Ultrahigh resolution protein structures using NMR chemical shift tensors

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NMR chemical shift tensors (CSTs) in proteins, as well as their orientations, represent an important new restraint class for protein structure refinement and determination. Here, we present the first determination of both CST magnitudes and orientations for $^{13}C\alpha$ and ¹⁵N (peptide backbone) groups in a protein, the β 1 lgG binding domain of protein G from Streptococcus spp., GB1. Site-specific $^{13}C\alpha$ and ^{15}N CSTs were measured using synchronously evolved recoupling experiments in which ¹³C and ¹⁵N tensors were projected onto the ¹H-¹³C and ¹H-¹⁵N vectors, respectively, and onto the $^{15}N^{-13}C$ vector in the case of $^{13}C\alpha$. The orientations of the $^{13}C\alpha$ CSTs to the ¹H-¹³C and ¹³C-¹⁵N vectors agreed well with the results of ab initio calculations, with an rmsd of approximately 8°. In addition, the measured ¹⁵N tensors exhibited larger reduced anisotropies in α -helical versus β -sheet regions, with very limited variation (18 \pm 4°) in the orientation of the *z*-axis of the ¹⁵N CST with respect to the ¹H-¹⁵N vector. Incorporation of the ¹³C α CST restraints into structure calculations, in combination with isotropic chemical shifts, transferred echo double resonance ¹³C-¹⁵N distances and vector angle restraints, improved the backbone rmsd to 0.16 Å (PDB ID code 2LGI) and is consistent with existing X-ray structures (0.51 Å agreement with PDB ID code 2QMT). These results demonstrate that chemical shift tensors have considerable utility in protein structure refinement, with the best structures comparable to 1.0-Å crystal structures, based upon empirical metrics such as Ramachandran geometries and χ^1/χ^2 distributions, providing solid-state NMR with a powerful tool for de novo structure determination.

magic-angle spinning | dihedral angles | cross validation | nanocrystal | quantum chemistry

he chemical shift is an exquisite and powerful probe of molecular structure, deriving from the interaction of molecular orbitals with an external magnetic field, B_0 . Understanding the relationships between chemical shifts and protein structure has substantial implications for modern nuclear magnetic resonance (NMR) spectroscopy, chemistry, and structural biology (1–12). The chemical shift tensor (CST) is rich with information, even when two-thirds of it is averaged to zero by molecular tumbling in solution or magic-angle spinning (MAS) of solid samples. The remaining isotopic chemical shifts remain an excellent resource for structure determination and validation, and higher-order interactions of the CST have substantial contributions to NMR relaxation (13-19). Therefore, detailed knowledge of CSTs permits a precise analysis of motion (20-22). Solid-state NMR (SSNMR) of fully aligned samples exploits amide ¹⁵N tensor information to determine the orientations of helices relative to the bilayer (23, 24). We have previously shown that use of a force field in which experimental ${}^{13}C\alpha$ CSTs are compared with ab initio CSTs [generated as a function of backbone conformation (ϕ, ψ)] significantly improves the precision and accuracy of SSNMR-computed protein structures (10). In addition to determination of NMR-based structures and dynamics, CST datasets are invaluable for the continued development of quantum chemical techniques to compute isotropic and anisotropic chemical shifts,

furthering our understanding of appropriate basis sets and functions for accurate MO theory of proteins (4, 6, 11, 25–27).

Over the past decade, protein structure determination by SSNMR has progressed substantially in terms of the rate of data collection and analysis, as well as in the resolution and complexity of the resulting structures (10, 28-36). In most cases, structures have been determined by using a combination of semiquantitative distance restraints (comparable to solution NOEs) together with semiempirical dihedral angle restraints, obtained from isotropic chemical shifts and chemical shift databases. More recently, we have demonstrated that tensor recoupling is also a powerful route to structure refinement since incorporating relative dipolar tensor orientations (36), CSTs (10), and precise z-filtered transferred echo double resonance (zf-TEDOR) distances (35) as restraint classes in annealing algorithms substantially enhances the precision and accuracy of the resulting protein structures. The next logical, yet technically challenging and heretofore unprecedented, step is to extend this approach by incorporating both tensor magnitudes as well as orientations into simulated annealing calculations. This approach promises further enhancement of structure quality and provides key internal controls for both backbone and side-chain conformations.

Here we present such results, first by determining the relative site-specific orientations of ¹H-¹⁵N (and ¹H-¹³C) dipolar tensors relative to the axis system of the ¹⁵N (and ¹³C) CST, building upon prior MAS studies of proteins (37) and static peptide samples (38–40). These NMR tensorial parameters are obtained using a set of three-dimensional (3D) synchronous recoupling pulse sequences. These datasets enable the accurate determination of CST orientations relative to the molecular frame for the majority of the backbone ¹⁵N and ¹³C α sites in a crystalline protein, GB1 (41). The ¹³C α tensors' orientations are then incorporated, along with other restraint classes, into simulated annealing calculations, resulting in structures exhibiting especially high precision [defined by the backbone rmsd (bbrmsd)] and accuracy (defined by the agreement with crystal structures and structure validation metrics).

Results and Discussion

Determination of ¹⁵N and ¹³C α CST Magnitudes, Orientations and Order Parameters. We first carried out a series of 3D [Rec]-¹⁵N-¹³C correlation experiments each consisting of a 2D NCA plane with a tensor recoupling period in the third dimension. The resulting lineshapes depend upon the CST as well as its relative orientation to the dipole vector (Fig. 1), consistent with conventions of prior studies (37). Further details regarding the tensor notation

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Data deposition: The 10 lowest energy protein structures have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 2LGI).

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Fig. 1. Dipolar:CST correlation spectra for both ${}^{13}C\alpha$ and ${}^{15}N$ sites. Experimental spectrum is presented in black, with simulations in red. Ratios provided are the ratio of dipolar to CST evolution. Row two of *A* indicates ratio of ${}^{15}N{}^{13}C\alpha$ dipolar:CST evolution. (*A*) Fit lineshapes for $[{}^{1}H{}^{-13}C]$: [${}^{13}CCST$] correlation spectra for lysines with different secondary structures are presented. K4 is located in a β -sheet, K28 in the α -helix, and K50 in a β -turn with an unusual positive value of ϕ . (*b*) Fit ensemble of $[{}^{1}H{}^{-15}N]$: [${}^{15}NCST$] correlation spectra. Fit is representative of limited variations of ${}^{15}N$ tensors throughout GB1.

(Fig. S1) and pulse sequence (Fig. S2) are provided in Materials and Methods and SI Text. The key elements of the data collection are ROCSA recoupling of the CST (42) and R18₁⁷ heteronuclear dipolar recoupling (43). Using a 2-¹³C-glycerol,U-¹⁵N GB1 sample (28, 36, 44) all 53 observable ¹⁵N and ¹³C resonances were resolved in the NCA isotropic 2D plane (Leu Ca is unlabeled). Four of the experiments measured tensor magnitudes for the ¹⁵N and ¹³C CSTs and the ¹H-¹⁵N and ¹H-¹³C vectors. The seven remaining experiments correlated the CST with these vectors using ROCSA and R18¹⁷ periods, evolved synchronously with different ratios of evolution time. To orient the ${}^{13}C\alpha$ tensor, ${}^{1}H{}^{-13}C{}^{2}CST$ ratios of 1:1, 1:2, and 1:3 were utilized, with an additional ¹³C CST: ¹⁵N-¹³C ratio of 1:1 to break mirror-plane degeneracies; to orient the ¹⁵N CST, ratios of ${}^{1}H - {}^{15}N$:CST of 1:1, 2:1, and 1:2 were acquired. Together these experiments yielded 563 lineshapes (>10 per residue), reporting uniquely upon each tensor magnitude and the orientation with respect to the molecular frame. Order parameters for <100 microsecond motions, derived directly from analysis of the ${}^{1}H{}^{-13}C\alpha$ and ${}^{1}H{}^{-15}N$ dipolar couplings (Fig. S3), indicate a rigid backbone. Aside from G41, we anticipate minimal motional averaging (approximately 1.5%) of CST magnitudes, based upon path integral calculations of Tang et al. (26). Further examples of fitted lineshapes are provided in SI Text (Figs. S4–S6), along with the full compilation of orientations (Tables S1 and S2).

These datasets together report upon the ${}^{13}C\alpha$ CST orientation with respect to the ¹H-¹³C α (α_1 , α_2 , α_3) and ¹⁵N-¹³C α (β_1 , β_2 , β_3) bond vectors. Tensor magnitudes were reported in our previous study (10); the results we report here, to restrain orientations, were acquired under identical experimental conditions. The ${}^{13}C\alpha$ exhibits a broad range of total magnitude and rhombicity, depending upon residue type and secondary structure. Beyond the changes in tensor anisotropy (δ) and asymmetry (n) discussed previously (7, 10), the orientation of each element to the molecular frame varies greatly (Fig. 2 and Fig. S7). For example, in a typical β -sheet conformation the δ_{11} element is oriented from 0 to 25° of the ¹H-¹³C bond vector, and closer to perpendicular to this vector in α-helical conformations. The orientation of the ¹³C CST to the ¹⁵N-¹³C bond is also a strong reporter of backbone torsion, in most cases a transposition of the β_1 and β_2 angles, often accompanied by a conversion to the complement of the angle. All angles fitted are provided in Table S1.

We investigated the trends and consistency of the ${}^{13}C\alpha$ orientations in two ways. First, we compared our measurements with ab initio chemical shielding surfaces that are available for all 20 common amino acids (http://feh.scs.uiuc.edu/amino_acid.php), (4, 6, 11) where we find excellent overall agreement. The theoryversus-experimental correlations are presented in Fig. 3 *A* and *B*, where it can be seen that all experimental values are within 30°



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Fig. 2. Analysis of $[{}^{1}H{-}{}^{13}C]$ dipolar: ${}^{13}C$ CST correlation spectra. (A) Fit α angles, defining orientation of each tensor element to the HC dipole, as a function of residue number. All angles over 180° were converted to their <90° complement for clarity. Clear trends are observed where δ_{11} is oriented within 20° of the dipole in β -strands but moves within 30° of the bond normal in the α -helix. δ_{22} and δ_{33} are near perpendicular to the HC bond in the β -sheet, while δ_{11} and δ_{22} reorient up to 80° in the α -helix. (B) Fit β angles defining the orientation of each tensor element to the NC bond vector. While overall variation of orientation is not as pronounced, there is a strong shift in the β_2 angle between helical and sheet conformations with a concerted, smaller adjustment of β_1 and β_3 .

(depicted in blue) of the predicted values. The overall agreement for each dataset is very good with an rmsd for (α_1 , α_2 , α_3) of 8.0° and an $R^2 = 0.97$ (Fig. 3*A*). In the case of (β_1 , β_2 , β_3), the rmsd and R^2 values are 8.5° and 0.95 (Fig. 3*B*), comparable to previously reported results for small peptides (6). Second, we computed the values for $\Delta \sigma^*$, defined as the difference between the shielding parallel to the ¹H-¹³C dipole and the shielding perpendicular to the dipole; this parameter was previously measured in solution for ubiquitin and calmodulin (15).

Observed outliers, highlighted in Fig. 3, are K10, K28, K31, and V21. In the case of K10 and V21, the agreement is actually quite good when the dihedral angles from the 2GI9 crystal structure are used, suggesting these deviations might represent a real, small difference between the microcrystal formulation used and that from the 2QMT crystal structure. In the case of the helical lysines, the measured values fit regions of the ab initio surface that are within 15° of those found in the 2QMT structure. Overall, the greatest deviations between theory and experiment are for angles near 90°, a known weak region for tensor correlation experiments, and a disproportionate number of these angles are in the α -helix. It is possible that some effects not included in the ab initio calculations, such as the helix dipole, might make a contribution to the ${}^{13}C\alpha$ shielding in this region, and such effects are indeed important in computing helical ¹⁵N shifts (45). This issue might be addressed in the future by implementing even stronger ¹³C (CST)-(¹⁵N-¹³C) correlations, by using ROCSA-REDOR type correlations.

