Full wwPDB NMR Structure Validation Report (i)

Jun 3, 2023 – 02:03 PM EDT

PDB ID	:	6NAN
BMRB ID	:	30549
Title	:	NMR structure determination of Ixolaris and Factor X interaction reveals a noncanonical mechanism of Kunitz inhibition
Authors	:	De Paula, V.S.; Sgourakis, N.G.; Francischetti, I.M.B.; Almeida, F.C.L.; Monteiro, R.Q.; Valente, A.P.
Deposited on	:	2018-12-06

This is a Full wwPDB NMR Structure Validation 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:

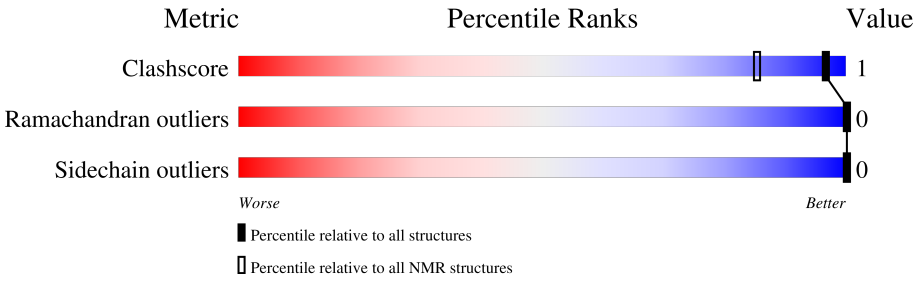
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal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

1 Overall quality at a glance

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

The overall completeness of chemical shifts assignment is 77%.

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	140	76% · 6% 16%

2 Ensemble composition and analysis

This entry contains 10 models. Model 4 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:14-A:26, A:31-A:70 (53)	0.54	7
2	A:74-A:129 (56)	0.45	4

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, 3, 4, 5, 8, 10
2	2, 6, 7, 9

3 Entry composition

There is only 1 type of molecule in this entry. The entry contains 1721 atoms, of which 807 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Ixolaris.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	118	1721	552	807	154	197	11	0

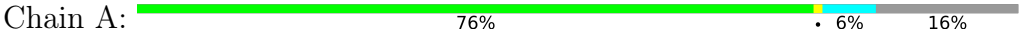
4 Residue-property plots (i)

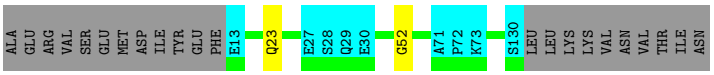
4.1 Average score per residue in the NMR ensemble

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: Ixolaris

Chain A:





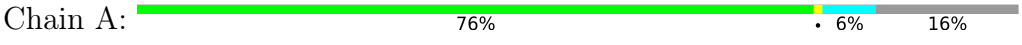
4.2 Scores per residue for each member of the ensemble

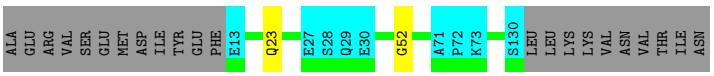
Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Ixolaris

Chain A:

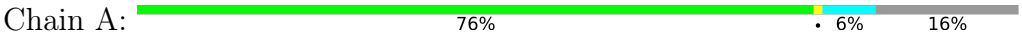


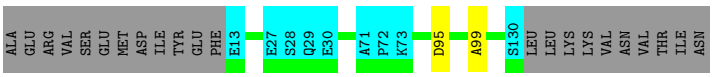


4.2.2 Score per residue for model 2

• Molecule 1: Ixolaris

Chain A:

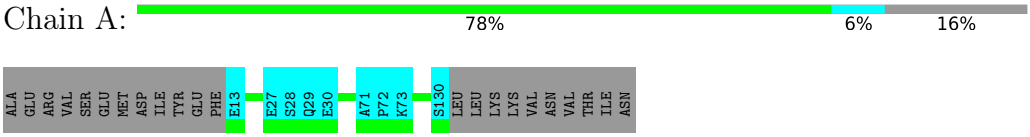




4.2.3 Score per residue for model 3

• Molecule 1: Ixolaris

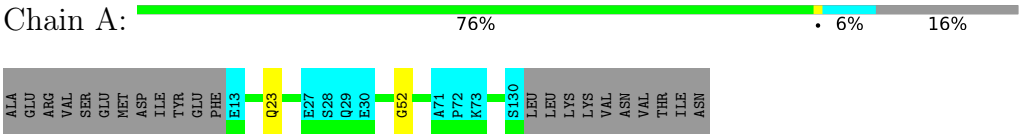
Chain A:



4.2.4 Score per residue for model 4 (medoid)

• Molecule 1: Ixolaris

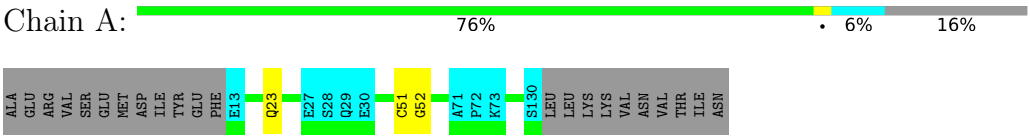
Chain A:



4.2.5 Score per residue for model 5

• Molecule 1: Ixolaris

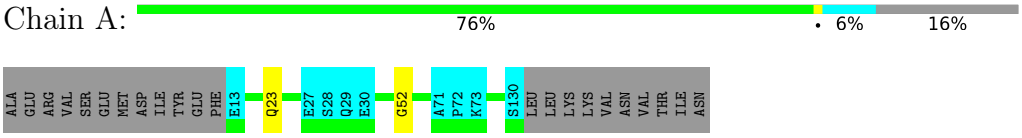
Chain A:



4.2.6 Score per residue for model 6

• Molecule 1: Ixolaris

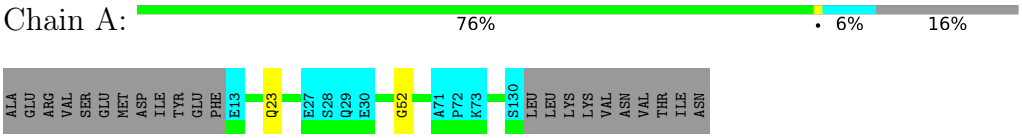
Chain A:



4.2.7 Score per residue for model 7

• Molecule 1: Ixolaris

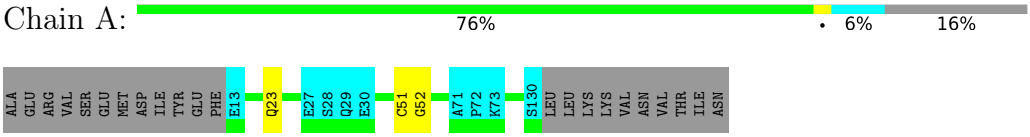
Chain A:



4.2.8 Score per residue for model 8

• Molecule 1: Ixolaris

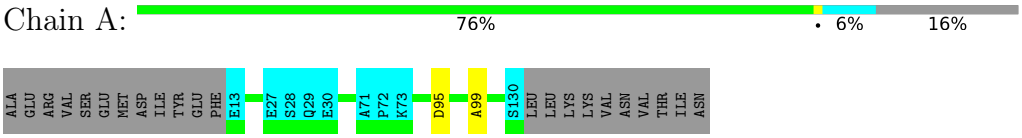
Chain A:



4.2.9 Score per residue for model 9

• Molecule 1: Ixolaris

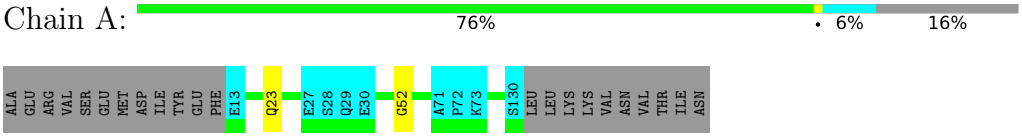
Chain A:



4.2.10 Score per residue for model 10

• Molecule 1: Ixolaris

Chain A:



5 Refinement protocol and experimental data overview

The models were refined using the following method: *na*.

