wwPDB NMR Structure Validation Summary Report (i)

Jun 5, 2023 – 04:32 AM EDT

PDB ID	:	2LNK
BMRB ID	:	18169
Title	:	Solution structure of Ca-bound S100A4 in complex with non-muscle myosin IIA
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Deposited on	:	2011-12-30

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:

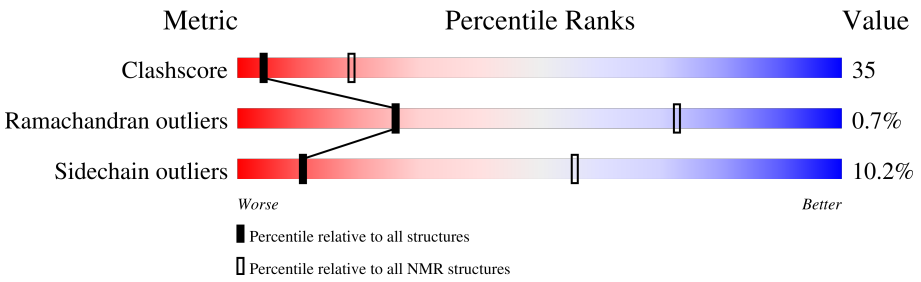
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.33

1 Overall quality at a glance (i)

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

The overall completeness of chemical shifts assignment is 46%.

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	158937	12864
Ramachandran outliers	154571	11451
Sidechain outliers	154315	11428

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	C	39	23% 51% 8% 18%
2	A	113	33% 36% • 18% 11%
2	B	113	35% 35% • 16% 11%

2 Ensemble composition and analysis (i)

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	C:1899-C:1922, C:1926-C:1933, A:2-A:21, A:28-A:88, B:2-B:21, B:28-B:90 (196)	0.51	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 2 clusters. No single-model clusters were found.

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

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 3895 atoms, of which 1943 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Myosin heavy chain, non-muscle IIa.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	C	39	635	189	321	62	61	2	0

- Molecule 2 is a protein called Protein S100-A4.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
2	A	101	1630	519	811	135	156	9	0
2	B	101	1630	519	811	135	156	9	0

There are 24 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-11	MET	-	expression tag	UNP P26447
A	-10	ARG	-	expression tag	UNP P26447
A	-9	GLY	-	expression tag	UNP P26447
A	-8	SER	-	expression tag	UNP P26447
A	-7	HIS	-	expression tag	UNP P26447
A	-6	HIS	-	expression tag	UNP P26447
A	-5	HIS	-	expression tag	UNP P26447
A	-4	HIS	-	expression tag	UNP P26447
A	-3	HIS	-	expression tag	UNP P26447
A	-2	HIS	-	expression tag	UNP P26447
A	-1	GLY	-	expression tag	UNP P26447
A	0	SER	-	expression tag	UNP P26447
B	-11	MET	-	expression tag	UNP P26447
B	-10	ARG	-	expression tag	UNP P26447
B	-9	GLY	-	expression tag	UNP P26447
B	-8	SER	-	expression tag	UNP P26447
B	-7	HIS	-	expression tag	UNP P26447
B	-6	HIS	-	expression tag	UNP P26447
B	-5	HIS	-	expression tag	UNP P26447
B	-4	HIS	-	expression tag	UNP P26447
B	-3	HIS	-	expression tag	UNP P26447
B	-2	HIS	-	expression tag	UNP P26447
B	-1	GLY	-	expression tag	UNP P26447

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Chain	Residue	Modelled	Actual	Comment	Reference
B	0	SER	-	expression tag	UNP P26447

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: Myosin heavy chain, non-muscle IIa

Chain C: 23% 51% 8% 18%

Q1897 R1898 E1899 L1900 E1901 D1902 A1903 T1904 E1905 T1906 A1907 M1910 N1911 V1914 S1915 S1916 L1917 K1918 N1919 K1920 L1921 R1922 R1923 G1924 D1925 L1926 P1927 F1928 V1929 V1930 R1933 M1934 A1935

• Molecule 2: Protein S100-A4

Chain A: 33% 36% • 18% 11%

MET ARG GLY SER HIS HIS HIS HIS HIS HIS GLY SER M1 P4 L5 E6 K7 D10 V11 M12 T15 F16 S20 G21 K22 E23 G24 D25 K26 F27 K28 L29 L34 K35 L38 T39 R40 E41 L42 P43 L46 G47 K48 R49 T50 A54 F55 Q56 K57 L58 N61

L62 D63 R66 V70 D71 F72 Q73 E74 Y75 C76 V77 F78 L79 S80 C81 I82 A83 M84 M85 F89 F90 E91 G92 F93 P94 D95 K96 Q97 P98 R99 K100 K101

• Molecule 2: Protein S100-A4

Chain B: 35% 35% • 16% 11%

MET ARG GLY SER HIS HIS HIS HIS HIS HIS GLY SER M1 L5 E6 K7 A8 L9 D10 V11 M12 F16 S20 G21 K22 E23 G24 D25 K26 F27 K28 L29 N30 K31 L34 K35 L38 T39 R40 E41 L42 P43 S44 F45 L46 T50 F55 Q56 K57 L58 M59 S60 N61

L62 N68 E69 V70 D71 F72 Q73 E74 Y75 C76 V77 F78 L79 S80 C81 M84 M85 C86 N87 E91 G92 F93 P94 D95 K96 Q97 P98 R99 K100 K101

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

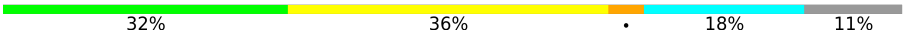
The representative model is number 6. Colouring as in section 4.1 above.

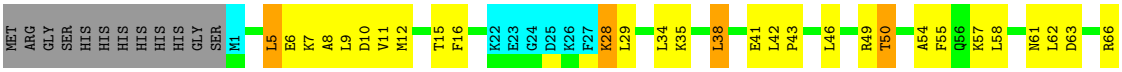
• Molecule 1: Myosin heavy chain, non-muscle IIa

Chain C: 28% 41% 13% 18%

- Molecule 2: Protein S100-A4

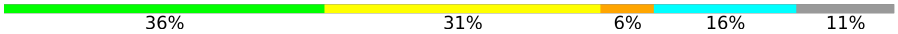
Chain A:

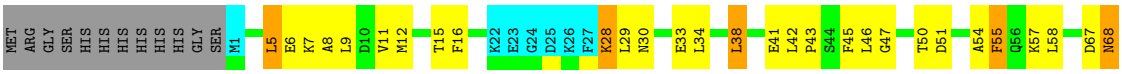


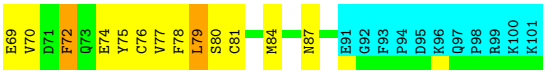


- Molecule 2: Protein S100-A4

Chain B:







5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *torsion angle dynamics*.

Of the 200 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
CNS	structure solution	1.1
CNS	refinement	1.1
ARIA	structure solution	1.2
ProcheckNMR	geometry optimization	

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	3
Total number of shifts	2776
Number of shifts mapped to atoms	2776
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	46%

6 Model quality (i)

6.1 Standard geometry (i)

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	C	0.50±0.09	0±1/260 (0.1± 0.2%)	0.60±0.03	0±0/351 (0.0± 0.0%)
2	A	0.34±0.01	0±0/655 (0.0± 0.0%)	0.46±0.01	0±0/879 (0.0± 0.0%)
2	B	0.34±0.01	0±0/679 (0.0± 0.0%)	0.44±0.01	0±0/911 (0.0± 0.0%)
All	All	0.37	4/31880 (0.0%)	0.48	0/42820 (0.0%)

All 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	C	1928	PHE	CE1-CZ	7.02	1.50	1.37	10	3
1	C	1928	PHE	CE2-CZ	-6.12	1.25	1.37	10	1

There are no bond-angle outliers.

There are no chirality outliers.

There are no planarity outliers.

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	C	257	264	264	44±5
2	A	645	640	639	55±4
2	B	667	658	657	53±7
All	All	31380	31240	31200	2217

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 35.

