# A Tensor-Free Method for the Structural and Dynamical Refinement of Proteins using Residual Dipolar Couplings

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ABSTRACT: Residual dipolar couplings (RDCs) are parameters measured in nuclear magnetic resonance spectroscopy that can provide exquisitely detailed information about the structure and dynamics of biological macromolecules. We describe here a method of using RDCs for the structural and dynamical refinement of proteins that is based on the observation that the RDC between two atomic nuclei depends directly on the angle ϑ between the internuclear vector and the external magnetic field. For every pair of nuclei for which an RDC is available experimentally, we introduce a structural restraint to minimize the deviation from the value of the angle ϑ derived from the measured RDC and that calculated in the refinement protocol. As each restraint involves only the calculation of the angle ϑ of the corresponding internuclear vector, the method does not require the definition of an overall alignment tensor to describe the preferred orientation of the protein with respect to the alignment medium. Application to the case of ubiquitin demonstrates that this method enables an accurate refinement of the structure and dynamics of this protein to be obtained.
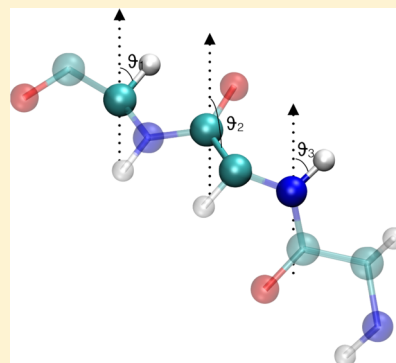
INTRODUCTION

Residual dipolar couplings (RDCs) have emerged as one of the most useful parameters in biomolecular nuclear magnetic resonance (NMR) spectroscopy.[1,2] They have been applied to a wide range of different problems, ranging from the determination of the structure of proteins,[1−8] nucleic acids,[9−12] and carbohydrates,[13−16] to the characterization of their dynamics.[17−32]

The RDC between two atomic nuclei depends on the angle between the internuclear vector and the external magnetic field.[33,34] In isotropic media RDCs average to zero because of orientational averaging, but when the rotational symmetry is broken, either through the introduction of an alignment medium[1,2] or for molecules with highly anisotropic paramagnetic susceptibility,[35] RDCs become measurable. In order to translate the information provided by RDCs into molecular structures, several accurate methods for calculating the RDCs corresponding to given conformations have been developed.[36−44] These methods are based on the introduction of an alignment tensor to describe the preferential orientation of a molecule with respect to the alignment medium.[1,45,46]

In addition to their usefulness in protein structure determination, RDCs can also be used for characterizing the dynamics of proteins. These NMR parameters, however, tend to have a strong structural dependence and, hence, to experience large fluctuations as a protein explores its conformational space,[47,48] which is an aspect that complicates the extraction of the information about dynamics from them. When conformational fluctuations of large amplitude are present, even the most accurate methods for calculating the RDCs for a given structure[36−44] may not provide values that can be expected to match the experimental ones. A close agreement between calculated and experimental RDCs can in these cases be obtained by averaging the calculated RDCs over an ensemble of structures representing the motions of the protein.[17−28,44,49−51]

As the calculation of the alignment tensor requires procedures of a certain complexity, which in some cases, in particular when electrostatic alignment media are used, can be very challenging,[36−44] it is interesting to explore alternative "tensor-free" methods that do not require the introduction of an alignment tensor.[52] For this purpose, here we describe a method for protein structural and dynamical refinement based on the direct dependence of the RDC between two atomic nuclei on the angle ϑ between the internuclear vector and the external magnetic field. In this protocol, called the "ϑ method", one introduces in the refinement protocol a structural restraint that minimizes the deviation from the experimental and calculated values of the angle ϑ.

