SUPPORTING INFORMATION

Paramagnetic-Based NMR Restraints Lift Residual Dipolar Coupling Degeneracy in Multidomain Detergent-Solubilized Membrane Proteins

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Structure calculation

In the first protocol, we started from a fully extended structure of AFA-PLN and carried out simulated annealing calculations starting at 6000 K and cooled to 0 K in Cartesian and torsion-angle space. The NOEs were modeled using a soft-square potential with a force constant ramping from 2 to 50 kcal $Å^{-2}$, while torsion angles were restrained by a square-well potential with a force constant of 200 kcal rad^{-2}. In addition, the torsion angle database potential 'RAMA' was used to bias the conformational search in the most allowed regions of the Ramachandran plot.

For the protocol two, residual dipolar coupling data were introduced during the lower temperature simulated annealing (3000 K) starting from the folded PLN structures obtained from protocol one. The system was cooled down to 0 K with a cooling rate of 5 K/step using 4000 steps of internal dynamics and 4000 steps of Cartesian dynamics. Only RDCs derived from residues in the helical regions with order parameters $S^2 > 0.6$ were used. The force constants for RDC restraints were ramped from 0.05 to 0.5 kcal Hz^{-2}, which was determined by compromising RDC agreement and geometrical penalties. After obtaining the tensor magnitude, only tensor orientations were allowed to vary during the internal and Cartesian dynamics.

For protocol three, additional MTSSL derived distance restraints are added to the restrains used in protocol two. The MTSSL restraints were imposed by a force constant ramped from 2 to 50 kcal $Å^{-2}$. The parameter and topology files for MTSSL were taken from the library available in XPLOR-NIH.[1] All calculations were performed on a Linux cluster at the Minnesota Supercomputing Institute.

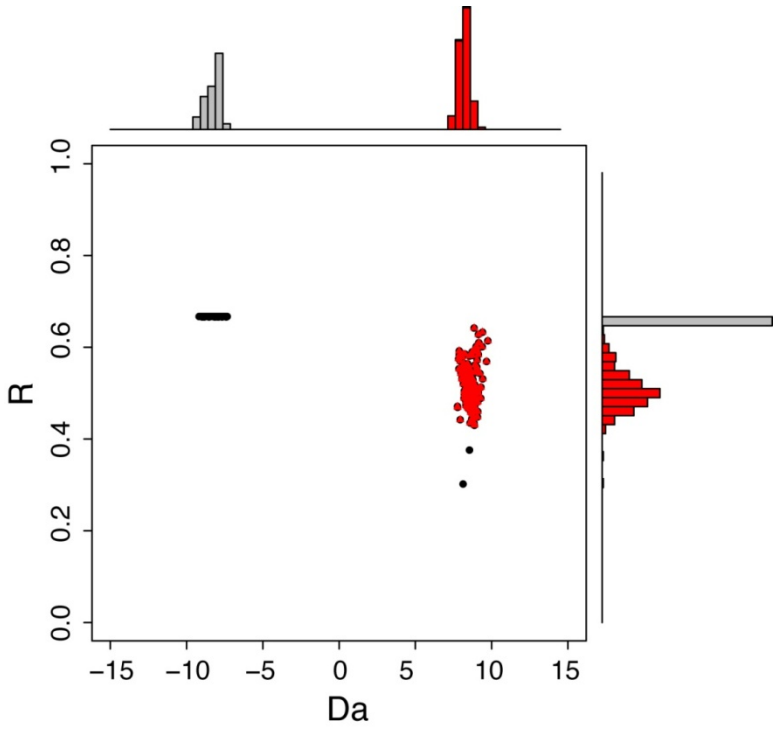

Figure S1. The (D_a, R) clustering (black) of structures refined with NOE and RDCs with variable tensor magnitude. Structures with $D_a > 0$ and $R > 0.4$ are selected for further structural calculation and analysis (red).

