WORLDWIDE wwPDB PROTEIN DATA BANK

wwPDB NMR Structure Validation Summary Report (i)

Dec 24, 2024 – 08:14 PM EST

PDB ID	:	2M55
BMRB ID	:	19036
Title	:	NMR structure of the complex of an N-terminally acetylated alpha-synuclein peptide with calmodulin
Authors	:	Gruschus, J.M.; Yap, T.; Pistolesi, S.; Maltsev, A.S.; Lee, J.C.
Deposited on	:	2013-02-13

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
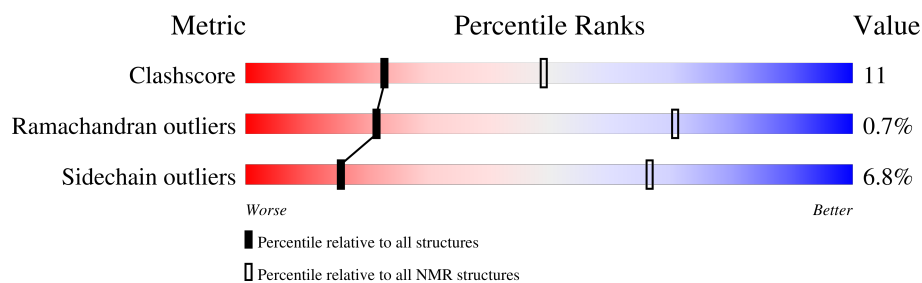http://www.wwpdb.org/validation/2017/FAQs#types.

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 38%.

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	Percentile Ranks	Value
Clashscore		11
Ramachandran outliers		0.7%
Sidechain outliers		6.8%

Worse   *Better*

▮ Percentile relative to all structures

▯ Percentile relative to all NMR structures

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	148	74% 20% • 5%
2	B	21	100%

2 Ensemble composition and analysis ⓘ

This entry contains 20 models. Model 12 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:4-A:144 (141)	0.75	12

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

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

NmrClust was unable to cluster the ensemble.

Error message: Inconsistent models

3 Entry composition ⓘ

There are 3 unique types of molecules in this entry. The entry contains 2552 atoms, of which 1244 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Calmodulin.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	148	2260	714	1094	188	255	9	0

- Molecule 2 is a protein called Alpha-synuclein.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	B	21	288	88	150	23	25	2	1

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	200	ACE	-	acetylation	UNP P37840
B	220	NH2	-	amidation	UNP P37840

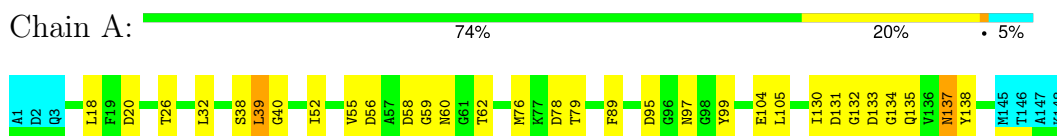- Molecule 3 is CALCIUM ION (three-letter code: CA) (formula: Ca).

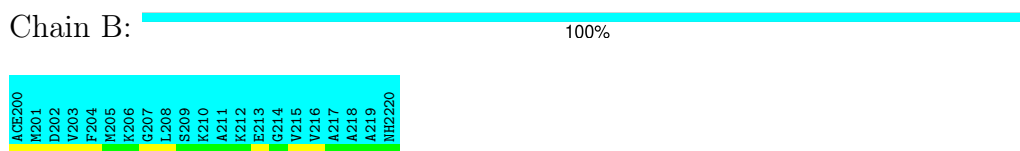| Mol	Chain	Residues	Atoms	
			Total	Ca
3	A	4	4	4

4   Residue-property plots ⓘ

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.

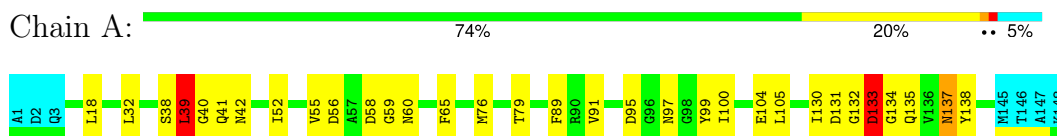- Molecule 1: Calmodulin

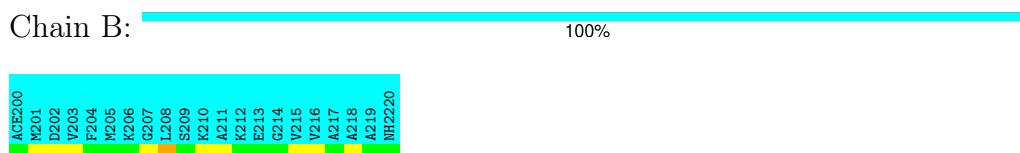- Molecule 2: Alpha-synuclein

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

The representative model is number 12. Colouring as in section 4.1 above.

- Molecule 1: Calmodulin

- Molecule 2: Alpha-synuclein

5 Refinement protocol and experimental data overview ⓘ

The models were refined using the following method: *DGSA-distance geometry simulated annealing.*

Of the 100 calculated structures, 20 were deposited, based on the following criterion: *structures with the least restraint violations.*

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

Software name	Classification	Version
X-PLOR NIH	structure solution	
X-PLOR NIH	refinement	

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	806
Number of shifts mapped to atoms	806
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	38%

6   Model quality ⓘ

6.1   Standard geometry ⓘ

Bond lengths and bond angles in the following residue types are not validated in this section: NH2, ACE, CA

There are no covalent bond-length or bond-angle outliers.

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

There are no planarity outliers.

6.2   Too-close contacts ⓘ

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	1114	1043	1043	24±5
2	B	0	0	0	0±0
All	All	22360	20860	20860	476

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 11.