The main advantage of the ϑ method is its simplicity. The relationship between an RDC and the structure of a protein is described in a straightforward manner by the orientation of the corresponding internuclear vector with respect to the external magnetic field (Figure 1). In this sense, the ϑ method requires just the calculation of the angles ϑ for the interatomic bonds for which RDCs have been measured, and not that of the overall alignment tensor. We illustrate the ϑ method by presenting its application to the refinement of the structure and dynamics of the protein ubiquitin, showing that it leads to results essentially as accurate as those obtained by standard NMR approaches.
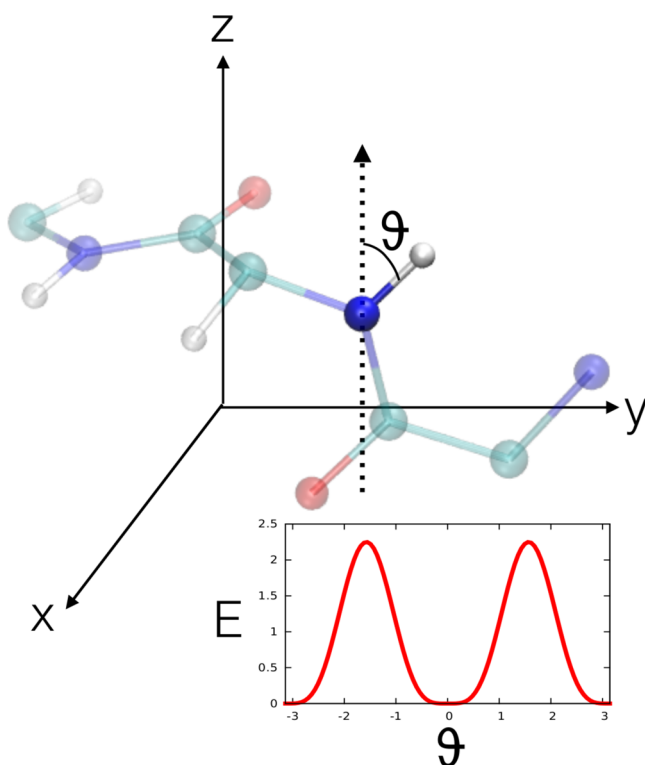
Figure 1. Illustration of the ϑ angle used in the ϑ method. The ϑ angle is defined as the angle between the direction of the internuclear vector between the two atoms for which an RDC is measured and the direction of the external magnetic field, which is conventionally assumed to be along the z axis. In the figure, the ϑ angle is shown for a backbone NH bond. The inset shows an example of a penalty function of the type used in eq 3 [i.e., $E = (D^{exp} - D^{cal})^2$], for a case in which the RDC takes its maximum possible value (corresponding to $\vartheta = 0$ or $\vartheta = \pi$); this penalty function restrains the angle ϑ to the value of the corresponding experimental RDC.

■ METHODS

RDC between Two Nuclear Spins. The RDC between two nuclear spins of gyromagnetic ratios γ_1 and γ_2 at a given distance r can be written as[34]

$$D = D_{max}\langle(3\cos^2\vartheta - 1)/2\rangle \quad (1)$$

where ϑ is the angle between the internuclear vector and the external magnetic field, $D_{max} = -\mu_0\gamma_1\gamma_2 h/8\pi^3 r^3$ is the maximal value of the dipolar coupling for the two nuclear spins, μ_0 is the magnetic constant, and h is the Planck constant. We note that the angle ϑ should not be confused with one of the two polar angles describing the position of the internuclear vector in the eigenframe of the alignment tensor, which is sometimes also indicated by ϑ. The averaging specified by the angular brackets describes the variations in the orientation of the internuclear vector with respect to the external magnetic field caused by thermal motions. In isotropic solutions, the RDCs average to zero because all directions are equivalent. By contrast, if the solution is anisotropic, as in the case of the addition of alignment media, the orientational symmetry is broken, and nonzero values of the RDCs may appear.[1,2,34,35,45,46,53,54]

Calculation of RDCs Using Alignment Tensor Methods. When a structural model of the protein is available, there are several ways to carry out the average in eq 1 to estimate the corresponding RDCs. The most common approaches involve the definition of an alignment tensor, either explic-

itly[4,34,45,46,53−58] or implicitly,[59,60] a procedure that is particularly convenient if a protein populates a rigid structure, so that the only important degrees of freedom in eq 1 concern the relative orientation of the molecule with respect to the alignment medium. In this case, one should consider just 5 degrees of freedom for the rotations and 3 further degrees of freedom for the translations of a protein molecule. More generally, if a protein undergoes conformational fluctuations, it is still possible to define an alignment tensor, although in this case the averaging has to be carried out not only over the rotations and translation of the molecule with respect to the alignment medium but also with respect to its internal degrees of freedom.

The alignment tensor of a given protein conformation can be obtained through fitting procedures, such as the singular-value decomposition (SVD) method,[55] in which the alignment tensor is chosen to optimize the agreement between calculated and experimental RDCs. Alternatively, the alignment tensor can be determined by structure-based procedures in which this quantity is calculated on the basis of the shape and charge of the protein molecule and the alignment medium,[27,28,36−44,51] without reference to experimentally measured RDCs.