The tensor magnitudes and orientations were used to reconstruct $\Delta \sigma^*$ values, as measured in solution (Fig. 3*C*), reproducing the observed trends (14). Here, β -sheet $\Delta \sigma^*$ values range from 20 to 33 ppm. The largest $\Delta \sigma^*$ value is for K50, which has a positive value of ϕ in all available crystal structures, which is unusual for a nonglycine residue. $\Delta \sigma^*$ values in the α -helix (residues 23 to 36) range from -6 to 8 ppm. Turns with near α -helical conformation exhibit near-helical values of $\Delta \sigma^*$ but are slightly larger (by 2 to



Fig. 3. Agreement of fit ¹³C α CST orientations with ab initio predictions and reconstructed $\Delta\sigma^*$ values as a function of residue number. For most sites, fits are within 30° of the predicted orientation, within the maximum experimental error. (A) Experimental ¹³C CST orientations α_1 , α_2 , and α_3 (black dots) and (B) experimental orientations β_1 , β_2 , and β_3 (black dots) plotted against theoretical angles predicted by ab initio surfaces of Sun et al. assumig 2QMT crystal structure geometries. Blue lines indicate a deviation of $\pm 30^\circ$. (c) Reconstructed $\Delta\sigma^*$ magnitudes. β -sheets sites range from 20–33 ppm and α -helical from -6–8 ppm, largely consistent with values reported by Tjandra and Bax.

3 ppm) as compared to those in the α -helix. Previously, it was observed that most Thr and Ser residues exhibited large negative values of $\Delta \sigma^*$. This is not observed in the present dataset; however, only one Thr is in the α -helix (T25), making this finding inconclusive. The chemical shift of the C β of T25 is nearly identical to the C α resonance frequency, so it is possible that there is some leakage to the C β resonance, which is partially labeled in the 2-¹³C-glycerol preparation, accounting for this effect.

GB1 Structure Refined with ¹³C Tensor Orientations. A series of structures were generated using ¹³C CST tensor magnitudes and orientations as structural constraints in an Xplor-NIH calculation, following the protocol described previously (10). These structures were solved using combinations of seven different restraint force fields, each abbreviated using a single initial for simplicity; all calculations are summarized in Table 1. They include distances from spin diffusion or ¹H-¹H couplings (N) (34, 36), TEDOR distances (T) (35), TALOS dihedral angles (D), CST magnitudes (C) (10), CST orientations (O), vector angles (VEAN, V) (36), and semiempirical database potentials (SE) (46-48). When compared with structures without CST or VEAN information, the tensor-refined structures possessed higher precision and better agreement with the crystal structures than those computed without these restraints. In addition to better precision and accuracy of the lowest energy structures, all 200 structures refined with CST restraints had an overall bbrmsd of 0.23–0.5 Å, and agreement with the 2QMT crystal structure of 0.51 to 1.1 Å, depending upon the distance restraints used. In all cases, the 10 lowest energy structures (of 200 total structures generated) composed the structural ensemble.

To improve continuity of the spline function used to create the CST potential from the ab initio surfaces, and to eliminate any ambiguity from mirror symmetries of the CST orientations measurements, all angles were converted to the complement $<90^{\circ}$. This greatly improved the continuity of the constructed energy surface and thus the convergence of the annealing algorithm. In the first two calculations, distance tables from our previous CST structure refinement were used. In the first, only CST orientations and distances were used (NO) and in the second, CST magnitudes and TALOS dihedrals were added (NDCO). The bbrmsd for the NO structures was 0.4 Å, with an rmsd agreement of 1.06 Å with the 2QMT crystal structure. This is comparable in precision but a significant improvement in accuracy relative to the previously published NC structure (10). The inclusion of TALOS dihedral angles and CST magnitudes and orientations improved the resolution of the structure to 0.19 Å with comparable accuracy (1.02-Å agreement with 2QMT). If the CST magnitudes are removed (i.e., distances and CST orientations only) there was a negligible difference in the results; however, the comparable calculation lacking orientations (structure 4 of ref. 10) yields less precise structures. The major advantage of using orientations in this case is that they are independent of the tensor scaling and offset effects observed in the comparison of theoretical and experimental CST magnitudes; thus they are immune to common errors from-e.g., motional averaging and/or pulse sequence imperfections. In all cases, the structures with CST information improved upon structures solved with only distance information.

After these control structures were refined, to confirm the effectiveness of these restraints, more structures were generated using all of the restraint types defined above. These include highly precise distances from TEDOR (T) (49, 50), a technique already shown to greatly improve protein structures and described in detail elsewhere (35). A calculation including TALOS restraints and all available distances (NTD) produced a structure with a bbrmsd of 0.19 Å and an agreement with the 2QMT crystal structure of 0.69 Å. Once the CST magnitudes and orientations were added to the calculations (NTDCO), the bbrmsd improved to 0.15 Å, and the agreement with the 2QMT structure improved to 0.59 Å. Inclusion of VEAN restraints (NTDCOV) resulted in a slightly better structure (0.14-Å bbrmsd, 0.57 vs. 2QMT). A structure with all available distances and CST restraints (NTCO) showed a slight deterioration in statistical quality (0.18-Å bbrmsd, 0.60 vs. 2QMT) compared to the NTDCOV structure but still an improvement upon the NTD ensemble. We also found that allowing the calibration factors for the CST surface to vary during annealing gave slightly better results than using the previously calibrated conversion factors (though by at most 0.04 Å).

Table 1. Structural ensemble statistical agreement internally and against the closest related crystal structure

Restraints U	sed*								rmsd	
Structure	Ν	Т	D	С	0	V	SE	Backbone	vs. 2QMT	All Heavy Atom
1	Х				Х			0.40 ± 0.07	1.06 ± 0.05	1.07 ± 0.08
2	Х		Х	Х	Х			0.19 ± 0.04	1.02 ± 0.02	0.98 ± 0.04
3	Х	Х	Х					0.19 ± 0.06	0.69 ± 0.03	0.68 ± 0.04
4	Х	Х		Х	Х			0.18 ± 0.03	0.60 ± 0.02	0.72 ± 0.03
5	Х	Х	Х	Х	Х			0.15 ± 0.03	0.59 ± 0.02	0.71 ± 0.04
6	Х	Х	Х	Х	Х	Х		0.14 ± 0.03	0.57 ± 0.02	0.68 ± 0.03
7	Х	Х	Х			Х	Х	0.19 ± 0.03	0.55 ± 0.01	0.71 ± 0.04
8	Х	Х		Х	Х	Х	Х	0.22 ± 0.03	0.57 ± 0.04	0.75 ± 0.04
9	Х	Х		Х	Х		Х	0.18 ± 0.03	0.55 ± 0.02	0.71 ± 0.04
10	Х	Х	Х	Х	Х		Х	0.17 ± 0.06	0.54 ± 0.03	0.73 ± 0.03
11	Х	Х	Х	Х	х	х	Х	0.16 ± 0.03	0.51 ± 0.02	0.72 ± 0.03

*Abbreviations for restraints used: ¹H-¹H couplings/¹³C-¹³C DARR distances (N); TEDOR distances (T), TALOS dihedral angles (D), CST magnitudes (C), CST orientations (O), vector angles (V), semiempirical database potentials (SE).

At this point, semiempirical Ramachandran database (46, 47) and hydrogen bonding energy database (SE) (48) potentials were included. This further improved the overall structural quality (0.16-Å bbrmsd, 0.51 Å vs. 2QMT) for the ensemble with all currently available restraints (NTDCOV-SE, Fig. 4). Removal of the vector angles (NTDCO-SE) or the CST restraints (NTDV-SE) yielded similar structures (0.17 Å/0.54 Å and to 0.19 Å/0.55 Å) in which the structure retaining CST information has slightly better agreement with the crystal structure, but the vector-angle structure has higher precision. Removal of TALOS and VEAN restraints (NTCO-SE) yielded a structure with 0.18-Å bbrmsd and 0.55-Å rmsd with 2QMT. Unlike TALOS (which relies upon a database of highly resolved X-ray structures), there is no explicit or implicit biasing toward favorable regions of Ramachandran space in the CST restraints presented here. Thus, it appears that employing the RAMA potential with CST restraints yields the closest accord with the X-ray results. This could of course indicate bias toward the X-ray results through the use of semiempirical database potentials; however, since the results of these calculations also exhibit the best overall agreement with all experimental restraints, this possibility seems remote. The structures (4, 8 and 9) with CST restraints, but without TALOS, exhibit improved accuracy over the structure (3) solved using only TALOS to restrain dihedral geometry. The TALOS restraints improve the convergence properties of calculations but are not required in the final stages of refinement.

The quality of each structure was evaluated using several metrics. First, the internal consistency defined by the backbone and all heavy atom rmsds, followed by the agreement of each ensemble mean to the most relevant crystal structure (Table 1) were determined. Second, each structural ensemble was evaluated with PROCHECK_NMR (51). We report four metrics: regions of Ramachandran space populated by percentage, the average χ^1 and χ^2 deviations, and hydrogen bonding energy (Table S3). The χ^1 and χ^2 deviations present further evidence of overall improvements in structure quality, serving as a cross



Fig. 4. GB1 structure calculated using all CST information, vector angles, TALOS dihedrals, and all distances. (A) The 10 lowest energy structures (out of 200) are presented in blue with 2QMT crystal structure represented in red; bbrmsd = 0.16 Å and agreement with 2QMT is 0.51 Å. (B) The lowest 10 energy structures are presented in CPK to illustrate the overall heavy atom order. The all heavy atom rmsd is 0.72 Å.

validation to illustrate that improvements in backbone geometry lead to tighter overall folds and improved geometries. Third, we determined the overall agreement of each ensemble with both CST (Table S4) and vector angle (Table S5) restraints. Finally, shifts were generated for each structure using the chemical shift prediction program SPARTA (52) and compared to the experimentally measured chemical shifts (Table S6). In all cases, these analyses indicated that all structures were highly consistent with one another having backbone resolution comparable to X-ray structures of 1.0–2.0-Å resolution. Not only do these SSNMR structures exhibit excellent structure validation metrics, the agreement of our highest-resolved structure with the 2QMT crystal structure is on the order of agreement among the four deposited crystal structures of GB1 (Table S7).

During these calculations, violations in several previously published distance, TEDOR, and TALOS dihedral restraints were identified and relaxed. In total, 20 TALOS error bars were doubled at some point during the calculations (though only 10 of the original restraints would have violated in the best final structures, within the originally published uncertainties), and the error estimates for 11 spin diffusion, 2 ¹H-¹H, and 26 TEDOR distances were increased. The TEDOR distances that violated had predicted error bars of 0.5 Å or less, and were lengthened by 1 Å. Most often, these restraints violated in the direction of geometries in agreement with the 2QMT crystal structure. These altered restraints are given in Tables S8 and S9.