5 of 114 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:110:THR:HG23	1:A:115:LYS:NZ	0.74	1.98	10	2
1:A:110:THR:HG23	1:A:115:LYS:HZ3	0.66	1.50	10	1
1:A:49:GLN:HE22	1:A:53:ASN:ND2	0.64	1.91	6	4
1:A:89:PHE:CD1	1:A:138:TYR:CZ	0.64	2.86	16	20
1:A:137:ASN:OD1	1:A:137:ASN:N	0.64	2.31	9	20

6.3 Torsion angles ⓘ

6.3.1 Protein backbone ⓘ

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	141/148 (95%)	137±2 (97±1%)	3±2 (2±1%)	1±0 (1±0%)	21	71
2	B	0	-	-	-		-
All	All	2820/3380 (83%)	2743 (97%)	56 (2%)	21 (1%)	21	71

All 3 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	39	LEU	18
1	A	78	ASP	2
1	A	133	ASP	1

6.3.2 Protein sidechains ⓘ

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	121/126 (96%)	113±1 (93±1%)	8±1 (7±1%)	16	66
2	B	0	-	-		-
All	All	2420/2780 (87%)	2256 (93%)	164 (7%)	16	66

5 of 19 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	18	LEU	20
1	A	130	ILE	20
1	A	137	ASN	20
1	A	32	LEU	19
1	A	79	THR	18

6.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

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

6.5 Carbohydrates ⓘ

There are no oligosaccharides in this entry.

6.6 Ligand geometry ⓘ

Of 4 ligands modelled in this entry, 4 are monoatomic - leaving 0 for Mogul analysis.

6.7 Other polymers ⓘ

There are no such molecules in this entry.

6.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.

7 Chemical shift validation ⓘ

The completeness of assignment taking into account all chemical shift lists is 38% for the well-defined parts and 37% 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	806
Number of shifts mapped to atoms	806
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
$^{13}C_\alpha$	160	-0.29 ± 0.08	None needed (< 0.5 ppm)
$^{13}C_\beta$	148	0.03 ± 0.11	None needed (< 0.5 ppm)
$^{13}C'$	0	—	None (insufficient data)
^{15}N	157	0.17 ± 0.35	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 38%, i.e. 700 atoms were assigned a chemical shift out of a possible 1855. 0 out of 16 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	570/712 (80%)	291/291 (100%)	140/282 (50%)	139/139 (100%)
Sidechain	130/1038 (13%)	0/663 (0%)	130/339 (38%)	0/36 (0%)

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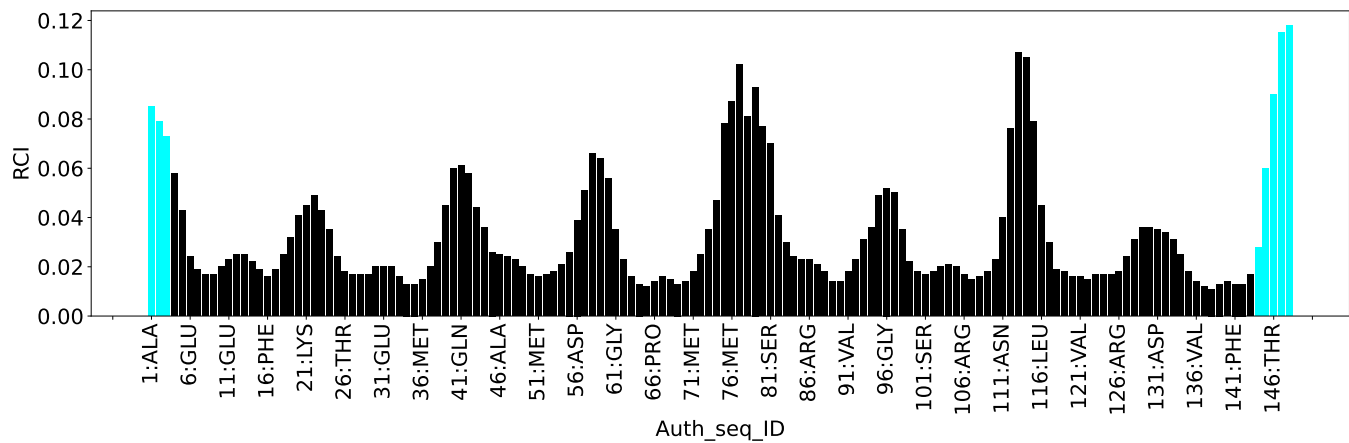|	Total	^{1}H	^{13}C	^{15}N
Aromatic	0/105 (0%)	0/52 (0%)	0/52 (0%)	0/1 (0%)
Overall	700/1855 (38%)	291/1006 (29%)	270/673 (40%)	139/176 (79%)

7.1.4 Statistically unusual chemical shifts ⓘ

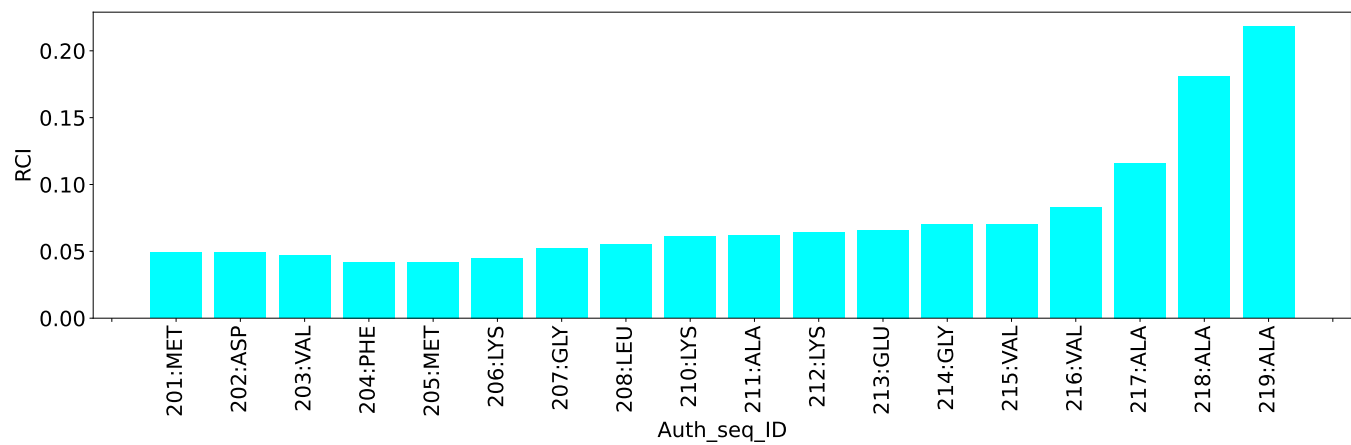There are no statistically unusual chemical shifts.

