wwPDB NMR Structure Validation Summary Report

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PDB ID	:	2N8Y
BMRB ID	:	25870
Title	:	Holo form of Calmodulin-Like Domain of Human Non-Muscle alpha-actinin 1
Authors	:	Drmota Prebil, S.; Slapsak, U.; de Almeida Ribeiro, E.; Pavsic, M.; Ilc, G.; Zielinska, K.; Hartl, M.; Backman, L.; Plavec, J.; Lenarcic, B.; Djinovic-Carugo, K.
Deposited on	:	2015-10-28

This is a wwPDB NMR Structure Validation Summary Report for a publicly released PDB entry.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

The types of validation reports are described at
http://www.wwpdb.org/validation/2017/FAQs#types.

The following versions of software and data (see references (1)) were used in the production of this report:

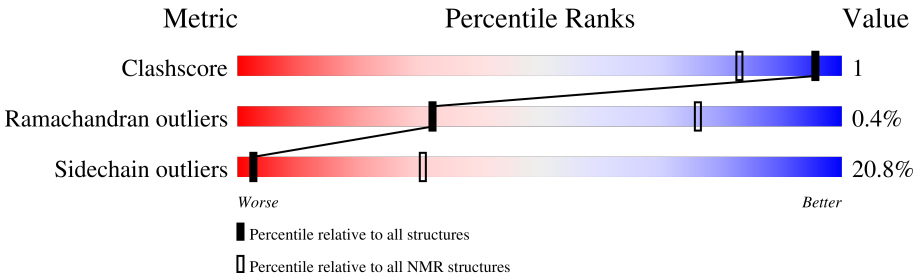
MolProbity	:	4.02b-467
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.40

1 Overall quality at a glance

The following experimental techniques were used to determine the structure:
SOLUTION NMR

The overall completeness of chemical shifts assignment is 86%.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	NMR archive (#Entries)
Clashscore	210492	14027
Ramachandran outliers	207382	12486
Sidechain outliers	206894	12463

The table below summarises the geometric issues observed across the polymeric chains and their fit to the experimental data. The red, orange, yellow and green segments indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. A cyan segment indicates the fraction of residues that are not part of the well-defined cores, and a grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5%

Mol	Chain	Length	Quality of chain
1	A	153	74% 14% 12%

2 Ensemble composition and analysis

This entry contains 20 models. Model 6 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *lowest energy*.

The following residues are included in the computation of the global validation metrics.

Well-defined (core) protein residues			
Well-defined core	**Residue range (total)**	**Backbone RMSD (Å)**	**Medoid model**
1	A:742-A:867, A:875-A:882 (134)	0.74	6

Ill-defined regions of proteins are excluded from the global statistics.

Ligands and non-protein polymers are included in the analysis.

The models can be grouped into 3 clusters and 2 single-model clusters were found.

Cluster number	Models
1	1, 2, 4, 5, 6, 7, 16, 19
2	8, 9, 10, 13, 20
3	12, 14, 15, 17, 18
Single-model clusters	3; 11

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2305 atoms, of which 1114 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Alpha-actinin-1.

Mol	Chain	Residues	Atoms						Trace
1	A	153	Total	C	H	N	O	S	0
			2304	742	1114	195	244	9	

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	740	GLY	-	expression tag	UNP P12814
A	741	SER	-	expression tag	UNP P12814
A	742	SER	-	expression tag	UNP P12814

- Molecule 2 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms	
2	A	1	Total	Ca
			1	1

4 Residue-property plots (i)

4.1 Average score per residue in the NMR ensemble

These plots are provided for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic is the same as shown in the summary in section 1 of this report. The second graphic shows the sequence where residues are colour-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. Stretches of 2 or more consecutive residues without any outliers are shown as green connectors. Residues which are classified as ill-defined in the NMR ensemble, are shown in cyan with an underline colour-coded according to the previous scheme. Residues which were present in the experimental sample, but not modelled in the final structure are shown in grey.

- Molecule 1: Alpha-actinin-1

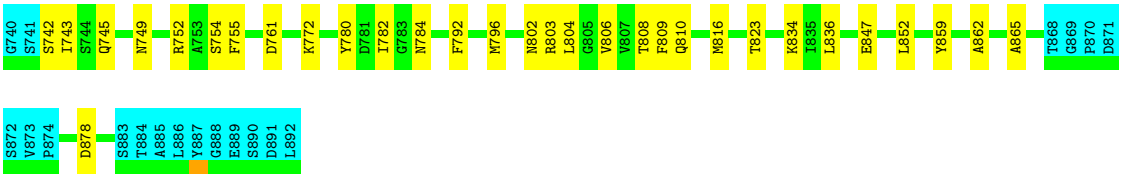
Chain A:

4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 6. Colouring as in section 4.1 above.

- Molecule 1: Alpha-actinin-1

Chain A:



5 Refinement protocol and experimental data overview

The models were refined using the following method: *simulated annealing, water refinement.*

Of the 20 calculated structures, 20 were deposited, based on the following criterion: *structures with the lowest energy.*

The following table shows the software used for structure solution, optimisation and refinement.

Software name	Classification	Version
CYANA	structure solution	3.0
YASARA	refinement	
CYANA	refinement	3.0

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 7 of this report.

Chemical shift file(s)	working_cs.cif
Number of chemical shift lists	1
Total number of shifts	1690
Number of shifts mapped to atoms	1690
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	86%

6 Model quality

6.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: CA

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the (average) root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	#Z>5	RMSZ	#Z>5
1	A	0.81±0.11	1±2/1083 (0.1± 0.2%)	0.89±0.13	3±3/1463 (0.2± 0.2%)
All	All	0.81	20/21660 (0.1%)	0.90	61/29260 (0.2%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	Chirality	Planarity
1	A	0.0±0.0	0.1±0.2
All	All	0	1

5 of 15 unique bond outliers are listed below. They are sorted according to the Z-score of the worst occurrence in the ensemble.

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)	Models	
								Worst	Total
1	A	809	PHE	CE1-CZ	14.54	1.65	1.37	10	2
1	A	809	PHE	CE2-CZ	12.84	1.61	1.37	19	1
1	A	809	PHE	CD2-CE2	11.77	1.62	1.39	19	1
1	A	809	PHE	CG-CD2	10.35	1.54	1.38	10	3
1	A	755	PHE	CD2-CE2	9.78	1.58	1.39	20	1

5 of 23 unique angle outliers are listed below. They are sorted according to the Z-score of the worst occurrence in the ensemble.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	A	807	VAL	O-C-N	-28.05	77.81	122.70	18	1
1	A	807	VAL	CA-C-N	18.94	158.86	117.20	18	1
1	A	807	VAL	CA-C-O	-18.57	81.09	120.10	18	1

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	A	809	PHE	CG-CD1-CE1	12.95	135.05	120.80	19	1
1	A	812	PHE	CB-CG-CD2	-10.88	113.18	120.80	20	4

There are no chirality outliers.

All unique planar outliers are listed below.

Mol	Chain	Res	Type	Group	Models (Total)
1	A	807	VAL	Mainchain	1

6.2 Too-close contacts (i)

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in each chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes averaged over the ensemble.