These two approaches are generally applicable to different situations. This aspect can be understood in particular in the presence of conformational fluctuations of large amplitude. In this case, the calculation of the average RDCs corresponding to an ensemble of conformations involves the definition of a different alignment tensor for each conformation in the ensemble. In approaches in which the RDCs are fitted to a structure, to simplify the calculations, one can assume that all the conformations in the ensemble have the same alignment tensor, which, however, is often not an accurate approximation.[28] Alternatively, to achieve greater accuracy, one can obtain the alignment tensor of each individual conformation by a separate fitting to the experimental RDCs. In this case, however, an impractically large number of experimental RDCs is required in order to avoid overfitting. Therefore, fitting methods are at risk of failing to capture the full changes in the alignment tensor during the conformational fluctuations.[27,28,44,51]

In the presence of conformational fluctuations, it is more effective to use structure-based methods.[27,28,44,50,51,61−63] In this case, each member in a structural ensemble can be associated with its own alignment tensor without the need of using experimental data. In practice, the averaging in eq 1 is carried out both over the external degrees of freedom, which involve rotations and translations, and the internal ones, which involve conformational fluctuations of a protein.

The Method of Structural Refinement. As mentioned above, it is not necessary to recast eq 1 in the framework of the internal coordinates and hence as a function of an alignment tensor, as this equation is well-defined as a function of the angle ϑ between the internuclear vector and the magnetic field, whose direction is usually taken as that of the z axis (Figure 1). One can thus use the information about the ϑ angles provided by the RDCs to refine the structures of proteins. In this approach, one asks if there is a structure that satisfies at the same time all the internuclear vector orientations specified from the ϑ angles with respect to the z axis.

The advantage of using the definition of the RDCs without recasting the equations in a tensor-dependent way is that of removing the need of calculating the alignment tensor, either implicitly by means of the single value decomposition or

Table 1. Assessment of the Structure of Ubiquitin (2MOR) Obtained Using the ϑ Method in Comparison with High-Resolution X-ray (1UBQ[70]) and NMR (1D3Z[77]) Structures[a]

	1UBQ (X-ray)	1D3Z (4159 restraints)	2MOR (381 restraints)
	Q Factor for the RDCs[71] Used in This Work as Restraints (SVD)		
N−H (AA 1−70/1−76)	0.16/0.21	(0.05)/0.19	(0.12)/0.18
Cα−Hα (AA 1−70/1−76)	0.30/0.28	(0.10)/0.13	(0.13)/0.13
Cα−C′ (AA 1−70/1−76)	0.22/0.31	(0.17)/0.27	(0.12)/0.24
C′−N (AA 1−70/1−76)	0.22/0.21	(0.17)/0.23	(0.14)/0.34
C′−H (AA 1−70/1−76)	0.29/0.32	(0.13)/0.29	(0.16)/0.29
	PROCHECK Structure Quality Check		
φ/ψ	1.0	1.0	1.0
H bonds	1.7	1.4	1.6
χ_1	2.0	1.0	1.0
χ_2	1.4	1.0	1.0
ω	1.0	1.0	1.0
	Q Factor for Additional 36 Sets of RDCs[84]		
AA 1−70	0.21 ± 0.03	0.17 ± 0.05	0.19 ± 0.04
AA 1−76	0.29 ± 0.06	0.29 ± 0.07	0.29 ± 0.07
	Q Factor for Squalamine RDCs[85]		
N−H (AA 1−70/1−76)	0.21/0.29	0.14/0.24	0.23/0.40
Cα−Hα (AA 1−70/1−76)	0.39/0.42	0.26/0.40	0.36/0.43
Cα−C′ (AA 1−70/1−76)	0.23/0.32	0.14/0.28	0.20/0.23
C′−N (AA 1−70/1−76)	0.22/0.33	0.20/0.28	0.25/0.37
C′−H (AA 1−70/1−76)	0.38/0.47	0.30/0.51	0.28/0.43
	Agreement with Other NMR Observables		
$^3J_{HNC'}$ RMSD (HZ)	0.22	0.30	0.26
$^3J_{HNC'}$ R	0.74	0.41	0.79
no. NOE violations	62/1320	(0/1320)	43/1320
Cα chemical shifts RMSD (ppm)	0.5	0.7	0.6
Cβ chemical shifts RMSD (ppm)	0.6	0.8	1.0
C′ chemical shifts RMSD (ppm)	0.6	0.6	0.6
HN chemical shifts RMSD (ppm)	0.2	0.3	0.3
Hα chemical shifts RMSD (ppm)	0.1	0.2	0.1
N chemical shifts RMSD (ppm)	1.9	2.2	2.2
methyl chemical shifts RMSD (ppm)	0.1	0.1	0.1

[a]Q factors were obtained using the SVD method to back-calculate the RDCs from the structures. Numbers in parentheses indicate parameters used as restraints in the structure determination protocol; Q factors are given separately for the protein without the C-terminal tail (AA 1−70) and the full-length protein (AA 1−76). The PROCHECK method[78] was used to quantify the structural quality for backbone (φ/ψ) and side-chain (χ_1, χ_2, and ω) dihedral angles and hydrogen-bond geometries (H bonds). Scalar coupling through hydrogen bond have been calculated using a simple geometric relation (see text). NOE have been calculated using the PROSESS web server.[79] Backbone chemical shifts are calculated with SHIFTX2[80] and methyl ^{1}H chemical shifts using CH3Shifts[82].

explicitly by modeling the alignment media, which are procedures that add approximations as well as computational burden. The method discussed in this work does not require the knowledge of the alignment tensor, and its results do not depend on the properties (as for example the axial symmetry or the rhombicity) of the alignment tensor itself.