7.1.5 Random Coil Index (RCI) plots ⓘ

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:

Random coil index (RCI) for chain B:

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	3452
Intra-residue ($|i\text{-}j|$=0)	1130
Sequential ($|i\text{-}j|$=1)	912
Medium range ($|i\text{-}j|$>1 and $|i\text{-}j|$<5)	832
Long range ($|i\text{-}j|$≥5)	529
Inter-chain	49
Hydrogen bond restraints	0
Disulfide bond restraints	0
Total dihedral-angle restraints	282
Number of unmapped restraints	0
Number of restraints per residue	21.6
Number of long range restraints per residue[1]	3.1

[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)	208.4	0.2
0.2-0.5 (Medium)	175.9	0.5
>0.5 (Large)	28.1	2.59

8.2.2   Average number of dihedral-angle violations per model ⓘ

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

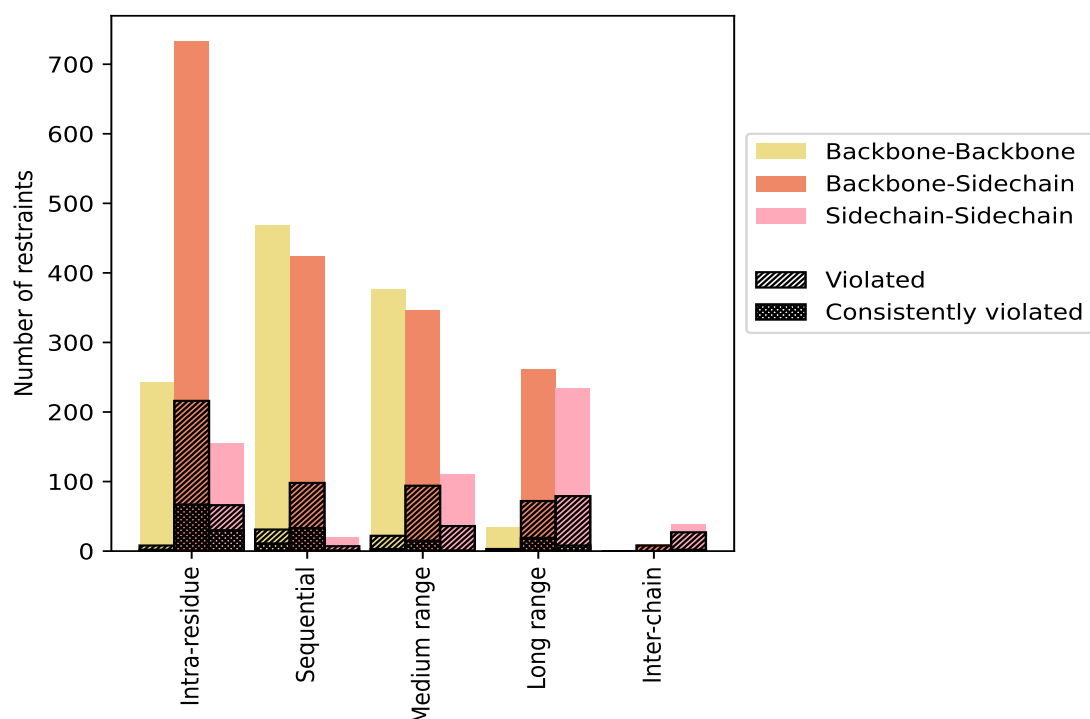| Bins (°)	Average number of violations per model	Max (°)
1.0-10.0 (Small)	11.0	8.12
10.0-20.0 (Medium)	None	None
>20.0 (Large)	None	None

9 Distance violation analysis ⓘ

9.1 Summary of distance violations ⓘ

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)	1130	32.7	290	25.7	8.4	99	8.8	2.9
Backbone-Backbone	242	7.0	8	3.3	0.2	2	0.8	0.1
Backbone-Sidechain	733	21.2	216	29.5	6.3	67	9.1	1.9
Sidechain-Sidechain	155	4.5	66	42.6	1.9	30	19.4	0.9
Sequential (|i-j|=1)	912	26.4	136	14.9	3.9	45	4.9	1.3
Backbone-Backbone	468	13.6	31	6.6	0.9	11	2.4	0.3
Backbone-Sidechain	424	12.3	98	23.1	2.8	33	7.8	1.0
Sidechain-Sidechain	20	0.6	7	35.0	0.2	1	5.0	0.0
Medium range (|i-j|>1 & |i-j|<5)	832	24.1	152	18.3	4.4	19	2.3	0.6
Backbone-Backbone	376	10.9	22	5.9	0.6	3	0.8	0.1
Backbone-Sidechain	346	10.0	94	27.2	2.7	15	4.3	0.4
Sidechain-Sidechain	110	3.2	36	32.7	1.0	1	0.9	0.0
Long range (|i-j|≥5)	529	15.3	154	29.1	4.5	27	5.1	0.8
Backbone-Backbone	34	1.0	3	8.8	0.1	0	0.0	0.0
Backbone-Sidechain	261	7.6	72	27.6	2.1	19	7.3	0.6
Sidechain-Sidechain	234	6.8	79	33.8	2.3	8	3.4	0.2
Inter-chain	49	1.4	35	71.4	1.0	2	4.1	0.1
Backbone-Backbone	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	10	0.3	8	80.0	0.2	0	0.0	0.0
Sidechain-Sidechain	39	1.1	27	69.2	0.8	2	5.1	0.1
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	3452	100.0	767	22.2	22.2	192	5.6	5.6
Backbone-Backbone	1120	32.4	64	5.7	1.9	16	1.4	0.5
Backbone-Sidechain	1774	51.4	488	27.5	14.1	134	7.6	3.9
Sidechain-Sidechain	558	16.2	215	38.5	6.2	42	7.5	1.2