Mol	Chain	Non-H	H(model)	H(added)	Clashes
1	A	1060	996	995	2±2
All	All	21220	19920	19900	39

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 1.

5 of 27 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:799:VAL:HA	1:A:811:ALA:HB1	0.91	1.42	15	2
1:A:771:PHE:HB2	1:A:807:VAL:HG11	0.70	1.62	14	1
1:A:807:VAL:CG2	1:A:812:PHE:CZ	0.65	2.80	14	1
1:A:799:VAL:CA	1:A:811:ALA:HB1	0.64	2.20	15	2
1:A:752:ARG:O	1:A:755:PHE:HB3	0.57	1.99	20	1

6.3 Torsion angles (i)

6.3.1 Protein backbone (i)

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the backbone conformation

was analysed and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
1	A	134/153 (88%)	123±1 (92±1%)	10±2 (8±1%)	1±1 (0±1%)	32 76
All	All	2680/3060 (88%)	2465 (92%)	204 (8%)	11 (0%)	32 76

5 of 6 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	A	742	SER	3
1	A	812	PHE	2
1	A	759	ASP	2
1	A	875	GLY	2
1	A	821	ALA	1

6.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the sidechain conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	113/128 (88%)	90±2 (79±2%)	23±2 (21±2%)	2 31
All	All	2260/2560 (88%)	1791 (79%)	469 (21%)	2 31

5 of 57 unique residues with a non-rotameric sidechain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	A	743	ILE	20
1	A	749	ASN	20
1	A	772	LYS	20
1	A	780	TYR	20
1	A	782	ILE	20

6.3.3 RNA (i)

There are no RNA molecules in this entry.

6.4 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

6.5 Carbohydrates (i)

There are no oligosaccharides in this entry.

6.6 Ligand geometry (i)

Of 1 ligands modelled in this entry, 1 is monoatomic - leaving 0 for Mogul analysis.

6.7 Other polymers (i)

There are no such molecules in this entry.

6.8 Polymer linkage issues (i)

There are no chain breaks in this entry.

7 Chemical shift validation

The completeness of assignment taking into account all chemical shift lists is 86% for the well-defined parts and 85% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *assigned_chem_shift_list_1*

7.1.1 Bookkeeping

The following table shows the results of parsing the chemical shift list and reports the number of nuclei with statistically unusual chemical shifts.

Total number of shifts	1690
Number of shifts mapped to atoms	1690
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	151	-0.09 ± 0.09	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	141	0.34 ± 0.12	None needed (< 0.5 ppm)
$^{13}C'$	0	—	None (insufficient data)
^{15}N	140	0.27 ± 0.24	None needed (< 0.5 ppm)

7.1.3 Completeness of resonance assignments

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 86%, i.e. 1526 atoms were assigned a chemical shift out of a possible 1782. 0 out of 12 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	530/666 (80%)	269/270 (100%)	134/268 (50%)	127/128 (99%)
Sidechain	848/957 (89%)	579/619 (94%)	258/300 (86%)	11/38 (29%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	148/159 (93%)	74/78 (95%)	74/79 (94%)	0/2 (0%)
Overall	1526/1782 (86%)	922/967 (95%)	466/647 (72%)	138/168 (82%)

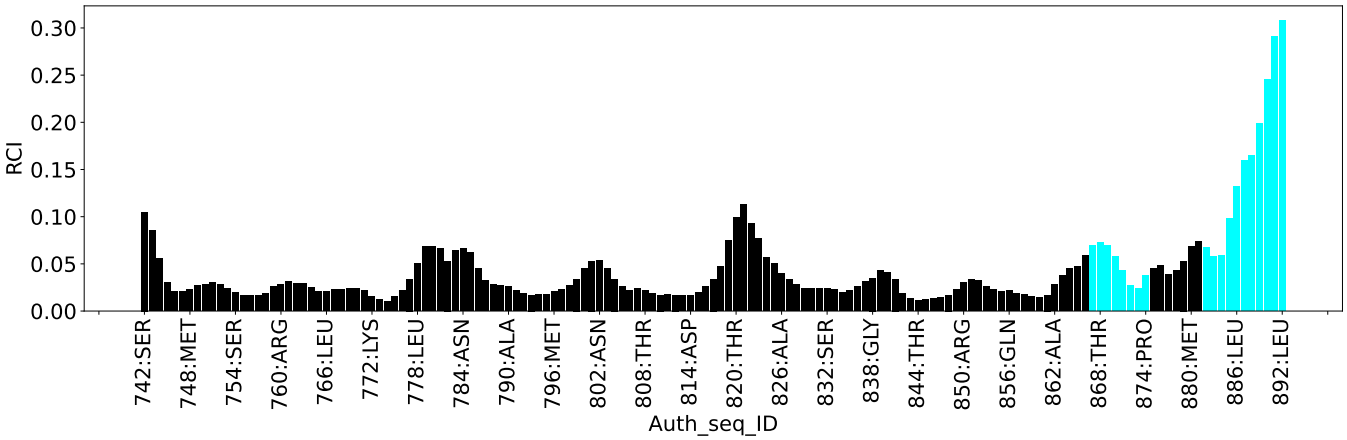
7.1.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

7.1.5 Random Coil Index (RCI) plots (i)

The image below reports *random coil index* values for the protein chains in the structure. The height of each bar gives a probability of a given residue to be disordered, as predicted from the available chemical shifts and the amino acid sequence. A value above 0.2 is an indication of significant predicted disorder. The colour of the bar shows whether the residue is in the well-defined core (black) or in the ill-defined residue ranges (cyan), as described in section 2 on ensemble composition.If well-defined core and ill-defined regions are not identified then it is shown as gray bars.

Random coil index (RCI) for chain A:



8 NMR restraints analysis

8.1 Conformationally restricting restraints

The following table provides the summary of experimentally observed NMR restraints in different categories. Restraints are classified into different categories based on the sequence separation of the atoms involved.

Description	Value
Total distance restraints	2612
Intra-residue (|i-j|=0)	560
Sequential (|i-j|=1)	666
Medium range (|i-j|>1 and |i-j|<5)	711
Long range (|i-j|≥5)	675
Inter-chain	0
Hydrogen bond restraints	0
Disulfide bond restraints	0
Total dihedral-angle restraints	208
Number of unmapped restraints	0
Number of restraints per residue	18.3
Number of long range restraints per residue[1]	4.4

[1]Long range hydrogen bonds and disulfide bonds are counted as long range restraints while calculating the number of long range restraints per residue

8.2 Residual restraint violations

This section provides the overview of the restraint violations analysis. The violations are binned as small, medium and large violations based on its absolute value. Average number of violations per model is calculated by dividing the total number of violations in each bin by the size of the ensemble.

8.2.1 Average number of distance violations per model

Distance violations less than 0.1 Å
are not included in the calculation.

Bins (Å)	Average number of violations per model	Max (Å)
0.1-0.2 (Small)	11.6	0.2
0.2-0.5 (Medium)	19.4	0.5
>0.5 (Large)	124.0	6.64

8.2.2 Average number of dihedral-angle violations per model (i)

Dihedral-angle violations less than 1° are not included in the calculation.