5 of 512 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
2:A:46:LEU:HD22	2:A:50:THR:HG21	0.97	1.37	20	19
1:C:1927:PRO:HB2	2:B:50:THR:HA	0.96	1.36	12	16
2:B:20:SER:HB3	2:B:29:LEU:HG	0.93	1.41	3	11
2:A:20:SER:HB3	2:A:29:LEU:HG	0.92	1.41	3	13
2:A:70:VAL:HG13	2:A:74:GLU:HB2	0.91	1.41	15	18

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	C	32/39 (82%)	28±1 (87±3%)	4±1 (12±3%)	0±1 (1±2%)	29	74
2	A	81/113 (72%)	70±2 (86±2%)	11±2 (13±2%)	0±1 (1±1%)	29	74
2	B	83/113 (73%)	71±2 (85±2%)	12±2 (14±2%)	1±1 (1±1%)	21	69
All	All	3920/5300 (74%)	3365 (86%)	527 (13%)	28 (1%)	26	73

5 of 9 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
2	B	49	ARG	7
2	B	47	GLY	6
2	A	63	ASP	5
2	A	48	LYS	4
2	B	68	ASN	2

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	C	29/34 (85%)	24±1 (84±2%)	5±1 (16±2%)	5 41
2	A	74/102 (73%)	68±1 (92±2%)	6±1 (8±2%)	16 63
2	B	76/102 (75%)	68±2 (90±2%)	8±2 (10±2%)	11 56
All	All	3580/4760 (75%)	3215 (90%)	365 (10%)	11 56

5 of 52 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	C	1906	THR	20
1	C	1928	PHE	20
2	A	5	LEU	20
2	B	5	LEU	20
2	B	79	LEU	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 monosaccharides in this entry.

6.6 Ligand geometry (i)

There are no ligands in this entry.

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 (i)

The completeness of assignment taking into account all chemical shift lists is 46% for the well-defined parts and 45% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *S100A4_chain_A*

7.1.1 Bookkeeping (i)

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	1270
Number of shifts mapped to atoms	1270
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	1

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	100	-0.71 ± 0.14	Should be checked
$^{13}C_{\beta}$	96	0.21 ± 0.14	None needed (< 0.5 ppm)
$^{13}C'$	98	-0.39 ± 0.14	None needed (< 0.5 ppm)
^{15}N	96	0.49 ± 0.40	None needed (< 0.5 ppm)

7.1.3 Completeness of resonance assignments (i)

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. 1025 atoms were assigned a chemical shift out of a possible 2701. 0 out of 37 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	401/972 (41%)	162/390 (42%)	160/392 (41%)	79/190 (42%)
Sidechain	562/1547 (36%)	382/999 (38%)	174/486 (36%)	6/62 (10%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	62/182 (34%)	31/89 (35%)	31/89 (35%)	0/4 (0%)
Overall	1025/2701 (38%)	575/1478 (39%)	365/967 (38%)	85/256 (33%)

7.1.4 Statistically unusual chemical shifts (i)

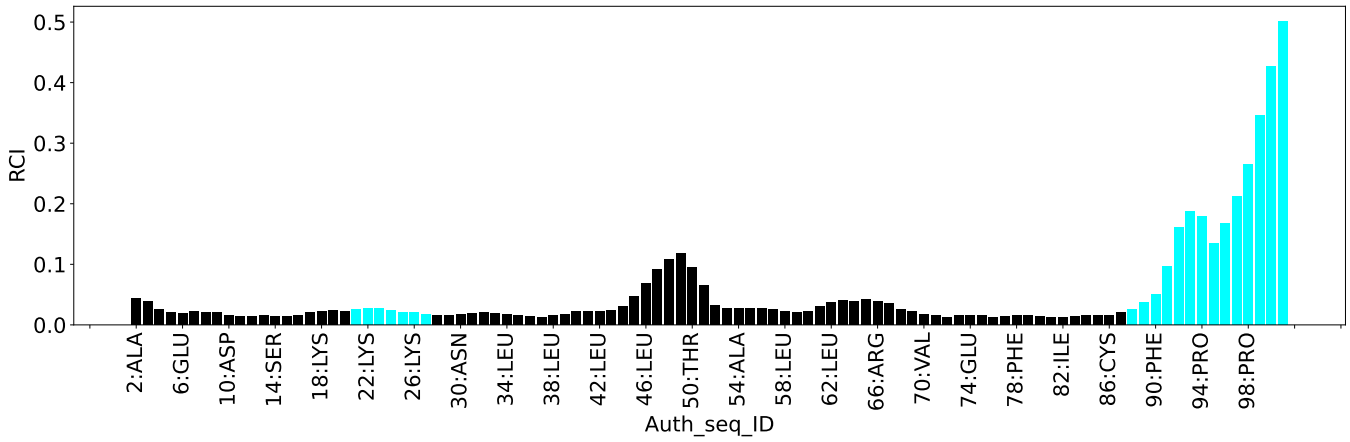
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	20	SER	HB3	2.17	2.49 – 5.20	-6.2

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:



7.2 Chemical shift list 2

File name: working_cs.cif

Chemical shift list name: *S100A4_chain_B*

7.2.1 Bookkeeping (i)

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	1266
Number of shifts mapped to atoms	1266
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	1

7.2.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	100	-0.86 ± 0.22	Should be checked
$^{13}C_{\beta}$	95	0.23 ± 0.07	None needed (< 0.5 ppm)
$^{13}C'$	97	-0.50 ± 0.14	Should be applied
^{15}N	96	0.48 ± 0.46	None needed (< 0.5 ppm)

7.2.3 Completeness of resonance assignments (i)

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. 1022 atoms were assigned a chemical shift out of a possible 2701. 0 out of 37 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	401/972 (41%)	162/390 (42%)	160/392 (41%)	79/190 (42%)
Sidechain	557/1547 (36%)	378/999 (38%)	173/486 (36%)	6/62 (10%)
Aromatic	64/182 (35%)	32/89 (36%)	32/89 (36%)	0/4 (0%)
Overall	1022/2701 (38%)	572/1478 (39%)	365/967 (38%)	85/256 (33%)

7.2.4 Statistically unusual chemical shifts (i)

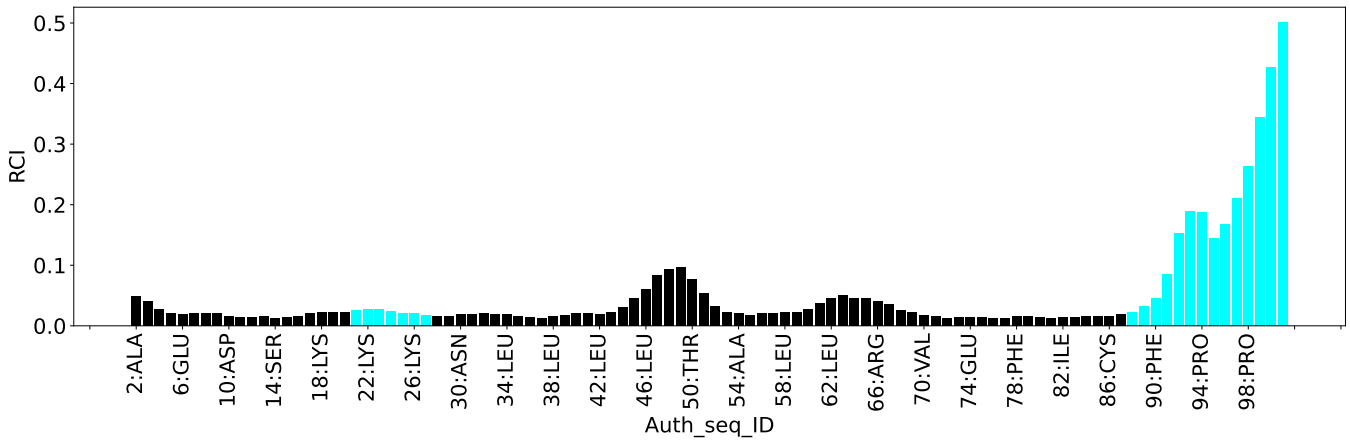
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
2	A	20	SER	HB3	2.27	2.49 – 5.20	-5.8

7.2.5 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:



7.3 Chemical shift list 3

File name: working_cs.cif

Chemical shift list name: *myosin_chain_C*

7.3.1 Bookkeeping (i)

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	240
Number of shifts mapped to atoms	240
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

7.3.2 Chemical shift referencing (i)

No chemical shift referencing corrections were calculated (not enough data).

