wwPDB NMR Structure Validation Summary Report (i)

Jun 3, 2023 – 11:19 PM EDT

PDB ID	:	2N1M
BMRB ID	:	25567
Title	:	NMR structure of the apo-form of the flavoprotein YP_193882.1 from Lactobacillus acidophilus NCFM
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Deposited on	:	2015-04-08

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 (i)) were used in the production of this report:

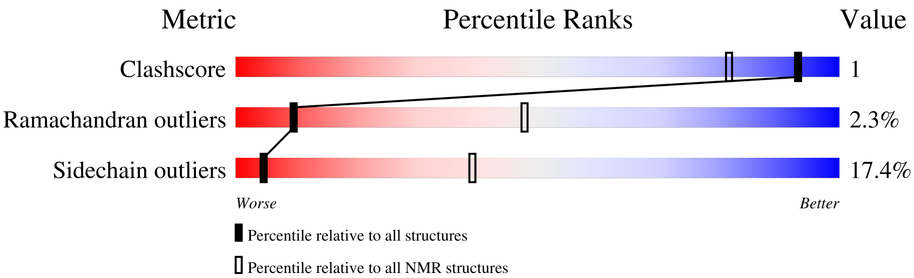
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal 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 88%.

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	A	151	

2 Ensemble composition and analysis

This entry contains 20 models. Model 2 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *closest to the average*.

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:3-A:11, A:16-A:39, A:54-A:151 (131)	0.88	2

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 4 clusters and 2 single-model clusters were found.

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

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 2174 atoms, of which 972 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Putative trp repressor binding protein.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	151	2174	764	972	193	241	4	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	GLY	-	expression tag	UNP Q5FKC3

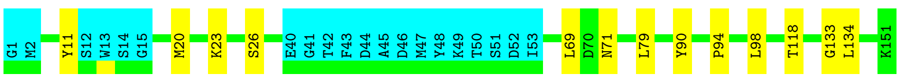
4 Residue-property plots (i)

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: Putative trp repressor binding protein

Chain A:

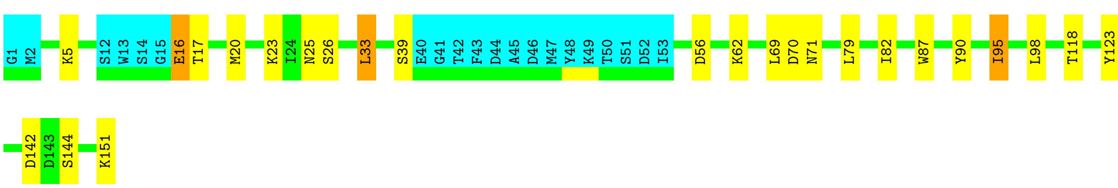


4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 2. Colouring as in section 4.1 above.

- Molecule 1: Putative trp repressor binding protein

Chain A:



5 Refinement protocol and experimental data overview (i)

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

Of the 80 calculated structures, 20 were deposited, based on the following criterion: *target function*.

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

Software name	Classification	Version
CYANA	refinement	
CANDID	refinement	
OPAL	refinement	
OPAL	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	1
Total number of shifts	1660
Number of shifts mapped to atoms	1497
Number of unparsed shifts	0
Number of shifts with mapping errors	163
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	88%

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	A	0.62±0.01	0±0/1072 (0.0± 0.0%)	1.05±0.03	1±1/1450 (0.1± 0.1%)
All	All	0.62	0/21440 (0.0%)	1.06	24/29000 (0.1%)

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	1.6±1.0
All	All	0	32

There are no bond-length outliers.

5 of 17 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(°)	Ideal(°)	Models	
								Worst	Total
1	A	106	ARG	NE-CZ-NH2	-6.98	116.81	120.30	17	1
1	A	105	TYR	CB-CG-CD1	-6.85	116.89	121.00	11	2
1	A	141	ARG	NE-CZ-NH2	-6.30	117.15	120.30	6	3
1	A	141	ARG	NE-CZ-NH1	6.30	123.45	120.30	13	2
1	A	90	TYR	CB-CG-CD2	-6.14	117.31	121.00	15	4

There are no chirality outliers.

5 of 13 unique planar outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Group	Models (Total)
1	A	11	TYR	Sidechain,Peptide	7
1	A	90	TYR	Sidechain	5
1	A	74	TYR	Sidechain	5

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Mol	Chain	Res	Type	Group	Models (Total)
1	A	77	TYR	Sidechain	4
1	A	106	ARG	Sidechain	2

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	1050	861	1031	3±2
All	All	21000	17220	20620	54

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:9:LEU:HD13	1:A:34:LYS:HG3	0.69	1.64	19	1
1:A:9:LEU:HD13	1:A:34:LYS:CG	0.68	2.19	19	1
1:A:25:ASN:HB2	1:A:33:LEU:HD13	0.62	1.70	1	5
1:A:102:MET:O	1:A:134:LEU:HD11	0.62	1.94	11	2
1:A:11:TYR:CA	1:A:36:VAL:HG13	0.61	2.25	15	2

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	130/151 (86%)	106±4 (81±3%)	21±3 (16±3%)	3±1 (2±1%)	9	48
All	All	2600/3020 (86%)	2110 (81%)	429 (16%)	61 (2%)	9	48

5 of 21 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	133	GLY	11
1	A	16	GLU	7
1	A	54	ALA	6
1	A	150	SER	5
1	A	132	ASP	4

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	115/131 (88%)	95±2 (83±2%)	20±2 (17±2%)	4	39
All	All	2300/2620 (88%)	1900 (83%)	400 (17%)	4	39

5 of 75 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	23	LYS	20
1	A	90	TYR	19
1	A	98	LEU	17
1	A	79	LEU	16
1	A	20	MET	13

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 88% for the well-defined parts and 81% 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 (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	1660
Number of shifts mapped to atoms	1497
Number of unparsed shifts	0
Number of shifts with mapping errors	163
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	20

The following errors were found when reading this chemical shift list.