Bins (°)	Average number of violations per model	Max (°)
1.0-10.0 (Small)	5.4	9.92
10.0-20.0 (Medium)	0.1	16.72
>20.0 (Large)	None	None

9 Distance violation analysis (i)

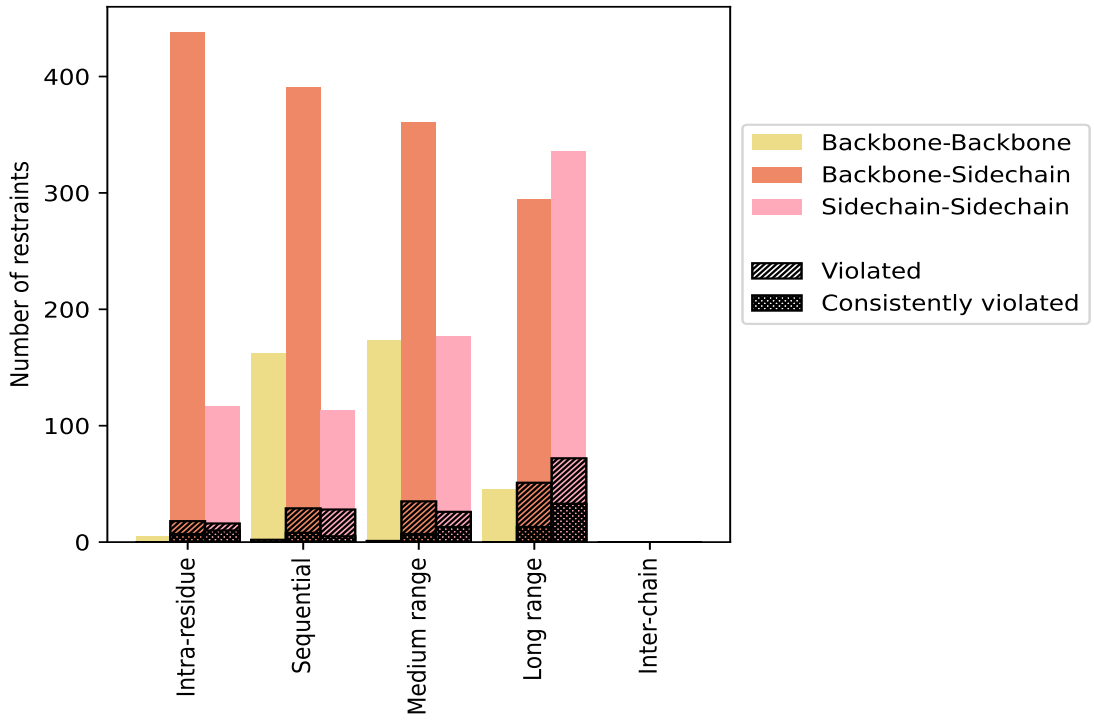
9.1 Summary of distance violations (i)

The following table shows the summary of distance violations in different restraint categories based on the sequence separation of the atoms involved. Each category is further sub-divided into three sub-categories based on the atoms involved. Violations less than 0.1 Å are not included in the statistics.

Restraints type	Count	%[1]	Violated[3]			Consistently Violated[4]		
			Count	%[2]	%[1]	Count	%[2]	%[1]
Intra-residue (|i-j|=0)	560	21.4	34	6.1	1.3	17	3.0	0.7
Backbone-Backbone	5	0.2	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	438	16.8	18	4.1	0.7	7	1.6	0.3
Sidechain-Sidechain	117	4.5	16	13.7	0.6	10	8.5	0.4
Sequential (|i-j|=1)	666	25.5	59	8.9	2.3	13	2.0	0.5
Backbone-Backbone	162	6.2	2	1.2	0.1	0	0.0	0.0
Backbone-Sidechain	391	15.0	29	7.4	1.1	8	2.0	0.3
Sidechain-Sidechain	113	4.3	28	24.8	1.1	5	4.4	0.2
Medium range (|i-j|>1 & |i-j|<5)	711	27.2	62	8.7	2.4	20	2.8	0.8
Backbone-Backbone	173	6.6	1	0.6	0.0	0	0.0	0.0
Backbone-Sidechain	361	13.8	35	9.7	1.3	7	1.9	0.3
Sidechain-Sidechain	177	6.8	26	14.7	1.0	13	7.3	0.5
Long range (|i-j|≥5)	675	25.8	123	18.2	4.7	46	6.8	1.8
Backbone-Backbone	45	1.7	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	294	11.3	51	17.3	2.0	13	4.4	0.5
Sidechain-Sidechain	336	12.9	72	21.4	2.8	33	9.8	1.3
Inter-chain	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Backbone	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Sidechain-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Hydrogen bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Disulfide bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Total	2612	100.0	278	10.6	10.6	96	3.7	3.7
Backbone-Backbone	385	14.7	3	0.8	0.1	0	0.0	0.0
Backbone-Sidechain	1484	56.8	133	9.0	5.1	35	2.4	1.3
Sidechain-Sidechain	743	28.4	142	19.1	5.4	61	8.2	2.3

[1] percentage calculated with respect to the total number of distance restraints, [2] percentage calculated with respect to the number of restraints in a particular restraint category, [3] violated in at least one model, [4] violated in all the models

9.1.1 Bar chart : Distribution of distance restraints and violations



Violated and consistently violated restraints are shown using different hatch patterns in their respective categories. The hydrogen bonds and disulfied bonds are counted in their appropriate category on the x-axis

9.2 Distance violation statistics for each model

The following table provides the distance violation statistics for each model in the ensemble. Violations less than 0.1 Å
are not included in the statistics.

Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
1	22	19	24	73	0	138	1.78	6.57	1.26	1.57
2	20	18	24	64	0	126	1.83	6.62	1.26	1.74
3	28	28	38	80	0	174	1.74	6.56	1.29	1.53
4	24	18	27	77	0	146	1.72	6.58	1.29	1.6
5	23	23	32	78	0	156	1.92	6.58	1.28	1.79
6	26	26	31	77	0	160	1.87	6.57	1.28	1.81
7	25	25	34	76	0	160	1.89	6.5	1.26	1.81
8	24	28	36	81	0	169	1.81	6.59	1.3	1.69
9	25	25	33	80	0	163	1.85	6.58	1.26	1.74
10	24	22	30	75	0	151	1.95	6.55	1.25	1.87