7.3.3 Completeness of resonance assignments (i)

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 7%, i.e. 202 atoms were assigned a chemical shift out of a possible 2701. 0 out of 37 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	54/972 (6%)	54/390 (14%)	0/392 (0%)	0/190 (0%)
Sidechain	143/1547 (9%)	143/999 (14%)	0/486 (0%)	0/62 (0%)
Aromatic	5/182 (3%)	5/89 (6%)	0/89 (0%)	0/4 (0%)
Overall	202/2701 (7%)	202/1478 (14%)	0/967 (0%)	0/256 (0%)

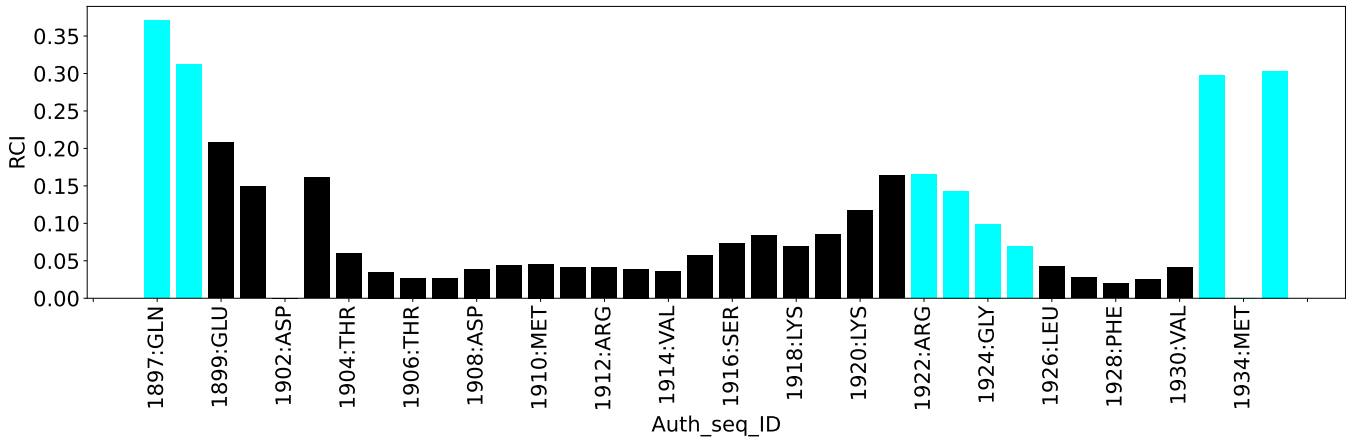
7.3.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

7.3.5 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 C:



8 NMR restraints analysis (i)

8.1 Conformationally restricting restraints (i)

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	7075
Intra-residue (|i-j|=0)	2473
Sequential (|i-j|=1)	1491
Medium range (|i-j|>1 and |i-j|<5)	1187
Long range (|i-j|≥5)	708
Inter-chain	1216
Hydrogen bond restraints	0
Disulfide bond restraints	0
Total dihedral-angle restraints	368
Number of unmapped restraints	0
Number of restraints per residue	28.1
Number of long range restraints per residue[1]	2.7

[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 (i)

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 (i)

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)	94.5	0.2
0.2-0.5 (Medium)	54.4	0.5
>0.5 (Large)	8.6	4.94

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)	21.4	8.1
10.0-20.0 (Medium)	None	None
>20.0 (Large)	None	None

9 Distance violation analysis (i)

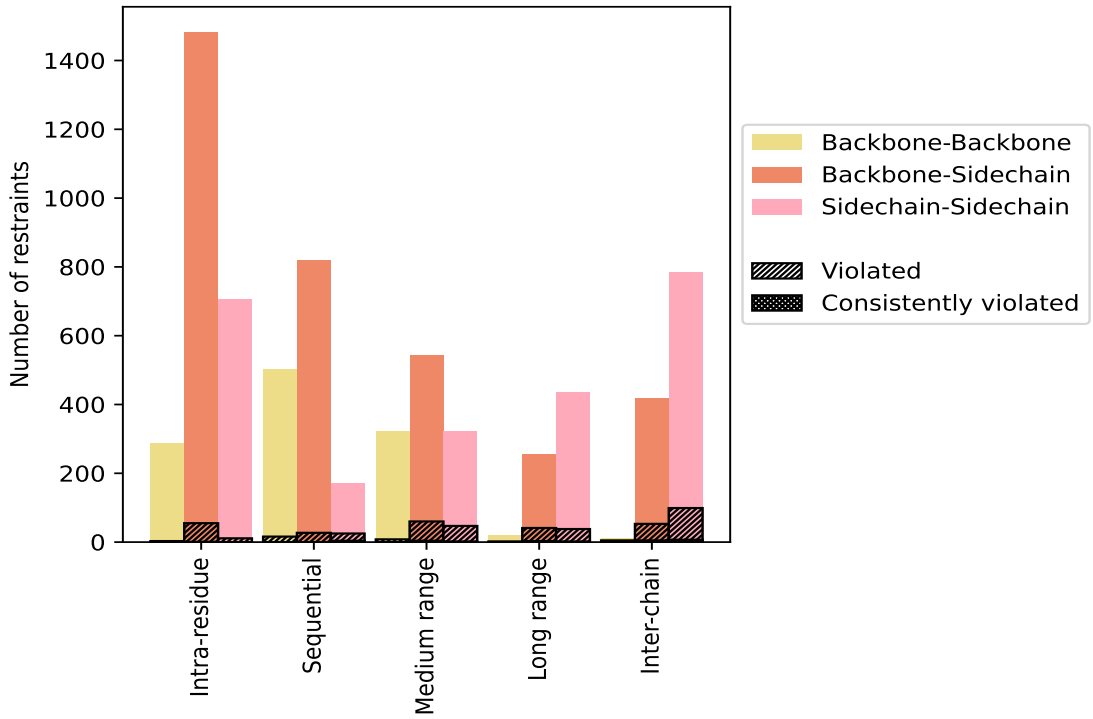
9.1 Summary of distance violations (i)

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)	2473	35.0	69	2.8	1.0	1	0.0	0.0
Backbone-Backbone	286	4.0	3	1.0	0.0	0	0.0	0.0
Backbone-Sidechain	1482	20.9	55	3.7	0.8	1	0.1	0.0
Sidechain-Sidechain	705	10.0	11	1.6	0.2	0	0.0	0.0
Sequential (|i-j|=1)	1491	21.1	68	4.6	1.0	4	0.3	0.1
Backbone-Backbone	502	7.1	16	3.2	0.2	0	0.0	0.0
Backbone-Sidechain	819	11.6	27	3.3	0.4	0	0.0	0.0
Sidechain-Sidechain	170	2.4	25	14.7	0.4	4	2.4	0.1
Medium range (|i-j|>1 & |i-j|<5)	1187	16.8	115	9.7	1.6	6	0.5	0.1
Backbone-Backbone	321	4.5	8	2.5	0.1	0	0.0	0.0
Backbone-Sidechain	543	7.7	60	11.0	0.8	4	0.7	0.1
Sidechain-Sidechain	323	4.6	47	14.6	0.7	2	0.6	0.0
Long range (|i-j|≥5)	708	10.0	81	11.4	1.1	5	0.7	0.1
Backbone-Backbone	19	0.3	2	10.5	0.0	0	0.0	0.0
Backbone-Sidechain	254	3.6	41	16.1	0.6	3	1.2	0.0
Sidechain-Sidechain	435	6.1	38	8.7	0.5	2	0.5	0.0
Inter-chain	1216	17.2	157	12.9	2.2	12	1.0	0.2
Backbone-Backbone	12	0.2	5	41.7	0.1	0	0.0	0.0
Backbone-Sidechain	419	5.9	53	12.6	0.7	5	1.2	0.1
Sidechain-Sidechain	785	11.1	99	12.6	1.4	7	0.9	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	7075	100.0	490	6.9	6.9	28	0.4	0.4
Backbone-Backbone	1140	16.1	34	3.0	0.5	0	0.0	0.0
Backbone-Sidechain	3517	49.7	236	6.7	3.3	13	0.4	0.2
Sidechain-Sidechain	2418	34.2	220	9.1	3.1	15	0.6	0.2