In order to implement this strategy for structural refinement, we included an additional term to the CHARMM22* force field[64] using PLUMED 2[65] to maximize the correlation, ρ, between the calculated, D^{calc}, and the experimental, D^{exp}, RDCs

$$V_\vartheta = -K_\vartheta[\rho(D^{calc}, D^{exp}) - 1] \tag{2}$$

Once a high correlation is obtained, it is possible to find the scaling factor for the RDCs as the slope of the line that fits D^{exp} as a function of D^{calc} and hence apply a simpler restraining potential of the form

$$E_\vartheta = K_\vartheta\left[\sum_i \left(D_i^{calc} - D_i^{exp}\right)^2\right] \tag{3}$$

where i runs over the experimental RDCs. In the implementation presented in eq 2, the ϑ method can be applied to multiple bonds measured in a single alignment medium. Subject to further developments, however, it may be possible to extend its use to multiple alignment media.

In the calculations, we also added a potential on the ω angles of the peptide bonds

$$V_\omega = \frac{K_\omega}{2}[1 + \cos(\omega - \omega_{ref})] \tag{4}$$

with K_ω set to 2500 kJ/mol. This term was introduced because in unrestrained simulations of ubiquitin we noticed that using the CHARMM22* force field resulted in a distribution of the values of the ω angles slightly wider than expected from X-ray structures in the PDB.

The Method of Dynamical Refinement. In order to extract the information about dynamics provided by RDCs, we incorporated them as replica-averaged structural restraints in molecular dynamics simulations.[17,19,21,27,28,44,49−51] This approach generates an ensemble of conformations consistent with the maximum entropy principle.[66−69] In this view, the generated ensemble is the most probable one, given the force

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field and the experimental data included, that reproduces at the same time the conformational dynamics of the system under study and the distribution of the orientations with respect to the alignment media employed to measure the RDCs. To this effect, in eq 3 we averaged the calculated RDCs over 8 replicas of the protein molecule. In this respect, the structural refinement procedure can be seen as a limiting case in which the dynamics can be well-represented by a single average structure. In the case of the refinement of the dynamics, the additional restraint in eq 4 was not added.

■ RESULTS AND DISCUSSION

Refinement of the Structure of Ubiquitin Using the ϑ Method. To illustrate the use of the ϑ method, we applied the structure refinement protocol described in the Method section starting from an X-ray structure of ubiquitin (1UBQ[70]). We selected a set of experimental RDCs measured in a liquid crystalline phase for the N–H, Cα–Hα, Cα–C′, C′–N, and C′–H bond vectors;[71] only the data for the first 70 residues were used because the last 6 residues belong to a flexible tail (i.e., in total, we used 381 restraints, see Table 1). We prepared the system using GROMACS,[72] adding hydrogen atoms and explicit solvent. We used the CHARMM22* force field,[64] a cubic box of 6.5 nm of side with 8700 TIP3P water molecules.[73] A time step of 2 fs was used together with LINCS constraints.[74] The van der Waals interactions were cutoff at 0.9 nm, and long-range electrostatic effects were treated with the particle mesh Ewald method.[75] All simulations were carried out keeping the volume fixed and by thermosetting the system with the Bussi thermostat.[76]

The energy of the system was first minimized without accounting for the additional term. Then the temperature was raised to 300 K by a linear increase in 300 ps. In this phase, together with the temperature, the RDC restraint constant K_Θ was also increased linearly from 100 to 5000 kJ/mol. The system was then evolved for further 200 ps at constant temperature. After that, K_Θ was further increased linearly from 5000 to 15000 kJ/mol in 200 ps. Then the simulations were run for further 1.3 ns. In addition to the RDCs restraint, we have also employed a restraint on the ω angles of the peptide bonds as illustrated in eq 4 in Methods. At the end of the 2 ns simulation, the correlation between experimental and calculated RDCs was about 0.995 and it was then possible to evaluate the scaling factor using a linear fit of the data. In this way, it was possible to directly compare the values of the calculated RDCs with the corresponding experimental values using the Q factor

$$Q = \sqrt{\sum (D_{calc} - D_{exp})^2 / \sum D_{exp}^2} \qquad (5)$$

The time evolution during the structure refinement of the Q factor for the N–H bonds indicates that the experimental RDCs are closely reproduced at the end of the procedure (Figure 2, black line).