- Chemical shift has been reported more than once. First 5 (of 163) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	69	LEU	HD11	0.789	.	.
1	A	69	LEU	HD12	0.789	.	.
1	A	69	LEU	HD13	0.789	.	.
1	A	7	LEU	HD11	0.384	.	.
1	A	7	LEU	HD12	0.384	.	.
1	A	7	LEU	HD13	0.384	.	.
1	A	55	LEU	HD11	0.800	.	.
1	A	55	LEU	HD12	0.800	.	.
1	A	55	LEU	HD13	0.800	.	.
1	A	79	LEU	HD11	0.785	.	.
1	A	79	LEU	HD12	0.785	.	.
1	A	79	LEU	HD13	0.785	.	.
1	A	9	LEU	HD11	0.784	.	.
1	A	9	LEU	HD12	0.784	.	.

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	9	LEU	HD13	0.784	.	.
1	A	33	LEU	HD11	0.840	.	.
1	A	33	LEU	HD12	0.840	.	.
1	A	33	LEU	HD13	0.840	.	.
1	A	98	LEU	HD11	0.731	.	.
1	A	98	LEU	HD12	0.731	.	.
1	A	98	LEU	HD13	0.731	.	.
1	A	81	LEU	HD11	0.798	.	.
1	A	81	LEU	HD12	0.798	.	.
1	A	81	LEU	HD13	0.798	.	.

The following assigned chemical shifts were not mapped to the molecules present in the coordinate file.

- No matching atom found in the structure. First 5 (of 163) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	55	LEU	HB2	1.613	.	.
1	A	58	ILE	HG12	0.899	.	.
1	A	59	GLN	HB2	1.969	.	.
1	A	59	GLN	HG2	2.408	.	.
1	A	61	ASN	HB2	2.502	.	.
1	A	65	PRO	HB2	1.918	.	.
1	A	66	GLU	HB2	1.929	.	.
1	A	66	GLU	HG2	2.167	.	.
1	A	68	GLN	HB2	1.711	.	.
1	A	69	LEU	HB2	1.416	.	.
1	A	70	ASP	HB2	2.311	.	.
1	A	71	ASN	HB2	2.624	.	.
1	A	72	ILE	HG12	0.722	.	.
1	A	73	ASP	HB2	2.351	.	.
1	A	74	TYR	HB2	2.836	.	.
1	A	75	ASN	HB2	2.864	.	.
1	A	76	ASN	HB2	2.275	.	.
1	A	77	TYR	HB2	3.148	.	.
1	A	78	ASP	HB2	2.873	.	.
1	A	79	LEU	HB2	1.821	.	.
1	A	80	ILE	HG12	1.725	.	.
1	A	81	LEU	HB2	1.884	.	.
1	A	85	PRO	HB2	1.969	.	.
1	A	87	TRP	HB2	3.196	.	.

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	88	SER	HB2	3.911	.	.
1	A	91	PRO	HB2	1.9	.	.
1	A	91	PRO	HG2	1.24	.	.
1	A	95	ILE	HG12	1.699	.	.
1	A	96	LYS	HB2	0.778	.	.
1	A	98	LEU	HB2	1.1	.	.
1	A	99	LEU	HB2	0.962	.	.
1	A	100	ASP	HB2	2.701	.	.
1	A	101	GLN	HB2	2.311	.	.
1	A	101	GLN	HG2	2.469	.	.
1	A	102	MET	HB2	1.62	.	.
1	A	102	MET	HG2	1.875	.	.
1	A	103	LYS	HB2	1.85	.	.
1	A	104	ASN	HB2	2.89	.	.
1	A	108	GLU	HB2	2.34	.	.
1	A	108	GLU	HG2	2.463	.	.
1	A	111	SER	HB2	2.564	.	.
1	A	112	PHE	HB2	3.492	.	.
1	A	113	PHE	HB2	2.215	.	.
1	A	115	SER	HB2	3.787	.	.
1	A	119	ASN	HB2	2.861	.	.
1	A	120	HIS	HB2	2.903	.	.
1	A	121	LYS	HB2	1.732	.	.
1	A	123	TYR	HB2	2.159	.	.
1	A	125	SER	HB2	3.485	.	.
1	A	126	HIS	HB2	2.366	.	.
1	A	127	PHE	HB2	2.241	.	.
1	A	128	ASN	HB2	2.83	.	.
1	A	129	GLU	HB2	2.24	.	.
1	A	129	GLU	HG2	2.464	.	.
1	A	130	TRP	HB2	2.773	.	.
1	A	132	ASP	HB2	2.807	.	.
1	A	134	LEU	HB2	1.826	.	.
1	A	135	ASN	HB2	3.177	.	.
1	A	137	ILE	HG12	0.668	.	.
1	A	141	ARG	HB2	1.093	.	.
1	A	141	ARG	HD2	2.979	.	.
1	A	143	ASP	HB2	3.049	.	.
1	A	144	SER	HB2	3.905	.	.
1	A	147	ASP	HB2	2.418	.	.
1	A	148	LYS	HB2	1.709	.	.

