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Evaluation of the influence of anisotropic indirect nuclear spin-spin coupling tensors on effective residual dipolar couplings for model peptides

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Abstract

Residual dipolar couplings (RDCs) observed between nuclear spins in molecules in partially oriented media have become a valuable source of information for NMR spectroscopists seeking to structurally characterize biological macromolecules. Examination of the form of the direct (**D**) and indirect (**J**) nuclear spin-spin coupling Hamiltonians indicates that all observed RDCs contain an unknown contribution from the anisotropic part of **J** (ΔJ) in addition to the direct dipolar contribution, D_{PQ}. Here, we evaluate the influence of ΔJ on RDCs through a series of DFT calculations on model peptides. Very small corrections to one-bond RDCs measured between heavy atoms in peptides and proteins are recommended: +0.51% for N-C′ spin pairs, and +0.45% for C^{α}-C′ spin pairs. The corrections to RDCs involving at least one proton are negligible. This latter point is likely to be equally applicable to nucleic acids and oligosaccharides in addition to peptides and proteins. Finally, the orientations of the **J**(N, C′) and **J**(C^{α}, C′) tensors in the molecular framework are reported for glycylglycine.

The measurement and interpretation of residual dipolar coupling constants (RDCs) between spatially proximate spin-pairs in biopolymers such as proteins, nucleic acids, and oligosaccharides in partially oriented environments has become a popular method for structure determination, refinement, and validation (Tolman et al., 1995; Tjandra and Bax, 1997; Prestegard, 1998; Prestegard et al., 2000, 2002; Brunner, 2001; Tolman, 2001; Simon and Sattler, 2002; de Alba and Tjandra, 2002; MacDonald and Lu, 2002; Bax et al., 2002). In this communication, we address the heretofore overlooked issue of the influence of anisotropic indirect nuclear spin-spin coupling tensors on RDCs in biopolymers, with a focus on model peptides. The influence of anisotropic indirect nuclear spin-spin coupling tensors on observed dipolar coupling constants has been investigated for many small molecules dissolved in liquid crystalline solvents (Emsley and Lindon, 1975; Lounila and Jokisaari, 1982; Vaara et al., 2002).

The total nuclear spin-spin coupling Hamiltonian for two nuclear spins $\mathbf{I}_P$ and $\mathbf{I}_Q$ may be written in Cartesiar form as:

$$\mathcal{H}_{DD,J} = h\mathbf{I}_P \cdot \mathbf{D} \cdot \mathbf{I}_Q + hJ_{iso}\mathbf{I}_P\mathbf{I}_Q + h\mathbf{I}_P \cdot \mathbf{J}' \cdot \mathbf{I}_Q, \quad (1)$$

where **D** is the axially symmetric direct dipolar coupling tensor, J_{iso} is the isotropic indirect nuclear spin-spin ('scalar') coupling constant, and $\mathbf{J}'$ is the second-rank anisotropic indirect nuclear spin-spin coupling tensor. Examination of the form of this Hamiltonian indicates that while J_{iso} may be measured independently of the other contributions, the effects of the **D** and $\mathbf{J}'$ tensors *cannot be measured separately* (Emsley and Lindon, 1975; Wasylishen, 1996, 2002; Bryce and Wasylishen, 2000). This fact is summarized in the effective dipolar coupling constant, D_{eff},

$$D_{eff} = D_{PQ} - \frac{\Delta J}{3}, \quad (2)$$

where D_{PQ} is the familiar direct dipolar coupling constant arising from the **D** tensor,

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$$D_{\mathrm{PQ}} = \frac{\mu_0}{4\pi}\frac{\hbar\gamma_{\mathrm{P}}\gamma_{\mathrm{Q}}}{2\pi}\left\langle r_{\mathrm{PQ}}^{-3}\right\rangle \tag{3}$$

and ΔJ is the anisotropy of the **J** tensor,

$$\Delta J = J_{33} - \frac{J_{11}+J_{22}}{3}. \tag{4}$$

The principal components of the **J** tensor are defined and ordered as $|J_{33}-J_{\mathrm{iso}}| \geq |J_{11}-J_{\mathrm{iso}}| \geq |J_{22}-J_{\mathrm{iso}}|$.