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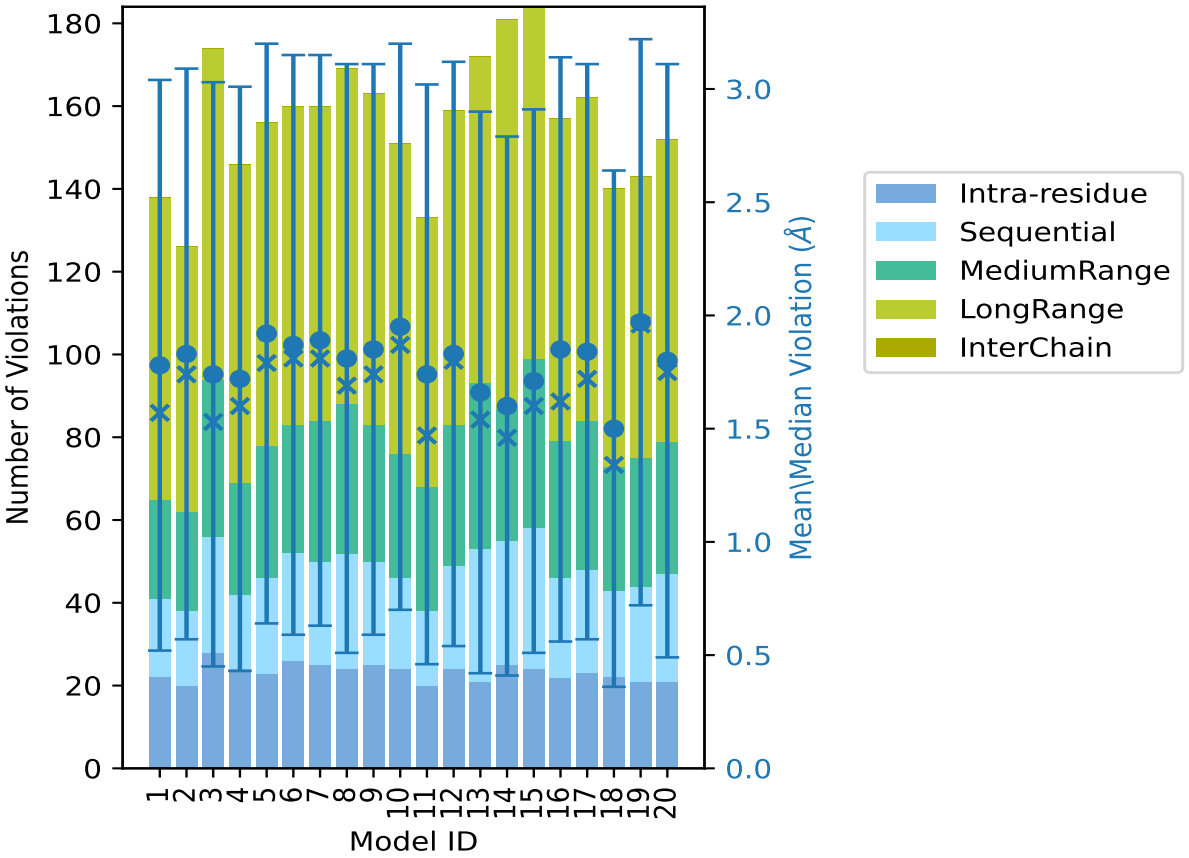
Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
11	20	18	30	65	0	133	1.74	6.47	1.28	1.47
12	24	25	34	76	0	159	1.83	6.5	1.29	1.8
13	21	32	40	79	0	172	1.66	6.38	1.24	1.54
14	25	30	32	94	0	181	1.6	6.31	1.19	1.46
15	24	34	41	85	0	184	1.71	6.43	1.2	1.6
16	22	24	33	78	0	157	1.85	6.49	1.29	1.62
17	23	25	36	78	0	162	1.84	6.52	1.27	1.72
18	22	21	30	67	0	140	1.5	4.13	1.14	1.34
19	21	23	31	68	0	143	1.97	6.52	1.25	1.96
20	21	26	32	73	0	152	1.8	6.64	1.31	1.75

[1]Intra-residue restraints, [2]Sequential restraints, [3]Medium range restraints, [4]Long range restraints, [5]Inter-chain restraints, [6]Standard deviation

9.2.1 Bar graph : Distance Violation statistics for each model



The mean(dot),median(x) and the standard deviation are shown in blue with respect to the y axis on the right

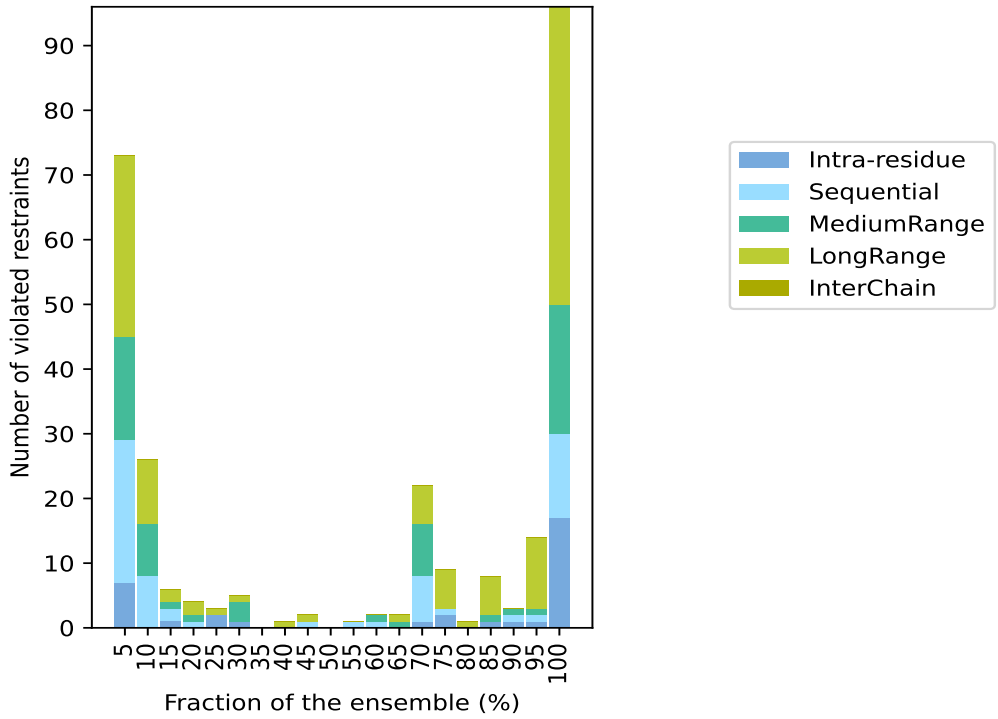
9.3 Distance violation statistics for the ensemble (i)

Violation analysis may find that some restraints are violated in few models and some are violated in most of models. The following table provides this information as number of violated restraints for a given fraction of the ensemble. In total, 2334(IR:526, SQ:607, MR:649, LR:552, IC:0) restraints are not violated in the ensemble.