¹⁵N CST Magnitudes and Orientations. The magnitudes of the principal elements of the ¹⁵N tensors are presented in Fig. 5A (and Table S2). Agreement between these measured values of δ_{11} $(\delta_{11} = \delta + \delta_{iso}$ in this instance) and our previous slow-MAS study (depicted in red in Fig. 5A) showed an rmsd between the two datasets of 1.6 ppm and an R^2 of 0.95. The tensor anisotropy was larger for the α -helical residues than for the β -sheet residues, as found also in thioredoxin (53). Similarly, the measured values of η (the tensor anisotropy) were slightly smaller in α -helical $(\eta = 0.23)$ than in β -sheet residues $(\eta = 0.27)$. Plotting each tensor element (in the "full" representation) vs. the isotropic chemical shift reveals that, unlike C' tensors, ¹⁵N isotropic chemical shift perturbations result from concerted shifts of all tensor elements rather than large shifts of a single element (Fig. 5B). The most deshielded ¹⁵N shift tensor element, δ_{11} , is oriented 9–24° from the ¹H-¹⁵N bond (Fig. 5*C*), δ_{22} is approximately 97° ± 12° from the ¹H-¹⁵N vector and δ_{33} is oriented near the N-C α bond, in or within 10° of the peptide plane (approximately $75^{\circ} \pm 12^{\circ}$ from the ¹H-¹⁵N bond). All CST orientations are shown in Table S2 and are in good accord with recent ab initio studies (45). Of particular interest is the observation that our tensor measurements indicate the ¹⁵N tensor deviates from ideal prolate symmetry, with η ranging from 0.15 to 0.32. Our original hypothesis was that this discrepancy between our data and other studies



Fig. 5. Amide chemical shift tensor analysis for protein GB1. (A) Principal elements of ¹⁵N tensor in the traceless representation compared to previously published slow-MAS data. Principal elements are presented in black with error bars corresponding to one standard deviation. Tensor values from previous work are presented in red. The rmsd between δ_{11} elements from both datasets is 1.6 ppm, and 6 ppm for δ_{22} and δ_{33} values corresponding to a deviation of η of 0.13. (*B*) Chemical shift tensor elements plotted against isotropic chemical shift. The correlation reveals that the changes in the isotropic chemical shift result from largely correlated shifts of all three principal elements. R^2 for each element (δ_{11} , δ_{22} , δ_{33}) are 0.82, 0.75, and 0.62, respectively. (*C*) The angle β (a1) as a function of residue number. The angle β defines the orientation of the δ_{11}/δ_{zz} tensor element to the HN bond dipole.

might result from the high covariance between relaxation and η when $\eta < \sim 0.5$. However, recent density functional theory calculations of ¹⁵N CSTs in the α -helix of GB3 by Cai et al. (45) agree well with all of our tensor elements (Fig. S84), ($R^2 = 0.99$ and rmsd of 6.5 ppm) and a recent solution NMR study presents even larger tensor asymmetry (54). It should be noted that δ_{22} and δ_{33} are significantly smaller in magnitude than δ_{11} in the traceless representation, leading to greater uncertainty in the fitted orientation. The smaller magnitudes of δ_{22} and δ_{33} in the traceless representation, and their near perpendicular orientation to the ¹H-¹⁵N vector, suggest that uncertainties in the magnitude of these elements might be inconsequential for most solution NMR relaxation studies but might be more important in the interpretation of PISEMA spectra, making a better understanding of these tensors (especially in α -helical conformations) of interest. Recent solution NMR work also reveals strong statistical agreement with our results (Fig. S8B) for the full tensor, albeit with some outliers.

Conclusions

The chemical shift tensors of all ¹⁵N and ¹³C sites in a protein report upon a vast range of molecular properties, including electronic structure, backbone conformation, steric clashes, electrostatics, side-chain packing, and dynamics. Specifically, a detailed knowledge of backbone amide and Ca tensors is relevant to an increasing range of structural and dynamics work throughout NMR and structural biology in general. In this paper, we have provided a unique example of the determination of ${}^{13}C\alpha$ and ¹⁵N chemical shift tensor magnitudes and orientations throughout a protein, using SSNMR tensor correlation techniques. As a first example of their utility, ${}^{13}C\alpha$ CST information was used to refine the structure of a 6-kDa protein. The ${}^{13}C\alpha$ shift tensor orientations are in good accord with ab initio quantum chemical prediction and provide an important parameter with which to refine SSNMR structures not currently available from experimental databases. These methods complement already established tensor refinement methods in SSNMR and further the pursuit of atomic-resolution structure determination by SSNMR. Validation of these structures reveals a quality on par with 1-Å X-ray structures. The experimental backbone amide tensors are in accordance with recent density functional theory predictions of tensor orientations in the helical residues of GB1, illustrating the importance of such measurements to macromolecular electronic structure calculations.

The methodology and information described here lays the groundwork for future CST studies determining both the structure and dynamics of a range of systems. Overall, we have established that SSNMR is a powerful tool for readily measuring important tensor quantities with site-resolution without the need for conservative mutagenesis and multiple molecular alignments. These results were achieved by using three-dimensional spectroscopy and, in principle, can be implemented on much larger systems given that spectrometers operating at twice the field used in this work are now available. Now that precise orientations and magnitudes have been determined for a range of residue types and secondary structural motifs, future structural work might include static ${}^{13}C\alpha$ spectra of oriented samples as a complement to the well-established PISEMA experiment. In addition, now that more tensor orientation information is known, the chemical shift can be incorporated into an even wider array of relaxation/ dynamics measurements in solution NMR.

Materials and Methods

Sample Preparation. Samples of GB1 were prepared using 2-¹³C-glycerol as the ¹³C source in the minimal growth media. Protein was precipitated as described previously and packed wet into the central 80% of a limited-speed (thin wall) 3.2-mm Varian rotor (Varian, Inc.).

NMR Spectroscopy. All spectra were acquired using a 500-MHz Varian InfinityPlus spectrometer and 3.2-mm T3 Balun[™] ¹H-¹³C-¹⁵N probe. Pulse widths ($\pi/2$) for ¹H, ¹³C, and ¹⁵N were 1.9 μ s, 2.5 μ s, and 5.0 μ s, respectively. Spinning was maintained at 11.111 \pm 0.002 kHz. Periods recoupling $^{1}\text{H-}^{15}\text{N/}^{13}\text{C}$ dipoles and ¹⁵N/¹³C CST line shapes were inserted into a 3D experiment using an NCA plane for site resolution, following the model used in our previous studies. Dipolar recoupling was achieved using the R1817 recoupling element and CST interactions were recoupled using ROCSA. Optimal resolution and sensitivity of the ¹⁵N-¹³C planes were achieved using SPECIFIC CP and TPPM decoupling during acquisition with approximately 75 kHz B_1 on ¹H. Eleven total spectra were acquired: one each for the ¹⁵N and ¹³C CST, the ¹H-¹⁵N and ¹H-¹³C dipoles, and seven dipole-CST correlated spectra. In these spectra ROCSA and dipolar dimensions were evolved synchronously in the same dimension, with different ratios of evolution time, in units of rotor periods. [¹H-¹⁵N]:[¹⁵N CST] ratios included 1:1, 2:1 and 1:2. [¹H-¹³C]:[¹³C CST] ratios included 1:1, 1:2, and 1:3. In addition a ¹³C CST recoupling experiment was performed where ¹⁵N was not decoupled, providing coevolution of the ¹³C CST and ¹³C-¹⁵N dipolar Hamiltonians.

Data Analysis. All eleven spectra were processed using NMRPipe. Lorentzianto-Gaussian apodization functions were applied in the two isotropic chemical shift dimensions, with a net line broadening of 30 Hz for ¹³C and 15 Hz for ¹⁵N. Peak intensities in each ¹⁵N-¹³C (F1–F3) plane were determined and trajectories of each correlation spectrum (t_2) extracted using the autoFit.tcl package in NMRPipe. All trajectories for each site were fit to exact spin simulations assuming a 2 spin basis. A subset of each experimental set were tested using a basis with all ¹H within 2.5 Å; however, this only impacted the relaxation matrix and had little to no impact upon the fit angles and dipolar magnitudes. The fitting procedure was facilitated by in-house FORTRAN-77 code

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Supporting Information

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SI Text

The Chemical Shift Tensor. The electronic environment surrounding the nucleus gives rise to the CST. In the presence of an external magnetic field, B_0 , the electrons in the orbitals surrounding these nuclei orient themselves either with the field or against the field, shifting the observed frequency of precession. The chemical shift, δ^{CS} , is typically represented as a Cartesian tensor composed of three orthogonal axes.

$$\delta^{\text{CS}} = \begin{pmatrix} \delta_{11} & \delta_{12} & \delta_{13} \\ \delta_{21} & \delta_{22} & \delta_{23} \\ \delta_{31} & \delta_{32} & \delta_{33} \end{pmatrix}$$
[S1]

where δ_{ii} are the nine components of the CST. In solution, isotropic molecular tumbling averages this tensor and the familiar isotropic "chemical shift" (δ_{iso}) is one third the trace of the above tensor:

$$\delta_{\rm iso} = \frac{1}{3} (\delta_{11} + \delta_{22} + \delta_{33}).$$
 [S2]

Here, δ_{11} is the most downfield tensor element, δ_{33} the furthest upfield, with δ_{22} between these two extremes. The orientation of these elements to the molecular frame can be described by a series of angles. Often, these are a set of Euler angles in a common coordinate system. In the convention presented, here these angles are defined as α_n , the angle between the nth tensor element and the ¹H-¹³C or ¹⁵N-H vector, and β_n , the orientation of the nth tensor element and the ¹³C α -¹⁵N vector, illustrated in Fig. 1. An alternate description of the CST was popularized by Haeberlen, Merhing, and Waugh. In this convention, very convenient for conversion from a Cartesian to spherical tensor basis set, labels the three axes δ_{xx} , δ_{yy} , δ_{zz} . They are then ordered by their deviation from the isotropic chemical shift, with δ_{zz} having the greatest deviation, followed by δ_{xx} and δ_{yy} closest to the δ_{iso} . In this convention tensor magnitude and rhombicity are defined by two parameters δ (or $\delta_{aniso} = \delta_{zz} - \delta_{iso}$) and η ($\eta = (\delta_{xx} - \delta_{yy})/\delta$).

Order Parameters. The experimentally determined ¹H-¹⁵N and ¹H-¹³C vector orientation report upon both bond distances as well as molecular motion. The assumption is that if the ¹H-¹⁵N bond length is approximately 1.04 Å and the ¹H-¹³C bond length is approximately 1.12 Å, in the presence of fast rigid motions, the order parameter S is the ratio of the measured dipolar coupling to the ideal dipolar coupling. This approach is the same as that used in recent studies of both ubiquitin (1) and thioredoxin (2). The order parameters for both NH and CH are presented in Fig. S3. The values of S measured from the H α -C α dipole (Fig. S3A) reveal a rigid backbone, where $S \sim 0.95$ for most sites, comparable to values measured in solid thioredoxin (2) but larger than the S values observed in solid ubiquitin (1). It is known that L12 and G41 in GB1 experience significant motional averaging; however, L12 is not labeled in this sample and the glycine ¹H-¹³C trajectories were not fit because of the high degeneracy of solutions arising from the presence of two directly bound protons; however, the residues adjacent to these sites (T11, K13, D40) do exhibit motional averaging greater than 1 standard deviation from the other sites. Based upon a recent study by Case et al., (3) dipolar averaging of this magnitude would result only a very small motional averaging of the ¹³C and ¹⁵N CSTs. For example, this study revealed that a Lipari–Szabo order parameter, S^2 , determined for F52 in GB3 using HN dipolar terms would be 0.897, however when CSA is considered the value rises to 0.975, this is largely attributed to the motion of the lighter proton relative to the heavier peptide backbone. This indicates there is only small motional averaging of CST magnitudes at sites away from the loop containing G41 in GB1 at ambient temperatures. The order parameters measured from the backbone 1H-15N couplings (Fig. S3B) follow a nearly identical pattern to that seen in the ¹H-¹³C results. Here, the G41 ¹H-¹⁵N tensor dipole can be measured, and reveals significant motional averaging, comparable to that reported by Barchi et al. (4). The main differences relative to the ¹H-¹³C scalings are in the turn near A20, and the turn near T49, due most likely to slightly elongated intermolecular and intramolecular hydrogen bonds. However, in the ¹H-¹⁵N recoupling experiment, A20 is overlapped with N8, and the signal-to-noise ratio at E19 is poor, so this cannot be confirmed. Overall, however, these measurements reveal a relatively rigid, well-ordered backbone.

Data Analysis and Fitting. The data that was acquired resulted in a total of six trajectories for each fit site in the ¹³C correlation experiments and five trajectories for each set of ¹⁵N correlation experiments. During the fitting first the CST magnitudes were fixed (in the case of ¹³C previously reported values were used). Following this the $R18_1^7$ trajectory was fit for both effective bond length and relaxation. In the next step angles are fit holding all magnitudes, relaxation parameters, and angles are allowed to vary. It was found during this procedure that modeling of cross polarization helped improve the fit quality, especially in the case of ¹H-¹³C trajectories and [¹H-¹³C]:[¹³CCST] correlation spectra.