Residual dipolar couplings observed in, e.g., dilute liquid crystalline solutions, between nuclei P and Q, are typically expressed as follows (Tjandra and Bax, 1997):

$$D_{\mathrm{PQ}}^{\mathrm{RDC}}(\theta^{\mathrm{D}},\phi^{\mathrm{D}}) = D_{\mathrm{PQ}}\mathbf{S}\left[A_{\mathrm{a}}(3\cos^2\theta^{\mathrm{D}}-1) + \frac{3}{2}A_{\mathrm{r}}\,\sin^2\theta^{\mathrm{D}}\,\cos 2\phi^{\mathrm{D}}\right], \tag{5}$$

where **S** is a generalized order parameter (Lipari and Szabo, 1982), A_{a} denotes the axially symmetric component of the molecular alignment tensor, **A**, and A_{r} denotes the asymmetric or rhombic component of the molecular alignment tensor. The angles θ^{D} and ϕ^{D} describe the orientation of the principal axis system (PAS) of the **D** tensor in the PAS of **A**. As described above, any observed direct dipolar coupling in fact contains a contribution from the anisotropy in **J**; thus Equation 5 may be written in modified form as:

$$D_{\mathrm{PQ}}^{\mathrm{eRDC}}(\theta^{\mathrm{D}},\phi^{\mathrm{D}},\theta^{\mathrm{J}},\phi^{\mathrm{J}}) = \begin{bmatrix} D_{\mathrm{PQ}}\left(A_{\mathrm{a}}(3\,\cos^2\theta^{\mathrm{D}}-1)+\frac{3}{2}A_{\mathrm{r}}\sin^2\theta^{\mathrm{D}}\,\cos 2\phi^{\mathrm{D}}\right) \\ -\frac{\Delta J}{3}\left(A_{\mathrm{a}}(3\cos^2\theta^{\mathrm{J}}-1)+\frac{3}{2}A_{\mathrm{r}}\,\sin^2\theta^{\mathrm{J}}\,\cos 2\phi^{\mathrm{J}}\right) \end{bmatrix}, \tag{6}$$

where the 'eRDC' superscript is used to denote the *effective* residual dipolar coupling. Since the PAS of **J** is not *a priori* coincident with the PAS of **D**, the angles θ^{J} and ϕ^{J} must be introduced to describe the orientation of **J** in the PAS of **A**. For simplicity, the assumption that **J** is axially symmetric has been made in Equation 6. A more general equation which incorporates the asymmetry of the **J** tensor requires three Euler angles, and may be formulated in a manner analogous to that used to describe the relative orientations of non-coincident asymmetric chemical shift and electric field gradient tensors given by Chu and Gerstein (1989), Cheng et al. (1990), and Power et al. (1990).

If one makes the further simplifying assumption that the PASs of **D** and **J** are coincident, then one may express the eRDC incorporating ΔJ in a form analogous to Equation 5:

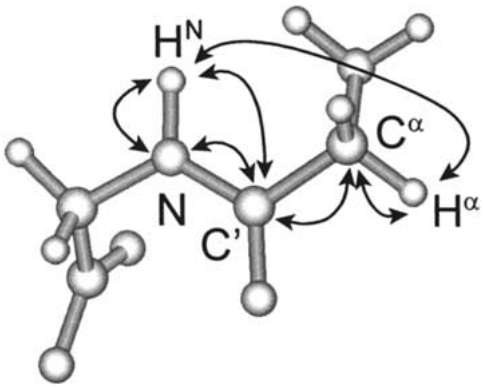

Figure 1. Structure of α-helical glycylglycine ($^{-}$OOC-CH_2-N(H)-C(O)-CH_2-NH_2) and selected spin pairs for which residual dipolar couplings are typically measured in larger peptides and proteins.