During a short transient time (300 ps) under the effect of the RDC restraints defined in eq 2, the protein experiences an overall rotation with respect to the z axis (Figure 2, black line), but once the optimal orientation is found, the further optimization of individual bond orientations with the ϑ method results in a low value of the Q factor. This value is comparable with that calculated using the SVD method as implemented in PALES.[36,41] (Figure 2, red curve). We note that since the SVD method is insensitive to the overall rotations, the 300 ps transient time exhibits lower Q values (Figure 1, red curve).
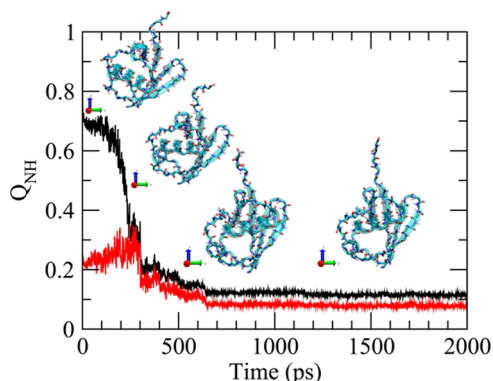

Figure 2. Minimisation of the Q factor for the N–H bonds during the structure refinement procedure of ubiquitin using the ϑ method (black curve). For comparison, the Q factor calculated using the standard SVD method for the structures in the same trajectory is also shown (red curve).

To further test the ϑ method, we applied the same protocol starting from a poor quality structure at about 2.5 Å from the reference X-ray structure of ubiquitin (1UBQ, shown in turquoise in Figure 3). We found that after about 4.5 ns the
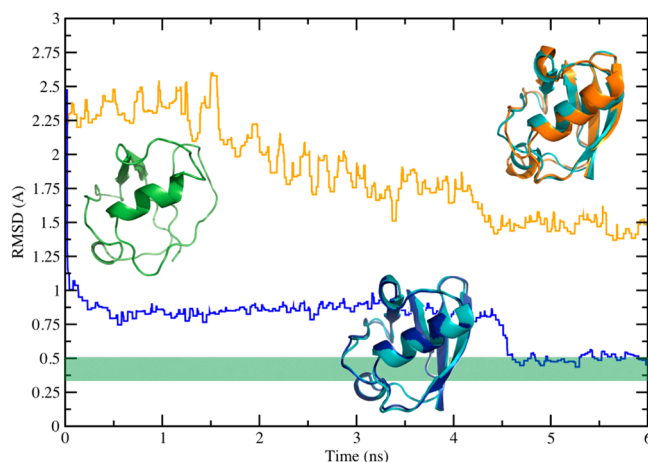

Figure 3. Refinement using the ϑ method from a starting structure of poor quality. Starting from a structure (shown in green) at about 2.5 Å from a reference X-ray structure of ubiquitin (1UBQ,[70] shown in turquoise), we applied the ϑ method, finding that after about 4.5 ns, the RMSD with 1UBQ (shown in blue) became comparable with that of that of the high-resolution reference NMR structure (1D3Z[77]); the green band indicates the RMSD between the 10 models in the 1D3Z file and the 1UBQ reference structure. For comparison, we show the RMSD resulting from an unrestrained simulation using the same force field but without the RDC restraints (shown in yellow).

RMSD with 1UBQ became comparable with that of the high-resolution reference NMR structure (1D3Z[77]). For comparison, the RMSD resulting from an unrestrained simulation using the same force field but without the RDC restraints did not decrease below about 1.3 Å during the simulation.

Validation of the Structure of Ubiquitin. From the refinement procedure described above, we selected the structure with the best average Q factor over all RDCs used as restraints (Table 1). This structure (2MOR, shown in green in Figure 4), which was obtained with 381 restraints, is very close to a high-resolution structure of ubiquitin obtained using a total of 4159 restraints, including RDCs, NOEs, and J-
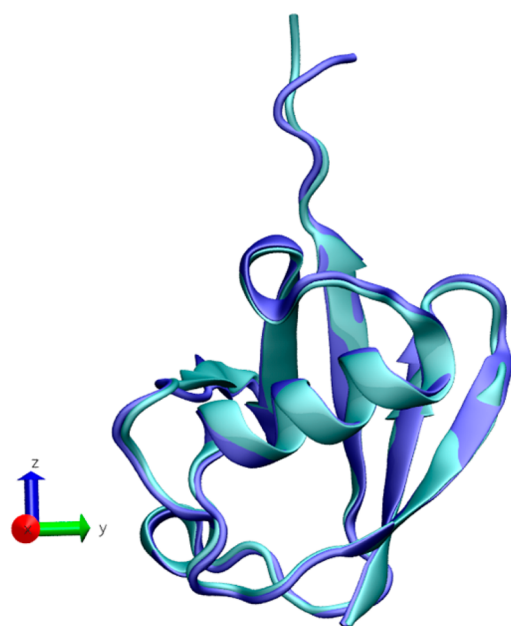
Figure 4. Comparison of the structure obtained in this work (2MOR, in turquoise) using the ϑ method with a high-resolution NMR structure (1D3Z,[77] in blue).