Of the 100 calculated structures, 10 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
CS-ROSETTA	structure calculation	RASREC

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	1401
Number of shifts mapped to atoms	1134
Number of unparsed shifts	0
Number of shifts with mapping errors	267
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	77%

6 Model quality

6.1 Standard geometry

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	845	746	746	1±1
All	All	8450	7460	7460	11

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.

All unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:51:CYS:SG	1:A:51:CYS:O	0.54	2.66	5	2
1:A:23:GLN:NE2	1:A:52:GLY:O	0.49	2.46	7	7
1:A:95:ASP:O	1:A:99:ALA:N	0.43	2.51	9	2

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	109/140 (78%)	100±2 (91±2%)	9±2 (9±2%)	0±0 (0±0%)	100	100
All	All	1090/1400 (78%)	997 (91%)	93 (9%)	0 (0%)	100	100

There are no Ramachandran outliers.

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	94/123 (76%)	94±0 (100±0%)	0±0 (0±0%)	100	100
All	All	940/1230 (76%)	940 (100%)	0 (0%)	100	100

There are no protein residues with a non-rotameric sidechain to report.

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 monosaccharides in this entry.

6.6 Ligand geometry

There are no ligands in this entry.

6.7 Other polymers

There are no such molecules in this entry.

6.8 Polymer linkage issues (i)

There are no chain breaks in this entry.

7 Chemical shift validation

The completeness of assignment taking into account all chemical shift lists is 77% for the well-defined parts and 78% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *starch_ output*

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	1401
Number of shifts mapped to atoms	1134
Number of unparsed shifts	0
Number of shifts with mapping errors	267
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	4

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

- No matching atom found in the structure. All 267 occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	1	ALA	C	178.143	0.3	1
1	A	1	ALA	CA	53.413	0.3	1
1	A	1	ALA	CB	20.083	0.3	1
1	A	1	ALA	H	8.524	0.020	1
1	A	1	ALA	HA	4.38	0.020	1
1	A	1	ALA	HB1	1.457	0.020	1
1	A	1	ALA	HB2	1.457	0.020	1
1	A	1	ALA	HB3	1.457	0.020	1
1	A	1	ALA	N	125.481	0.3	1
1	A	2	GLU	C	176.31	0.3	1
1	A	2	GLU	CA	57.483	0.3	1
1	A	2	GLU	CB	30.659	0.3	1
1	A	2	GLU	CG	36.95	0.3	1
1	A	2	GLU	H	8.276	0.020	1

Continued on next page...

Continued from previous page...