$$D_{\mathrm{PQ}}^{\mathrm{eRDC}}(\theta^{\mathrm{D}},\phi^{\mathrm{D}}) = D_{\mathrm{eff}}\mathbf{S}\left[A_{\mathrm{a}}(3\cos^2\theta^{\mathrm{D}}-1) + \frac{3}{2}A_{\mathrm{r}}\,\sin^2\theta^{\mathrm{D}}\,\cos 2\phi^{\mathrm{D}}\right]. \tag{7}$$

The important point is that whenever a residual dipolar splitting is measured, e.g., for a protein in a dilute liquid crystalline solution, this splitting contains an unknown contribution from ΔJ. While in many cases, especially for light atoms, the contribution due to ΔJ may be small compared to D_{PQ}, there are also many cases known where ΔJ is comparable to D_{PQ} (Bryce and Wasylishen, 2000; Bryce et al., 2002; Vaara et al., 2002). Lack of knowledge of the value of ΔJ therefore introduces uncertainty in relating observed eRDCs to structure, i.e., bond lengths and bond vector orientations.

Over the past few years, density-functional theory (DFT) has become established as a successful technique for investigating J-couplings in peptides and proteins (Bagno, 2000; Cornilescu et al., 2000; Case et al., 2000; Barfield, 2002). Presently, we evaluate the influence of ΔJ on eRDCs employed for protein structure refinement through DFT calculations of the complete **J** tensors for N-H^{N}, N-C′, C^{α}-H^{α}, H^{α}-H^{N}, C^{α}-C′, and C′-H^{N} spin pairs in model dipeptides (Figure 1), where C′ is the amide carbonyl carbon. Glycylglycine was constructed using the mean bond lengths for polypeptides recommended by Engh and Huber (1991): 1.329 Å for $r_{\mathrm{NC'}}$, 1.231 Å for $r_{\mathrm{OC'}}$, and 1.516 Å for $r_{\mathrm{C'C^{\alpha}}}$. For calculations on serylserine, a value of 1.525 Å was used for $r_{\mathrm{C'C^{\alpha}}}$. The amide N-$H^{\mathrm{N}}$ bond lengths were set to 1.02 Å in accordance with neutron diffraction studies (Kvick et al., 1977;

Roberts et al., 1987; Jeffrey, 1992), and a standard carbon-hydrogen bond length of 1.09 Å was used at C^{α}. In separate calculations, standard α-helix angles, $\phi = -58°$ and $\psi = -47°$, as well as extended chain angles, $\phi = \psi = 180°$, were employed. In Ser-Ser, the sidechain was oriented in a staggered conformation such that the C^{β}-OH bond vector bisects the C'-C^{α}-H^{α} angle. The C-terminal ends of the dipeptides were in the ionized form COO^{-}, while the N-terminal ends were converted to a neutral NH_2 group to facilitate convergence of the calculations.

DFT calculations of **J** coupling tensors were carried out using the CPL module (Dickson and Ziegler, 1996; Khandogin and Ziegler, 1999; Autschbach and Ziegler, 2000a, b) of the Amsterdam Density Functional (ADF 2000.01, 2000.02) software package (Baerends et al., 1973; Versluis and Ziegler, 1988; te Velde and Baerends, 1992; Fonseca Guerra et al., 1998). The XC functional employed the local density approximation (LDA) of Vosko, Wilk, and Nusair (VWN) (Vosko et al., 1980), and the Becke-Perdew generalized gradient approximation (GGA) (Becke, 1988; Perdew, 1986). All contributions to the spin-spin coupling tensors have been included in the calculations: Fermi-contact (FC), diamagnetic spin-orbit (DSO), paramagnetic spin-orbit (PSO), and spin-dipolar (SD). Consideration of terms other than FC is essential since the FC contribution to **J** is isotropic and thus ΔJ(FC) is zero. Calculations were carried out with the ADF double-ζ 'II', double-ζ plus polarization 'III', core double-ζ valence triple-ζ polarized 'IV', and core double-ζ valence triple-ζ double-polarized 'V' basis sets. The highest-quality 'V' results are discussed herein.