[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	193	73	71	82	11	430	0.25	1.84	0.2	0.2
2	180	74	70	76	10	410	0.25	2.13	0.19	0.2
3	193	76	64	73	12	418	0.26	2.26	0.22	0.2
4	189	73	71	81	10	424	0.26	1.97	0.24	0.2
5	181	79	64	78	14	416	0.26	1.68	0.2	0.2
6	187	71	72	77	15	422	0.26	1.8	0.2	0.21
7	184	76	72	74	13	419	0.25	2.17	0.21	0.19
8	176	71	71	75	12	405	0.26	2.27	0.23	0.2
9	180	74	71	80	10	415	0.25	1.61	0.17	0.2
10	194	71	72	79	12	428	0.25	2.14	0.2	0.2

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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	189	75	73	75	14	426	0.25	2.27	0.2	0.21
12	196	74	72	78	10	430	0.26	2.07	0.21	0.2
13	193	76	70	82	16	437	0.25	2.44	0.22	0.2
14	190	74	72	73	12	421	0.24	1.9	0.19	0.2
15	195	80	69	74	16	434	0.25	2.26	0.21	0.2
16	191	79	65	80	17	432	0.25	2.31	0.22	0.19
17	186	75	70	72	15	418	0.25	1.82	0.21	0.2
18	186	77	74	78	12	427	0.26	2.59	0.24	0.2
19	195	72	76	75	15	433	0.25	2.15	0.22	0.19
20	179	72	75	71	15	412	0.25	2.0	0.22	0.2

[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

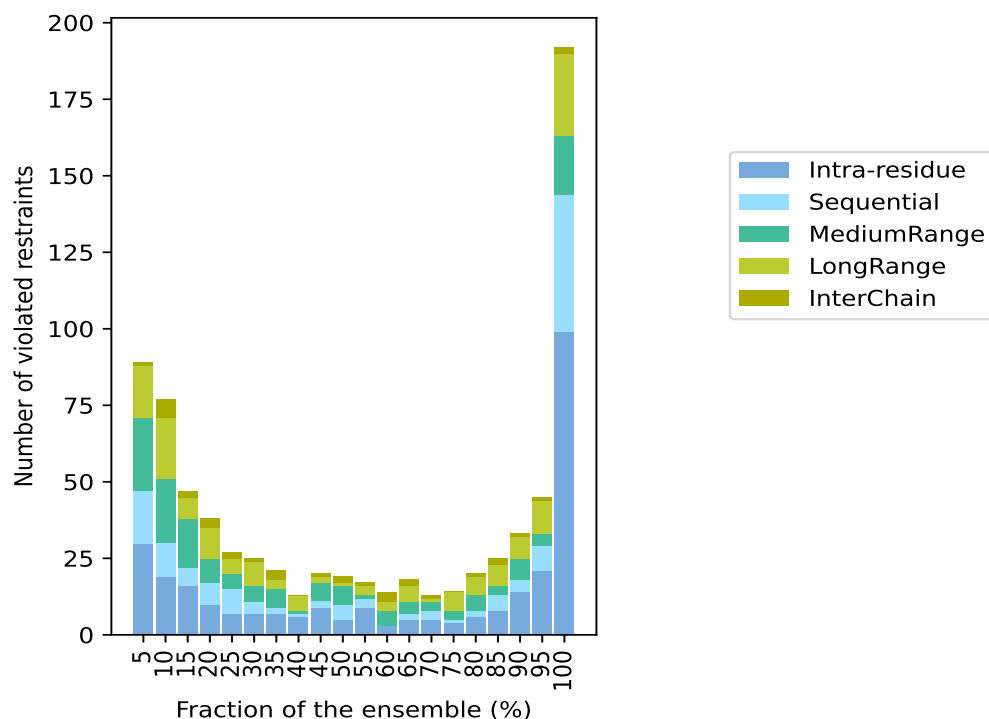## 9.3 Distance violation statistics for the ensemble (i)

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, 2685(IR:840, SQ:776, MR:680, LR:375, IC:14) 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]	%
30	17	24	17	1	89	1	5.0
19	11	21	20	6	77	2	10.0
16	6	16	7	2	47	3	15.0
10	7	8	10	3	38	4	20.0
7	8	5	5	2	27	5	25.0
7	4	5	8	1	25	6	30.0
7	2	6	3	3	21	7	35.0
6	1	1	5	0	13	8	40.0
9	2	6	2	1	20	9	45.0
5	5	6	1	2	19	10	50.0
9	3	1	3	1	17	11	55.0
3	0	5	3	3	14	12	60.0
5	2	4	5	2	18	13	65.0
5	3	3	1	1	13	14	70.0
4	1	3	6	0	14	15	75.0
6	2	5	6	1	20	16	80.0
8	5	3	7	2	25	17	85.0
14	4	7	7	1	33	18	90.0
21	8	4	11	1	45	19	95.0
99	45	19	27	2	192	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
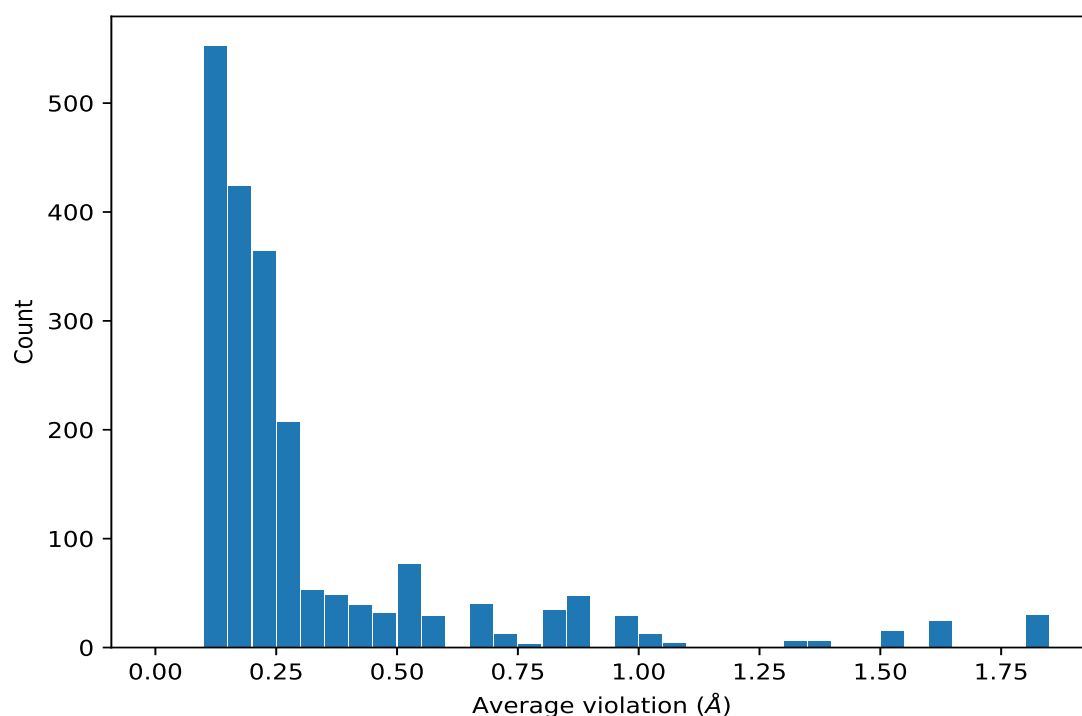
9.3.1   Bar graph : Distance violation statistics for the ensemble ⓘ