[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 (i)



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 (i)

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	17	12	38	26	64	157	0.23	1.44	0.19	0.17
2	11	14	33	23	63	144	0.23	1.47	0.19	0.17
3	12	11	34	33	66	156	0.21	1.41	0.16	0.16
4	17	15	30	26	69	157	0.22	1.28	0.14	0.18
5	17	17	38	34	75	181	0.25	1.62	0.24	0.17
6	11	12	38	29	58	148	0.26	4.94	0.43	0.17
7	10	16	39	29	63	157	0.21	0.84	0.11	0.19
8	13	16	34	33	66	162	0.24	1.51	0.21	0.17
9	14	19	40	26	71	170	0.27	1.57	0.23	0.19
10	14	17	37	33	71	172	0.26	1.5	0.25	0.18
11	14	20	32	26	58	150	0.21	0.96	0.13	0.17

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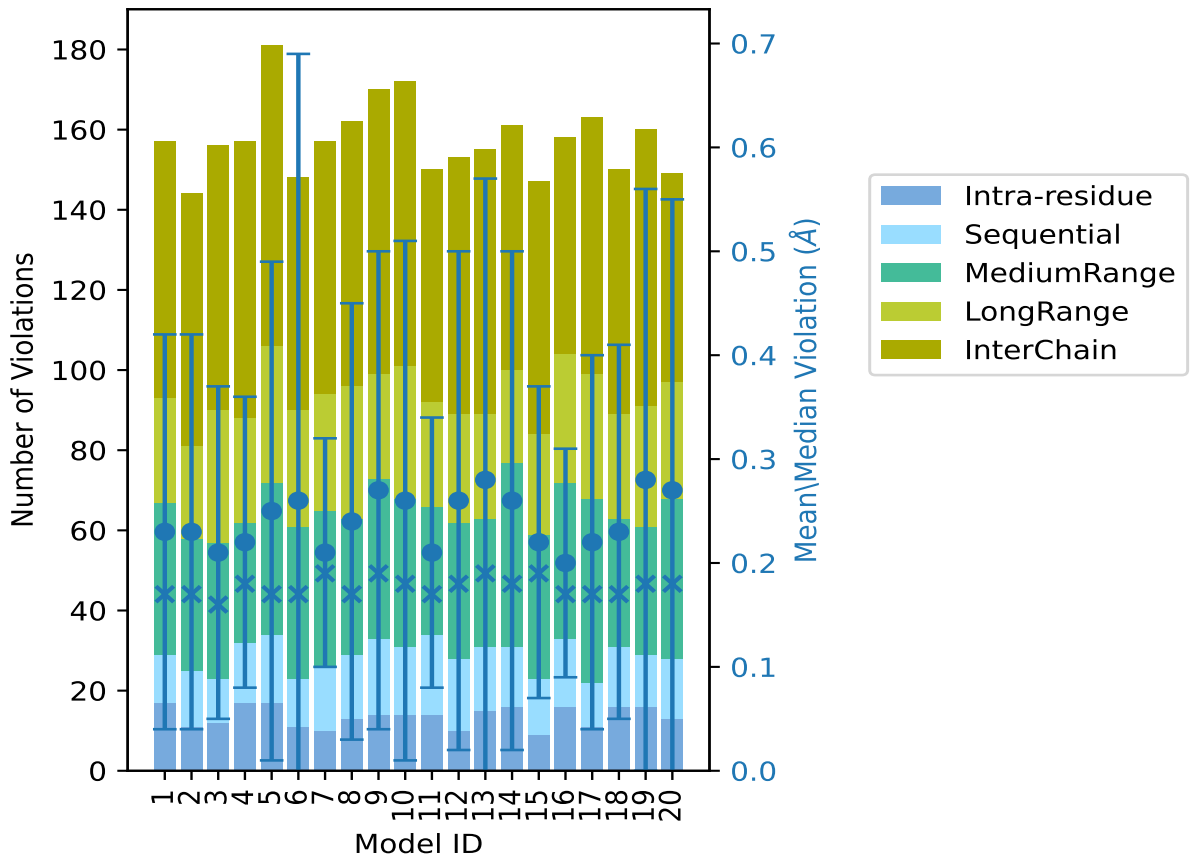
Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
12	10	18	34	27	64	153	0.26	1.82	0.24	0.18
13	15	16	32	26	66	155	0.28	1.94	0.29	0.19
14	16	15	46	23	61	161	0.26	1.61	0.24	0.18
15	9	14	36	25	63	147	0.22	1.21	0.15	0.19
16	16	17	39	32	54	158	0.2	0.87	0.11	0.17
17	11	11	46	31	64	163	0.22	1.61	0.18	0.17
18	16	15	32	26	61	150	0.23	1.28	0.18	0.17
19	16	13	32	30	69	160	0.28	1.79	0.28	0.18
20	13	15	40	29	52	149	0.27	2.27	0.28	0.18

[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 (i)



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

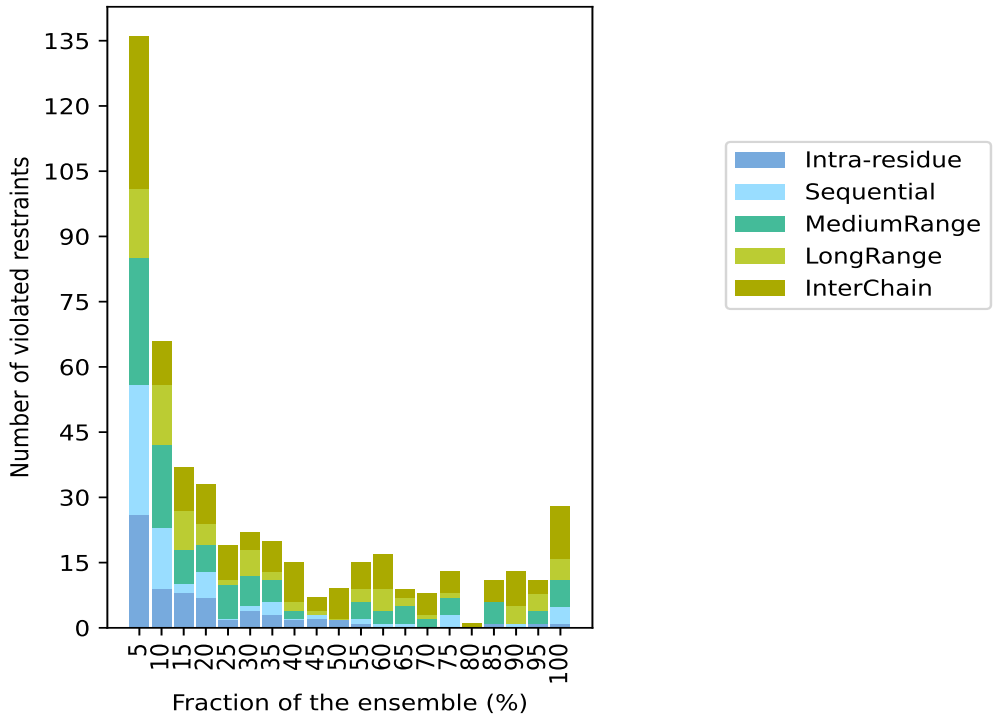
9.3 Distance violation statistics for the ensemble (i)

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, 6585(IR:2404, SQ:1423, MR:1072, LR:627, IC:1059) 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]	%
26	30	29	16	35	136	1	5.0
9	14	19	14	10	66	2	10.0
8	2	8	9	10	37	3	15.0
7	6	6	5	9	33	4	20.0
2	0	8	1	8	19	5	25.0
4	1	7	6	4	22	6	30.0
3	3	5	2	7	20	7	35.0
2	0	2	2	9	15	8	40.0
2	1	0	1	3	7	9	45.0
2	0	0	0	7	9	10	50.0
1	1	4	3	6	15	11	55.0
0	1	3	5	8	17	12	60.0
0	1	4	2	2	9	13	65.0
0	0	2	1	5	8	14	70.0
0	3	4	1	5	13	15	75.0
0	0	0	0	1	1	16	80.0
1	0	5	0	5	11	17	85.0
0	1	0	4	8	13	18	90.0
1	0	3	4	3	11	19	95.0
1	4	6	5	12	28	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