Shown in Table 1 are the calculated values of ΔJ in α-helical and extended chain glycylglycine for a variety of spin pairs for which eRDCs are typically observed experimentally in proteins. Also shown are the calculated values for J_{iso}; these values are generally in good agreement with experimentally known values, e.g., in small peptides, $^{1}J_{iso}(^{15}N, H^{N})$ is known to range from −89.3 to −94.5 Hz (Witanowski et al., 1977; Martin et al., 1981), $^{1}J_{iso}(^{15}N, ^{13}C')$ varies from −17.7 to −18.9 Hz in Gly-Gly (Irving et al., 1976; Martin et al., 1981), and $^{1}J_{iso}(^{13}C^{\alpha}, ^{13}C')$ generally lies between +50 and +60 Hz for amino acids, depending on the pH (Tran-Dihn et al., 1974, 1975; Wasylishen, 1977). The signs of typical $^{1}J_{iso}(^{15}N,H)$, $^{1}J_{iso}(^{15}N, ^{13}C)$, and $^{1}J_{iso}(^{13}C, ^{13}C)$ coupling constants, as indicated above, are experimentally established (Lynden-Bell and Sheppard, 1962; McFarlane, 1966, 1967; Grant, 1967; Chuck et al., 1969; Randall and Gillies, 1970; Yeh et al., 1972; Friesen and Wasylishen, 1982; Jameson, 1987).

Table 1. Calculated[a] indirect nuclear spin-spin coupling tensors for selected spin pairs[b] in α-helix (first row of data for each spin pair) and extended chain (second row of data for each spin pair) glycylglycine and the corresponding direct dipolar coupling constants[c]

Spin pair	J_{iso}/Hz	ΔJ/Hz	D_{PQ}/Hz	$(-\Delta J/3)/D_{eff}$ (%)
N-H^{N}	−83.1	13.0	−11480	0.038
	−92.6	16.7	−11480	0.048
N-C'	−19.0	−19.8	−1305	−0.508
	−17.2	−20.4	−1305	−0.524
C^{α}-H^{α}	158.1	−19.0	23330	0.027
	142.0	−19.0	23330	0.027
H^{α}-H^{N}	0.23	1.67	2605	−0.021
	−0.53	−0.73	6991	0.003
C^{α}-C'	53.5	29.5	2181	−0.453
	48.1	28.9	2181	−0.444
C'-H^{N}	4.8	4.2	3610	−0.039
	5.3	4.9	3610	−0.045

[a] ADF program, 'V' basis set.
[b] Coupling constants are reported for ^{1}H, ^{13}C, and ^{15}N isotopes.
[c] For completeness, the asymmetry parameters, $\eta_J = (J_{22} - J_{11})/(J_{33} - J_{iso})$ are for α-helical GlyGly: 0.45 for N-H^{N}, 0.73 for N-C', 0.08 for H^{α}-C^{α}, 0.29 for H^{α}-H^{N}, 0.07 for C^{α}-C' and 0.64 for C'-H^{N}; for extended chain Gly Gly: 0.28 for N-H^{N}, 0.81 for N-C', 0.13 for H^{α}-C^{α}, 0.73 for H^{α}-H^{N}, 0.11 for C^{α}-C', and 0.58 for C'-H^{N}.