While powerful, these experiments possess a few complexities that needed to be addressed. Tensor correlation experiments in SSNMR are most accurate when the correlated vectors are oriented at 30° or less. To partially overcome this shortcoming, each CST orientation was constrained relative to two different vectors, resulting in an improvement in the fit quality for these near orthogonal orientations. Unlike the ¹H-¹³C dipolar tensor, the Ca CST deviates significantly from axial symmetry, and a single correlation of one tensor to another can be ambiguous in isolated regions of conformational space. To address this, we acquired several different ratios of ROCSA to $R18_1^7$ evolution times. This allowed for tighter constraints on the C α tensor, and also alleviated errors arising from ¹H-¹³C distance measurements. Using this approach we found that varying the ¹H-¹³C dipolar coupling by up to 1 kHz resulted in minimal perturbation $(x-y^{\circ})$ in the fitted orientations.

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Fig. S1. Orientation of the ¹⁵N and ¹³C α chemical shift tensors to the molecular frame. Typically the tensor may be described using sets of angles to orient each tensor element to the molecular bonds. Tensor elements are ordered by furthest downfield (11) to furthest upfield (33). In the text presented here these angles are defined as α_n , the angle between the nth tensor element and the ¹H-¹³C or ¹⁵N dipole, and β_n , the orientation of the nth tensor element to the ¹³C α -¹⁵N dipole. This convention was used because it was the most convenient to compare measured CST information to ab initio surfaces. Figure was made using SIMMOL package.



Fig. 52. ROCSA:R1817 CST-dipole correlation sequence. Polarization is created on ¹H and transferred to ¹⁵N by adiabatic cross-polarization with a 1-ms contact time. Following a ¹⁵N chemical shift evolution period (t_1), polarization is transferred to ¹³C using SPECIFIC cross polarization. The ¹³C α CST is recoupled under ROCSA followed by a *z*-filter and a synchronously evolved R1817 period that recouples the ¹H-¹³C dipolar interaction. This is followed by a second *z*-filter and acquisition. Phase cycle is as follows: $\phi_3 = 0$, 180, 0, 180, 0, 180; $\phi_4 = 0$, 0, 180, 180, 0, 0, 180, 180; $\phi_5 = 270$, 270, 90, 90, 90, 90, 270, 270; $\phi_6 = 90$, 90, 270, 270, 270, 270, 90, 90; $\phi_7 = 0$, 180, 90, 270, 270, 90, 90; $\phi_8 = 270$, 270, 90, 90; $\phi_9 = 0$, 0, 90, 90, 180, 180, 270, 270. The following receiver cycle was used: 0, 2, 3, 1, 0, 2, 3, 1, 2, 0, 1, 3, 0, 2, 3, 1.



Fig. S3. Order parameters (*S*) measured from backbone ${}^{1}H{-}{}^{13}C$ (*A*) and ${}^{1}H{-}{}^{15}N$ (*B*) effective dipolar couplings. Equilibrium bond lengths for ${}^{1}H{-}{}^{13}C$ and ${}^{1}H{-}{}^{15}N$ were assumed to be 1.12 Å and 1.04 Å, respectively. Dipolar interaction was recoupled using $R18_{1}^{7}$ pulse sequence element applied at 11.111 kHz spinning (100 kHz B_{1} field). ${}^{1}H{-}{}^{13}C$ and ${}^{1}H{-}^{15}N$ couplings were read from the third dimension of a 3D experiment implementing NCA 2D plane to provide site resolution. Data were acquired at VT set temperature of 0 °C and ${}^{1}H$ Larmor frequency of 500 MHz.



Fig. 54. Analysis of ${}^{1}H_{-}{}^{13}C$ dipole: ${}^{13}C$ CST correlation spectra. Fit lineshapes for $[{}^{1}H_{-}{}^{13}C]$: $[{}^{13}C$ CST] correlation spectra for alanine and valine with different secondary structures are presented. Experimental data are presented in black and best fit are in red. Ratios correspond to ratio of dipolar evolution time to CST evolution time. A20 is located in a turn with β -sheet geometry, A26 and A34 are located in the α -helix, and A48 is in a turn with α -helical geometry. V21 is in a turn with near helical geometry, V29 is in a helix, V39 is in a turn with mixed geometry, and V54 is β -sheet.



Fig. S5. Analysis of ¹H-¹³C dipole: ¹³C CST correlation spectra. Fit lineshapes for [¹H-¹³C]: [¹³C CST] correlation spectra for threonine, tyrosine, and aspartic acid residues with different secondary structures are presented. Experimental data are presented in black and best fit are in red. Ratios correspond to ratio of dipolar evolution time: CST evolution time. In the examples provided, T18 and T51 have β -sheet geometry. T25 is located in the α -helix and T49 is in the turn between β 3 and β 4. Y3 is β -sheet and Y33 is α -helical. D36 is located at the end of the α -helix and D47 is at the end of β 3 leading into a β -turn.



Fig. S6. Amide chemical shift tensor analysis for protein GB1. Fit ensemble of $[^{1}H_{-15}N]:[^{15}N CST]$ correlation spectra. Data is presented in black with best fit in red. The ratio is the ratio of dipolar:CST evolution time for each lineshape. Typical rmsd between theory and experiment is less than 2%.



Fig. 57. Expanded view of Fig. 2. Analysis of ¹H-¹³C dipolar:¹³C CST correlation spectra. In the left column, fit α angles, defining orientation of each tensor element to the HC dipole, as a function of residue number. All angels over 180° were converted to their <90° compliment for clarity. Clear trends are observed where δ_{11} is oriented within 20° of dipole in β -strands but moves within 30° of bond normal in the α -helix. δ_{22} and δ_{33} are near perpendicular to the HC bond in the β -sheet, while δ_{22} reorients up to 80° in the α -helix. In the right column, fit β angles defining the orientation of each tensor element to the NC bond vector. While overall variation of orientation is not as pronounced, there is a strong shift in the β_2 angle between helical and sheet conformations with a concerted, smaller adjustment of β_1 and β_3 .



Fig. S8. ¹⁵N tensor data compared to solution NMR studies and ab initio calculations. (*A*) Theoretical chemical shielding tensor calculations of helical residues 26–33 in GB3 plotted against measured chemical shift tensor measurements. Calculations presented are from Cai and Fushaman. Overall statistical agreement is good, $R^2 = 0.993$, rmsd = 6.5 ppm. Least squares fit slope of -0.944 with y intercept of 235.7 ppm. Once E27 outlier is removed agreement improves to $R^2 = 0.997$, rmsd = 4.3 ppm, with a least squares slope of -0.938, and y intercept of 236.6 ppm. Statistical agreement is within combined experimental and computational error for all sites but E27. For comparison, the recent solution NMR study of Yao et al. shows $R^2 = 0.986$, rmsd = 9.1 ppm, least squares slope of -0.916, with y intercept of 233.5 ppm. (*B*) Chemical Shift Tensor Principal elements measured by Yao et al. plotted against our measured values. Overall statistical agreement is good when considering the full tensor. Overall rmsd is 8.1 ppm, with R^2 of 0.989 and a slope near unity with y offset of 1.5 ppm.