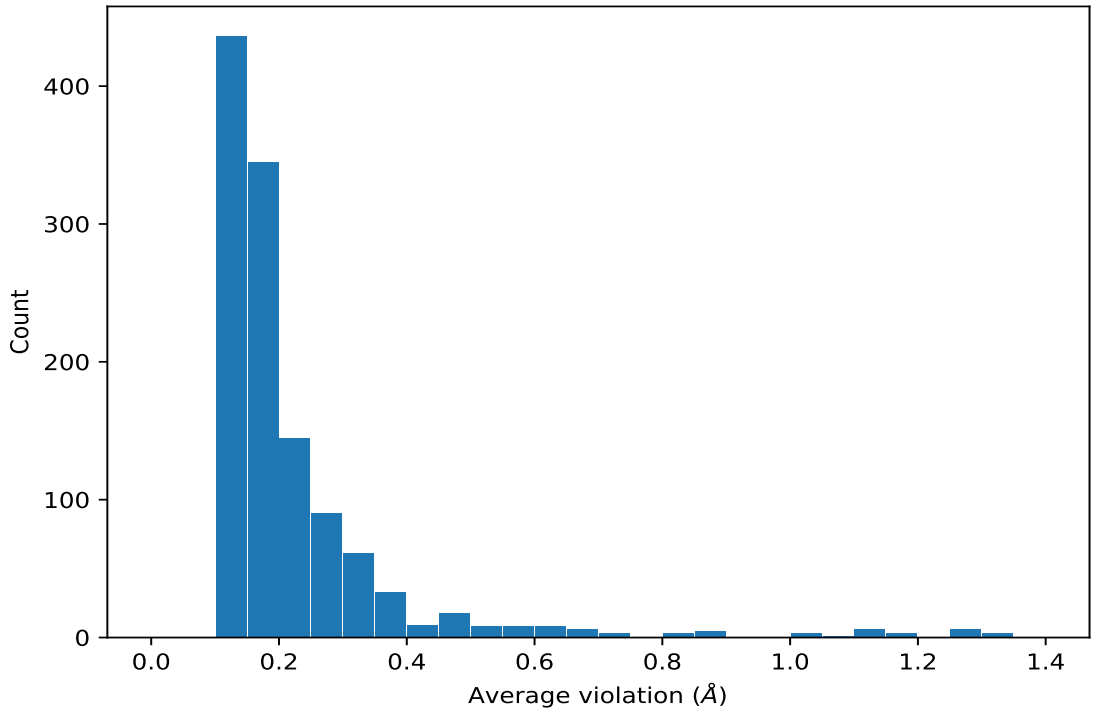
9.3.1 Bar graph : Distance violation statistics for the ensemble (i)



9.4 Most violated distance restraints in the ensemble (i)

9.4.1 Histogram : Distribution of mean distance violations (i)

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 (i)

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)
(1,5026)	1:C:1907:ALA:H	2:A:85:MET:HG3	20	0.89	0.53	0.56
(1,5026)	1:C:1907:ALA:H	2:A:85:MET:HB2	20	0.89	0.53	0.56
(1,5026)	1:C:1907:ALA:H	2:B:76:CYS:HB3	20	0.89	0.53	0.56
(1,4720)	2:A:82:ILE:HG21	1:C:1900:LEU:HB2	20	0.6	0.16	0.6
(1,4720)	2:A:82:ILE:HG22	1:C:1900:LEU:HB2	20	0.6	0.16	0.6
(1,4720)	2:A:82:ILE:HG23	1:C:1900:LEU:HB2	20	0.6	0.16	0.6
(1,5360)	1:C:1917:LEU:HD21	1:C:1918:LYS:HE3	20	0.53	0.52	0.3
(1,5360)	1:C:1917:LEU:HD22	1:C:1918:LYS:HE3	20	0.53	0.52	0.3
(1,5360)	1:C:1917:LEU:HD23	1:C:1918:LYS:HE3	20	0.53	0.52	0.3
(1,5360)	1:C:1917:LEU:HD21	1:C:1918:LYS:HE2	20	0.53	0.52	0.3
(1,5360)	1:C:1917:LEU:HD22	1:C:1918:LYS:HE2	20	0.53	0.52	0.3
(1,5360)	1:C:1917:LEU:HD23	1:C:1918:LYS:HE2	20	0.53	0.52	0.3
(1,5818)	2:B:79:LEU:HD21	2:B:80:SER:HB3	20	0.43	0.08	0.42
(1,5818)	2:B:79:LEU:HD21	2:B:77:VAL:HA	20	0.43	0.08	0.42
(1,5818)	2:B:79:LEU:HD21	2:B:45:PHE:HB2	20	0.43	0.08	0.42
(1,5818)	2:B:79:LEU:HD22	2:B:45:PHE:HB2	20	0.43	0.08	0.42

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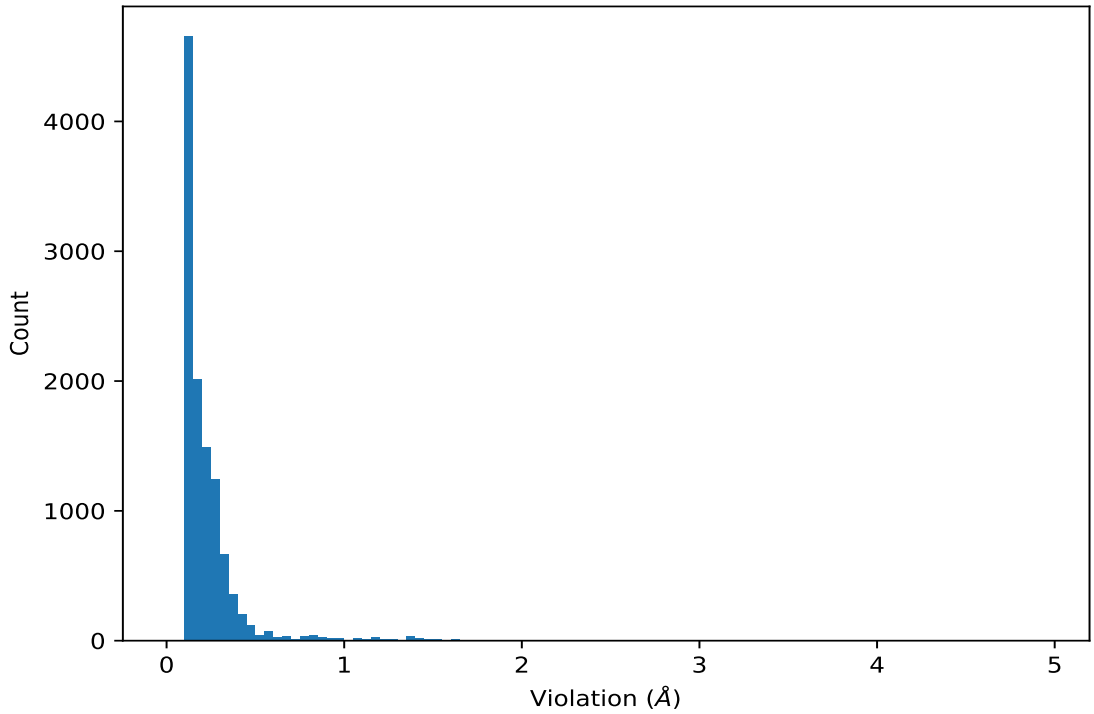
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,2056)	2:B:27:PHE:HA	2:B:75:TYR:HD1	20	0.4	0.26	0.35
(1,2056)	2:B:27:PHE:HA	2:B:75:TYR:HD2	20	0.4	0.26	0.35
(1,6072)	2:B:39:THR:HG23	2:B:36:GLU:HB3	20	0.37	0.07	0.34
(1,6072)	2:B:39:THR:HG22	2:B:36:GLU:HB3	20	0.37	0.07	0.34
(1,6072)	2:B:39:THR:HG21	2:B:36:GLU:HB3	20	0.37	0.07	0.34
(1,6072)	2:B:39:THR:HG23	2:B:40:ARG:HB2	20	0.37	0.07	0.34
(1,6072)	2:B:39:THR:HG21	2:B:40:ARG:HB2	20	0.37	0.07	0.34
(1,6072)	2:B:39:THR:HG22	2:B:40:ARG:HB2	20	0.37	0.07	0.34
(1,2024)	2:A:29:LEU:HD13	2:A:72:PHE:HE1	20	0.35	0.31	0.24
(1,2024)	2:A:29:LEU:HD13	2:A:72:PHE:HE2	20	0.35	0.31	0.24
(1,2024)	2:A:29:LEU:HD12	2:A:72:PHE:HE1	20	0.35	0.31	0.24
(1,2024)	2:A:29:LEU:HD12	2:A:72:PHE:HE2	20	0.35	0.31	0.24
(1,2024)	2:A:29:LEU:HD11	2:A:72:PHE:HE1	20	0.35	0.31	0.24
(1,2024)	2:A:29:LEU:HD11	2:A:72:PHE:HE2	20	0.35	0.31	0.24
(1,5537)	1:C:1907:ALA:HA	2:B:73:GLN:HB3	20	0.34	0.07	0.32
(1,5537)	1:C:1907:ALA:HA	1:C:1905:GLU:HB3	20	0.34	0.07	0.32
(1,5537)	1:C:1907:ALA:HA	2:A:73:GLN:HB3	20	0.34	0.07	0.32
(1,5537)	1:C:1907:ALA:HA	1:C:1905:GLU:HB2	20	0.34	0.07	0.32
(1,636)	2:B:76:CYS:HA	2:B:62:LEU:HD11	20	0.32	0.04	0.32
(1,636)	2:B:76:CYS:HA	2:B:62:LEU:HD13	20	0.32	0.04	0.32
(1,636)	2:B:76:CYS:HA	2:B:62:LEU:HD12	20	0.32	0.04	0.32
(1,174)	2:B:85:MET:HE2	2:B:85:MET:HA	20	0.31	0.05	0.32