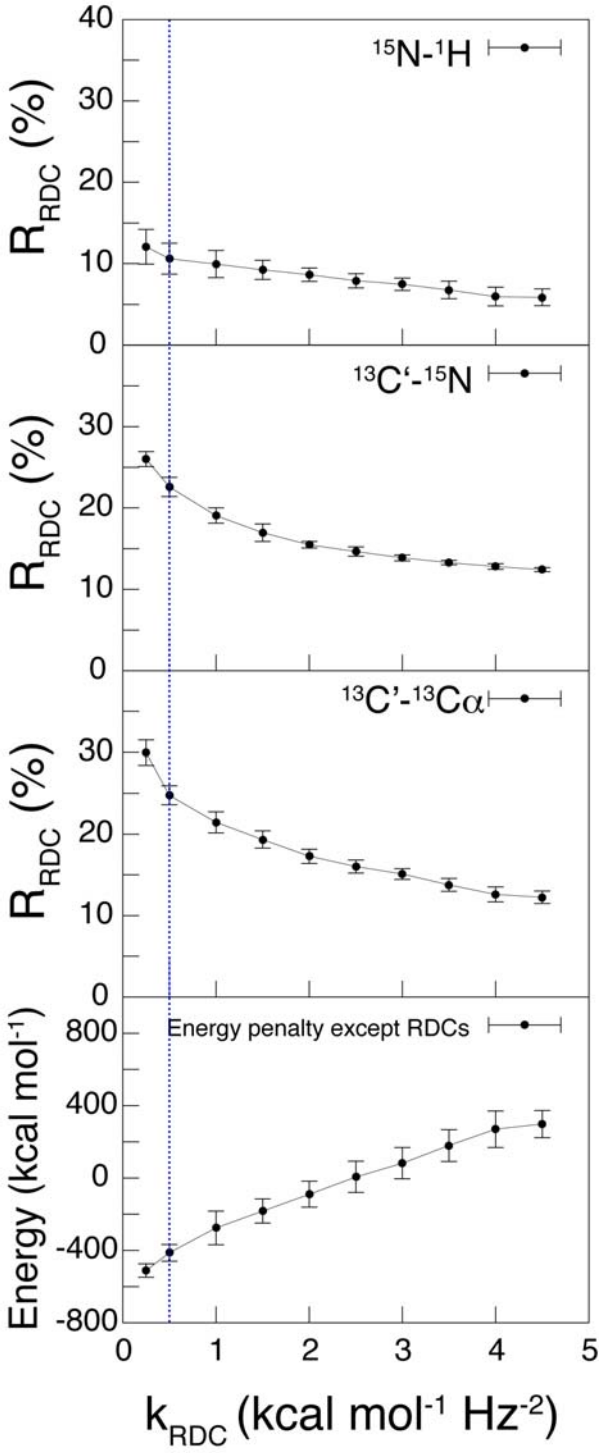

Figure S2. RDC R factors and energy value as functions of force constant used in the simulated annealing. The force constant for RDC was ramped from 0.25 kcal mol^{-1} Hz^{-2} to 4.5 kcal mol^{-1} Hz^{-2}, while the force constants for other energy terms such as NOEs and torsion angles were fixed. RDCs were modeled using flat-well potentials with the relative weight between ^{15}N-^{1}H, ^{13}C'-^{15}N and ^{13}C'-^{13}Cα was set to be 1:1:1. The energy values are summations from different terms in Equation 1 other than RDCs and the magnitude indicates penalties from other solution NMR data (NOEs and torsion angles) and ideal geometry (bond, angle *etc.*).