Number of violated restraints						Fraction of the ensemble	
IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total	Count[6]	%
7	22	16	28	0	73	1	5.0
0	8	8	10	0	26	2	10.0
1	2	1	2	0	6	3	15.0
0	1	1	2	0	4	4	20.0
2	0	0	1	0	3	5	25.0
1	0	3	1	0	5	6	30.0
0	0	0	0	0	0	7	35.0
0	0	0	1	0	1	8	40.0
0	1	0	1	0	2	9	45.0
0	0	0	0	0	0	10	50.0
0	1	0	0	0	1	11	55.0
0	1	1	0	0	2	12	60.0
0	0	1	1	0	2	13	65.0
1	7	8	6	0	22	14	70.0
2	1	0	6	0	9	15	75.0
0	0	0	1	0	1	16	80.0
1	0	1	6	0	8	17	85.0
1	1	1	0	0	3	18	90.0
1	1	1	11	0	14	19	95.0
17	13	20	46	0	96	20	100.0

[1]Intra-residue restraints, [2]Sequential restraints, [3]Medium range restraints, [4]Long range restraints, [5]Inter-chain restraints, [6] Number of models with violations

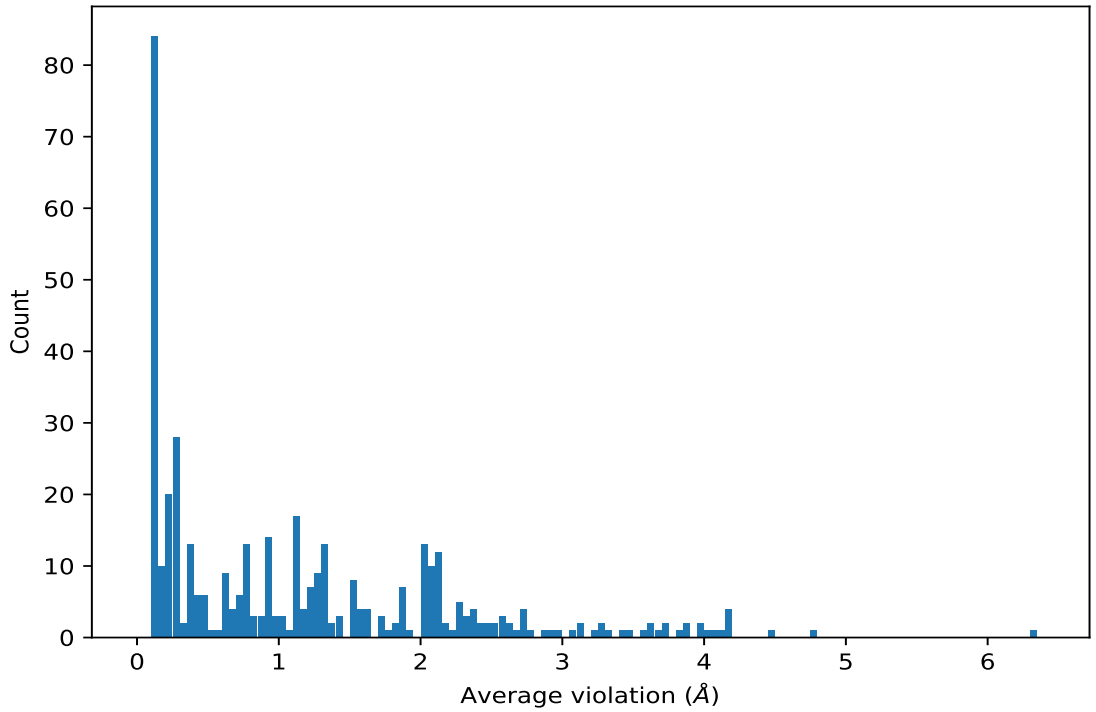
9.3.1 Bar graph : Distance violation statistics for the ensemble



9.4 Most violated distance restraints in the ensemble

9.4.1 Histogram : Distribution of mean distance violations

The following histogram shows the distribution of the average value of the violation. The average is calculated for each restraint that is violated in more than one model over all the violated models in the ensemble



9.4.2 Table: Most violated distance restraints

The following table provides the mean and the standard deviation of the violations for the 10 worst performing restraints, sorted by number of violated models and the mean violation value. The Key (restraint list ID, restraint ID) is the unique identifier for a given restraint. Rows with same key represent combinatorial or ambiguous restraints and are counted as a single restraint.

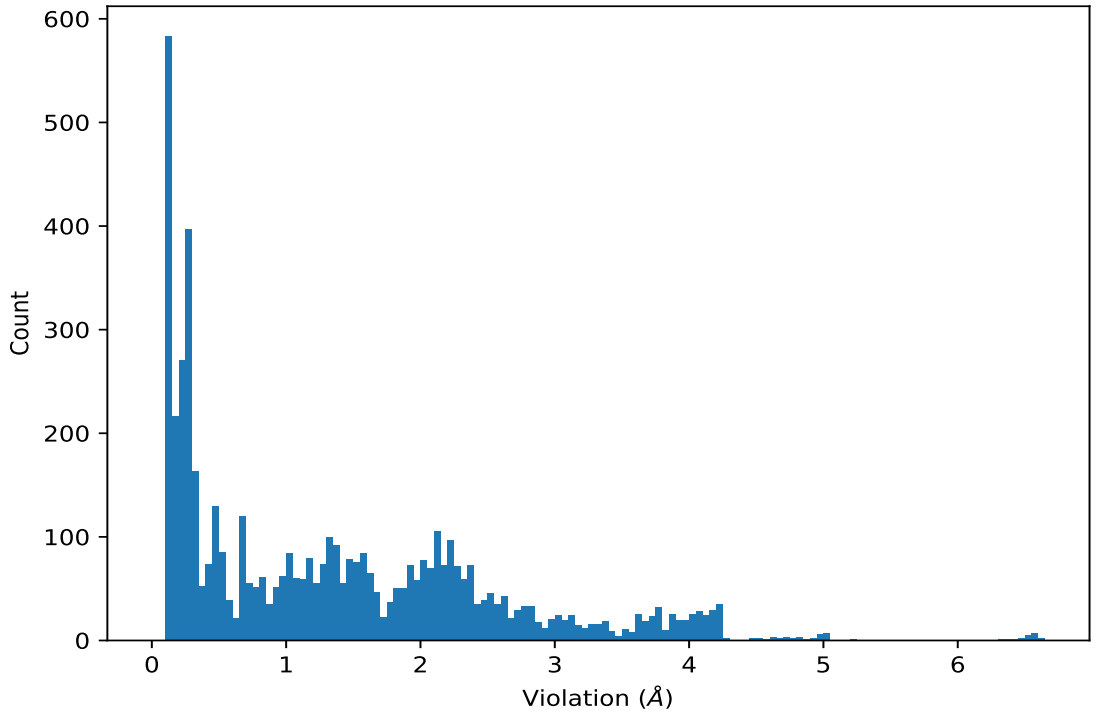
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	20	6.35	0.78	6.54
(1,1192)	1:771:A:PHE:HD2	1:812:A:PHE:HE2	20	4.76	0.94	4.99
(1,1218)	1:842:A:TYR:HD1	1:879:A:TYR:HD1	20	4.46	0.29	4.54
(1,1195)	1:792:A:PHE:HA	1:815:A:PHE:HE1	20	4.16	0.1	4.19
(1,1183)	1:775:A:LEU:HG	1:812:A:PHE:HD2	20	4.14	0.15	4.22
(1,1193)	1:775:A:LEU:HG	1:812:A:PHE:HE2	20	4.1	0.09	4.14
(1,477)	1:795:A:ILE:HB	1:815:A:PHE:HD1	20	3.96	0.15	4.01
(1,1201)	1:815:A:PHE:HD2	1:819:A:GLU:HA	20	3.96	0.15	3.99
(1,646)	1:809:A:PHE:HB2	1:812:A:PHE:HD1	20	3.87	0.07	3.89
(1,478)	1:795:A:ILE:HB	1:815:A:PHE:HE1	20	3.86	0.1	3.88

[1]Number of violated models, [2]Standard deviation

9.5 All violated distance restraints (i)

9.5.1 Histogram : Distribution of distance violations (i)

The following histogram shows the distribution of the absolute value of the violation for all violated restraints in the ensemble.