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)
(1,2632)	1:51:A:MET:HE3	2:204:B:PHE:HE1	20	1.38	0.4	1.37
(1,2632)	1:51:A:MET:HE3	2:204:B:PHE:HE2	20	1.38	0.4	1.37
(1,2632)	1:51:A:MET:HE2	2:204:B:PHE:HE1	20	1.38	0.4	1.37
(1,2632)	1:51:A:MET:HE2	2:204:B:PHE:HE2	20	1.38	0.4	1.37
(1,2632)	1:51:A:MET:HE1	2:204:B:PHE:HE1	20	1.38	0.4	1.37
(1,2632)	1:51:A:MET:HE1	2:204:B:PHE:HE2	20	1.38	0.4	1.37
(1,2745)	1:71:A:MET:HE1	2:204:B:PHE:HE1	20	1.32	0.18	1.32
(1,2745)	1:71:A:MET:HE1	2:204:B:PHE:HE2	20	1.32	0.18	1.32
(1,2745)	1:71:A:MET:HE2	2:204:B:PHE:HE1	20	1.32	0.18	1.32
(1,2745)	1:71:A:MET:HE2	2:204:B:PHE:HE2	20	1.32	0.18	1.32
(1,2745)	1:71:A:MET:HE3	2:204:B:PHE:HE1	20	1.32	0.18	1.32
(1,2745)	1:71:A:MET:HE3	2:204:B:PHE:HE2	20	1.32	0.18	1.32
(1,1574)	1:140:A:GLU:H	1:136:A:VAL:HG21	20	1.0	0.06	1.0
(1,1574)	1:140:A:GLU:H	1:136:A:VAL:HG22	20	1.0	0.06	1.0
(1,1574)	1:140:A:GLU:H	1:136:A:VAL:HG23	20	1.0	0.06	1.0
(1,1574)	1:140:A:GLU:H	1:130:A:ILE:HD11	20	1.0	0.06	1.0

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Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,1574)	1:140:A:GLU:H	1:130:A:ILE:HD12	20	1.0	0.06	1.0
(1,1574)	1:140:A:GLU:H	1:130:A:ILE:HD13	20	1.0	0.06	1.0
(1,3444)	1:108:A:VAL:HG23	1:112:A:LEU:HG	20	0.88	0.24	0.84
(1,3444)	1:108:A:VAL:HG22	1:112:A:LEU:HG	20	0.88	0.24	0.84
(1,3444)	1:108:A:VAL:HG22	1:112:A:LEU:HB3	20	0.88	0.24	0.84
(1,3444)	1:108:A:VAL:HG21	1:112:A:LEU:HG	20	0.88	0.24	0.84
(1,295)	1:27:A:ILE:H	1:64:A:ASP:HB3	20	0.86	0.05	0.88
(1,295)	1:27:A:ILE:H	1:20:A:ASP:HB3	20	0.86	0.05	0.88
(1,2670)	1:55:A:VAL:HG11	1:71:A:MET:HB3	20	0.76	0.21	0.81
(1,2670)	1:55:A:VAL:HG13	1:71:A:MET:HB3	20	0.76	0.21	0.81
(1,2670)	1:55:A:VAL:HG12	1:71:A:MET:HB3	20	0.76	0.21	0.81
(1,3436)	1:35:A:VAL:HG21	1:39:A:LEU:HB2	20	0.74	0.19	0.75
(1,3436)	1:35:A:VAL:HG23	1:39:A:LEU:HB2	20	0.74	0.19	0.75
(1,3436)	1:35:A:VAL:HG23	1:39:A:LEU:HG	20	0.74	0.19	0.75
(1,3436)	1:35:A:VAL:HG22	1:39:A:LEU:HB2	20	0.74	0.19	0.75
(1,3436)	1:35:A:VAL:HG22	1:39:A:LEU:HG	20	0.74	0.19	0.75
(1,1073)	1:100:A:ILE:H	1:101:A:SER:HA	20	0.66	0.06	0.68
(1,1073)	1:100:A:ILE:H	1:135:A:GLN:HA	20	0.66	0.06	0.68
(1,889)	1:83:A:GLU:H	1:79:A:THR:HA	20	0.66	0.08	0.65
(1,889)	1:83:A:GLU:H	1:79:A:THR:HB	20	0.66	0.08	0.65
(1,762)	1:68:A:PHE:H	1:63:A:ILE:HG12	20	0.54	0.05	0.54