$\begin{array}{c ccccccccccccccccccccccccccccccccccc$							
M1Q210100891163666Y36959410529114K47879611140121L5	Residue	α ₁ (± 10°)	α ₂ (± 10°)	α ₃ (± 10°)	β ₁ (± 15°)	β_2 (± 15°)	β ₃ (± 15°)
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	M1						
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Q2	10	100	89	116	36	66
K47879611140121L51617585896726103L7181768691692676G9111173968669148112L12173968669144112L12111173968669144112L12111173968611442122G14112101103147601161710210110314760T16171021011031476011514862122118A20797931131173772118169110610514862L2079793113117377211467344241201509411251133L222368951146734341241251331331331331331331331331331331331331331341341441421221341441	Y3	6	95	94	105	29	114
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	K4	7	87	96	111	40	121
16 175 85 89 67 26 103 L7	L5						
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	16	175	85	89	67	26	103
N8 176 86 91 69 26 76 G9 53 138 73 110 32 66 T11 173 96 86 69 148 112 L12 114 42 122 G14 7 84 86 114 42 122 G14 7 87 112 151 72 T16 17 102 101 103 147 60 T17 4 87 87 112 151 72 T18 16 91 106 105 148 62 E19 15 100 100 105 32 118 A20 7 97 93 113 117 37 V21 158 91 112 51 133	L7						
G9 $K10$ 53138731103266T11173968669148112L12 $K13$ 7848611442122K137848611442122E1510898011657136T161710210110314760T174878711215172T18169110610514862E191510010010532118A207979311311737V211589111211940115D222368951146734A2311714810612068142A241201509411555134E27100162751275556K285814576593771V29951601093062102F307716176477149K317916379527144Q32105154691377252Y33931495945556543N35119141661083462D361281	N8	176	86	91	69	26	76
K10 53 138 73 110 32 66 T11 173 96 86 69 148 112 L12	G9						
T11 173 96 86 69 148 112 K13 7 84 86 114 42 122 G14 2 122 114 42 122 E15 10 89 80 116 57 136 T16 17 102 101 103 147 60 T17 4 87 87 112 151 72 T18 16 91 106 105 148 62 E19 15 100 100 105 32 118 A20 7 97 93 113 117 37 V21 158 91 112 119 40 115 D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 115 55 134 E27 100 162 75 127 55 <td>K10</td> <td>53</td> <td>138</td> <td>73</td> <td>110</td> <td>32</td> <td>66</td>	K10	53	138	73	110	32	66
L12 K13 7 84 86 114 42 122 G14 E15 10 89 80 116 57 136 T16 17 102 101 103 147 60 T17 4 87 87 112 151 72 T18 16 91 106 105 148 62 E19 15 100 100 105 32 118 A20 7 97 93 113 117 37 V21 158 91 112 119 40 115 D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 112 51 133 T25 83 173 91 29 66 106 A26 120 150 94 115 55 134 E27 100 162 75 127 55 56 K28 58 145 76 59 37 71 V29 95 160 109 30 62 102 F30 77 161 76 47 71 49 K31 79 163 79 52 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 29 71	T11	173	96	86	69	148	112
K13 7 84 86 114 42 122 G14	L12						
G14 E15 10 89 80 116 57 136 T16 17 102 101 103 147 60 T17 4 87 87 112 151 72 T18 16 91 106 105 148 62 E19 15 100 100 105 32 118 A20 7 97 93 113 117 37 V21 158 91 112 119 40 115 D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 112 51 133 T25 83 173 91 29 66 106 A26 120 150 94 115 55 134 E27 100 162 75 127 55 56 K28 58 145 76 </td <td>K13</td> <td>7</td> <td>84</td> <td>86</td> <td>114</td> <td>42</td> <td>122</td>	K13	7	84	86	114	42	122
E15 10 89 80 116 57 136 T16 17 102 101 103 147 60 T17 4 87 87 112 151 72 T18 16 91 106 105 148 62 E19 15 100 100 105 32 118 A20 7 97 93 113 117 37 V21 158 91 112 119 40 115 D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 112 51 133 T25 83 173 91 29 66 106 A26 120 150 94 115 55 134 E27 100 162 75 127 55 56 K28 58 145 76 59 <td>G14</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	G14						
T161710210110314760T174878711215172T18169110610514862E191510010010532118A207979311311737V211589111211940115D222368951146734A2311714810612068424A241201509411251133T2583173912966106A261201509411555134E27100162751275556K285814576593771V29951601093062102F307716176477149K317916379527144Q32105154691377252Y339314959455565A34871691011226543N35119141661083462D3612813671953952N3717585896914766G387765287777<	E15	10	89	80	116	57	136
T17 4 87 87 112 151 72 T18 16 91 106 105 148 62 E19 15 100 100 105 32 118 A20 7 97 93 113 117 37 V21 158 91 112 119 40 115 D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 112 51 133 T25 83 173 91 29 66 106 A26 120 150 94 115 55 134 E27 100 162 75 127 55 56 K28 58 145 76 59 37 71 V29 95 160 109 30 62 102 K31 79 163 79 52	T16	17	102	101	103	147	60
T18169110610514862E191510010010532118A207979311311737V211589111211940115D222368951146734A2311714810612068142A241201509411251133T2583173912966106A261201509411555134E27100162751275556K285814576593771V29951601093062102F307716176477149K3179154691377252Y339314959455565A34871691011226543N35119141661083462D3612813671953952N3717585896914766G38770401778789692971	T17	4	87	87	112	151	72
E191510010010532118A207979311311737V211589111211940115D222368951146734A2311714810612068142A241201509411251133T2583173912966106A261201509411555134E27100162751275556K285814576593771V29951601093062102F307716176477149K317916379527144Q32105154691377252Y339314959455565A34871691011226543N35119141661083462D3612813671953952N3717585896914766G3877265287777D401778789692971	T18	16	91	106	105	148	62
A20 7 97 93 113 117 37 V21 158 91 112 119 40 115 D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 112 51 133 T25 83 173 91 29 66 106 A26 120 150 94 115 55 134 E27 100 162 75 127 55 56 K28 58 145 76 59 37 71 V29 95 160 109 30 62 102 F30 77 161 76 47 71 49 K31 79 163 79 52 71 44 Q32 105 154 69	E19	15	100	100	105	32	118
V21 158 91 112 119 40 115 D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 112 51 133 T25 83 173 91 29 66 106 A26 120 150 94 115 55 134 E27 100 162 75 127 55 56 K28 58 145 76 59 37 71 V29 95 160 109 30 62 102 F30 77 161 76 47 71 49 K31 79 163 79 52 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122	A20	7	97	93	113	117	37
D22 23 68 95 114 67 34 A23 117 148 106 120 68 142 A24 120 150 94 112 51 133 T25 83 173 91 29 66 106 A26 120 150 94 115 55 134 E27 100 162 75 127 55 56 K28 58 145 76 59 37 71 V29 95 160 109 30 62 102 F30 77 161 76 47 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66	V21	158	91	112	119	40	115
A2311714810612068142A241201509411251133T2583173912966106A261201509411555134E27100162751275556K285814576593771V29951601093062102F307716176477149K317916379527144Q32105154691377252Y339314959455565A34871691011226543N35119141661083462D3612813671953952N3717585896914766G38770401778789692971	D22	23	68	95	114	67	34
A241201509411251133T25 83 173912966106A261201509411555134E27100162751275556K285814576593771V29951601093062102F307716176477149K317915379527144Q32105154691377252Y339314959455565A34871691011226543N35119141661083462D3612813671953952N3717585896914766G38770401778789692971	A23	117	148	106	120	68	142
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	A24	120	150	94	112	51	133
A26 120 150 94 115 55 134 $E27$ 100 162 75 127 55 56 $K28$ 58 145 76 59 37 71 $V29$ 95 160 109 30 62 102 $F30$ 77 161 76 47 71 49 $K31$ 79 163 79 52 71 44 $Q32$ 105 154 69 137 72 52 $Y33$ 93 149 59 45 55 65 $A34$ 87 169 101 122 65 433 $N35$ 119 141 66 108 34 62 $D36$ 128 136 71 95 39 52 $N37$ 175 85 89 69 147 66 $G38$ V V V 97 87 89 69 29 71	T25	83	173	91	29	66	106
L27 100 162 75 127 55 56 K28 58 145 76 59 37 71 V29 95 160 109 30 62 102 F30 77 161 76 47 71 49 K31 79 163 79 52 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 U U 177 87 89 69 29 71	A26	120	150	94	115	55	134
K28 58 145 76 59 37 71 V29 95 160 109 30 62 102 F30 77 161 76 47 71 49 K31 79 163 79 52 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 U U 167 86 102 65 28 77 D40 177 87 89 69 29 71	F27	100	162	75	127	55	56
V29 95 160 109 30 62 102 F30 77 161 76 47 71 49 K31 79 163 79 52 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 U U U 177 87 89 69 29 71	K28	58	145	76	59	37	71
F30 77 161 76 47 71 49 K31 79 163 79 52 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 V V39 167 86 102 65 28 77 D40 177 87 89 69 29 71	V29	95	160	109	30	62	102
K31 79 163 79 52 71 44 Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 V V V39 167 86 102 65 28 77 D40 177 87 89 69 29 71	F30	77	161	76	47	71	49
Q32 105 154 69 137 72 52 Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 77 87 89 69 29 71 D40 177 87 89 69 29 71	K31	79	163	79	52	71	44
Y33 93 149 59 45 55 65 A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38	032	105	154	69	137	72	52
A34 87 169 101 122 65 43 N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 V39 167 86 102 65 28 77 D40 177 87 89 69 29 71	Y33	93	149	59	45	55	65
N35 119 141 66 108 34 62 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38	A34	87	169	101	122	65	43
N35 115 111 05 105 114 05 D36 128 136 71 95 39 52 N37 175 85 89 69 147 66 G38 77 940 167 86 102 65 28 77 D40 177 87 89 69 29 71	N35	119	141	66	108	34	62
N37 175 85 89 69 147 66 G38	D36	128	136	71	95	39	52
King King <th< td=""><td>N37</td><td>175</td><td>85</td><td>89</td><td>69</td><td>147</td><td>66</td></th<>	N37	175	85	89	69	147	66
V3916786102652877D401778789692971	G38	175	05	05	05		00
D40 177 87 89 69 29 71	V39	167	86	102	65	28	77
	D40	177	87	89	69	20	71
G41	G41	.,,	0,	05	05	LJ	<i>,</i> ,
F42 7 97 89 99 27 65	F42	7	97	89	99	27	65
W43 1 89 89 108 147 64	W/43	1	89	89	108	147	64
T44 12 84 101 119 133 57	T44	12	84	101	119	133	57
Y45 175 86 93 68 30 70	Y45	175	86	93	68	30	70
D46 6 88 84 109 34 117	D46	6	88	84	109	34	117
D47 142 128 90 104 37 56	D47	142	128	90	103	37	56
Δ48 136 128 109 83 45 46	Δ48	136	128	109	83	45	46
T49 170 95 81 64 148 107	T40	170	95	81	64	148	107
K50 172 98 93 68 95 157	K50	172	92	92	68	95	157
T51 6 84 97 113 77 76	T51	6	90 84	92	113	25 27	76
F52 17 104 100 90 31 121	F52	17	104	100	90	21	171
T53 12 97 100 119 140 64	T53	12	97	100	119	140	64
V54 6 88 95 118 3/ 102	V54	6	88	95	118	34	108
T55 12 91 107 117 155 77	T55	17	Q1	102	117	155	77
E56	E56	12	51	102	112		.,

Table S1. ¹³C CST orientation to ¹H-¹³C and ¹⁵N-¹³C dipoles

The angles $\alpha_{(11,22,33)}$ define the angle between each tensor element and the ¹H-¹³C dipole. The angles $\beta_{(11,22,33)}$ define the angle between each tensor element and the ¹⁵N-¹³C dipole.