9.5.2 Table : All distance violations (i)

The following table provides the 10 worst performing restraints, sorted by the violation value. The Key (restraint list ID, restraint ID) is the unique identifier for a given restraint. Rows with same key represent combinatorial or ambiguous restraints and are counted as a single restraint.

Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	20	6.64
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	2	6.62
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	8	6.59
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	4	6.58
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	5	6.58
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	9	6.58
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	1	6.57
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	6	6.57
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	3	6.56
(1,1191)	1:771:A:PHE:HE2	1:812:A:PHE:HE2	10	6.55

10 Dihedral-angle violation analysis

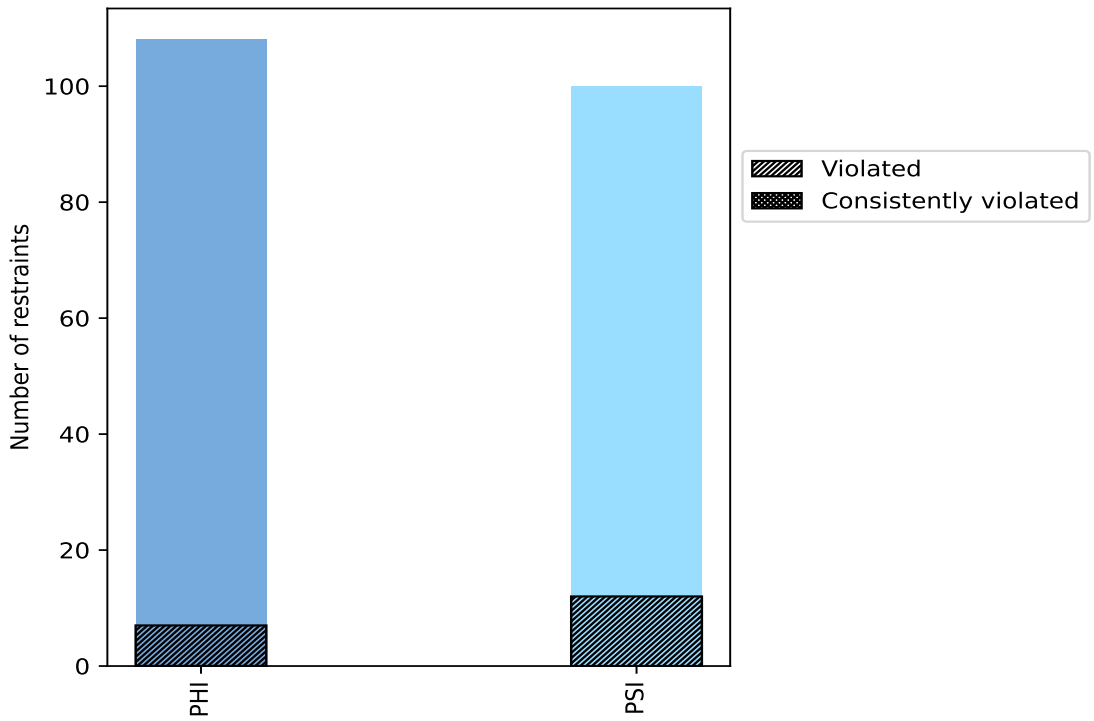
10.1 Summary of dihedral-angle violations

The following table provides the summary of dihedral-angle violations in different dihedral-angle types. Violations less than 1° are not included in the calculation.

Angle type	Count	%[1]	Violated[3]			Consistently Violated[4]		
			Count	%[2]	%[1]	Count	%[2]	%[1]
PHI	108	51.9	7	6.5	3.4	0	0.0	0.0
PSI	100	48.1	12	12.0	5.8	0	0.0	0.0
Total	208	100.0	19	9.1	9.1	0	0.0	0.0

[1] percentage calculated with respect to total number of dihedral-angle restraints, [2] percentage calculated with respect to number of restraints in a particular dihedral-angle type, [3] violated in at least one model, [4] violated in all the models

10.1.1 Bar chart : Distribution of dihedral-angles and violations



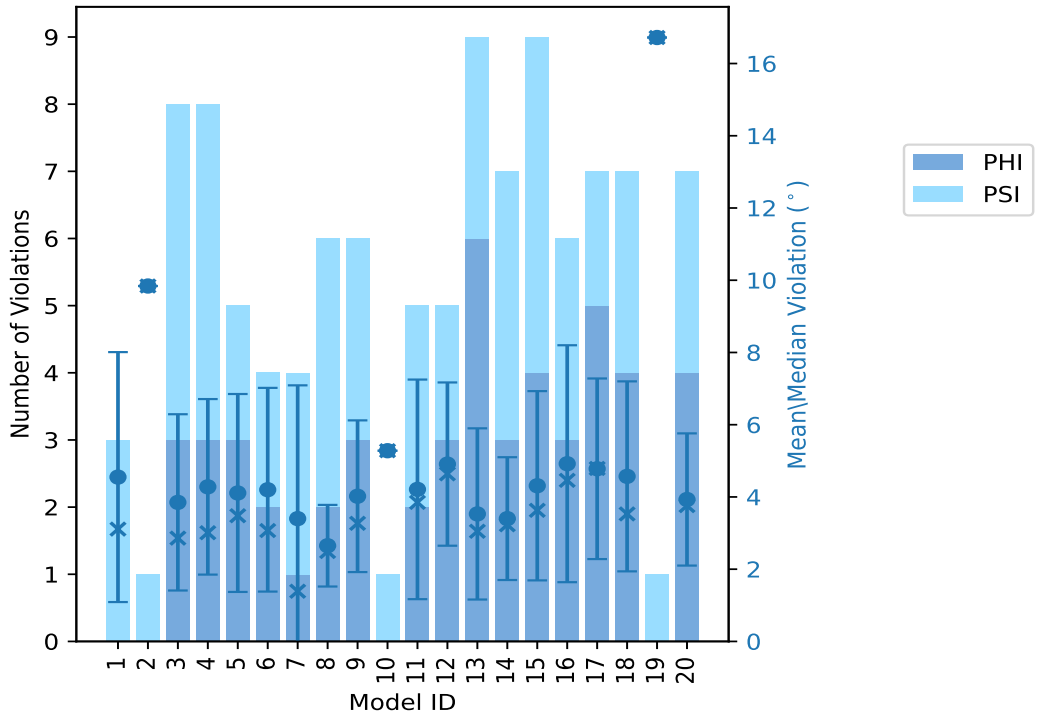
Violated and consistently violated restraints are shown using different hatch patterns in their respective categories

10.2 Dihedral-angle violation statistics for each model (i)

The following table provides the dihedral-angle violation statistics for each model in the ensemble. Violations less than 1° are not included in the statistics.