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	2	GLU	HA	4.406	0.020	1
1	A	2	GLU	HB2	2.195	0.020	1
1	A	2	GLU	HB3	2.195	0.020	1
1	A	2	GLU	HG2	2.645	0.020	1
1	A	2	GLU	HG3	2.645	0.020	1
1	A	2	GLU	N	118.931	0.3	1
1	A	3	ARG	C	176.174	0.3	1
1	A	3	ARG	CA	57.22	0.3	1
1	A	3	ARG	CB	32.962	0.3	1
1	A	3	ARG	CG	27.91	0.3	1
1	A	3	ARG	CD	44.107	0.3	1
1	A	3	ARG	HA	4.389	0.020	1
1	A	3	ARG	HB2	2.021	0.020	1
1	A	3	ARG	HB3	2.021	0.020	1
1	A	3	ARG	HG2	1.629	0.020	1
1	A	3	ARG	HD2	3.25	0.020	1
1	A	4	VAL	C	176.912	0.3	1
1	A	4	VAL	CA	63.0	0.3	1
1	A	4	VAL	CB	33.253	0.3	1
1	A	4	VAL	CG1	21.748	0.3	1
1	A	4	VAL	CG2	20.442	0.3	1
1	A	4	VAL	H	8.278	0.020	1
1	A	4	VAL	HA	4.347	0.020	1
1	A	4	VAL	HB	2.174	0.020	1
1	A	4	VAL	HG11	0.99	0.020	1
1	A	4	VAL	HG12	0.99	0.020	1
1	A	4	VAL	HG13	0.99	0.020	1
1	A	4	VAL	HG21	0.99	0.020	1
1	A	4	VAL	HG22	0.99	0.020	1
1	A	4	VAL	HG23	0.99	0.020	1
1	A	4	VAL	N	120.923	0.3	1
1	A	5	SER	C	176.93	0.3	1
1	A	5	SER	CA	59.301	0.3	1
1	A	5	SER	CB	64.826	0.3	1
1	A	5	SER	H	8.457	0.020	1
1	A	5	SER	HA	4.562	0.020	1
1	A	5	SER	HB2	3.933	0.020	1
1	A	5	SER	HB3	3.933	0.020	1
1	A	5	SER	N	118.842	0.3	1
1	A	6	GLU	C	176.921	0.3	1
1	A	6	GLU	CA	57.425	0.3	1

Continued on next page...

Continued from previous page...

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	6	GLU	CB	30.625	0.3	1
1	A	6	GLU	CG	36.418	0.3	1
1	A	6	GLU	H	8.496	0.020	1
1	A	6	GLU	HA	4.406	0.020	1
1	A	6	GLU	HB2	2.11	0.020	1
1	A	6	GLU	HB3	2.11	0.020	1
1	A	6	GLU	HG2	2.276	0.020	2
1	A	6	GLU	HG3	2.398	0.020	2
1	A	6	GLU	N	122.475	0.3	1
1	A	7	MET	C	176.481	0.3	1
1	A	7	MET	CA	56.367	0.3	1
1	A	7	MET	CB	34.335	0.3	1
1	A	7	MET	CG	32.89	0.3	1
1	A	7	MET	CE	17.75	0.3	1
1	A	7	MET	H	8.334	0.020	1
1	A	7	MET	HA	4.584	0.020	1
1	A	7	MET	HB2	2.136	0.020	1
1	A	7	MET	HB3	2.136	0.020	1
1	A	7	MET	HG2	2.657	0.020	1
1	A	7	MET	HG3	2.657	0.020	1
1	A	7	MET	HE1	1.98	0.020	1
1	A	7	MET	HE2	1.98	0.020	1
1	A	7	MET	HE3	1.98	0.020	1
1	A	7	MET	N	120.11	0.3	1
1	A	8	ASP	C	176.052	0.3	1
1	A	8	ASP	CA	55.275	0.3	1
1	A	8	ASP	CB	42.204	0.3	1
1	A	8	ASP	H	8.335	0.020	1
1	A	8	ASP	HA	4.735	0.020	1
1	A	8	ASP	HB2	2.664	0.020	1
1	A	8	ASP	HB3	2.664	0.020	1
1	A	8	ASP	N	121.388	0.3	1
1	A	9	ILE	C	175.704	0.3	1
1	A	9	ILE	CA	62.186	0.3	1
1	A	9	ILE	CB	40.021	0.3	1
1	A	9	ILE	CG1	26.936	0.3	1
1	A	9	ILE	CG2	18.395	0.3	1
1	A	9	ILE	CD1	14.006	0.3	1
1	A	9	ILE	H	8.038	0.020	1
1	A	9	ILE	HA	4.248	0.020	1
1	A	9	ILE	HB	1.809	0.020	1

Continued on next page...

Continued from previous page...