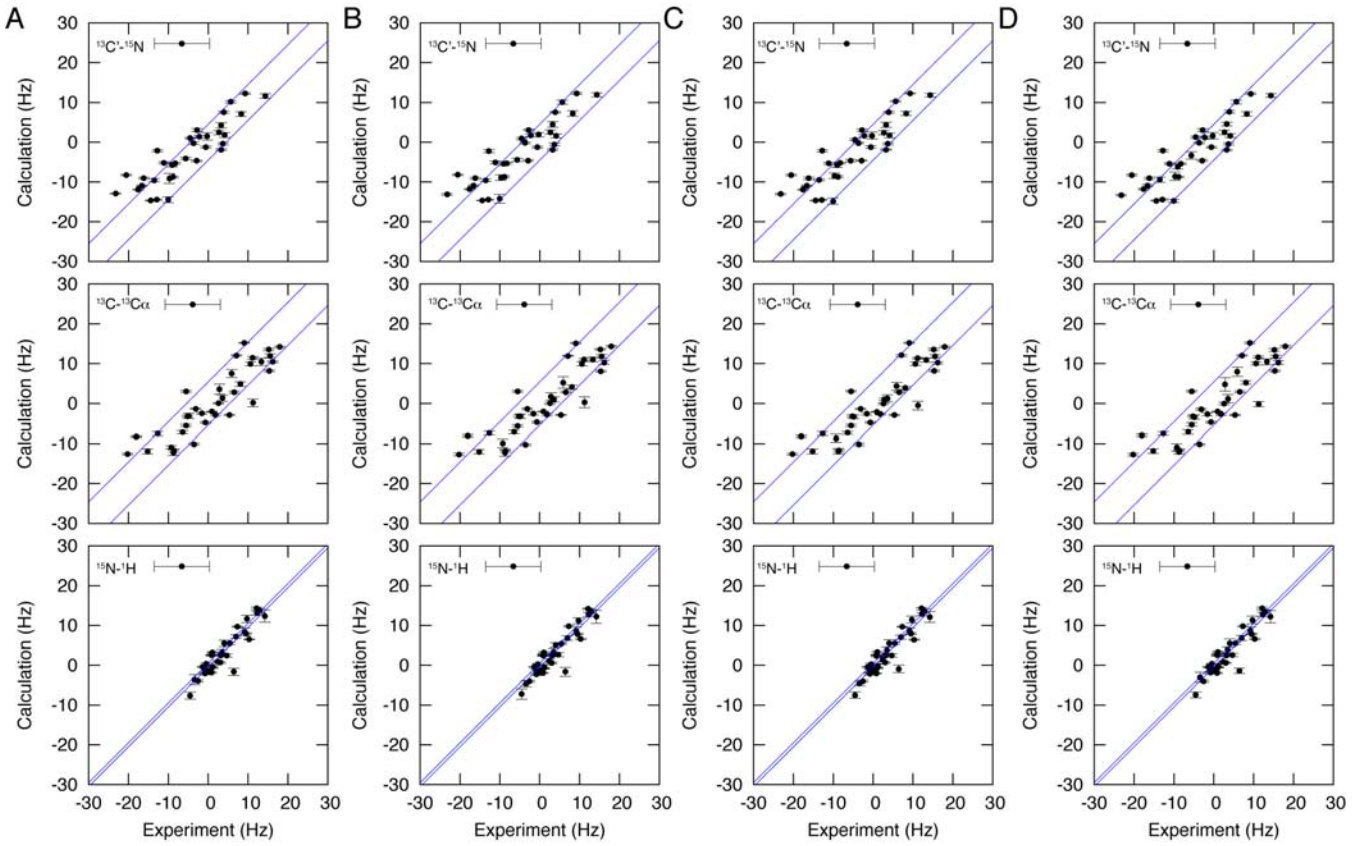

Figure S3. Correlation of experimental versus calculated RDCs of RDC ensemble family I, II, III and IV (15 monomers). Experimental errors of 3.0 Hz, 4.5 Hz and 0.5 Hz are used for ^{13}C'-^{15}N (A), ^{13}C'-^{13}Cα (B) and ^{15}N-^{1}H (C) respectively.

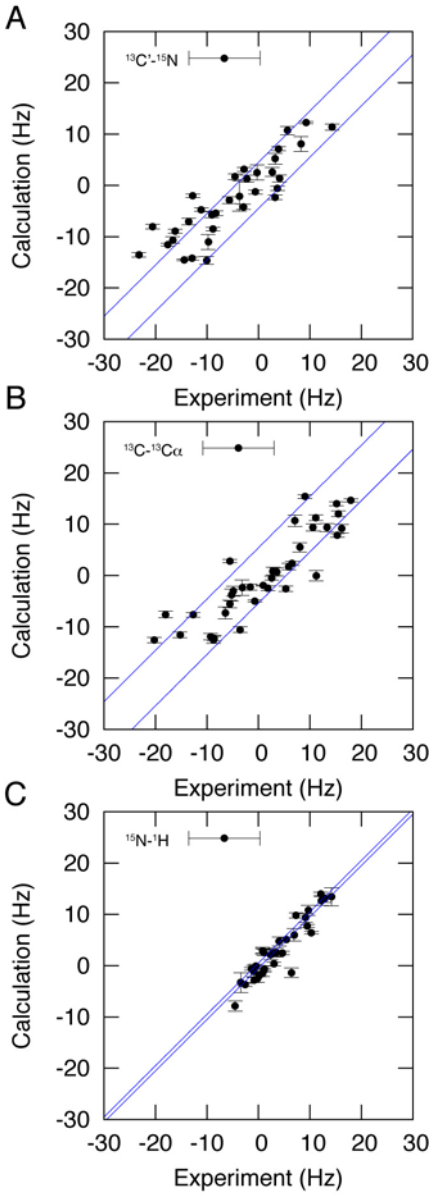

Figure S4. Correlation of experimental versus calculated RDCs from protocol three, the PRE ensemble (20 monomers). Experimental errors of 3.0 Hz, 4.5 Hz and 0.5 Hz are used for $^{13}C'$-^{15}N (A), $^{13}C'$-$^{13}C\alpha$ (B) and ^{15}N-^{1}H (C) respectively.