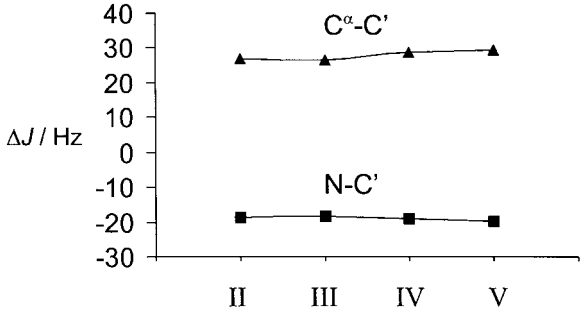

Figure 2. Basis set dependence of the indirect nuclear spin-spin coupling anisotropies for the N-C' (■) and C^{α}-C' (▲) spin pairs in α-helical glycylglycine. The computational methods are described in the text. For the N-C' coupling tensor, ΔJ has values of −18.7 Hz (II basis), −18.4 Hz (III basis), −19.2 Hz (IV basis), and −19.8 Hz (V basis). For the C^{α}-C' coupling tensor, ΔJ has values of 26.7 Hz (II basis), 26.5 Hz (III basis), 28.6 Hz (IV basis), and 29.5 Hz (V basis).

From the data in Table 1, ΔJ is clearly typically of significant magnitude compared to J_{iso}. Also shown in Table 1 are the equilibrium direct dipolar coupling constants determined *via* Equation 3 and the equi-

librium bond lengths. The ratio $(-\Delta J/3)/D_{\text{eff}}$ (cf. Equation 2) is found to be negligible for all spin pairs involving at least one proton. For the N-C′ and C^{α}-C′ spin pairs, in both α-helix and extended chain forms, this ratio is approximately −0.5%. A drastic departure from this order of magnitude for different sets of φ, ψ angles is not anticipated. The calculated values of ΔJ for these spin pairs are quite constant as a function of the basis set used, as shown in Figure 2.

The isotropic coupling constants are dominated by the FC and SD mechanisms, while ΔJ (N,C′) and $\Delta J(C^{\alpha},C')$ are dominated by the cross term between the FC and SD mechanisms. These mechanisms were also observed to be dominant for $^{2h}\mathbf{J}$(N, N) coupling tensors on the basis of high-level *ab initio* calculations (Bryce and Wasylishen, 2001).

The orientations of $\mathbf{J}$(N,C′) and $\mathbf{J}(C^{\alpha},C')$ may be compared with the typical orientations of the nitrogen and carbon chemical shift tensors in peptides. In contrast to the chemical shift tensor, which has an orientation in the molecular framework that depends primarily on the electronic structure and site symmetry about a single nucleus of interest, the properties of the $\mathbf{J}$ tensor depend on the electronic structure and molecular symmetry about two coupled nuclei. Shown in Figure 3 are the orientations of the $\mathbf{J}$(N,C′) and $\mathbf{J}(C^{\alpha},C')$ tensors in the glycylglycine molecular framework. In all cases, the J_{33} component lies along the internuclear vector and is therefore coincident with the largest component of $\mathbf{D}$. For nitrogen, the most shielded component of the chemical shift tensor, δ_{33}, typically lies closest to the N-C′ bond axis and the intermediate component δ_{22} is approximately perpendicular to the amide plane (Lumsden et al., 1994; Brender et al., 2001). Thus, in the case of nitrogen, the orientations of $\mathbf{J}$(N,C′) and the chemical shift tensor are similar. This is in contrast with the situation for C′, for which δ_{33} lies approximately perpendicular to the amide plane (Eichele et al., 1993; Takeda et al., 1999; Wei et al., 2001); the orientations of both $\mathbf{J}$(N,C′) and $\mathbf{J}(C^{\alpha},C')$ bear no resemblance to the orientation of the C′ chemical shift tensor. The orientation of $\mathbf{J}$(N,C′) in glycylglycine is analogous to that calculated for formamide by Vaara et al. (1997).