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	149	TRP	HB2	2.923	.	.
1	A	150	SER	HB2	3.694	.	.
1	A	151	LYS	HG2	1.397	.	.
1	A	151	LYS	HE2	2.902	.	.
1	A	151	LYS	HD2	1.604	.	.
1	A	121	LYS	HG2	1.267	.	.
1	A	121	LYS	HE2	2.937	.	.
1	A	121	LYS	HD2	1.597	.	.
1	A	96	LYS	HG2	-0.677	.	.
1	A	96	LYS	HD2	0.032	.	.
1	A	96	LYS	HE2	2.029	.	.
1	A	44	ASP	HB2	2.39	.	.
1	A	148	LYS	HD2	1.581	.	.
1	A	103	LYS	HE2	2.941	.	.
1	A	148	LYS	HG2	1.374	.	.
1	A	148	LYS	HE2	2.935	.	.
1	A	151	LYS	HB2	1.791	.	.
1	A	94	PRO	HD2	3.983	.	.
1	A	5	LYS	HB2	1.898	.	.
1	A	5	LYS	HG2	1.47	.	.
1	A	10	TYR	HB2	2.491	.	.
1	A	11	TYR	HB2	2.482	.	.
1	A	9	LEU	HB2	1.576	.	.
1	A	85	PRO	HD2	3.921	.	.
1	A	64	PHE	HB2	2.614	.	.
1	A	39	SER	HB2	3.715	.	.
1	A	4	LYS	HB2	1.487	.	.
1	A	4	LYS	HG2	1.284	.	.
1	A	4	LYS	HD2	1.523	.	.
1	A	8	ILE	HG12	1.59	.	.
1	A	19	LYS	HB2	1.732	.	.
1	A	19	LYS	HG2	1.352	.	.
1	A	19	LYS	HD2	1.609	.	.
1	A	19	LYS	HE2	2.854	.	.
1	A	22	GLU	HB2	2.026	.	.
1	A	22	GLU	HG2	1.979	.	.
1	A	23	LYS	HG2	1.182	.	.
1	A	23	LYS	HE2	2.744	.	.
1	A	25	ASN	HB2	2.652	.	.
1	A	26	SER	HB2	3.916	.	.
1	A	29	LYS	HB2	1.665	.	.

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	29	LYS	HG2	1.401	.	.
1	A	29	LYS	HE2	2.898	.	.
1	A	30	ASP	HB2	2.96	.	.
1	A	31	SER	HB2	3.778	.	.
1	A	32	GLU	HB2	1.918	.	.
1	A	32	GLU	HG2	2.356	.	.
1	A	33	LEU	HB2	1.772	.	.
1	A	34	LYS	HB2	1.684	.	.
1	A	34	LYS	HG2	1.269	.	.
1	A	34	LYS	HD2	1.499	.	.
1	A	34	LYS	HE2	2.798	.	.
1	A	35	GLU	HB2	1.656	.	.
1	A	24	ILE	HG12	0.361	.	.
1	A	37	LYS	HB2	1.676	.	.
1	A	37	LYS	HG2	1.314	.	.
1	A	37	LYS	HE2	2.901	.	.
1	A	40	GLU	HB2	1.98	.	.
1	A	40	GLU	HG2	2.305	.	.
1	A	105	TYR	HB2	3.028	.	.
1	A	84	SER	HB2	3.711	.	.
1	A	90	TYR	HB2	2.803	.	.
1	A	106	ARG	HB2	1.532	.	.
1	A	106	ARG	HD2	3.132	.	.
1	A	106	ARG	HG2	1.497	.	.
1	A	18	LYS	HB2	1.538	.	.
1	A	23	LYS	HB2	1.729	.	.
1	A	27	GLU	HB2	2.12	.	.
1	A	27	GLU	HG2	2.517	.	.
1	A	28	ILE	HG12	1.594	.	.
1	A	35	GLU	HG2	1.672	.	.
1	A	48	TYR	HB2	3.006	.	.
1	A	65	PRO	HD2	3.375	.	.
1	A	65	PRO	HG2	1.712	.	.
1	A	82	ILE	HG12	1.556	.	.
1	A	94	PRO	HG2	0.626	.	.
1	A	67	ILE	HG12	1.205	.	.
1	A	103	LYS	HD2	1.599	.	.
1	A	103	LYS	HG2	1.495	.	.
1	A	142	ASP	HB2	2.666	.	.
1	A	68	GLN	HG2	2.109	.	.
1	A	145	GLU	HG2	1.761	.	.