M1 U Q2 109.0 0.29 234.2 86.7 54.8 18 90 72 Y3 103.0 0.26 226.3 85.4 58.3 18 90 72 K4 106.0 0.25 228.7 82.8 56.6 19 101 80 L5 0.23 0.24 73 73 73 73 K10 109.6 0.22 218.2 67.5 43.1 22 102 73 K11 109.7 0.15 216.1 60.0 43.1 17 95 74 K12 0.15 216.1 60.5 36.0 22 95 96 K13 104.4 0.29 227.7 86.2 55.9 16 92 75 F11 109.7 0.23 220.2 74.9 50.5 73 73 73 T16 105.7 0.23 220.2 74.9 5	Res.	δ (± 2.5 ppm)	η (± 0.03)	δ_{11} (± 2.5 ppm)	$\delta_{ m 22}$ (± 3.0 ppm)	δ_{33} (± 4.0 ppm)	α ₁ (± 5°)	α ₂ (± 12°)	α ₃₃ (± 12°)
Q2 109.0 0.29 234.2 86.7 54.8 18 101 77 Y3 103.0 0.26 228.3 82.8 56.6 19 101 80 I6 104.6 0.25 228.7 82.8 56.6 19 101 80 I7 106.0 0.25 223.0 88.8 59.2 9 95 82.8 R 9.3 0.24 I 109 60.25 230.7 80.0 52.6 16 92 75 R 109.7 0.22 221.1 60.0 43.1 17 95 74 L12 73 230.8 79.0 53.5 17 93 73 L15 109.7 0.22 223.1 75.5 48.7 16 122 85 L107.0 0.26 222.1 77.3 49.5 16 102 80 L107.0 0.22 233.8 84.0	M1								
Y3 103.0 0.26 226.3 85.4 58.3 18 90 72 I6 106.0 0.25 228.7 82.8 56.6 19 101 80 L5 104.6 0.28 230.9 88.8 59.2 9 95 82 L7 7 7 7 7 7 7 7 K10 108.6 0.22 218.2 67.5 43.1 22 102 73 K11 109.7 0.15 216.1 60.0 43.1 17 95 74 L12 7 86.2 55.9 16 92 75 K13 104.4 0.29 220.2 74.9 50.5 77 K14 105.7 0.23 220.2 74.9 50.5 77 K16 106.0 0.23 220.2 74.9 50.7 73 93 66 K14 107.9 0.22 233.8 84.0 60 94 74 K21 107.8 0.6 <th< td=""><td>Q2</td><td>109.0</td><td>0.29</td><td>234.2</td><td>86.7</td><td>54.8</td><td>18</td><td>101</td><td>77</td></th<>	Q2	109.0	0.29	234.2	86.7	54.8	18	101	77
K4 106.0 0.25 228.7 82.8 56.6 19 101 80 16 104.6 0.28 230.9 88.8 59.2 9 95 82. N8 92.3 0.24 <td< td=""> 73 73 73 G9 108.6 0.22 218.2 67.5 43.1 22 102 73 K10 109.6 0.22 218.2 67.5 43.1 17 95 74 L12 0.15 216.1 60.0 43.1 17 95 74 L13 104.4 0.29 221.7 85.2 55.9 16 92 75 G14 105.7 0.32 220.2 74.9 50.5 11 93 73 73 L17 105.0 0.23 220.2 74.9 50.5 22 10.2 10.2 80 L17 0.16 23.0 66.3 47.4 9 74 <td< td=""><td>Y3</td><td>103.0</td><td>0.26</td><td>226.3</td><td>85.4</td><td>58.3</td><td>18</td><td>90</td><td>72</td></td<></td<>	Y3	103.0	0.26	226.3	85.4	58.3	18	90	72
L5 L7 0.28 230.9 88.8 59.2 9 95 82 L7 106.6 0.22 218.2 67.5 43.1 22 102 73 K10 109.6 0.22 218.2 67.5 43.1 17 95 74 L1 109.7 0.15 216.1 60.0 43.1 17 95 74 L12 - - - - 75 614 105.7 0.32 211.3 69.5 36.0 22 95 69 L14 105.7 0.32 220.2 74.9 50.5 17 73 73 L16 105.0 0.23 220.2 74.9 50.5 48.9 16 102 80 L10 107.0 0.22 223.5 74.7 50.7 23 92 67 L21 107.2 0.22 23.5 77.8 48.9 16 94 74	K4	106.0	0.25	228.7	82.8	56.6	19	101	80
I6 104.6 0.28 230.9 88.8 59.2 9 95 82 N8 92.3 0.24 73 G9 1086 0.22 218.2 67.5 43.1 22 102 73 K10 1096 0.25 230.7 80.0 52.6 16 98 76 L12 0.15 215.1 60.0 43.1 17 95 74 K13 104.4 0.29 227.7 86.2 55.9 16 92 75 G14 105.7 0.32 220.2 74.9 50.5 17 93 73 T16 105.0 0.22 223.1 76.5 48.7 66 88 A20 107.9 0.22 233.5 74.7 50.7 24 92 67 D22 117.5 0.16 233.0 66.3 47.2 24 92 67	L5								
L7 R8 92.3 0.24 G9 108.6 0.22 218.2 67.5 43.1 22 102 73 R11 109.7 0.15 216.1 60.0 43.1 17 95 74 L7 K13 104.4 0.29 227.7 86.2 55.9 16 92 75 G14 105.7 0.32 211.3 69.5 36.0 22 95 69 E15 109.7 0.32 220.2 74.9 50.7 T16 105.0 0.23 220.2 74.9 50.7 T17 105.8 0.26 223.1 77.3 49.5 16 102 80 A20 107.9 0.22 233.8 84.0 66.0 K13 105.8 0.26 223.1 77.3 49.5 16 102 80 A20 107.9 0.22 233.8 84.0 66.0 K13 107.2 0.22 233.8 84.0 66.0 K14 107.2 0.22 233.8 84.0 66.0 K11 107.2 0.22 233.5 74.7 50.7 2 97 67 D22 117.5 0.16 233.0 66.3 47.2 24 9 67 T25 114.2 0.19 23.5 52.3 K24 114.9 0.25 235.7 77.8 48.9 16 94 74 T25 114.2 0.19 23.16 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 T22 117.5 0.16 233.0 76.6 48.5 19 98 73 A24 114.9 0.25 235.7 77.8 47.4 19 97 73 K28 112.5 0.23 228.9 75.2 47.1 14 95 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 K28 112.5 0.23 228.7 77.8 48.9 16 94 74 A25 114.2 0.19 23.16 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 K28 112.5 0.23 228.9 75.2 47.1 14 95 77 K28 112.5 0.23 237.5 74.3 50.6 18 98 74 G32 113.7 0.24 235.5 74.3 50.6 18 98 74 G33 113.7 0.24 235.7 77.8 48.9 16 94.74 19 97 73 K28 112.5 0.23 228.9 75.2 47.1 14 95 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 K28 112.5 0.23 228.7 75.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K28 112.5 0.25 239.7 75.5 50.9 21 105 77 K29 109.4 0.22 240.7 76.5 50.9 21 105 77 K29 103.4 0.22 240.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 72.6 50.1 8 98 74 G32 113.7 0.24 235.7 77.23 55.4 18 98 74 G33 113.7 0.24 235.7 77.23 55.4 18 98 74 G34 116.7 0.17 225.1 60.1 40.0 22 110 83 G41 84.1 0.30 192.2 78.7 53.4 20 97 71 N35 113.1 0.20 231.3 76.6 53.4 20 97 71 N35 113.1 0.20 231.3 76.6 53.4 20 97 71 N35 113.1 0.20 231.3 76.5 53.4 20 97 71 N35 113.1 0.20 231.3 76.5 53.4 20 97 71 N35 113.1 0.20 231.3 77.2 35.4 3.1 00 73 H44 004.4 0.29 23.3 76.5 53.4 20 97 71 N35 113.1 0.30 192.2 78.7 53.4 20 97 71 N35 113.1 0.30 192.2 78.7 53.4 20 97 71 N36 112.2 0.21 233.3 76.6 53.1 1 N44 10.4 0.4 29 23.5 83.1 00.7 18	16	104.6	0.28	230.9	88.8	59.2	9	95	82
N8 92.3 0.24 G9 108.6 0.22 218.2 67.5 43.1 12 102 73 K10 109.7 0.15 216.1 60.0 43.1 17 95 74 L12 0.15 216.1 60.0 43.1 17 95 74 K13 104.4 0.29 227.7 86.2 55.9 16 92 93 73 G14 105.7 0.23 230.8 79.0 53.5 17 93 73 T16 105.0 0.23 220.2 74.9 50.5 717 73 75.5 48.7 T18 105.8 0.26 222.1 77.3 49.5 16 102 80 Q12 107.2 0.22 233.5 74.7 50.7 23 92 67 A23 112.7 0.25 235.5 80.5 52.3 74 93 66 Q22 117.5 0.16 233.0 66.5 52.3 77 78 43.9	L7								
G9 108.6 0.22 218.2 67.5 43.1 22 102 73 K10 109.6 0.25 230.7 80.0 52.6 16 98 76 T11 109.7 0.15 216.1 60.0 43.1 17 95 74 K13 104.4 0.29 227.7 86.2 55.9 16 92 75 G14 105.7 0.32 230.8 79.0 53.5 17 93 73 T16 105.0 0.23 220.2 74.9 50.5 T 73 49.5 16 102 80 T17 107.0 0.26 223.1 76.5 48.7 T 1 102.8 80 60.0 T 73 K20 107.9 0.22 233.8 84.0 60.0 T 73 K21 107.2 0.22 235.5 77.8 48.9 16 94 74	N8	92.3	0.24						
K10 109.6 0.25 230.7 80.0 52.6 16 98 76 L11 109.7 0.15 216.1 60.0 43.1 17 95 74 L12	G9	108.6	0.22	218.2	67.5	43.1	22	102	73
T11 109.7 0.15 216.1 60.0 43.1 17 95 74 K13 104.4 0.29 227.7 86.2 55.9 16 92 75 G14 105.7 0.32 211.3 69.5 36.0 22 95 69 E15 109.7 0.23 220.2 74.9 50.5 17 93 73 T16 105.0 0.23 220.2 74.9 50.5 16 102 80 E19 103.8 0.26 222.1 77.3 49.5 16 102 80 A20 107.9 0.22 233.8 84.0 60.0	K10	109.6	0.25	230.7	80.0	52.6	16	98	76
L12 K13 104.4 0.29 227.7 86.2 55.9 16 92 75 G14 105.7 0.32 211.3 69.5 36.0 22 95 69 E15 109.7 0.23 220.2 74.9 50.5 T17 107.0 0.26 223.1 76.5 48.7 T18 105.8 0.26 222.1 77.3 49.5 16 102 80 E19 103.8 0.32 229.2 90.0 57.0 6 96 88 A20 107.9 0.22 233.8 84.0 60.0 V21 107.2 0.22 223.5 74.7 50.7 23 92 67 D22 117.5 0.16 233.0 66.3 47.2 24 93 66 A23 112.7 0.25 235.5 80.5 52.3 A24 114.9 0.25 235.7 77.8 48.9 16 94 74 T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 75.2 47.1 14 95 77 K28 112.5 0.23 228.9 75.2 47.1 14 95 77 K29 109.4 0.22 237.7 76.9 51.0 14 93 76 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 G33 113.7 0.24 235.0 77.9 51.0 14 93 76 F33 113.7 0.24 235.7 77.8 49.5 1.3 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 G33 113.7 0.23 234.7 76.9 51.3 19 98 73 G34 418.0 0.22 110 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 G38 116.7 0.17 225.1 60.1 40.0 22 11 05 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.9 47.9 22 99 71 G37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 80.1 40.0 22 110 81 Y39 115.9 0.15 237.7 73.3 55.4 18 98 74 D40 102.6 0.19 233.7 85.3 10 00 23 T44 104.4 0.29 213.6 72.3 41.7 18 98 74 D40 102.6 0.19 233.7 85.3 10 31 31 00 83 G41 84.1 0.30 132.2 78.7 73.3 55.4 18 98 74 D40 102.6 0.19 233.7 73 53.4 20 94 71 E42 104.5 0.31 223.5 83.1 50.4 20 100 73 T44 104.4 0.29 213.6 72.3 41.7 18 98 74 D45 105.4 0.24 222.2 79.3 54.3 16 96 75 D46 106.6 0.22 232.9 84.8 61.3 17 103 79 D47 103.0 0.36 226.4 90.6 53.1 	T11	109.7	0.15	216.1	60.0	43.1	17	95	74
K13 104.4 0.29 227.7 86.2 55.9 16 92 75 614 105.7 0.32 211.3 65.5 36.0 22 95 69 E15 109.7 0.23 220.2 74.9 50.5 77 73 T16 105.0 0.23 220.2 74.9 50.5 77 6 96 88 E19 103.8 0.32 229.2 90.0 57.0 6 96 88 A20 107.9 0.22 233.8 84.0 60.0 74 74 V21 107.2 0.22 235.5 80.5 52.3 74.7 50.7 23 92 67 A23 112.7 0.25 235.7 77.8 48.9 16 94 74 T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 20.3 81.0 50.7 14 93 80 73 112.5 0.23	L12								
G14 105.7 0.22 211.3 69.5 36.0 22 95 69 F15 109.7 0.23 220.2 74.9 50.5 73 T16 107.0 0.26 223.1 76.5 48.7 73 T18 105.8 0.26 222.1 77.3 49.5 16 102 80 E19 103.8 0.32 229.2 90.0 57.0 6 96 88 A20 107.9 0.22 233.8 84.0 60.0 74 74 D22 117.5 0.16 233.0 66.3 47.2 24 93 66 A23 112.7 0.25 235.5 80.5 52.3 74.7 74 T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 228.9 72.8 47.4 19 97 73 K28 112.5 0.23 228.9 72.8 47.4 14 95 77 <	K13	104.4	0.29	227.7	86.2	55.9	16	92	75
E15 109.7 0.23 220.8 79.0 53.5 17 93 73 T16 105.0 0.23 220.2 74.9 50.5 50.5 T17 107.0 0.26 223.1 76.5 48.7 76.5 88.7 E19 103.8 0.32 229.2 90.0 57.0 6 96 88 A20 107.9 0.22 223.8 84.0 60.0 72 73 92 67 D22 117.5 0.16 233.0 66.3 47.2 24 93 66 A23 112.7 0.25 235.7 77.8 48.9 16 94 74 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 75.2 47.1 14 95 77 V29 109.4 0.22 228.7 76.5 52.8 14 99 80 73 K31 116.7 0.20 237.5 74.3 <td>G14</td> <td>105.7</td> <td>0.32</td> <td>211.3</td> <td>69.5</td> <td>36.0</td> <td>22</td> <td>95</td> <td>69</td>	G14	105.7	0.32	211.3	69.5	36.0	22	95	69
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	E15	109.7	0.23	230.8	79.0	53.5	17	93	73
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	T16	105.0	0.23	220.2	74.9	50.5			
T18 105.8 0.26 222.1 77.3 49.5 16 102 80 E19 103.8 0.32 229.2 90.0 57.0 6 96 88 A20 107.9 0.22 233.8 84.0 60.0	T17	107.0	0.26	223.1	76.5	48.7			
E19 103.8 0.32 229.2 90.0 57.0 6 96 88 A200 107.9 0.22 233.8 84.0 60.0 V21 107.2 0.22 223.5 74.7 50.7 23 92 67 D22 117.5 0.16 233.0 66.3 47.2 24 93 66 A23 112.7 0.25 235.5 80.5 52.3 74 725 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 75.2 47.1 14 95 77 V29 109.4 0.22 228.7 76.5 52.8 14 99 80 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 <	T18	105.8	0.26	222.1	77.3	49.5	16	102	80
A20 107.9 0.22 233.8 84.0 60. V21 107.2 0.22 233.5 74.7 50.7 23 92 67 D22 117.5 0.16 233.0 66.3 47.2 24 93 66 A23 112.7 0.25 235.5 80.5 52.3 77 A24 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 72.8 47.4 19 97 73 K28 112.5 0.25 229.7 76.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.23 234.7 76.9 51.3 19 98 73 M34 118.0 0.22 240.7 76.5 50.9 21 105 77	E19	103.8	0.32	229.2	90.0	57.0	6	96	88
V21 107.2 0.22 223.5 74.7 50.7 23 92 67 D22 117.5 0.16 233.0 66.3 47.2 24 93 66 A23 112.7 0.25 235.5 80.5 52.3 74.4 94 94 74 T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 72.8 47.1 14 95 77 V29 109.4 0.22 228.7 76.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.24	A20	107.9	0.22	233.8	84.0	60.0			
