel A: the dipolar couplings for $^1\text{H}_\text{N}-^{15}\text{N}$, $^1\text{H}_\text{Ni}-^{13}\text{C}'_{i-1}$, and $^1\text{H}_\alpha-^{13}\text{C}_\alpha$ were used in the calculation and simulated experimental values were computed using a symmetric tensor [$\vec{S} = (0, 0, 1, 0, 0)$]. Panel B: zoomed region of panel A for variations of the backbone torsion angles of $\pm 50^\circ$. Panel C: same as panel B, but only $^1\text{H}_\text{N}-^{15}\text{N}$ dipolar couplings were taken into account. Panel D: same as panel B, but an asymmetric orientation tensor [$\vec{S} = (1/\sqrt{6}, 0, 1, 0, 1/\sqrt{6})$] was used.

out using the program MATLAB (The MathWorks, Natick, MA, U.S.A.). In order to model different experimental situations, synthetic dipolar couplings were derived from the coordinates of the crystal structure of this protein (Vijay-Kumar et al., 1987). These couplings were calculated for the one- and two-bond $^1\text{H}_\text{N}-^{15}\text{N}$, $^1\text{H}_\text{Ni}-^{13}\text{C}'_{i-1}$, and $^1\text{H}_\alpha-^{13}\text{C}_\alpha$ interactions according to Equation 8, assuming either an orientation

tensor symmetric with respect to the z-axis [$\vec{S} = (0, 0, 1, 0, 0)$] or an orientation tensor with largest principal value in the z-direction and an asymmetry parameter η of 1 [$\vec{S} = (1/\sqrt{6}, 0, 1, 0, 1/\sqrt{6})$]. A value for g'_λ of 1 was used for all couplings and normally distributed random errors of standard variation 0.1 were added to all dipolar couplings.

In a structure calculation/refinement program such as XPLOR (Brünger, 1992) or DYANA (Günther et al., 1997), the information content of the residual tensorial couplings is usually added in the form of an energy penalty function (Tjandra et al., 1997) which is proportional to the sum of the squared deviations (χ^2) between measured and calculated couplings as given by Equation 9. In order to simulate the situation occurring near the global χ^2 -minimum, a number of different models were considered. In model 1 it was assumed that both the orientation and principal values of the orientation tensor were known completely. This amounts to the specification of all five components of the \vec{S} vector. In model 2, it was assumed that only the S'_{zz} value in the principal axis system and the asymmetry parameter η were known. Such a situation can be realized for an unknown structure by deriving these values under the assumption that the tensorial couplings follow an isotropic distribution (Clare et al., 1998a). In this case, the Euler angles describing the orientation of the Saupe orientation matrix are fitted implicitly by the energy minimization routine of the molecular dynamics program. In model 2, this situation was simulated by a simplex minimization of the energy with respect to three Euler angles (α, β, γ) in the case of an asymmetric orientation tensor or two Euler angles (β, γ) in the case of a symmetric tensor. Finally, model 3 assumes no prior knowledge of the orientation tensor at all and uses the pseudo-inverse as described above to calculate the energy penalty function according to Equation 10. This implicitly fits orientation and principal values of the orientation tensor for each given configuration of the molecule.

For these models and the two assumed orientation tensors, one-dimensional cross sections through the χ^2 hypersurface were calculated as functions of three different backbone torsion angles in ubiquitin: ϕ (L15), ψ (E34), and ϕ (T22). The direction of the bond vectors for these torsion angles, i.e. N-C $_\alpha$ for ϕ and C $_\alpha$ -C' for ψ , deviates by 89° (L15), 41° (E34), and 7° (T22) from the direction of the crystal coordinate z-axis. The torsion angle for each of these bonds was varied in steps of 3° in an interval of $\pm 180^\circ$ around the torsion angle given by the crystal structure. All other torsion

angles were kept unchanged from the crystal structure. χ^2 values were determined either for the $^1\text{H}_\text{N}$ - ^{15}N dipolar couplings alone or for the sum of the $^1\text{H}_\text{N}$ - ^{15}N , $^1\text{H}_{\text{Ni}}$ - $^{13}\text{C}'_{i-1}$, and $^1\text{H}_\alpha$ - $^{13}\text{C}_\alpha$ dipolar couplings for all the residues of ubiquitin.

Figure 1 shows examples of these simulations. As expected, all one-dimensional cross sections through the χ^2 hypersurface show a distinct minimum for torsion angles which are close to the crystal structure values ($\Delta\phi, \Delta\psi \approx 0^\circ$). Note, however, that even in the case of complete knowledge of the orientation tensor (Figure 1, dotted lines), the minimum positions sometimes deviate to some extent from the crystal structure because of the random errors incorporated into the synthetic data. The cross sections through the χ^2 surface are clearly influenced by the symmetry of the torsion bond vectors relative to the orientation tensor. For example, in the case of the ϕ -angle of residue L15, a second minimum appears in the χ^2 surface for $\Delta\phi = 180^\circ$ because the N-C $_\alpha$ bond vector of L15 is approximately perpendicular to the z-axis of the orientation tensor and a rotation by 180° around this axis leaves the calculated dipolar couplings approximately invariant. No such second minimum is observed when the torsion bond vector is not perpendicular to the z-axis [ψ (E34) and ϕ (T22), Figure 1]. On the other hand, a much shallower minimum is observed for the variation of ϕ (T22) in the case of the axially symmetric orientation tensor (Figure 1, panel A), since the N-C $_\alpha$ bond vector of T22 is approximately parallel to the symmetry axis of the orientation tensor.