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	145	GLU	HB2	1.368	.	.
1	A	51	SER	HB2	3.682	.	.
1	A	7	LEU	HB2	-0.216	.	.
1	A	29	LYS	HD2	1.604	.	.
1	A	23	LYS	HD2	1.369	.	.
1	A	18	LYS	HG2	0.924	.	.
1	A	18	LYS	HE2	2.892	.	.
1	A	18	LYS	HD2	1.603	.	.
1	A	91	PRO	HD2	2.55	.	.
1	A	94	PRO	HB2	0.775	.	.
1	A	4	LYS	HE2	2.794	.	.
1	A	5	LYS	HD2	1.651	.	.
1	A	5	LYS	HE2	2.828	.	.
1	A	20	MET	HB2	1.964	.	.
1	A	37	LYS	HD2	1.601	.	.
1	A	141	ARG	HG2	1.09	.	.

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	136	2.65 ± 0.30	Should be applied
$^{13}C_{\beta}$	128	2.71 ± 0.15	Should be applied
$^{13}C'$	99	2.76 ± 0.12	Should be applied
^{15}N	125	0.08 ± 0.37	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 88%, i.e. 1560 atoms were assigned a chemical shift out of a possible 1781. 0 out of 18 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	588/654 (90%)	250/265 (94%)	221/262 (84%)	117/127 (92%)
Sidechain	870/974 (89%)	593/629 (94%)	265/312 (85%)	12/33 (36%)
Aromatic	102/153 (67%)	62/74 (84%)	37/74 (50%)	3/5 (60%)
Overall	1560/1781 (88%)	905/968 (93%)	523/648 (81%)	132/165 (80%)

7.1.4 Statistically unusual chemical shifts (i)

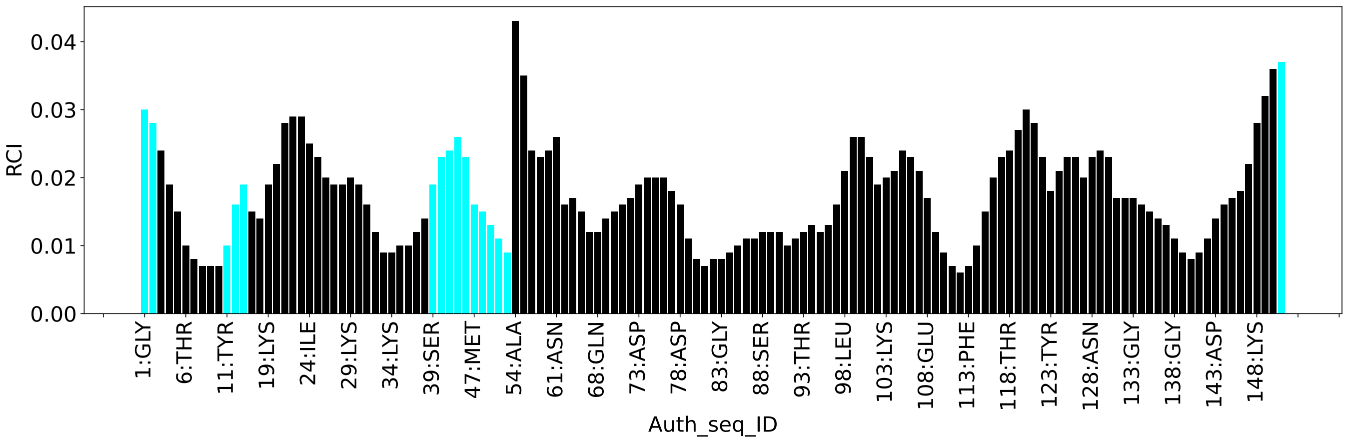
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	96	LYS	HB3	-0.67	0.46 – 3.04	-9.4
1	A	96	LYS	HD3	-0.29	0.54 – 2.65	-8.9
1	A	145	GLU	HB3	0.15	0.95 – 3.05	-8.8
1	A	96	LYS	HG2	-0.68	0.13 – 2.61	-8.2
1	A	96	LYS	HD2	0.03	0.58 – 2.64	-7.7
1	A	110	ALA	HB1	-0.41	0.14 – 2.58	-7.2
1	A	110	ALA	HB2	-0.41	0.14 – 2.58	-7.2
1	A	110	ALA	HB3	-0.41	0.14 – 2.58	-7.2
1	A	10	TYR	CD1	122.96	125.84 – 139.60	-7.1
1	A	96	LYS	HG3	-0.45	0.04 – 2.67	-6.9
1	A	83	GLY	HA2	1.93	2.15 – 5.77	-5.6
1	A	7	LEU	HB2	-0.22	-0.07 – 3.30	-5.4
1	A	92	ALA	HB1	0.06	0.14 – 2.58	-5.3
1	A	92	ALA	HB2	0.06	0.14 – 2.58	-5.3
1	A	92	ALA	HB3	0.06	0.14 – 2.58	-5.3
1	A	96	LYS	HE3	1.88	1.92 – 3.89	-5.2
1	A	127	PHE	CE1	123.91	124.17 – 137.29	-5.2
1	A	111	SER	HB2	2.56	2.61 – 5.13	-5.2
1	A	111	SER	HB3	2.44	2.49 – 5.20	-5.2
1	A	149	TRP	CZ3	113.34	113.48 – 129.28	-5.1

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:


RCI
0.00
0.01
0.02
0.03
0.04
1:GLY
6:THR
11:TYR
19:LYS
24:ILE
29:LYS
34:LYS
39:SER
47:MET
54:ALA
61:ASN
68:GLN
73:ASP
78:ASP
83:GLY
88:SER
93:THR
98:LEU
103:LYS
108:GLU
113:PHE
118:THR
123:TYR
128:ASN
133:GLY
138:GLY
143:ASP
148:LYS
Auth_seq_ID

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	2308
Intra-residue (|i-j|=0)	596
Sequential (|i-j|=1)	624
Medium range (|i-j|>1 and |i-j|<5)	383
Long range (|i-j|≥5)	705
Inter-chain	0
Hydrogen bond restraints	0
Disulfide bond restraints	0
Total dihedral-angle restraints	0
Number of unmapped restraints	665
Number of restraints per residue	15.3
Number of long range restraints per residue[1]	4.7

[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)	23.9	0.2
0.2-0.5 (Medium)	61.5	0.5
>0.5 (Large)	95.2	3.57

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

Dihedral-angle violations less than 1° are not included in the calculation.There are no dihedral-angle violations

9 Distance violation analysis (i)

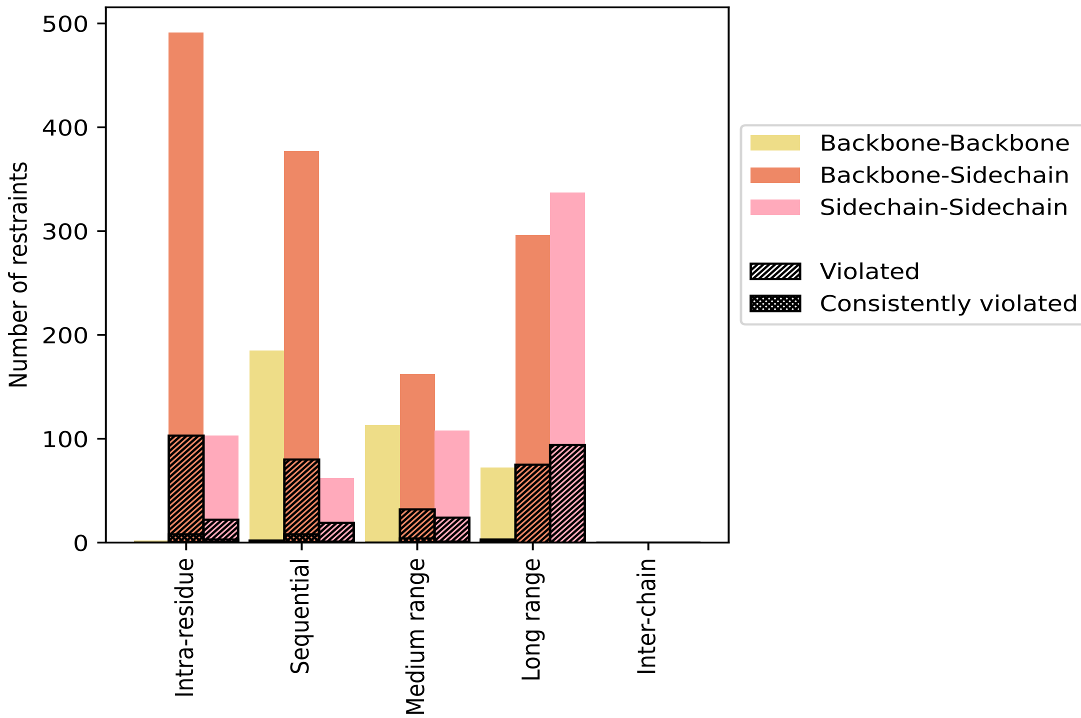
9.1 Summary of distance violations (i)

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)	596	25.8	125	21.0	5.4	11	1.8	0.5
Backbone-Backbone	2	0.1	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	491	21.3	103	21.0	4.5	8	1.6	0.3
Sidechain-Sidechain	103	4.5	22	21.4	1.0	3	2.9	0.1
Sequential (|i-j|=1)	624	27.0	101	16.2	4.4	9	1.4	0.4
Backbone-Backbone	185	8.0	2	1.1	0.1	0	0.0	0.0
Backbone-Sidechain	377	16.3	80	21.2	3.5	8	2.1	0.3
Sidechain-Sidechain	62	2.7	19	30.6	0.8	1	1.6	0.0
Medium range (|i-j|>1 & |i-j|<5)	383	16.6	56	14.6	2.4	5	1.3	0.2
Backbone-Backbone	113	4.9	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	162	7.0	32	19.8	1.4	4	2.5	0.2
Sidechain-Sidechain	108	4.7	24	22.2	1.0	1	0.9	0.0
Long range (|i-j|≥5)	705	30.5	172	24.4	7.5	1	0.1	0.0
Backbone-Backbone	72	3.1	3	4.2	0.1	1	1.4	0.0
Backbone-Sidechain	296	12.8	75	25.3	3.2	0	0.0	0.0
Sidechain-Sidechain	337	14.6	94	27.9	4.1	0	0.0	0.0
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	2308	100.0	454	19.7	19.7	26	1.1	1.1
Backbone-Backbone	372	16.1	5	1.3	0.2	1	0.3	0.0
Backbone-Sidechain	1326	57.5	290	21.9	12.6	20	1.5	0.9
Sidechain-Sidechain	610	26.4	159	26.1	6.9	5	0.8	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	56	48	25	52	0	181	0.69	3.06	0.51	0.55
2	52	48	19	36	0	155	0.65	2.48	0.46	0.53
3	56	45	23	60	0	184	0.68	2.32	0.48	0.6
4	41	39	26	60	0	166	0.71	3.09	0.48	0.62
5	55	47	24	66	0	192	0.7	2.38	0.53	0.56
6	52	45	22	54	0	173	0.68	2.91	0.56	0.47
7	54	44	21	48	0	167	0.66	3.57	0.55	0.51
8	57	46	24	61	0	188	0.7	2.85	0.56	0.52
9	58	49	24	57	0	188	0.69	2.57	0.51	0.54
10	51	45	27	52	0	175	0.71	2.88	0.54	0.54
11	51	45	26	65	0	187	0.73	2.82	0.6	0.54