Model ID	Number of violations			Mean (°)	Max (°)	SD (°)	Median (°)
	PHI	PSI	Total				
1	0	3	3	4.55	9.32	3.46	3.11
2	0	1	1	9.84	9.84	0.0	9.84
3	3	5	8	3.85	9.13	2.44	2.86
4	3	5	8	4.28	9.02	2.43	3.01
5	3	2	5	4.11	9.37	2.74	3.48
6	2	2	4	4.2	8.97	2.82	3.07
7	1	3	4	3.4	9.77	3.69	1.39
8	2	4	6	2.65	4.76	1.13	2.49
9	3	3	6	4.02	8.59	2.1	3.27
10	0	1	1	5.28	5.28	0.0	5.28
11	2	3	5	4.21	9.43	3.04	3.85
12	3	2	5	4.91	8.19	2.26	4.64
13	6	3	9	3.53	9.11	2.37	3.05
14	3	4	7	3.4	6.16	1.7	3.23
15	4	5	9	4.31	8.95	2.62	3.63
16	3	3	6	4.92	9.63	3.28	4.46
17	5	2	7	4.78	9.92	2.5	4.79
18	4	3	7	4.57	9.47	2.63	3.53
19	0	1	1	16.72	16.72	0.0	16.72
20	4	3	7	3.93	6.78	1.83	3.76

10.2.1 Bar graph : Dihedral violation statistics for each model



The mean(dot),median(x) and the standard deviation are shown in blue with respect to the y axis on the right

10.3 Dihedral-angle violation statistics for the ensemble

Violation analysis may find that some restraints are violated in very few models and some are violated in most of models. The following table provides this information as number of violated restraints for a given fraction of ensemble.

Number of violated restraints			Fraction of the ensemble	
PHI	PSI	Total	Count[1]	%
1	5	6	1	5.0
1	3	4	2	10.0
0	0	0	3	15.0
1	0	1	4	20.0
0	0	0	5	25.0
0	1	1	6	30.0
0	0	0	7	35.0
1	0	1	8	40.0
0	0	0	9	45.0
0	1	1	10	50.0
0	0	0	11	55.0

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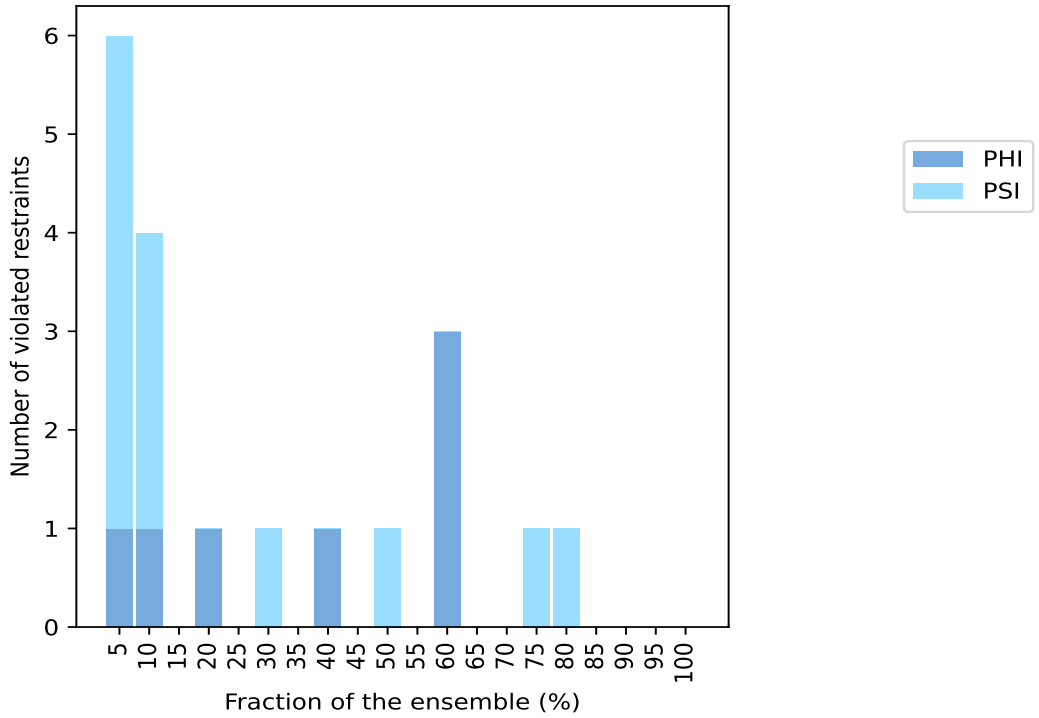
Number of violated restraints			Fraction of the ensemble	
PHI	PSI	Total	Count[1]	%
3	0	3	12	60.0
0	0	0	13	65.0
0	0	0	14	70.0
0	1	1	15	75.0
0	1	1	16	80.0
0	0	0	17	85.0
0	0	0	18	90.0
0	0	0	19	95.0
0	0	0	20	100.0

[1] Number of models with violations

10.3.1 Bar graph : Dihedral-angle Violation statistics for the ensemble

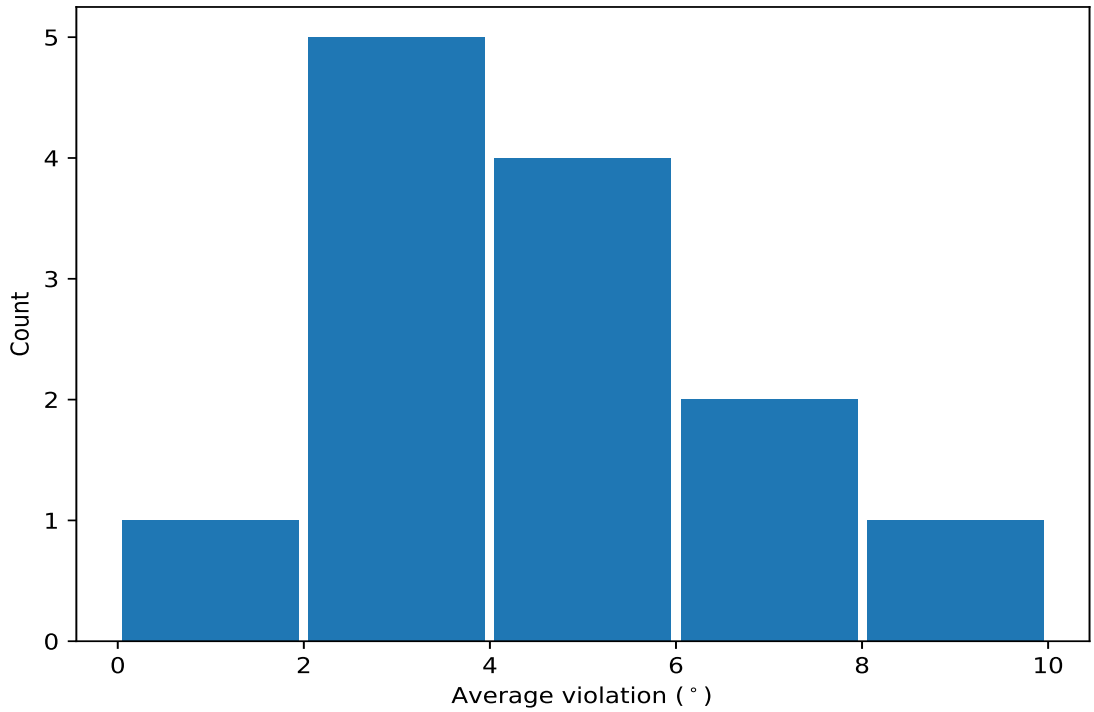


10.4 Most violated dihedral-angle restraints in the ensemble

10.4.1 Histogram : Distribution of mean dihedral-angle violations

The following histogram shows the distribution of the average value of the violation. The average is calculated for each restraint that is violated in more than one model over all the violated models

in the ensemble



10.4.2 Table: Most violated dihedral-angle restraints

The following table provides the mean and the standard deviation of the violations for the 10 worst performing restraints, sorted by number of violated models and the mean violation value. The Key (restraint list ID, restraint ID) is the unique identifier for a given restraint.