[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)
(1,4752)	2:A:58:LEU:H	1:C:1898:ARG:HD3	6	4.94
(1,4752)	2:A:53:ALA:H	1:C:1898:ARG:HD3	6	4.94
(1,4752)	2:A:53:ALA:H	1:C:1898:ARG:HD2	6	4.94
(1,5360)	1:C:1917:LEU:HD21	1:C:1918:LYS:HE3	20	2.27
(1,5360)	1:C:1917:LEU:HD22	1:C:1918:LYS:HE3	20	2.27
(1,5360)	1:C:1917:LEU:HD23	1:C:1918:LYS:HE3	20	2.27
(1,5360)	1:C:1917:LEU:HD21	1:C:1918:LYS:HE2	20	2.27
(1,5360)	1:C:1917:LEU:HD22	1:C:1918:LYS:HE2	20	2.27
(1,5360)	1:C:1917:LEU:HD23	1:C:1918:LYS:HE2	20	2.27
(1,5359)	1:C:1917:LEU:HD21	2:B:61:ASN:HB2	13	1.94
(1,5359)	1:C:1917:LEU:HD22	2:B:61:ASN:HB2	13	1.94
(1,5359)	1:C:1917:LEU:HD23	2:B:61:ASN:HB2	13	1.94
(1,4879)	1:C:1920:LYS:H	1:C:1926:LEU:HG	12	1.82
(1,5620)	1:C:1903:ALA:HB1	1:C:1898:ARG:HD3	6	1.81
(1,5620)	1:C:1903:ALA:HB2	1:C:1898:ARG:HD3	6	1.81
(1,5620)	1:C:1903:ALA:HB3	1:C:1898:ARG:HD3	6	1.81
(1,5620)	1:C:1903:ALA:HB1	2:A:89:PHE:HB2	6	1.81

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,5620)	1:C:1903:ALA:HB2	2:A:89:PHE:HB2	6	1.81
(1,5620)	1:C:1903:ALA:HB3	2:A:89:PHE:HB2	6	1.81
(1,5359)	1:C:1917:LEU:HD21	2:B:61:ASN:HB2	19	1.79
(1,5359)	1:C:1917:LEU:HD22	2:B:61:ASN:HB2	19	1.79
(1,5359)	1:C:1917:LEU:HD23	2:B:61:ASN:HB2	19	1.79
(1,4052)	2:B:85:MET:HE1	1:C:1929:VAL:HG11	5	1.62
(1,4052)	2:B:85:MET:HE1	1:C:1929:VAL:HG12	5	1.62
(1,4052)	2:B:85:MET:HE1	1:C:1929:VAL:HG13	5	1.62
(1,4052)	2:B:85:MET:HE2	1:C:1929:VAL:HG11	5	1.62
(1,4052)	2:B:85:MET:HE2	1:C:1929:VAL:HG12	5	1.62
(1,4052)	2:B:85:MET:HE2	1:C:1929:VAL:HG13	5	1.62
(1,5026)	1:C:1907:ALA:H	2:A:85:MET:HG3	14	1.61
(1,5026)	1:C:1907:ALA:H	2:A:85:MET:HB2	14	1.61
(1,5026)	1:C:1907:ALA:H	2:B:76:CYS:HB3	14	1.61
(1,5026)	1:C:1907:ALA:H	2:A:85:MET:HG3	17	1.61
(1,5026)	1:C:1907:ALA:H	2:A:85:MET:HB2	17	1.61
(1,5026)	1:C:1907:ALA:H	2:B:76:CYS:HB3	17	1.61
(1,5364)	1:C:1917:LEU:HD21	1:C:1920:LYS:HG3	9	1.57

10 Dihedral-angle violation analysis (i)

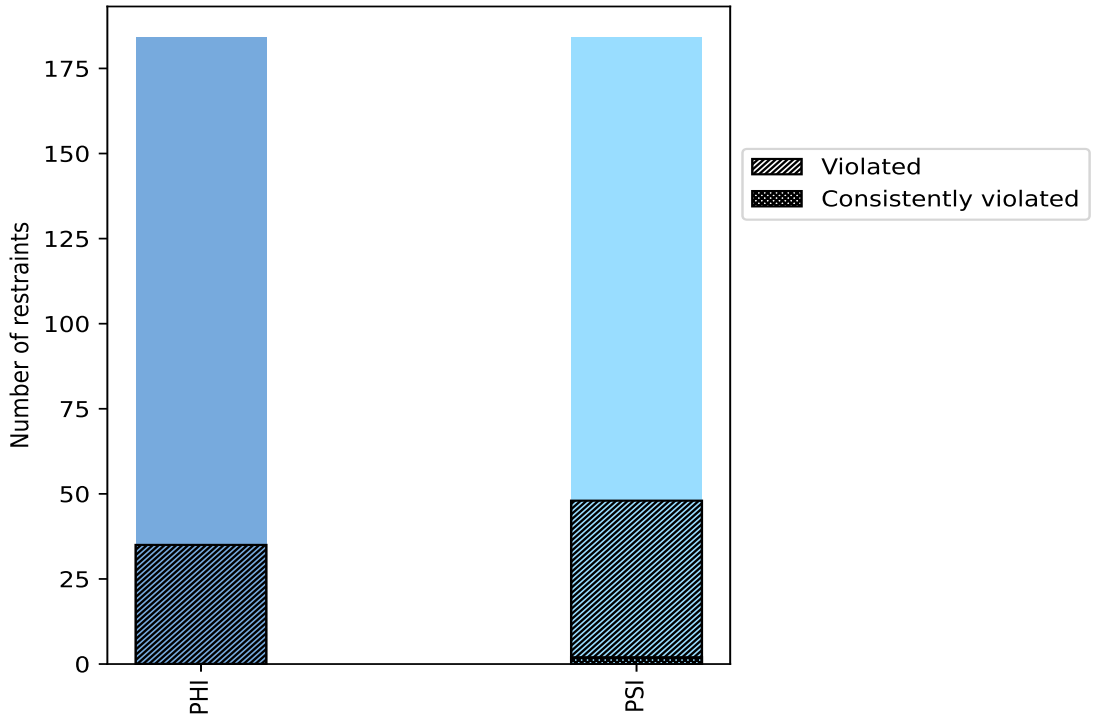
10.1 Summary of dihedral-angle violations (i)