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	9	ILE	HG12	1.11	0.020	1
1	A	9	ILE	HG13	1.11	0.020	1
1	A	9	ILE	HG21	0.898	0.020	1
1	A	9	ILE	HG22	0.898	0.020	1
1	A	9	ILE	HG23	0.898	0.020	1
1	A	9	ILE	HD11	0.848	0.020	1
1	A	9	ILE	HD12	0.848	0.020	1
1	A	9	ILE	HD13	0.848	0.020	1
1	A	9	ILE	N	119.22	0.3	1
1	A	10	TYR	C	175.736	0.3	1
1	A	10	TYR	CA	57.81	0.3	1
1	A	10	TYR	CB	41.463	0.3	1
1	A	10	TYR	H	8.216	0.020	1
1	A	10	TYR	HA	5.006	0.020	1
1	A	10	TYR	HB2	2.845	0.020	1
1	A	10	TYR	HB3	2.845	0.020	1
1	A	10	TYR	N	122.149	0.3	1
1	A	11	GLU	C	176.008	0.3	1
1	A	11	GLU	CA	55.214	0.3	1
1	A	11	GLU	CB	32.725	0.3	1
1	A	11	GLU	CG	35.819	0.3	1
1	A	11	GLU	H	8.697	0.020	1
1	A	11	GLU	HA	4.582	0.020	1
1	A	11	GLU	HB2	2.0	0.020	1
1	A	11	GLU	HB3	2.0	0.020	1
1	A	11	GLU	HG2	2.222	0.020	1
1	A	11	GLU	HG3	2.222	0.020	1
1	A	11	GLU	N	120.149	0.3	1
1	A	12	PHE	C	178.321	0.3	1
1	A	12	PHE	CA	57.885	0.3	1
1	A	12	PHE	CB	41.027	0.3	1
1	A	12	PHE	HA	5.003	0.020	1
1	A	12	PHE	HB2	2.824	0.020	1
1	A	12	PHE	HB3	2.824	0.020	1
1	A	131	LEU	C	177.732	0.3	1
1	A	131	LEU	CA	58.175	0.3	1
1	A	131	LEU	CB	44.291	0.3	1
1	A	131	LEU	CG	28.009	0.3	1
1	A	131	LEU	CD1	25.477	0.3	1
1	A	131	LEU	H	8.745	0.020	1
1	A	131	LEU	HA	4.176	0.020	1

Continued on next page...

Continued from previous page...

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	131	LEU	HB2	1.762	0.020	1
1	A	131	LEU	HB3	1.762	0.020	1
1	A	131	LEU	HG	1.604	0.020	1
1	A	131	LEU	HD11	1.008	0.020	1
1	A	131	LEU	HD12	1.008	0.020	1
1	A	131	LEU	HD13	1.008	0.020	1
1	A	131	LEU	N	123.561	0.3	1
1	A	132	LEU	C	178.018	0.3	1
1	A	132	LEU	CA	54.408	0.3	1
1	A	132	LEU	CB	44.42	0.3	1
1	A	132	LEU	CG	27.535	0.3	1
1	A	132	LEU	CD1	25.29	0.3	1
1	A	132	LEU	H	7.719	0.020	1
1	A	132	LEU	HA	4.922	0.020	1
1	A	132	LEU	HB2	1.667	0.020	1
1	A	132	LEU	HB3	1.667	0.020	1
1	A	132	LEU	HG	1.597	0.020	1
1	A	132	LEU	HD11	1.364	0.020	1
1	A	132	LEU	HD12	1.364	0.020	1
1	A	132	LEU	HD13	1.364	0.020	1
1	A	132	LEU	N	115.053	0.3	1
1	A	133	LYS	C	176.325	0.3	1
1	A	133	LYS	CA	56.129	0.3	1
1	A	133	LYS	CB	35.193	0.3	1
1	A	133	LYS	CG	25.331	0.3	1
1	A	133	LYS	CD	30.361	0.3	1
1	A	133	LYS	CE	42.907	0.3	1
1	A	133	LYS	H	8.991	0.020	1
1	A	133	LYS	HA	4.597	0.020	1
1	A	133	LYS	HB2	1.788	0.020	1
1	A	133	LYS	HB3	1.788	0.020	1
1	A	133	LYS	HG2	1.366	0.020	1
1	A	133	LYS	HG3	1.366	0.020	1
1	A	133	LYS	HD2	1.745	0.020	1
1	A	133	LYS	HE2	3.082	0.020	1
1	A	133	LYS	HE3	3.082	0.020	1
1	A	133	LYS	N	121.359	0.3	1
1	A	134	LYS	C	177.219	0.3	1
1	A	134	LYS	CA	56.417	0.3	1
1	A	134	LYS	CB	32.909	0.3	1
1	A	134	LYS	CG	25.565	0.3	1