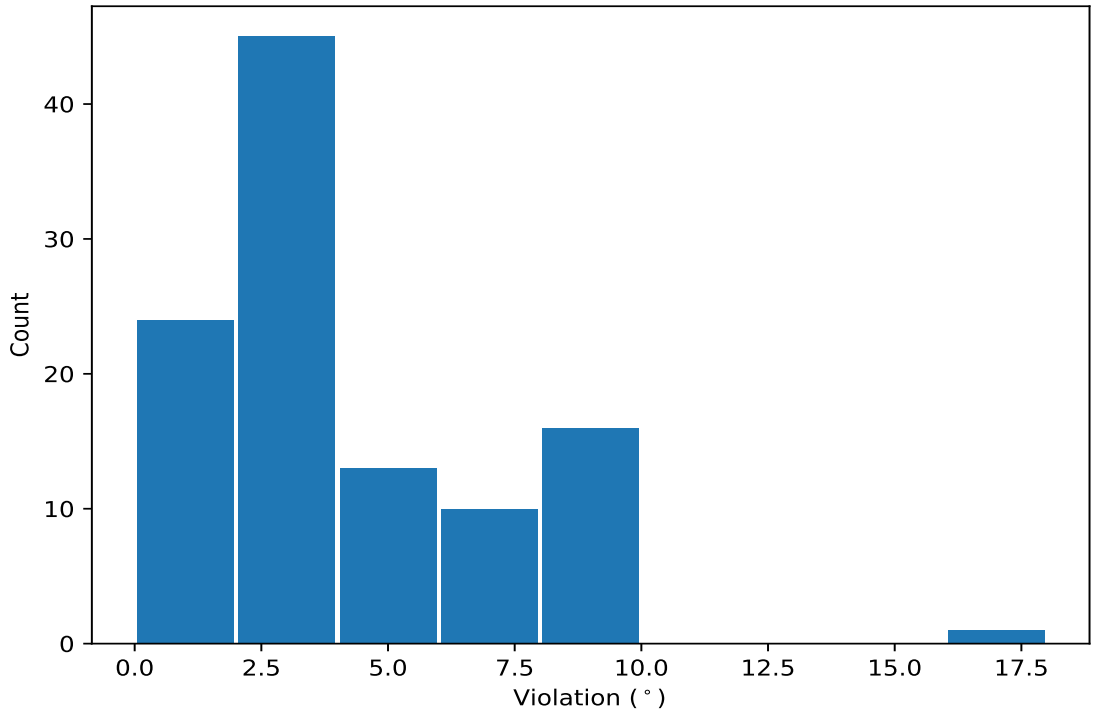
Key	Atom-1	Atom-2	Atom-3	Atom-4	Models[1]	Mean	SD[2]	Median
(1,49)	1:772:A:LYS:N	1:772:A:LYS:CA	1:772:A:LYS:C	1:773:A:ALA:N	16	2.22	0.73	2.16
(1,123)	1:825:A:THR:N	1:825:A:THR:CA	1:825:A:THR:C	1:826:A:ALA:N	15	5.22	1.81	5.5
(1,32)	1:760:A:ARG:C	1:761:A:ASP:N	1:761:A:ASP:CA	1:761:A:ASP:C	12	4.94	2.39	4.38
(1,202)	1:879:A:TYR:C	1:880:A:MET:N	1:880:A:MET:CA	1:880:A:MET:C	12	4.73	2.58	4.18
(1,104)	1:810:A:GLN:C	1:811:A:ALA:N	1:811:A:ALA:CA	1:811:A:ALA:C	12	2.97	1.49	2.42
(1,189)	1:867:A:TYR:N	1:867:A:TYR:CA	1:867:A:TYR:C	1:868:A:THR:N	10	7.81	2.53	9.23
(1,38)	1:764:A:GLY:C	1:765:A:THR:N	1:765:A:THR:CA	1:765:A:THR:C	8	2.13	0.8	2.24
(1,119)	1:818:A:ARG:N	1:818:A:ARG:CA	1:818:A:ARG:C	1:819:A:GLU:N	6	2.22	0.71	1.94
(1,146)	1:838:A:GLY:C	1:839:A:ASP:N	1:839:A:ASP:CA	1:839:A:ASP:C	4	6.44	2.56	6.38
(1,106)	1:811:A:ALA:C	1:812:A:PHE:N	1:812:A:PHE:CA	1:812:A:PHE:C	2	9.03	0.08	9.03

[1] Number of violated models, [2]Standard deviation, All angle values are in degree (°)

10.5 All violated dihedral-angle restraints (i)

10.5.1 Histogram : Distribution of violations (i)

The following histogram shows the distribution of the absolute value of the violation for all violated restraints in the ensemble.



10.5.2 Table: All violated dihedral-angle restraints (i)

The following table provides the list of violations for the 10 worst performing restraints, sorted by the violation value. The Key (restraint list ID, restraint ID) is the unique identifier for a given restraint.

Key	Atom-1	Atom-2	Atom-3	Atom-4	Model ID	Violation (°)
(1,170)	1:855:A:ASP:N	1:855:A:ASP:CA	1:855:A:ASP:C	1:856:A:GLN:N	19	16.72
(1,32)	1:760:A:ARG:C	1:761:A:ASP:N	1:761:A:ASP:CA	1:761:A:ASP:C	17	9.92
(1,189)	1:867:A:TYR:N	1:867:A:TYR:CA	1:867:A:TYR:C	1:868:A:THR:N	2	9.84
(1,189)	1:867:A:TYR:N	1:867:A:TYR:CA	1:867:A:TYR:C	1:868:A:THR:N	7	9.77
(1,189)	1:867:A:TYR:N	1:867:A:TYR:CA	1:867:A:TYR:C	1:868:A:THR:N	16	9.63
(1,202)	1:879:A:TYR:C	1:880:A:MET:N	1:880:A:MET:CA	1:880:A:MET:C	18	9.47
(1,189)	1:867:A:TYR:N	1:867:A:TYR:CA	1:867:A:TYR:C	1:868:A:THR:N	11	9.43
(1,146)	1:838:A:GLY:C	1:839:A:ASP:N	1:839:A:ASP:CA	1:839:A:ASP:C	5	9.37
(1,189)	1:867:A:TYR:N	1:867:A:TYR:CA	1:867:A:TYR:C	1:868:A:THR:N	1	9.32
(1,189)	1:867:A:TYR:N	1:867:A:TYR:CA	1:867:A:TYR:C	1:868:A:THR:N	3	9.13