couplings (1D3Z,[77] shown in blue in Figure 4). In the comparison shown in Figure 4, we did not perform a standard RMSD alignment of the two structures. Rather, we considered directly the orientation of the structure of ubiquitin resulting from the minimization with the ϑ method, and, to orient the structure of 1D3Z, we calculated using the SVD method its alignment tensor using all the bonds included in the refinement procedure. In this way, we found that the two structures are closely superimposable.

We assessed the quality of the structure obtained using the ϑ method by comparing it to the 1UBQ and 1D3Z structures (Table 1). We used PROCHECK[78] for the quality check of the structure obtained through the structure refinement using the ϑ method. The resulting values for the structural parameters considered by PROCHECK (Table 1) indicate that the restraints that we used do not introduce local distortions in the structure. For the validation of the structures using experimental data not used in the structure refinement, we used the web server PROSESS[79] for the evaluation of the NOE violations, and SHIFTX2[80] for the calculation of the differences between experimental and back-calculated backbone chemical shifts, although we should point out that essentially all the available methods for the back-calculation of chemical shifts are trained on the structure of ubiquitin. Scalar couplings across hydrogen bonds have been calculated as $^{h3}J_{NC} = (-357 \text{ Hz})$ $\exp(-3.2 r_{HO}/\text{Å})\cos^2\theta$, where θ represents the HO=C angle.[81] Methyl ^{1}H chemical shifts are calculated using CH3Shift,[82] and SVD calculation for RDCs have been performed with PALES.[36,41]

Overall, the structure that we obtained using the ϑ method showed a comparable quality with respect to 1UBQ and 1D3Z (Table 1) and represents an improvement over 1UBQ in terms of agreement with several independent experimental measurements, indicating that the refinement protocol that we used is effective in providing structures of high quality.

Refinement of the Dynamics of Ubiquitin Using the ϑ Method. To illustrate the use of the ϑ method within a

dynamical refinement protocol, we use the same simulation set up described for the structure refinement protocol, with the difference that the RDCs are now calculated as averages over 8 replicas of the protein molecule (see Methods) and that the simulations were performed at constant temperature (300 K). We used the same set of experimental RDCs described above for the structural refinement (381 RDCs) (i.e., N−H, Cα−Hα, Cα−C′, C′−N, and C′−H bond vectors measured in a liquid crystalline phase[71]), now including also the data for the last 6 residues belonging to the C-terminal flexible tail, and generated an ensemble of structures (the "ϑ 5-bonds" ensemble) by maximizing the agreement between experimental and back-calculated RDCs.

Eight starting structures for the replica-averaged RDCs restrained simulation were generated by running eight 1 ns simulations from the solvated 1UBQ structure without employing experimental restraints. During the first 1 ns in the replica-averaged restrained simulation, the RDCs restraint constant K_Θ was increased linearly from 100 kJ/mol to 50000 kJ/mol, applying the restraint in the form of a correlation (see eq 2). The simulation readily reached a region of the conformational space characterized by small violations of the RDC restraints, as illustrated in the case of the N−H RDCs in Figure 5. We evaluated the scaling factor using a linear fit of the
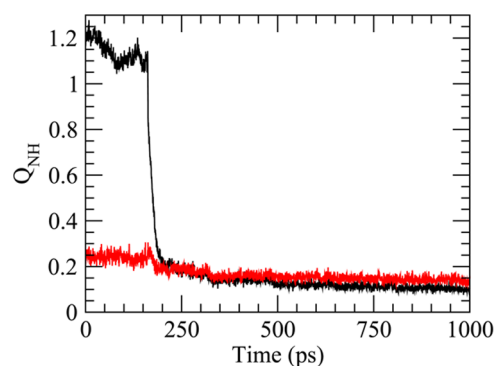

Figure 5. Convergence of the Q factor for the N−H bonds during the ensemble refinement procedure of ubiquitin using the ϑ method (black curve). For comparison, the Q factor calculated using the standard SVD method for the structures in the same trajectory is also shown (red curve).

experimental and calculated RDCs and switched the restraint in the form of eq 3. We then continued the simulations for another 100 ns per replica to sample the conformational space compatible with the averaged restraints and thus generate an ensemble of conformations consistent with the RDCs. As ubiquitin is a rather rigid molecule in its native state, the structures in the ensemble have a narrow distribution of pairwise root-mean-square (RMS) distances (Figure 6).