Clearly, the variations in χ^2 and therefore the discriminative power of the energy minimization are less pronounced for incomplete knowledge of the orientation tensor [Figure 1: model 2 (dashed line) and model 3 (solid line)] than for a complete knowledge of the orientation tensor [Figure 1: model 1 (solid line)]. However, the differences between the assumption of knowledge of only amplitude and rhombicity (model 2, dashed lines) and no prior knowledge of the orientation tensor (model 3, solid lines) are not very pronounced. In the vicinity of the global minimum, both χ^2 surfaces are almost identical. A quantitative comparison was carried out between the three models by using 15 different simulations of dipolar coupling constants for the backbone of ubiquitin with the incorporation of 10% random errors. This comparison shows that the root mean square difference between the χ^2 minimum positions of model 2 and 3 for the three torsion angles is 4.7° (1.3°) for the symmetric orientation tensor and 1.3° (0.2°) for the asymmetric

orientation tensor in the case of $^1\text{H}_\text{N}-^{15}\text{N}$ ($^1\text{H}_\text{N}-^{15}\text{N}$, $^1\text{H}_{\text{Ni}}-^{13}\text{C}'_{i-1}$, and $^1\text{H}_\alpha-^{13}\text{C}_\alpha$) couplings. These deviations between model 2 and 3 are smaller than the variations of the minimum positions of model 1 which are due to the presence of the 10% random error in the coupling constants.

Since the discriminative power of χ^2 depends on its steepness, a more meaningful comparison between the models is given by the variations in the χ^2 surface. Confidence intervals for parameters fitted from a χ^2 minimization for normally distributed errors can be defined as the region around the χ^2 minimum for which χ^2 does not change by more than a certain amount $\Delta\chi^2$ (Press et al., 1988). Therefore, in a suitably small region, the curvature at the χ^2 minimum provides an estimate for the precision by which a parameter will be defined by means of the χ^2 fit. It is obvious from Figure 1 that the curvature at the minimum position decreases continuously from model 1 to model 3 as the number of known parameters is decreased in the assumptions about the orientation tensor. Clearly, the largest reduction in curvature occurs between model 1 and 2. When the curvature is calculated from a parabolic fit in a region of $\pm 15^\circ$ around the X-ray torsion angles, the average reduction in curvature between model 1 and 2 ranges between 40 and 70%, depending on the torsion angle, the choice of the orientation tensor and the set of dipolar couplings ($^1\text{H}_\text{N}-^{15}\text{N}$ or $^1\text{H}_\text{N}-^{15}\text{N}$, $^1\text{H}_{\text{Ni}}-^{13}\text{C}'_{i-1}$, and $^1\text{H}_\alpha-^{13}\text{C}_\alpha$). The differences in curvature between model 2 and 3 are, however, much smaller: for the 15 simulations and the average over the three torsion angles considered, the reduction in curvature between model 2 and model 3 amounts to 6% (6%) for the symmetric orientation tensor and 8% (2%) for the asymmetric orientation tensor in the case of $^1\text{H}_\text{N}-^{15}\text{N}$ ($^1\text{H}_\text{N}-^{15}\text{N}$, $^1\text{H}_{\text{Ni}}-^{13}\text{C}'_{i-1}$, and $^1\text{H}_\alpha-^{13}\text{C}_\alpha$) couplings. It is therefore expected that the discriminative power of the energy penalty function given by Equation 10 is not much reduced in the vicinity of the global minimum compared to the method where magnitude and rhombicity are used as known parameters. Because of the high dimensionality of the χ^2 surface, it is difficult to extend this local observation to the global parameter space. It is, however, obvious from Figure 1A that even for the full range of one torsion angle, the reduction in the steepness of the energy function between model 2 and 3 is not very pronounced for two of the three cases considered [$\phi(\text{L15})$ and $\phi(\text{T22})$].

In summary, we have shown that an energy penalty function for structural calculations using residual ten-

sorial couplings can be defined without an explicit knowledge of the orientation tensor. The application of this type of penalty function should be beneficial in cases where the amplitude and rhombicity of the tensor cannot be determined accurately enough by other means. An obvious extension in the application of the method is the structural refinement with respect to relaxation data resulting from anisotropic diffusion which can be cast into a similar mathematical form (Brüschweiler et al., 1995; Lee et al., 1997).

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