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	184	50.0	35	19.0	9.5	0	0.0	0.0
PSI	184	50.0	48	26.1	13.0	2	1.1	0.5
Total	368	100.0	83	22.6	22.6	2	0.5	0.5

[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 (i)



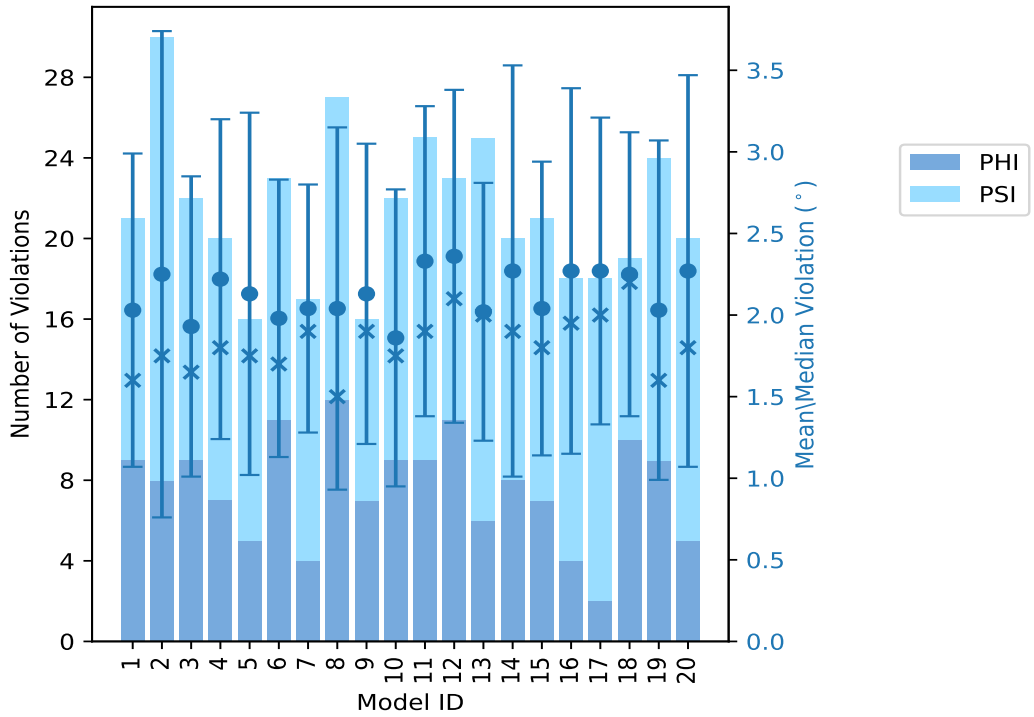
Violated and consistently violated restraints are shown using different hatch patterns in their respective categories

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	9	12	21	2.03	4.8	0.96	1.6
2	8	22	30	2.25	8.1	1.49	1.75
3	9	13	22	1.93	5.0	0.92	1.65
4	7	13	20	2.22	4.6	0.98	1.8
5	5	11	16	2.13	5.1	1.11	1.75
6	11	12	23	1.98	4.4	0.85	1.7
7	4	13	17	2.04	4.0	0.76	1.9
8	12	15	27	2.04	6.2	1.11	1.5
9	7	9	16	2.13	4.8	0.92	1.9
10	9	13	22	1.86	4.6	0.91	1.75
11	9	16	25	2.33	4.8	0.95	1.9
12	11	12	23	2.36	5.5	1.02	2.1
13	6	19	25	2.02	4.6	0.79	2.0
14	8	12	20	2.27	6.6	1.26	1.9
15	7	14	21	2.04	4.2	0.9	1.8
16	4	14	18	2.27	6.1	1.12	1.95
17	2	16	18	2.27	4.7	0.94	2.0
18	10	9	19	2.25	3.6	0.87	2.2
19	9	15	24	2.03	4.7	1.04	1.6
20	5	15	20	2.27	4.7	1.2	1.8

10.2.1 Bar graph : Dihedral violation statistics for each model (i)



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 (i)

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]	%
16	11	27	1	5.0
2	6	8	2	10.0
2	4	6	3	15.0
2	4	6	4	20.0
2	3	5	5	25.0
0	4	4	6	30.0
1	4	5	7	35.0
0	1	1	8	40.0
5	3	8	9	45.0
2	0	2	10	50.0
1	3	4	11	55.0

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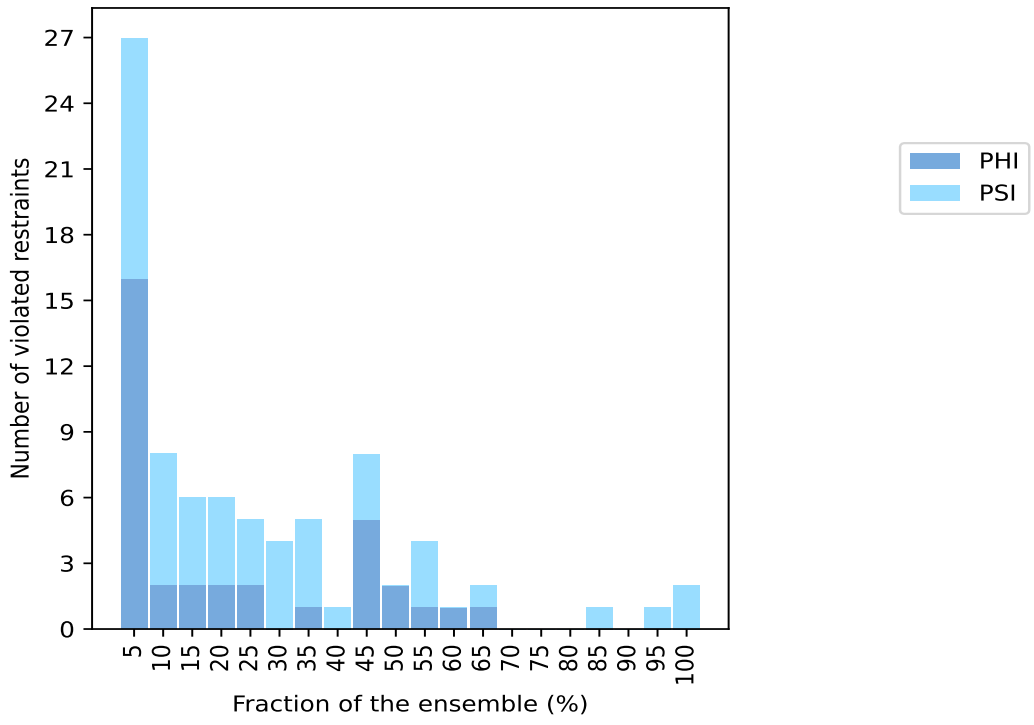
Number of violated restraints			Fraction of the ensemble	
PHI	PSI	Total	Count[1]	%
1	0	1	12	60.0
1	1	2	13	65.0
0	0	0	14	70.0
0	0	0	15	75.0
0	0	0	16	80.0
0	1	1	17	85.0
0	0	0	18	90.0
0	1	1	19	95.0
0	2	2	20	100.0