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

9.5 All violated distance restraints ⓘ

9.5.1 Histogram : Distribution of distance violations ⓘ

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 ⓘ

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)
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG11	18	2.59
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG12	18	2.59
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG13	18	2.59
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG21	18	2.59
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG22	18	2.59
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG23	18	2.59
(1,2632)	1:51:A:MET:HE1	2:204:B:PHE:HE1	13	2.44
(1,2632)	1:51:A:MET:HE1	2:204:B:PHE:HE2	13	2.44
(1,3423)	2:219:B:ALA:HB3	1:105:A:LEU:HD11	16	2.31
(1,3423)	2:219:B:ALA:HB3	1:105:A:LEU:HD12	16	2.31
(1,3423)	2:219:B:ALA:HB3	1:105:A:LEU:HD13	16	2.31
(1,3423)	2:219:B:ALA:HB3	1:105:A:LEU:HD21	16	2.31
(1,3423)	2:219:B:ALA:HB3	1:105:A:LEU:HD22	16	2.31
(1,3423)	2:219:B:ALA:HB3	1:105:A:LEU:HD23	16	2.31
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG11	11	2.27
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG12	11	2.27
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG13	11	2.27

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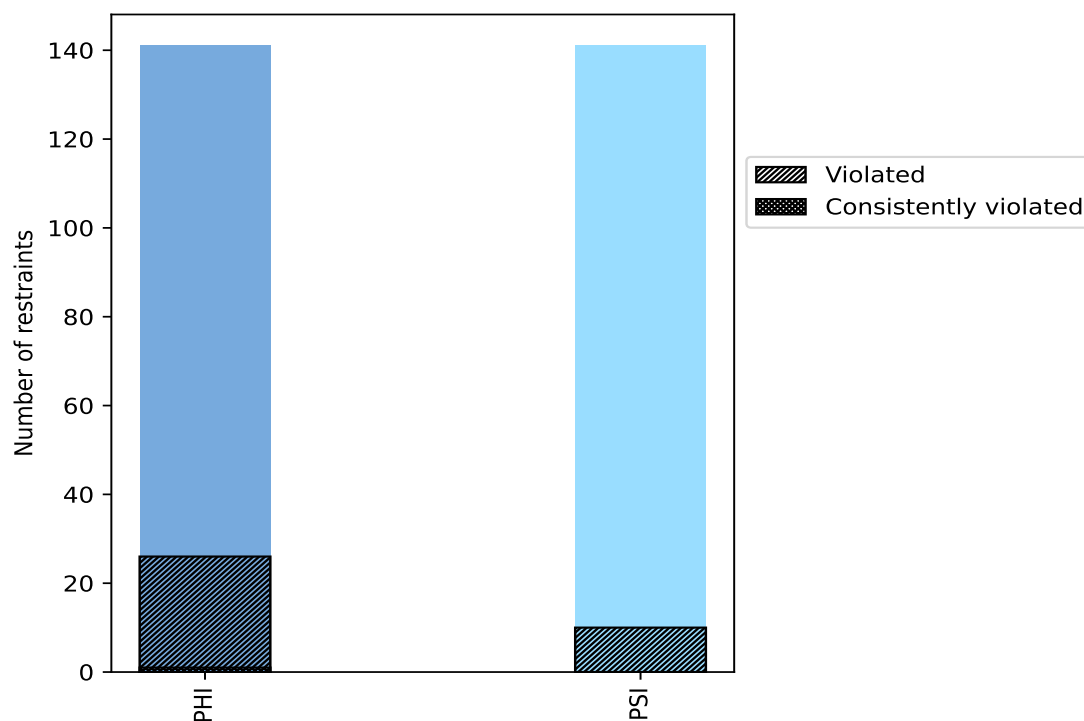| Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG21	11	2.27
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG22	11	2.27
(1,3411)	1:145:A:MET:HE2	2:216:B:VAL:HG23	11	2.27
(1,3326)	2:205:B:MET:HE3	2:208:B:LEU:HD11	8	2.27
(1,3326)	2:205:B:MET:HE3	2:208:B:LEU:HD12	8	2.27
(1,3326)	2:205:B:MET:HE3	2:208:B:LEU:HD13	8	2.27
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD11	15	2.26
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD12	15	2.26
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD13	15	2.26
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD21	15	2.26
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD22	15	2.26
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD23	15	2.26
(1,3411)	1:145:A:MET:HE3	2:216:B:VAL:HG11	3	2.26
(1,3411)	1:145:A:MET:HE3	2:216:B:VAL:HG12	3	2.26
(1,3411)	1:145:A:MET:HE3	2:216:B:VAL:HG13	3	2.26
(1,3411)	1:145:A:MET:HE3	2:216:B:VAL:HG21	3	2.26
(1,3411)	1:145:A:MET:HE3	2:216:B:VAL:HG22	3	2.26
(1,3411)	1:145:A:MET:HE3	2:216:B:VAL:HG23	3	2.26
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD11	7	2.17
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD12	7	2.17
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD13	7	2.17
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD21	7	2.17
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD22	7	2.17
(1,3423)	2:218:B:ALA:HB2	1:105:A:LEU:HD23	7	2.17
(1,3411)	1:145:A:MET:HE2	2:215:B:VAL:HG11	19	2.15
(1,3411)	1:145:A:MET:HE2	2:215:B:VAL:HG12	19	2.15
(1,3411)	1:145:A:MET:HE2	2:215:B:VAL:HG13	19	2.15
(1,3411)	1:145:A:MET:HE2	2:215:B:VAL:HG21	19	2.15
(1,3411)	1:145:A:MET:HE2	2:215:B:VAL:HG22	19	2.15
(1,3411)	1:145:A:MET:HE2	2:215:B:VAL:HG23	19	2.15
(1,3411)	1:145:A:MET:HE1	2:216:B:VAL:HG11	10	2.14

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	141	50.0	26	18.4	9.2	1	0.7	0.4
PSI	141	50.0	10	7.1	3.5	0	0.0	0.0
Total	282	100.0	36	12.8	12.8	1	0.4	0.4