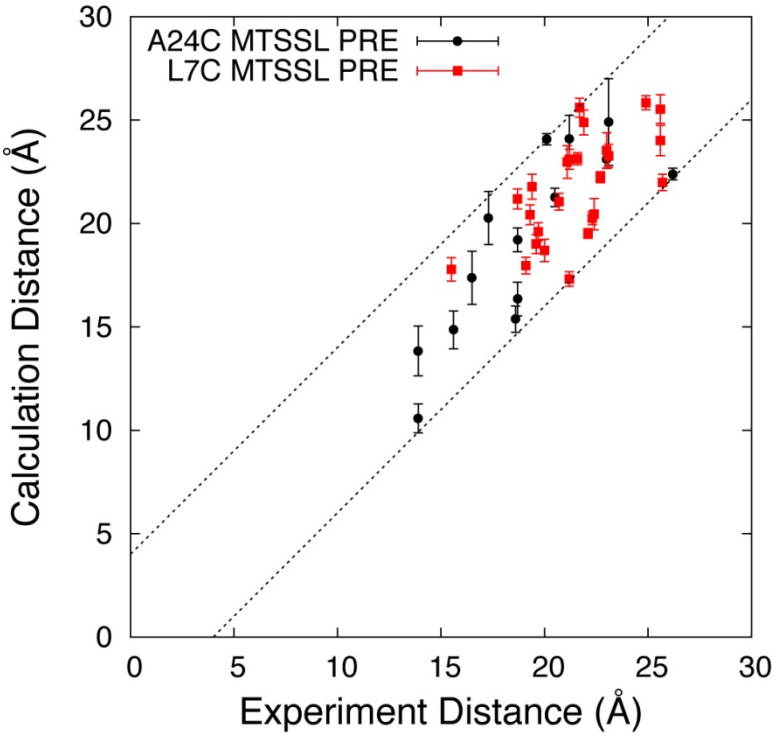

Figure S5. Correlation of experimental distances obtained from PREs (A24C MTSSL in black and L7C MTSSL in red) with calculated distances measured from the PRE ensemble (20 monomers). Experimental errors of 4 Å are plotted from the correlation line (dotted lines). The calculated distances (and error bars) reflect the average (and standard deviation) from the 20 structures in the PRE ensemble.