[1] Number of models with violations

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

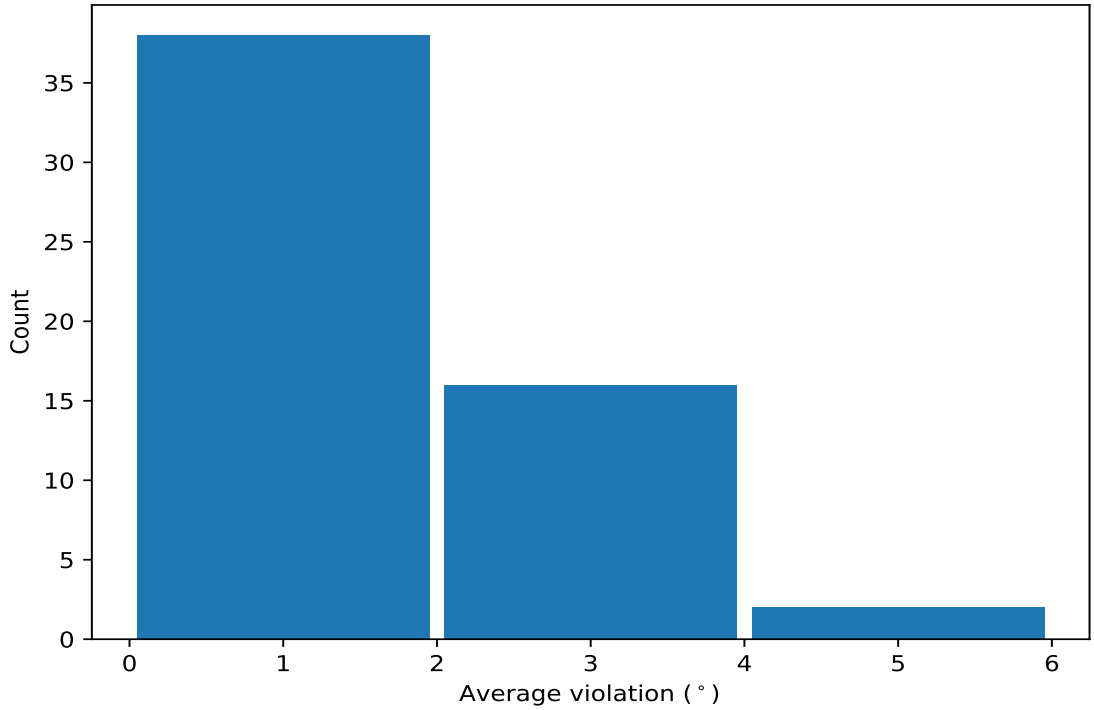


10.4 Most violated dihedral-angle restraints in the ensemble (i)

10.4.1 Histogram : Distribution of mean dihedral-angle violations (i)

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 (i)

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.

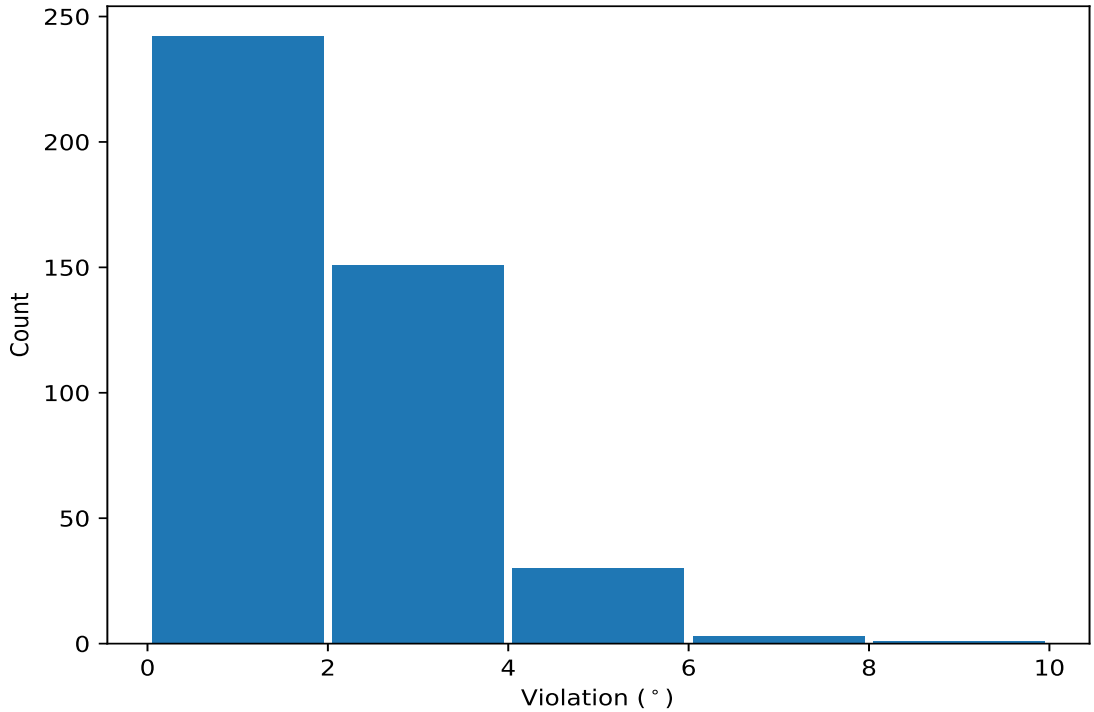
Key	Atom-1	Atom-2	Atom-3	Atom-4	Models[1]	Mean	SD[2]	Median
(1,232)	2:B:47:GLY:N	2:B:47:GLY:CA	2:B:47:GLY:C	2:B:48:LYS:N	20	3.88	0.93	3.9
(1,2)	2:A:4:PRO:N	2:A:4:PRO:CA	2:A:4:PRO:C	2:A:5:LEU:N	20	2.76	0.71	2.85
(1,160)	2:B:4:PRO:N	2:B:4:PRO:CA	2:B:4:PRO:C	2:B:5:LEU:N	19	2.29	0.64	2.2
(1,366)	1:C:1931:PRO:N	1:C:1931:PRO:CA	1:C:1931:PRO:C	1:C:1932:ARG:N	17	3.48	1.63	3.1
(1,24)	2:A:15:THR:N	2:A:15:THR:CA	2:A:15:THR:C	2:A:16:PHE:N	13	2.62	0.89	2.3
(1,135)	2:A:80:SER:C	2:A:81:CYS:N	2:A:81:CYS:CA	2:A:81:CYS:C	13	1.68	0.34	1.6
(1,79)	2:A:50:THR:C	2:A:51:ASP:N	2:A:51:ASP:CA	2:A:51:ASP:C	12	1.96	0.48	1.95
(1,72)	2:A:45:PHE:N	2:A:45:PHE:CA	2:A:45:PHE:C	2:A:46:LEU:N	11	2.92	1.39	2.6
(1,358)	1:C:1922:ARG:N	1:C:1922:ARG:CA	1:C:1922:ARG:C	1:C:1923:ARG:N	11	2.03	0.67	2.3
(1,270)	2:B:71:ASP:N	2:B:71:ASP:CA	2:B:71:ASP:C	2:B:72:PHE:N	11	1.64	0.53	1.4

[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 violationsfor 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 (°)
(1,366)	1:C:1931:PRO:N	1:C:1931:PRO:CA	1:C:1931:PRO:C	1:C:1932:ARG:N	2	8.1
(1,76)	2:A:47:GLY:N	2:A:47:GLY:CA	2:A:47:GLY:C	2:A:48:LYS:N	14	6.6
(1,76)	2:A:47:GLY:N	2:A:47:GLY:CA	2:A:47:GLY:C	2:A:48:LYS:N	8	6.2
(1,232)	2:B:47:GLY:N	2:B:47:GLY:CA	2:B:47:GLY:C	2:B:48:LYS:N	16	6.1
(1,359)	1:C:1923:ARG:C	1:C:1924:GLY:N	1:C:1924:GLY:CA	1:C:1924:GLY:C	12	5.5
(1,264)	2:B:67:ASP:N	2:B:67:ASP:CA	2:B:67:ASP:C	2:B:68:ASN:N	2	5.2
(1,232)	2:B:47:GLY:N	2:B:47:GLY:CA	2:B:47:GLY:C	2:B:48:LYS:N	5	5.1
(1,72)	2:A:45:PHE:N	2:A:45:PHE:CA	2:A:45:PHE:C	2:A:46:LEU:N	3	5.0
(1,76)	2:A:47:GLY:N	2:A:47:GLY:CA	2:A:47:GLY:C	2:A:48:LYS:N	2	4.8
(1,366)	1:C:1931:PRO:N	1:C:1931:PRO:CA	1:C:1931:PRO:C	1:C:1932:ARG:N	11	4.8