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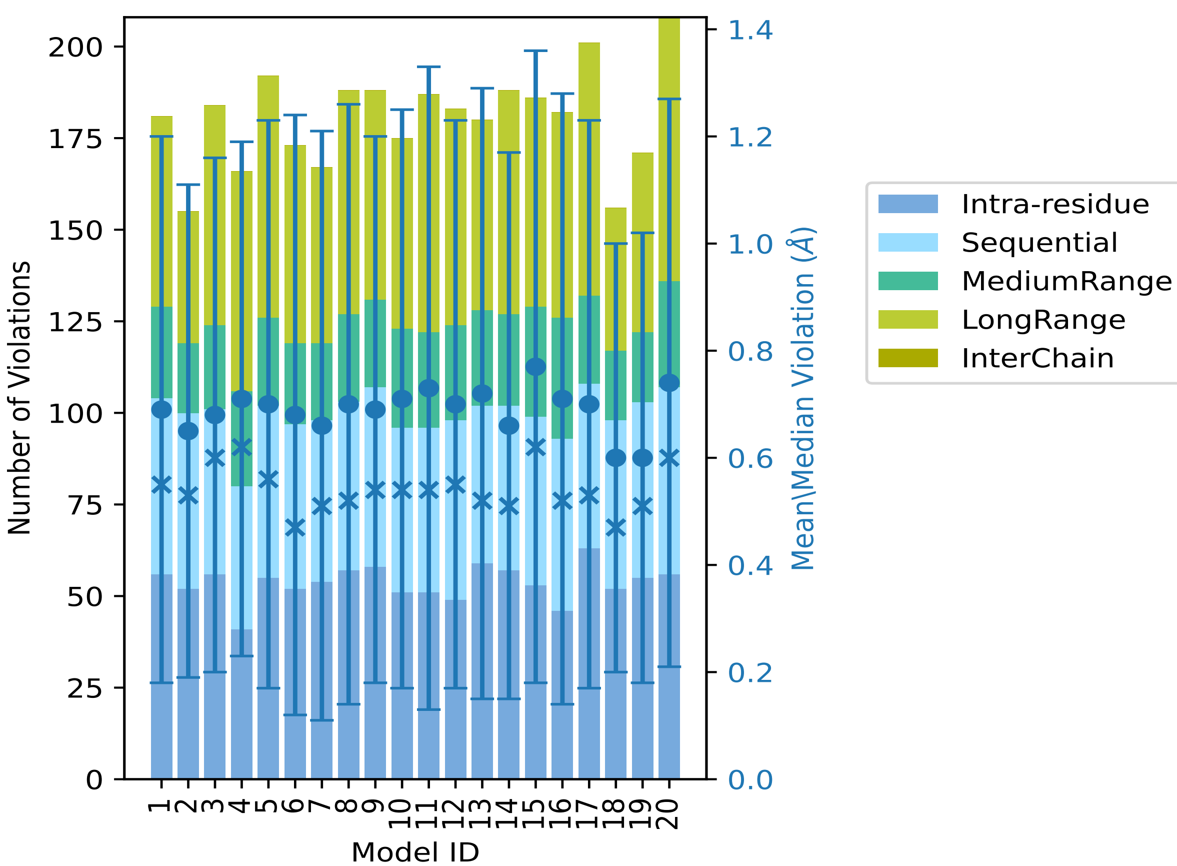
Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
12	49	49	26	59	0	183	0.7	2.83	0.53	0.55
13	59	43	26	52	0	180	0.72	3.05	0.57	0.52
14	57	45	25	61	0	188	0.66	2.96	0.51	0.51
15	53	46	30	57	0	186	0.77	3.27	0.59	0.62
16	46	47	33	56	0	182	0.71	3.13	0.57	0.52
17	63	45	24	69	0	201	0.7	2.89	0.53	0.53
18	52	46	19	39	0	156	0.6	2.07	0.4	0.47
19	55	48	19	49	0	171	0.6	1.87	0.42	0.51
20	56	51	29	72	0	208	0.74	2.94	0.53	0.6