In order to explore the robustness of the method, we then repeated the calculations by using only two bond vectors (N−H and Cα−Hα), obtaining a second ensemble of structures (the "ϑ 2-bonds" ensemble), which was structurally quite close to the "ϑ 5-bonds" ensemble (Tables 2 and 3).

Validation of the Dynamics of Ubiquitin. To assess the quality of the ϑ 5-bonds and the ϑ 2-bonds ensembles, we compared them with other existing high-resolution ensembles in the PDB, including three ensembles determined using RDC restraints (2LJS,[44] 2KOX,[21] and 2K39[20]), with an ensemble determined using NOEs and S2 order parameters (2NR2[83])
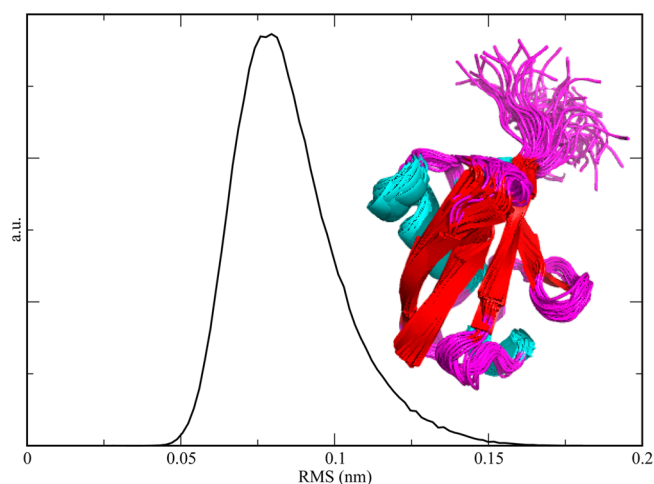

Figure 6. Representation of the structural heterogeneity of the "ϑ 5-bonds" ensemble of ubiquitin using the distribution of the root-mean-square (RMS) distances between pairs of structures in the ensemble. The ensemble is obtained by collecting the conformations generated during the sampling carried out with a 8-replica averaging of the RDCs to obtain the structural restraints (see Methods).

and with an ensemble (MD) obtained using a control simulation with the same procedure of the ϑ method but without RDC restraints (Tables 2 and 3).

We then calculated the Q factors for independent sets of RDCs, finding that both the ϑ 5-bonds and the ϑ 2-bonds ensembles reproduce quite well independent measurements (Table 2). Indeed, they satisfy these RDCs in a comparable manner to the high-quality ensembles described above, which in many cases used these RDCs as restraints in the calculations. Further, we used the PROCHECK method[78] (Table 3) to quantify the structural quality for backbone (φ/ψ) and side-chain (χ_1, χ_2, ω) dihedral angles and hydrogen-bond geometries (H bonds). Finally, we considered other sets of NMR measurements (Table 4), including hydrogen-bond J couplings ($^3J_{HNC'}$), both in terms of root-mean-square deviations (RMSD

Table 3. Assessment of the Quality of the Structures Comprising the Ensemble Representing the Dynamics of Ubiquitin Obtained Using the ϑ Method[a]

	2LJ5	2KOX	2K39	ϑ (5-bonds)	ϑ (2-bonds)	MD	2NR2
			PROCHECK Structure Quality Check				
φ/ψ	1.0	1.0	1.6	1.0	1.0	1.0	1.0
H bonds	1.5	1.8	2.0	1.5	1.5	1.6	2.0
χ_1	1.6	1.2	1.6	1.3	1.4	1.4	1.3
χ_2	1.3	1.0	1.2	1.0	1.0	1.0	1.0
ω	2.3	1.8	2.5	2.1	2.1	2.1	1.8

[a]The PROCHECK method[78] was used to quantify the structural quality for backbone (φ/ψ) and side-chain ($\chi_1, \chi_2,$ and ω) dihedral angles and hydrogen-bond geometries (H bonds). The two ensembles obtained by using 5 bonds (ϑ 5-bonds) and 2 bonds (ϑ 2-bonds) are compared with three ensembles in the PDB determined using RDC restraints (2LJ5, 2KOX, and 2K39), with an ensemble determined using NOEs and S2 order parameters (2NR2) and with an ensemble (MD) obtained using a control simulation with the same procedure of the ϑ method but without RDC restraints.

in Hz) and of coefficient of correlation (R) between experimental and calculated J couplings, the violations of NOE-derived distances, and violations of experimental and calculated chemical shifts (RMSD in ppm). The excellent results of these validations demonstrate that the ϑ method can be effectively used for the dynamical refinement of proteins.