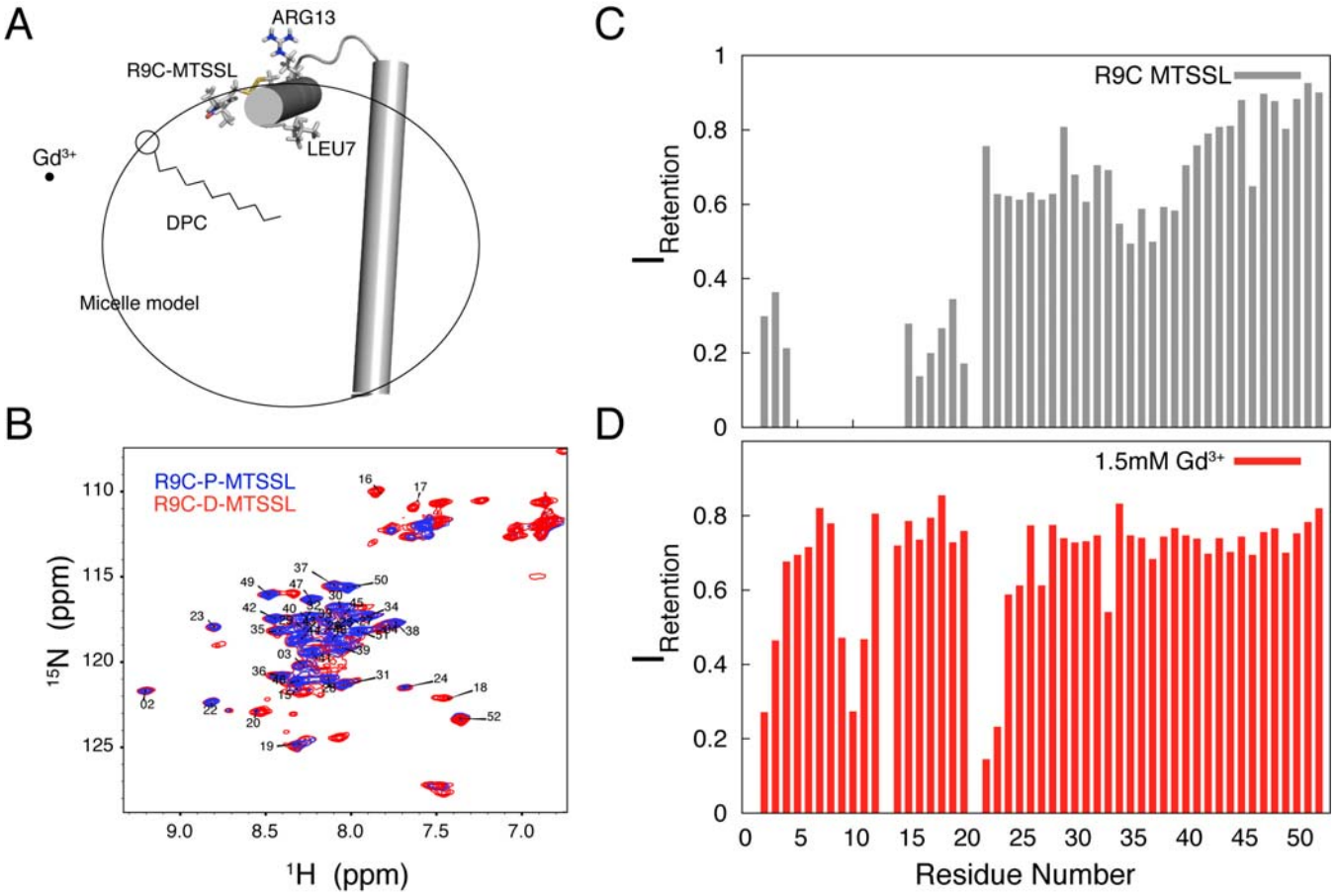

Figure S6. (A) Cartoon representation of R9C-AFA-PLN-MTSSL labeled AFA-PLN in a simplified micelle model. The model is supported by PRE data obtained from (B) NMR HSQC experiments. (C) Intensity retention from covalent attachment of MTSSL indicates that the hydrophobic spin label prefers to insert into the micelle, significantly quenching inserted residues around 35. (D) Intensity retention from 1.5 mM Gd^{3+} to the HSQC spectrum of R9C-AFA-PLN indicates that the cytoplasmic domain Ia is inserted into the micelle with preferential orientation for hydrophobic (e.g., L7) and hydrophilic residues (e.g., R13).