[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

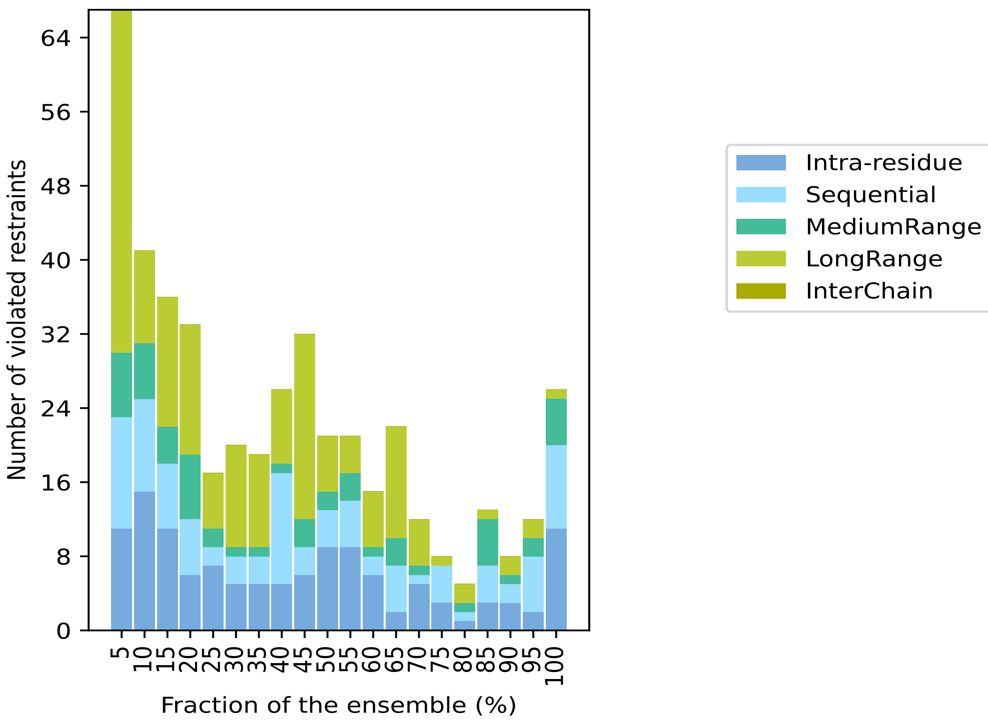
9.3 Distance violation statistics for the ensemble (i)

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, 1854(IR:471, SQ:523, MR:327, LR:533, 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]	%
11	12	7	37	0	67	1	5.0
15	10	6	10	0	41	2	10.0
11	7	4	14	0	36	3	15.0
6	6	7	14	0	33	4	20.0
7	2	2	6	0	17	5	25.0
5	3	1	11	0	20	6	30.0
5	3	1	10	0	19	7	35.0
5	12	1	8	0	26	8	40.0
6	3	3	20	0	32	9	45.0
9	4	2	6	0	21	10	50.0
9	5	3	4	0	21	11	55.0
6	2	1	6	0	15	12	60.0
2	5	3	12	0	22	13	65.0
5	1	1	5	0	12	14	70.0
3	4	0	1	0	8	15	75.0
1	1	1	2	0	5	16	80.0
3	4	5	1	0	13	17	85.0
3	2	1	2	0	8	18	90.0
2	6	2	2	0	12	19	95.0
11	9	5	1	0	26	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

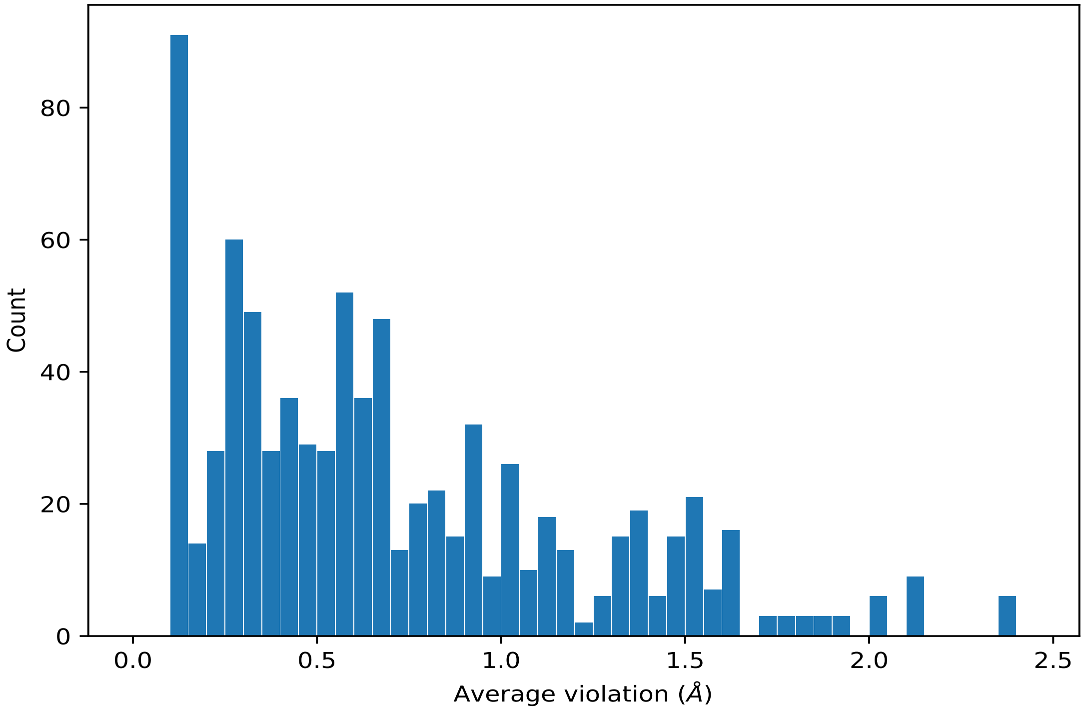
9.3.1 Bar graph : Distance violation statistics for the ensemble (i)



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.