D22 117.5 0.16 233.0 66.3 47.2 24 93 66 A23 112.7 0.25 235.5 80.5 52.3 77.8 48.9 16 94 74 T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 72.8 47.4 19 97 73 K28 112.5 0.25 229.9 75.2 47.1 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.23 234.7 76.9 51.3 19 98 73 A34 118.0 0.22 240.7 76.5 50.9 21 105 77 N35 113.1	V21	107.2	0.22	223.5	74.7	50.7	23	92	67
A23 112.7 0.25 235.5 80.5 52.3 A24 114.9 0.25 235.7 77.8 48.9 16 94 74 T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 K28 112.5 0.22 228.9 72.8 47.4 19 97 73 K28 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.23 234.7 76.9 51.3 19 98 73 A34 118.0 0.22 240.7 76.5 50.9 21 105 77 A34 118.0 0.28 20.3 76.9 47.9 22 10 81 V33 113.7 0.28 220.3 76.9 47.9 22	D22	117.5	0.16	233.0	66.3	47.2	24	93	66
A24 114.9 0.25 235.7 77.8 48.9 16 94 74 T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 72.8 47.1 14 95 77 V29 109.4 0.22 228.7 76.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 X31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.23 234.7 76.9 51.3 19 98 73 A34 118.0 0.22 240.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3	A23	112.7	0.25	235.5	80.5	52.3			
T25 114.2 0.19 231.6 70.9 49.7 21 105 77 A26 116.3 0.26 240.3 81.0 50.7 16 96 75 E27 112.5 0.23 228.9 72.8 47.1 14 95 77 V29 109.4 0.22 228.7 76.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.23 234.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 20.3	A24	114.9	0.25	235.7	77.8	48.9	16	94	74
A26116.30.26240.381.050.7169675E27112.50.23228.972.847.4199773K28112.50.25229.975.247.1149577V29109.40.22228.776.552.8149980F30112.30.25231.076.648.5199873K31116.70.20237.574.350.6189874Q32113.70.24235.077.951.0149376Y33113.70.22240.776.550.92110577N35113.10.20231.373.250.1189874D36112.20.21233.376.653.4209772N37105.30.28220.376.947.9229971G38116.70.17225.160.140.02211081V39115.90.15237.772.355.4189874D40102.60.19233.789.370.31310083G4184.10.30192.278.753.4209471E42104.50.31223.583.150.42010073W43105.40.24230.484.959.720 <t< td=""><td>T25</td><td>114.2</td><td>0.19</td><td>231.6</td><td>70.9</td><td>49.7</td><td>21</td><td>105</td><td>77</td></t<>	T25	114.2	0.19	231.6	70.9	49.7	21	105	77
E27 112.5 0.23 228.9 72.8 47.4 19 97 73 K28 112.5 0.25 229.9 75.2 47.1 14 95 77 V29 109.4 0.22 228.7 76.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.23 234.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 20.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1	A26	116.3	0.26	240.3	81.0	50.7	16	96	75
K28 112.5 0.25 229.9 75.2 47.1 14 95 77 V29 109.4 0.22 228.7 76.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.23 234.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7	E27	112.5	0.23	228.9	72.8	47.4	19	97	73
V29 109.4 0.22 228.7 76.5 52.8 14 99 80 F30 112.3 0.25 231.0 76.6 48.5 19 98 73 K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.22 240.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2	K28	112.5	0.25	229.9	75.2	47.1	14	95	77
F30112.30.25231.076.648.5199873K31116.70.20237.574.350.6189874Q32113.70.24235.077.951.0149376Y33113.70.23234.776.951.3199873A34118.00.22240.776.550.92110577N35113.10.20231.373.250.1189874D36112.20.21233.376.653.4209772N37105.30.28220.376.947.9229971G38116.70.17225.160.140.02211081V39115.90.15237.772.355.4189874D40102.60.19233.789.370.31310083G4184.10.30192.278.753.4209471E42104.50.31223.583.150.42010073W43105.40.24232.984.861.31710379D47103.60.24222.279.354.3169675D46106.60.22234.774.148.2189673T44104.40.29213.670.943.721 <td< td=""><td>V29</td><td>109.4</td><td>0.22</td><td>228.7</td><td>76.5</td><td>52.8</td><td>14</td><td>99</td><td>80</td></td<>	V29	109.4	0.22	228.7	76.5	52.8	14	99	80
K31 116.7 0.20 237.5 74.3 50.6 18 98 74 Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.23 234.7 76.9 51.3 19 98 73 A34 118.0 0.22 240.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2	F30	112.3	0.25	231.0	76.6	48.5	19	98	73
Q32 113.7 0.24 235.0 77.9 51.0 14 93 76 Y33 113.7 0.23 234.7 76.9 51.3 19 98 73 A34 118.0 0.22 240.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2 78.7 53.4 20 94 71 K42 104.5 0.31 223.5	K31	116.7	0.20	237.5	74.3	50.6	18	98	74
Y33 113.7 0.23 234.7 76.9 51.3 19 98 73 A34 118.0 0.22 240.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2 78.7 53.4 20 94 71 E42 104.5 0.31 223.5 83.1 50.4 20 100 73 W43 105.4 0.24 223.5	Q32	113.7	0.24	235.0	77.9	51.0	14	93	76
A34 118.0 0.22 240.7 76.5 50.9 21 105 77 N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2 78.7 53.4 20 94 71 E42 104.5 0.31 223.5 83.1 50.4 20 100 73 W43 105.4 0.24 230.4 84.9 59.7 20 100 73 V45 103.6 0.24 222.2	Y33	113.7	0.23	234.7	76.9	51.3	19	98	73
N35 113.1 0.20 231.3 73.2 50.1 18 98 74 D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2 78.7 53.4 20 94 71 E42 104.5 0.31 223.5 83.1 50.4 20 100 73 W43 105.4 0.24 230.4 84.9 59.7 20 100 73 V44 104.4 0.29 213.6 72.3 41.7 18 98 74 Y45 103.6 0.24 222.2	A34	118.0	0.22	240.7	76.5	50.9	21	105	77
D36 112.2 0.21 233.3 76.6 53.4 20 97 72 N37 105.3 0.28 220.3 76.9 47.9 22 99 71 G38 116.7 0.17 225.1 60.1 40.0 22 110 81 V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2 78.7 53.4 20 94 71 E42 104.5 0.31 223.5 83.1 50.4 20 100 73 W43 105.4 0.24 230.4 84.9 59.7 20 100 73 T44 104.4 0.29 213.6 72.3 41.7 18 98 74 V45 103.6 0.24 222.2 79.3 54.3 16 96 75 D46 106.6 0.22 232.9	N35	113.1	0.20	231.3	73.2	50.1	18	98	74
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	D36	112.2	0.21	233.3	76.6	53.4	20	97	72
G38116.70.17225.160.140.02211081 $V39$ 115.90.15237.772.355.4189874 $D40$ 102.60.19233.789.370.31310083G4184.10.30192.278.753.4209471E42104.50.31223.583.150.42010073W43105.40.24230.484.959.72010073T44104.40.29213.672.341.7189874Y45103.60.24222.279.354.3169675D46106.60.22232.984.861.31710379D47103.00.36226.490.653.179749169C44115.70.22234.774.148.2189673T49104.10.16208.360.543.7219169K50118.80.18238.570.949.7189975T51109.90.24221.970.343.7209170F52108.80.21239.187.564.22110273T53106.50.24218.771.646.21710076V54109.20.3123.386.252.8 <td>N37</td> <td>105.3</td> <td>0.28</td> <td>220.3</td> <td>76.9</td> <td>47.9</td> <td>22</td> <td>99</td> <td>71</td>	N37	105.3	0.28	220.3	76.9	47.9	22	99	71
V39 115.9 0.15 237.7 72.3 55.4 18 98 74 D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2 78.7 53.4 20 94 71 E42 104.5 0.31 223.5 83.1 50.4 20 100 73 W43 105.4 0.24 230.4 84.9 59.7 20 100 73 T44 104.4 0.29 213.6 72.3 41.7 18 98 74 Y45 103.6 0.24 222.2 79.3 54.3 16 96 75 D46 106.6 0.22 232.9 84.8 61.3 17 103 79 D47 103.0 0.36 226.4 90.6 53.1	G38	116.7	0.17	225.1	60.1	40.0	22	110	81
D40 102.6 0.19 233.7 89.3 70.3 13 100 83 G41 84.1 0.30 192.2 78.7 53.4 20 94 71 E42 104.5 0.31 223.5 83.1 50.4 20 100 73 W43 105.4 0.24 230.4 84.9 59.7 20 100 73 T44 104.4 0.29 213.6 72.3 41.7 18 98 74 Y45 103.6 0.24 222.2 79.3 54.3 16 96 75 D46 106.6 0.22 232.9 84.8 61.3 17 103 79 D47 103.0 0.36 226.4 90.6 53.1	V39	115.9	0.15	237.7	72.3	55.4	18	98	74
G4184.10.30192.278.753.4209471E42104.50.31223.583.150.42010073W43105.40.24230.484.959.72010073T44104.40.29213.672.341.7189874Y45103.60.24222.279.354.3169675D46106.60.22232.984.861.31710379D47103.00.36226.490.653.1	D40	102.6	0.19	233.7	89.3	/0.3	13	100	83
E42104.50.31223.583.150.42010073W43105.40.24230.484.959.72010073T44104.40.29213.672.341.7189874Y45103.60.24222.279.354.3169675D46106.60.22232.984.861.31710379D47103.00.36226.490.653.1	G41	84.1	0.30	192.2	/8./	53.4	20	94	/1
W43105.4 0.24 230.4 84.9 59.7 20 100 73 T44104.4 0.29 213.6 72.3 41.7 18 98 74 Y45103.6 0.24 222.2 79.3 54.3 16 96 75 D46106.6 0.22 232.9 84.8 61.3 17 103 79 D47103.0 0.36 226.4 90.6 53.1 $$	E4Z	104.5	0.31	223.5	83.1	50.4	20	100	/3
144 104.4 0.29 213.6 72.3 41.7 18 98 74 Y45 103.6 0.24 222.2 79.3 54.3 16 96 75 D46 106.6 0.22 232.9 84.8 61.3 17 103 79 D47 103.0 0.36 226.4 90.6 53.1 $A48115.70.22234.774.148.2189673T49104.10.16208.360.543.7219169K50118.80.18238.570.949.7189975T51109.90.24221.970.343.7209170F52108.80.21239.187.564.22110273T53106.50.24218.771.646.21710076V54109.20.31227.680.547.1209171T55109.20.31233.386.252.8229669$	VV43	105.4	0.24	230.4	84.9	59.7	20	100	/3
Y45 103.6 0.24 222.2 79.3 54.3 16 96 75 D46 106.6 0.22 232.9 84.8 61.3 17 103 79 D47 103.0 0.36 226.4 90.6 53.1	144	104.4	0.29	213.6	/2.3	41.7	18	98	74
D46 106.6 0.22 232.9 84.8 61.3 17 103 79 D47 103.0 0.36 226.4 90.6 53.1 53.1 74 A48 115.7 0.22 234.7 74.1 48.2 18 96 73 T49 104.1 0.16 208.3 60.5 43.7 21 91 69 K50 118.8 0.18 238.5 70.9 49.7 18 99 75 T51 109.9 0.24 221.9 70.3 43.7 20 91 70 F52 108.8 0.21 239.1 87.5 64.2 21 102 73 T53 106.5 0.24 218.7 71.6 46.2 17 100 76 V54 109.2 0.31 227.6 80.5 47.1 20 91 71 T55 109.2 0.31 233.3 86.2 52.8 22 96 69	Y45	103.6	0.24	222.2	/9.3	54.3	16	96	75
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	D46	106.6	0.22	232.9	84.8	61.3	17	103	79
A48115.7 0.22 234.7 74.1 48.2 18 96 73 T49104.10.16208.3 60.5 43.7 21 91 69 K50118.80.18 238.5 70.9 49.7 18 99 75 T51109.90.24221.9 70.3 43.7 20 91 70 F52108.80.21239.1 87.5 64.2 21 102 73 T53106.50.24218.7 71.6 46.2 17 100 76 V54109.20.31227.6 80.5 47.1 20 91 71 T55109.20.31233.3 86.2 52.8 22 96 69	D47	103.0	0.36	226.4	90.6	53.1	40		70
149104.10.16208.360.543.7219169K50118.80.18238.570.949.7189975T51109.90.24221.970.343.7209170F52108.80.21239.187.564.22110273T53106.50.24218.771.646.21710076V54109.20.31227.680.547.1209171T55109.20.31233.386.252.8229669	A48	115.7	0.22	234.7	/4.1	48.2	18	96	/3
K50118.80.18238.570.949.7189975T51109.90.24221.970.343.7209170F52108.80.21239.187.564.22110273T53106.50.24218.771.646.21710076V54109.20.31227.680.547.1209171T55109.20.31233.386.252.8229669	149	104.1	0.16	208.3	60.5	43.7	21	91	69
151109.90.24221.970.343.7209170F52108.80.21239.187.564.22110273T53106.50.24218.771.646.21710076V54109.20.31227.680.547.1209171T55109.20.31233.386.252.8229669	K50	118.8	0.18	238.5	/0.9	49.7	18	99	/5
F52108.80.21239.187.564.22110273T53106.50.24218.771.646.21710076V54109.20.31227.680.547.1209171T55109.20.31233.386.252.8229669	151	109.9	0.24	221.9	/0.3	43.7	20	91	/0
153 106.5 0.24 218.7 71.6 46.2 17 100 76 V54 109.2 0.31 227.6 80.5 47.1 20 91 71 T55 109.2 0.31 233.3 86.2 52.8 22 96 69	F52	108.8	0.21	239.1	8/.5	64.2	21	102	/3
V 54 109.2 0.31 227.6 80.5 47.1 20 91 71 T55 109.2 0.31 233.3 86.2 52.8 22 96 69	153	106.5	0.24	218.7	/1.6	46.2	17	100	/6
155 109.2 0.31 233.3 86.2 52.8 22 96 69	V54	109.2	0.31	227.6	80.5	47.1	20	91	/1
	155	109.2	0.31	233.3	86.2	52.8	22	96	69