■ CONCLUSIONS

We have presented a method of using RDCs for structural and dynamical refinement of proteins. This method is not based on the introduction of an alignment tensor but on the direct use of the information provided by RDCs about the angles between the internuclear vectors and the external magnetic field. Application to the case of ubiquitin has illustrated that this approach can achieve a structural accuracy comparable to that of other more standard NMR procedures. We anticipate that tensor-free approaches of the type discussed in this work will be

Table 2. Assessment of the Quality of the Ensemble of Structures Representing the Dynamics of Ubiquitin Obtained Using the ϑ Method[a]

	2LJ5	2KOX	2K39	ϑ 5-bonds	ϑ 2-bonds	MD	2NR2
			Q Factor for the RDCs[71] Used in This Work as Restraints (SVD/SB/ϑ)				
N−H	0.10/(0.19)	(0.09)/0.30	(0.20)/0.40	0.07/0.30/(0.04)	0.08/0.30/(0.03)	0.21/0.43	0.24/0.38
Cα−Hα	0.15/0.24	(0.21)/0.40	(0.16)/0.36	0.12/0.36/(0.04)	0.16/0.37/(0.03)	0.27/0.45	0.17/0.32
Cα−C′	0.11/(0.18)	(0.17)/0.30	(0.18)/0.27	0.09/0.30/(0.05)	0.20/0.34/0.21	0.31/0.38	0.20/0.30
C′−N	0.13/0.20	(0.15)/0.44	(0.18)/0.32	0.10/0.27/(0.05)	0.17/0.30/0.18	0.24/0.35	0.25/0.33
C′−H	0.16/(0.25)	(0.21)/0.45	(0.20)/0.40	0.13/0.38/(0.08)	0.22/0.44/0.22	0.32/0.54	0.27/0.41
			Q Factor for Additional 36 Sets of RDCs[84]				
<Q>	(0.13) ± 0.04	(0.15) ± 0.07	(0.15) ± 0.05	0.17 ± 0.06	0.18 ± 0.07	0.27 ± 0.05	0.27 ± 0.05
			Q Factor for Squalamine RDCs[85]				
N−H	0.26	0.21	0.22	0.19	0.23	0.30	0.22
Cα−Hα	0.26	0.32	0.27	0.29	0.36	0.41	0.27
Cα−C′	0.24	0.20	0.21	0.19	0.24	0.32	0.21
C′−N	0.27	0.23	0.25	0.23	0.26	0.31	0.25
C′−H	0.31	0.24	0.28	0.23	0.27	0.36	0.28

[a]The two ensembles obtained by using 5 bonds ('ϑ 5-bonds') and 2 bonds ('ϑ 2-bonds') are compared with three ensembles in the PDB determined using RDC restraints (2LJ5, 2KOX, and 2K39), with an ensemble determined using NOEs and S2 order parameters (2NR2) and with an ensemble (MD) obtained using a control simulation with the same procedure of the ϑ method but without RDC restraints. The Q factors were calculated using three different methods to back-calculate the RDCs from the structures: the SVD method (SVD), the structure-based method (SB), and the ϑ method (ϑ). Numbers in parentheses indicate parameters used as restraints in the ensemble determination protocol.

Table 4. Assessment of the Quality of the Ensemble of Structures Representing the Dynamics of Ubiquitin Obtained Using the ϑ Method[a]

				agreement with NMR observables not used as restraints in this work			
	2LJ5	2KOX	2K39	ϑ (5-bonds)	ϑ (2-bonds)	MD	2NR2
$^3J_{HNC'}$ RMSD (Hz)	0.12	0.12	0.14	0.08	0.09	0.11	0.14
$^3J_{HNC'}$ R	0.78	0.88	0.82	0.88	0.86	0.83	0.83
no. NOE violations	0	0	0	0	0	0	(0)
$\delta C\alpha$ RMSD (ppm)	0.5	0.6	0.6	0.5	0.5	0.5	0.6
$\delta C\beta$ RMSD (ppm)	0.7	0.7	0.8	0.7	0.7	0.8	0.9
$\delta C'$ RMSD (ppm)	0.6	0.6	0.6	0.6	0.6	0.6	0.6
δHN RMSD (ppm)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$\delta H\alpha$ RMSD (ppm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
δN RMSD (ppm)	1.9	1.9	2.0	1.9	2.2	2.4	1.9
methyl RMSD (ppm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[a]We considered the violations of hydrogen-bond J couplings ($^3J_{HNC'}$), both in terms of root mean square deviations (RMSD in Hz) and of coefficient of correlation (R) between experimental and calculated J couplings, the violations of NOE-derived distances, and violations of experimental and calculated chemical shifts (RMSD in ppm). For the 2NR2 ensemble 1320 NOE restraints were used in the ensemble determination protocol.

useful in situations where the calculation of the alignment tensors is challenging, as, for example, in the case of highly charged alignment media.

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Notes

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