[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 ⓘ
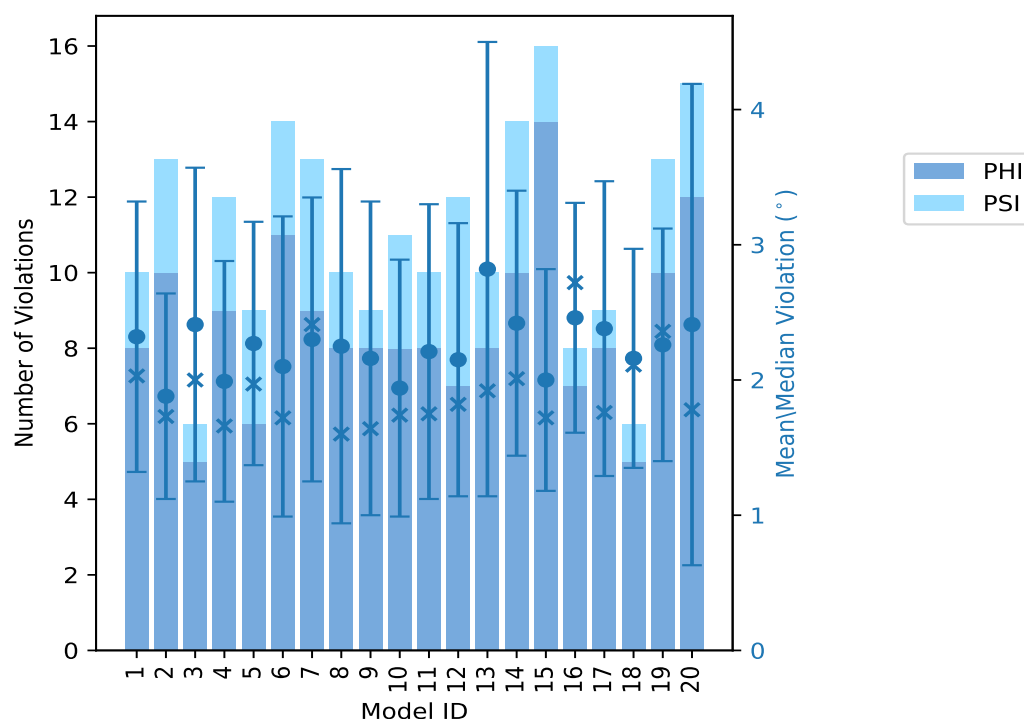

Violated and consistently violated restraints are shown using different hatch patterns in their respective categories

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	8	2	10	2.32	4.5	1.0	2.03
2	10	3	13	1.88	3.79	0.76	1.73
3	5	1	6	2.41	4.87	1.16	2.0
4	9	3	12	1.99	3.73	0.89	1.66
5	6	3	9	2.27	3.61	0.9	1.97
6	11	3	14	2.1	4.92	1.11	1.72
7	9	4	13	2.3	4.56	1.05	2.41
8	8	2	10	2.25	5.43	1.31	1.6
9	8	1	9	2.16	4.89	1.16	1.64
10	8	3	11	1.94	4.23	0.95	1.74
11	8	2	10	2.21	4.42	1.09	1.75
12	7	5	12	2.15	4.04	1.01	1.82
13	8	2	10	2.82	6.62	1.68	1.92
14	10	4	14	2.42	4.41	0.98	2.01
15	14	2	16	2.0	3.43	0.82	1.72
16	7	1	8	2.46	3.44	0.85	2.72
17	8	1	9	2.38	4.66	1.09	1.76
18	5	1	6	2.16	3.37	0.81	2.11
19	10	3	13	2.26	3.69	0.86	2.36
20	12	3	15	2.41	8.12	1.78	1.78

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]	%
10	1	11	1	5.0
2	1	3	2	10.0
2	2	4	3	15.0
0	2	2	4	20.0
1	1	2	5	25.0
1	0	1	6	30.0
1	0	1	7	35.0
1	2	3	8	40.0
1	0	1	9	45.0
1	0	1	10	50.0
0	1	1	11	55.0

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Number of violated restraints			Fraction of the ensemble	
PHI	PSI	Total	Count[1]	%
0	0	0	12	60.0
0	0	0	13	65.0
0	0	0	14	70.0
1	0	1	15	75.0
0	0	0	16	80.0
2	0	2	17	85.0
1	0	1	18	90.0
1	0	1	19	95.0
1	0	1	20	100.0

[1] Number of models with violations

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

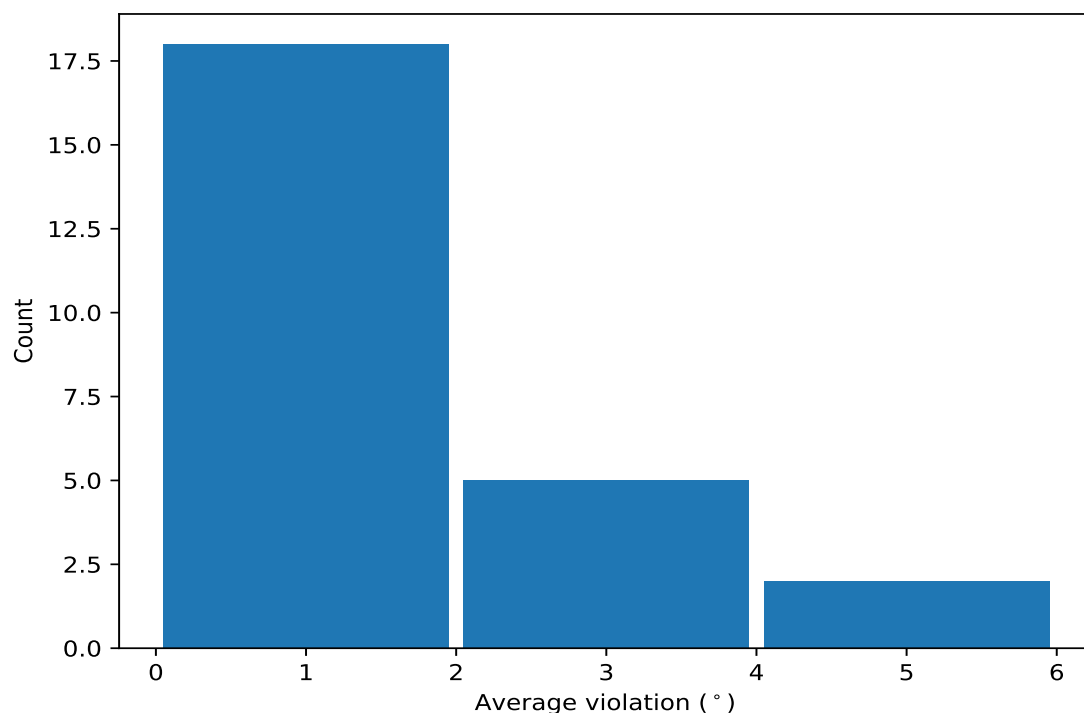## 10.4   Most violated dihedral-angle restraints in the ensemble ⓘ