To further investigate the properties of the $\mathbf{J}$(N,C′) and $\mathbf{J}(C^{\alpha},C')$ tensors, the geometrical dependence of ΔJ for the N-C′ and C^{α}-C′ spin pairs has been investigated by carrying out calculations at r_e, $r_e \pm 0.01$ Å, and $r_e \pm 0.02$ Å. In the region of r_e, the derivatives $\partial(\Delta J)/\partial r$ for the N-C′ and C^{α}-C′ spin pairs of α-helical Gly-Gly are found to be:

$$(\partial(\Delta J(\mathrm{N}, \mathrm{C}'))/\partial r)r_e = 4.71 \text{ Hz/Å}, \quad (8)$$

$$(\partial(\Delta J(\mathrm{C}^{\alpha}, \mathrm{C}'))/\partial r)r_e = 1.87 \text{ Hz/Å}, \quad (9)$$

The data indicate that ΔJ has a shallow distance dependence relative to D_{PQ}, which varies with the inverse cube of r_{PQ} (cf. Equation 3). Hence, the relative importance of ΔJ becomes marginally larger for greater N-C′ and C^{α}-C′ separations. The average equilibrium C^{α}-C′ bond length is known to be shorter in glycine residues (1.516 Å) than in residues with side chains (1.525 Å) (Engh and Huber, 1991). Calculations of the $\mathbf{J}(C^{\alpha}, C')$ tensor in the extended chain Ser-Ser dipeptide indicate that $\Delta J(C^{\alpha}, C')$ is 28.8 Hz, which is essentially identical to the value calculated for extended chain Gly-Gly, 28.9 Hz. In contrast, the value of $D_{C^{\alpha}C'}$ decreases by about 2% in Ser-Ser due to the longer C^{α}-C′ bond.

Beyond the peptide backbone, residual dipolar couplings measured between nuclei within protein sidechains may also be employed to gain important structural information (Chou and Bax, 2001; Cai et al., 2001; Chou et al., 2001). Most of the measured couplings have involved one proton, e.g., the C^{β}-H^{β} spin pair. Calculations on extended chain Ser-Ser indicate that the contribution from $\Delta J(C^{\beta}, H^{\beta 1})$ and $\Delta J(C^{\beta}, H^{\beta 2})$ to the overall effective dipolar coupling is only 0.027%, which is identical to the case for the C^{α}-H^{α} spin pair (Table 1). A significant change in the magnitude of $\Delta J(C^{\beta}, H^{\beta})$ for different sidechain rotamers is not expected.

As observed eRDCs are commonly used to define bond vector orientations, it is of interest to determine the extent to which ΔJ may influence the measurement of such orientations. In general, an error due to the neglect of ΔJ will have an effect analogous to any error introduced in the measurement of the eRDC, which depends on the digital resolution, line width, and signal-to-noise ratio of the relevant peaks in the NMR spectra (Bax et al., 2002), or due to vibrational averaging of the direct dipolar coupling tensor (Case, 1999). The effect of an error of 0.5% in a measured eRDC may be interpreted in terms of the most simple version of Equation 7, where one assumes that $\mathbf{A}$ is axially symmetric. In such a case, the magnitude of the error introduced into θ only exceeds one degree for extreme values of θ, i.e., $\theta \approx 0°$ or $\theta \approx 90°$. Between the angles $\theta \approx 33°$ and $\theta \approx 70°$, the magnitude of the error in θ does not exceed approximately 0.1°.

We note that all the calculations described herein have been carried out on isolated molecules, and therefore intermolecular effects on the coupling ten-