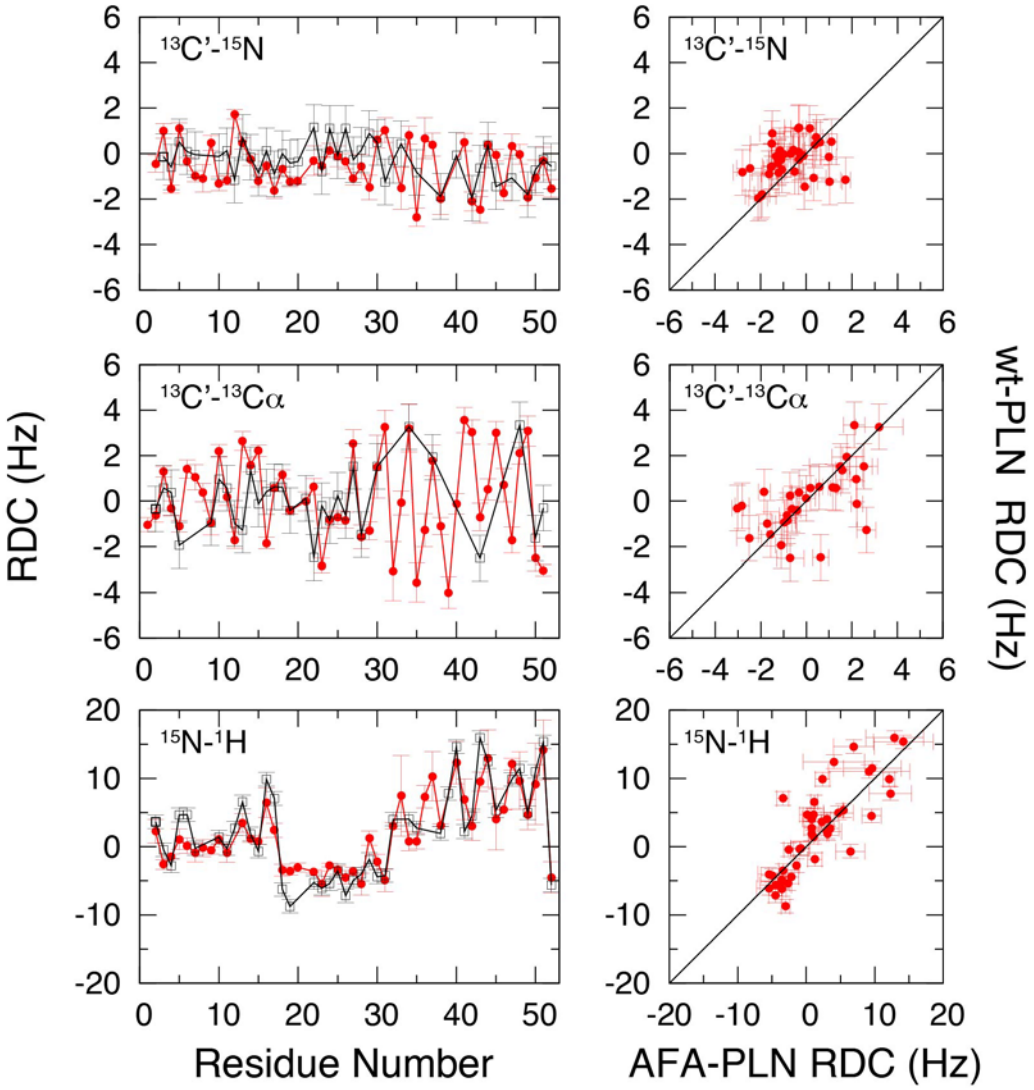

Figure S7. Comparison of ^{13}C'-^{15}N, ^{13}C'-^{13}Cα, and ^{15}N-^{1}H RDCs of AFA-PLN (monomer, red) and wt-PLN (pentamer, black) by residue number (left column) and as correlation plots (right column).

Table S1. NMR and Structural Refinement Statistics

	NOE+DIHE	RDC Ensemble				PRE Ensemble
		Family I	Family II	Family III	Family IV	
R.m.s. deviations from experimental restraints						
NOE/H-bond (Å) (431)	0.058	0.043	0.043	0.043	0.043	0.047
Torsion angle (º) (38)	0.072	0.739	0.724	0.746	0.741	1.160
MTSL PRE (Å) (43)	N/A	N/A	N/A	N/A	N/A	0.039
RDC R-factors (%)						
$^1D_{NH}$ (33)	N/A	10.23	10.19	9.20	9.88	10.68
$^1D_{NC'}$ (36)	N/A	22.04	22.16	21.97	22.13	23.55
$^1D_{CCA}$ (33)	N/A	24.75	22.68	25.33	25.09	25.86
R.m.s. deviations from idealized covalent geometry						
Bond (Å)	0.003	0.005	0.005	0.005	0.005	0.005
Angle (º)	0.397	0.655	0.647	0.648	0.650	0.736
Impropers (º)	0.278	0.629	0.612	0.619	0.621	0.684
Measure of structural quality						
% Residues in most favored region	92.5	90.7	91.7	87.1	90.1	85.3
%Residues in additional allowed region	7.5	9.1	7.5	12.3	9.6	10.2
%Residues in generously allowed region	0	0.2	0.8	0.6	0.3	4.5
%Residues in disallowed regions	0	0.0	0.0	0.0	0.0	0.0
Precision of atomic coordinates (Å)						
Backbone all (3-50)	4.402	1.6	1.5	0.9	0.9	1.6
Helix1 (residue 3-18)	0.621	0.2	0.1	0.1	0.1	0.2
Helix 2 (residue 24-50)	0.657	0.5	0.3	0.3	0.3	1.0

All the statistics were carried using Xplor-NIH software package.[1] Ramachandran analysis was carried out using PROCHECK_NMR.[2] Atom superposition was carried out using MOLMOL.[3]

References

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(3) Koradi, R.; Billeter, M.; Wuthrich, K. J. Mol. Graphics, **1996**, 14, 51-55.