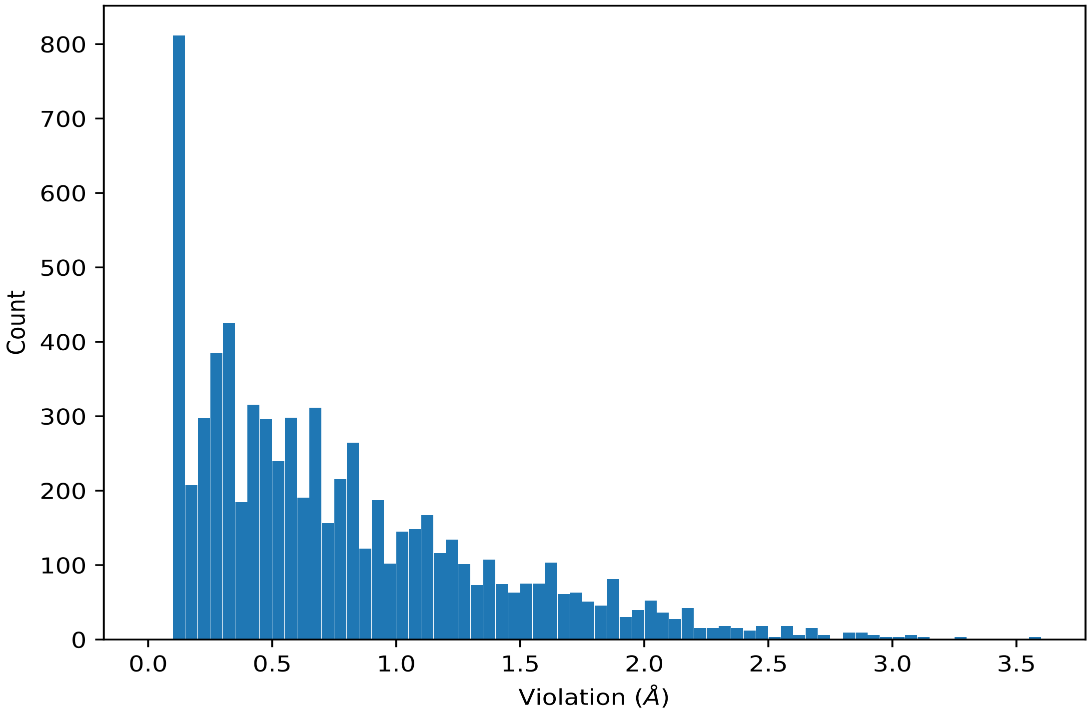
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	20	2.13	1.09	2.82
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	20	2.13	1.09	2.82
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	20	2.13	1.09	2.82
(1,577)	1:A:97:THR:H	1:A:99:LEU:HB3	20	1.57	0.22	1.64
(1,244)	1:A:98:LEU:H	1:A:99:LEU:HB3	20	1.39	0.12	1.43
(1,1219)	1:A:94:PRO:HD3	1:A:95:ILE:HG13	20	1.18	0.17	1.23
(1,1790)	1:A:96:LYS:HA	1:A:99:LEU:HB3	20	1.03	0.24	1.15
(1,780)	1:A:83:GLY:HA3	1:A:111:SER:HA	20	1.0	0.25	1.05
(1,1156)	1:A:129:GLU:H	1:A:130:TRP:HB3	20	0.98	0.18	0.96
(1,1161)	1:A:67:ILE:HA	1:A:68:GLN:HB3	20	0.82	0.1	0.82
(1,653)	1:A:135:ASN:H	1:A:135:ASN:HD22	20	0.77	0.05	0.77
(1,16)	1:A:28:ILE:HG13	1:A:29:LYS:H	20	0.71	0.07	0.74

[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)
(1,1118)	1:A:4:LYS:HD3	1:A:79:LEU:HD21	7	3.57
(1,1118)	1:A:4:LYS:HD3	1:A:79:LEU:HD22	7	3.57
(1,1118)	1:A:4:LYS:HD3	1:A:79:LEU:HD23	7	3.57
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	15	3.27
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	15	3.27
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	15	3.27
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	16	3.13
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	16	3.13
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	16	3.13
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	4	3.09
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	4	3.09

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	4	3.09
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	1	3.06
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	1	3.06
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	1	3.06
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	13	3.05
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	13	3.05
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	13	3.05
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	14	2.96
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	14	2.96
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	14	2.96
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	20	2.94
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	20	2.94
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	20	2.94
(1,1634)	1:A:86:VAL:HG11	1:A:89:GLY:HA3	6	2.91
(1,1634)	1:A:86:VAL:HG12	1:A:89:GLY:HA3	6	2.91
(1,1634)	1:A:86:VAL:HG13	1:A:89:GLY:HA3	6	2.91
(1,1637)	1:A:36:VAL:HG11	1:A:67:ILE:HB	17	2.89

10 Dihedral-angle violation analysis (i)

No dihedral-angle restraints found