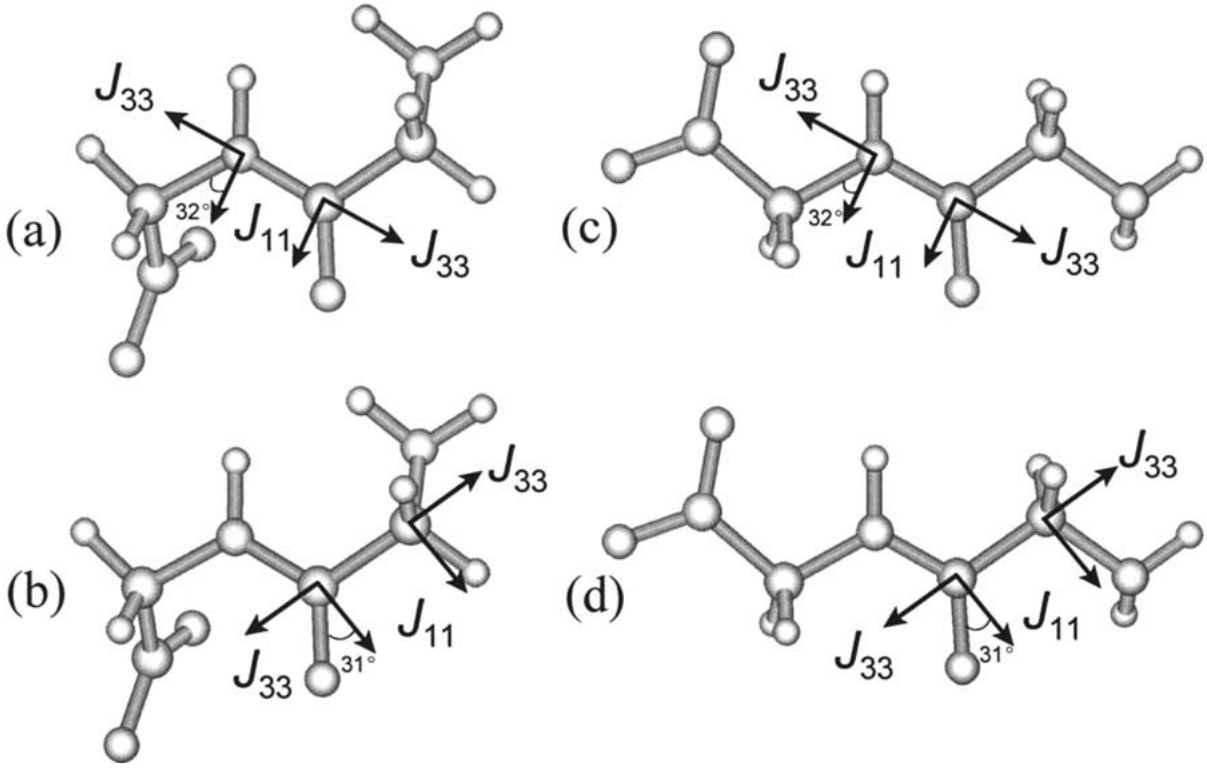

Figure 3. Calculated orientations of (a) the **J**(N,C′) and (b) **J**(C^{α},C′) coupling tensors in α-helical glycylglycine ($^{-}$OOC-CH_2-N(H)-C(O)-CH_2-NH_2) and (c) the **J**(N,C′) and (d) **J**(C^{α},C′) coupling tensors in extended chain glycylglycine. In all cases, the J_{33} component lies along the internuclear vector, and the J_{22} component is perpendicular to the amide plane.

sors, such as those arising from hydrogen bonding or solvation, have not been taken into account. While these effects are likely to induce subtle changes to the calculated coupling tensors, the general conclusions reached will not be altered.

In conclusion, this work has addressed the issue of the influence of anisotropic indirect nuclear spin-spin coupling tensors on observed residual dipolar coupling constants. Small corrections to measured eRDCs in peptides and proteins, +0.45% for C^{α}-C′ spin pairs and +0.51% for N-C′ spin pairs, are recommended on the basis of DFT calculations on the model peptides Gly-Gly and Ser-Ser. While any improvements in protein structure refinement due to these corrections will certainly be minor, it is nevertheless important to be aware of the influence of ΔJ on observed eRDCs. All couplings involving at least one proton do not require any significant correction for the effects of ΔJ. The latter point will be equally applicable in the cases of other biopolymers such as nucleic acids and oligosaccharides. In contrast, residual dipolar couplings involving metals, e.g., ^{57}Fe, $^{111/113}$Cd, ^{195}Pt, and ^{13}C or ^{15}N in metalloproteins may have significant contributions from ΔJ. Finally, we emphasize that the influence of ΔJ on effective dipolar coupling constants (Equation 2) is a general phenomenon, in principle affecting any measurement involving direct dipolar coupling, e.g., relaxation of nuclear spins by the dipole-dipole mechanism.

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