Table S2. ¹⁵N CST elements and orientation to ¹H-¹⁵N dipole

The angles $\alpha_{(11,22,33)}$ define the angle between each tensor element and the ¹H-¹⁵N dipole.

Table S3. Structural quality as assessed by Procheck

Restraints	Used						Ramachandran	Quality %	% X-ray Structure Equiva			e
Structure	т	D	С	0	V	SE	Most Favored	Allowed	Rama	H-bondEnergy	χ1	χ2
1				х			77.0	22.2	2.5	2.9	1.8	1.0
2		Х	Х	Х			94.0	6.0	1.0	3.3	1.9	1.0
3	Х	Х					94.0	6.0	1.0	3.4	3.2	1.7
4	Х		Х	Х			86.0	14.0	1.8	2.7	2.3	1.3
5	Х	Х	Х	Х			93.8	6.2	1.0	3.0	2.2	1.4
6	Х	Х	Х	Х	Х		96.0	4.0	1.0	3.0	2.4	1.2
7	Х	Х			Х	Х	100.0	0.0	1.0	2.9	2.0	1.0
8	Х		Х	Х	Х	Х	95.8	4.2	1.0	2.8	1.9	1.0
9	Х		Х	Х		Х	93.2	6.8	1.0	2.6	1.9	1.0
10	Х	Х	Х	Х		Х	96.0	4.0	1.0	2.8	1.8	1.0
11	Х	Х	Х	Х	Х	Х	96.4	3.6	1.0	2.8	1.8	1.0
2QMT							96.0	4.0	1.0	1.5	1.0	2.1
2GI9							94.0	6.0	1.0	1.2	1.0	1.9
1PGA							92.0	8.0	1.0	1.4	1.4	2.1
1PGB							90.0	10.0	1.0	1.0	1.5	2.1
2JSV		х			Х		92.0	8.0	1.0	4.0	3.2	1.6

Table S4. Agreement of CST magnitudes and orientations with theory for all structures

							CST		α _{(1,2,3})	$\beta_{(1,2,3)}$	
Structure	Т	D	С	0	V	SE	RMSD(ppm)	R ²	RMSD (°)	R ²	RMSD (°)	R ²
1				Х			3.6	0.95	4.7	0.99	5.7	0.98
2		Х	Х	Х			2.1	0.98	5.6	0.98	6.6	0.97
3	Х	Х					3.1	0.96	11.0	0.93	9.6	0.93
4	Х		Х	Х			2.1	0.98	5.5	0.98	6.5	0.97
5	Х	Х	Х	Х			2.0	0.98	5.6	0.98	6.6	0.97
6	Х	Х	Х	Х	Х		2.1	0.98	5.8	0.98	6.7	0.97
7	Х	Х			Х	Х	2.9	0.96	9.3	0.96	9.0	0.95
8	Х		Х	Х	Х	Х	2.1	0.98	5.8	0.98	6.6	0.97
9	Х		Х	Х		Х	2.0	0.98	5.7	0.98	6.5	0.97
10	Х	Х	Х	Х		Х	2.0	0.98	5.6	0.98	6.6	0.97
11	Х	Х	Х	Х	Х	Х	2.1	0.98	5.5	0.98	6.7	0.97
2QMT							2.5	0.97	8.0	0.97	8.5	0.95

Table S5. Agreement of structures with measured pseudodihedral angles

Restraints	Used				VEAN						
Structure	Т	D	С	0	V	SE	rmsd (°)*	dev. > 5°*	dev. > 10°*		
1				Х			11.5	40	21		
2		Х	Х	Х			7.4	27	12		
3	Х	Х					8.1	41	17		
4	Х		Х	Х			9.1	31	16		
5	Х	Х	Х	Х			6.7	26	11		
6	Х	Х	Х	Х	Х		3.5	11	2		
7	Х	Х			Х	Х	3.0	12	4		
8	Х		Х	Х	Х	Х	3.0	11	2		
9	Х		Х	Х		Х	8.6	33	14		
10	Х	Х	Х	Х		Х	8.6	34	14		
11	Х	Х	Х	Х	Х	Х	3.0	13	2		
2QMT⁺							6.1	28	13		

*After accounting for experimental error.

[†]Assuming canonical ¹H bond lengths, bond angles, and positions.

Table S6. Agreement of experimental	isotropic	chemical	shifts	with	shifts	predicted by	/ SPARTA	for
each structure								

								Cα		Cβ		C'	
Structure	Ν	Т	D	С	0	V	SE	RMSD (ppm)	R ²	RMSD (°)	R ²	RMSD (°)	R ²
1	Х				Х			1.08	0.95	1.35	0.99	1.23	0.8
2	Х		Х	Х	Х			1.06	0.95	1.28	0.99	1.15	0.8
3	Х	Х	Х					1.04	0.96	1.25	0.99	1.14	0.8
4	Х	Х		Х	Х			1.01	0.96	1.13	0.99	1.13	0.8
5	Х	Х	Х	Х	Х			1.02	0.96	1.13	0.99	1.16	0.8
6	Х	Х	Х	Х	Х	Х		1.04	0.96	1.15	0.99	1.19	0.8
7	Х	Х	Х			Х	Х	1.04	0.96	1.27	0.99	1.16	0.8
8	Х	Х		Х	Х	Х	Х	1.01	0.96	1.13	0.99	1.13	0.8
9	Х	Х		Х	Х		Х	1.01	0.96	1.13	0.99	1.13	0.8
10	Х	Х	Х	Х	Х		Х	1.01	0.96	1.13	0.99	1.13	0.8
11	Х	Х	Х	Х	Х	Х	Х	1.03	0.96	1.14	0.99	1.14	0.8
2QMT								0.93	0.97	0.99	0.99	1.12	0.8

Table S7. Backbone rmsd among crystal structures and SSNMR structure

Structure	SSNMR	2QMT	2GI9	1PGA	1PGB	Crystal Avg.
SSNMR	_	0.51	0.58	0.54	0.53	0.54
2QMT	0.51	_	0.47	0.39	0.28	0.39
2GI9	0.58	0.47	_	0.24	0.36	0.37
1PGA	0.54	0.39	0.24	_	0.25	0.30
1PGB	0.53	0.28	0.36	0.25	_	0.30
Crystal Avg	0.54	0.39	0.37	0.30	0.30	_

Table S8. Table of TALOS restraints that violated at some point during annealing and refinement

Residue	Angle	TALOS Prediction (°)	Doubled TALOS error (°)
Q2	φ	-109	34.0
N8	ϕ	-100	34.0
T11	ϕ	-94.0	34.0
E19	ϕ	-117.0	20.0
A24	ϕ	-65.0	24.0
E27	ϕ	-66.0	16.0
N37	ϕ	-105.0	32.0
V39	ϕ	-101.0	30.0
W43	ϕ	-122.0	32.0
T49	ϕ	-110.0	36.0
T11	Ψ	-6.0	28.0
T18	Ψ	137.0	28.0
A26	Ψ	-41.0	10.0
Y33	Ψ	-37.0	18.0
D36	Ψ	-31.0	16.0
W43	Ψ	160.0	20.0
Y45	Ψ	126.0	48.0
T49	Ψ	10.0	26.0
T51	Ψ	134.0	32.0
T53	Ψ	144.0	34.0

Table S9. Dista	nces lengthened	during strue	ture calculations
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					Low	Upper
Residue	Resonance	Residue	Resonance	Distance	error	Error
A23	СВ	Y3	CZ	2.75	1.75	3.00
16	CG1	T53	CB	3.05	2.05	3.05
M1	CE	E19	CG	3.55	2.55	3.55
Q2	CD	K50	CA	4.25	3.25	5.00
16	CG1	T53	CB	3.55	2.55	3.55
T18	С	Y3	CZ	4.25	3.25	4.25
Y33	С	L7	CD(1,2)	3.55	2.55	4.55
16	CG1	T53	CB	3.70	2.70	3.70
T53	CB	L7	CD(1,2)	3.70	2.70	5.00
Y33	С	L7	CD(1,2)	3.83	2.83	4.00
N8	CA	L12	CG	4.25	3.25	4.25
E42	HN	V54	HN	4.75	3.75	5.75
V54	HN	E42	HN	3.25	2.25	3.25
A26	Ν	A20	CB	4.43	1.00	1.00
F30	Ν	Y33	CB	3.82	0.28	1.28
G38	Ν	L12	CD1	4.31	2.00	2.00
N37	ND2	L12	CD1	3.06	1.00	1.00
E15	Ν	L7	CD2	4.76	1.00	1.00
K13	Ν	L7	CD2	4.79	1.00	1.00
K13	NZ	L7	CD2	6.73	1.00	1.00
A23	Ν	M1	CE	4.20	1.04	2.10
M1	Ν	M1	CE	3.77	1.60	1.60
K31	N	E27	CG	4.19	1.60	2.60
K28	N	K28	CG	2.67	1.22	1.22
Q2	N	M1	CG	3.01	1.00	1.00
F30	N	V29	CG1	2.81	1.00	1.00
N37	N	V39	CG1	5.64	1.39	1.39
Q32	N	V54	CG1	4.50	3.50	4.50
G41	N	V54	CG2	3.05	0.10	1.00
T18	N	E19	CD	6.86	0.35	1.35
G9	N	K13	CE	3.65	1.38	1.38
T11	N	K13	CE	4.36	0.67	1.67
T44	N	W43	CZ3	5.04	0.43	1.43
Q2	N	M1	CG	2.86	1.00	1.00
T17	N	T16	CG2	3.90	1.10	1.10
E19	N	T17	CG2	5.45	1.00	2.00
W43	N	T55	CG2	5.72	1.10	2.10
G41	N	V54	CG2	2.93	0.10	1.00
N37	N	Y33	CG	5.27	1.00	1.00