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.
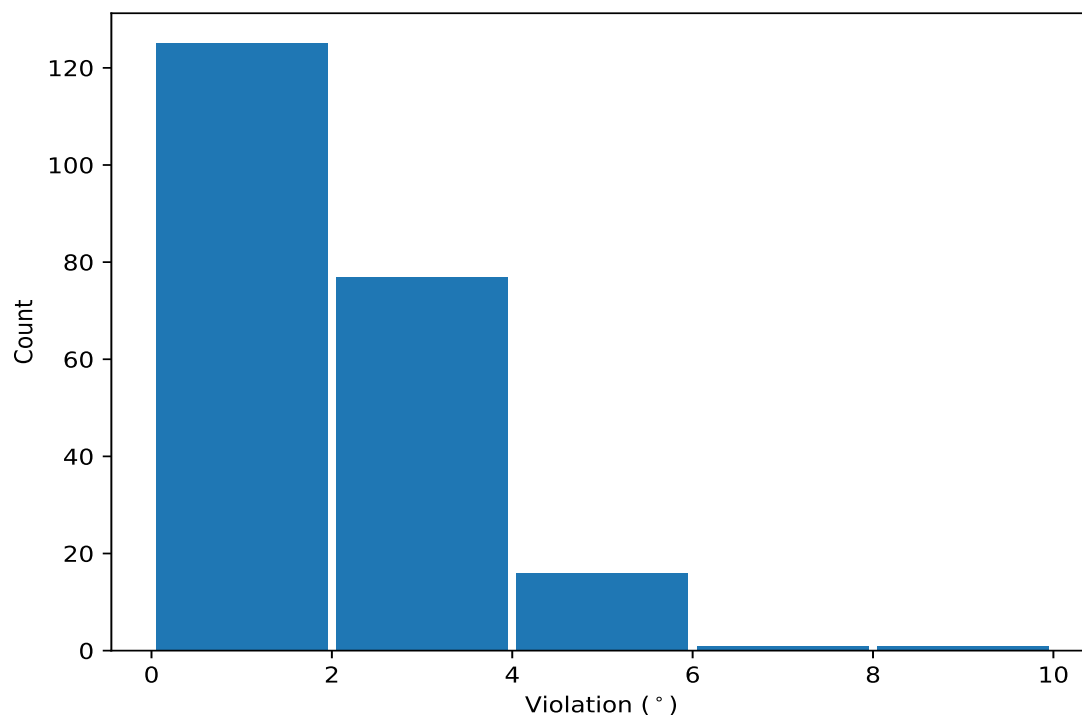
Key	Atom-1	Atom-2	Atom-3	Atom-4	Models[1]	Mean	SD[2]	Median
(1,39)	1:21:A:LYS:C	1:22:A:ASP:N	1:22:A:ASP:CA	1:22:A:ASP:C	20	3.15	0.57	3.26
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	19	4.11	0.76	4.14
(1,19)	1:11:A:GLU:C	1:12:A:PHE:N	1:12:A:PHE:CA	1:12:A:PHE:C	18	2.54	0.61	2.49
(1,131)	1:72:A:MET:C	1:73:A:ALA:N	1:73:A:ALA:CA	1:73:A:ALA:C	17	1.82	0.63	1.6
(1,103)	1:56:A:ASP:C	1:57:A:ALA:N	1:57:A:ALA:CA	1:57:A:ALA:C	17	1.64	0.36	1.59
(1,257)	1:146:A:THR:C	1:147:A:ALA:N	1:147:A:ALA:CA	1:147:A:ALA:C	15	1.52	0.36	1.43
(1,166)	1:95:A:ASP:N	1:95:A:ASP:CA	1:95:A:ASP:C	1:96:A:GLY:N	11	2.72	0.65	2.86
(1,139)	1:80:A:ASP:C	1:81:A:SER:N	1:81:A:SER:CA	1:81:A:SER:C	10	1.82	0.62	1.78
(1,165)	1:94:A:LYS:C	1:95:A:ASP:N	1:95:A:ASP:CA	1:95:A:ASP:C	9	1.6	0.4	1.71
(1,256)	1:146:A:THR:N	1:146:A:THR:CA	1:146:A:THR:C	1:147:A:ALA:N	8	1.94	0.55	1.91

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

10.5 All violated dihedral-angle restraints ⓘ

10.5.1 Histogram : Distribution of violations ⓘ

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 ⓘ

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 (°)
(1,140)	1:81:A:SER:N	1:81:A:SER:CA	1:81:A:SER:C	1:82:A:GLU:N	20	8.12
(1,140)	1:81:A:SER:N	1:81:A:SER:CA	1:81:A:SER:C	1:82:A:GLU:N	13	6.62
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	8	5.43
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	6	4.92
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	9	4.89
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	3	4.87
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	17	4.66
(1,140)	1:81:A:SER:N	1:81:A:SER:CA	1:81:A:SER:C	1:82:A:GLU:N	7	4.56
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	1	4.5
(1,5)	1:4:A:LEU:C	1:5:A:THR:N	1:5:A:THR:CA	1:5:A:THR:C	11	4.42