Continued on next page...

Continued from previous page...

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	134	LYS	CD	27.835	0.3	1
1	A	134	LYS	CE	44.547	0.3	1
1	A	134	LYS	H	8.606	0.020	1
1	A	134	LYS	HA	4.517	0.020	1
1	A	134	LYS	HB2	1.922	0.020	1
1	A	134	LYS	HB3	1.922	0.020	1
1	A	134	LYS	HD2	1.379	0.020	1
1	A	134	LYS	HD3	1.379	0.020	1
1	A	134	LYS	HE2	2.939	0.020	1
1	A	134	LYS	HE3	2.939	0.020	1
1	A	134	LYS	N	124.038	0.3	1
1	A	135	VAL	C	176.268	0.3	1
1	A	135	VAL	CA	62.877	0.3	1
1	A	135	VAL	CB	33.922	0.3	1
1	A	135	VAL	CG1	21.785	0.3	1
1	A	135	VAL	CG2	20.56	0.3	1
1	A	135	VAL	H	8.281	0.020	1
1	A	135	VAL	HA	4.23	0.020	1
1	A	135	VAL	HB	2.1	0.020	1
1	A	135	VAL	HG11	1.02	0.020	1
1	A	135	VAL	HG12	1.02	0.020	1
1	A	135	VAL	HG13	1.02	0.020	1
1	A	135	VAL	HG21	1.02	0.020	1
1	A	135	VAL	HG22	1.02	0.020	1
1	A	135	VAL	HG23	1.02	0.020	1
1	A	135	VAL	N	120.755	0.3	1
1	A	136	ASN	C	175.519	0.3	1
1	A	136	ASN	CA	53.959	0.3	1
1	A	136	ASN	CB	39.926	0.3	1
1	A	136	ASN	H	8.639	0.020	1
1	A	136	ASN	HA	4.824	0.020	1
1	A	136	ASN	HB2	2.821	0.020	1
1	A	136	ASN	HB3	2.821	0.020	1
1	A	136	ASN	HD21	7.665	0.020	1
1	A	136	ASN	HD22	7.014	0.020	1
1	A	136	ASN	N	122.739	0.3	1
1	A	136	ASN	ND2	112.78	0.3	1
1	A	137	VAL	C	176.81	0.3	1
1	A	137	VAL	CA	62.879	0.3	1
1	A	137	VAL	CB	34.221	0.3	1
1	A	137	VAL	CG1	21.803	0.3	1

Continued on next page...

Continued from previous page...

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	137	VAL	CG2	20.876	0.3	1
1	A	137	VAL	H	8.255	0.020	1
1	A	137	VAL	HA	4.304	0.020	1
1	A	137	VAL	HB	2.174	0.020	1
1	A	137	VAL	HG11	0.993	0.020	1
1	A	137	VAL	HG12	0.993	0.020	1
1	A	137	VAL	HG13	0.993	0.020	1
1	A	137	VAL	HG21	0.993	0.020	1
1	A	137	VAL	HG22	0.993	0.020	1
1	A	137	VAL	HG23	0.993	0.020	1
1	A	137	VAL	N	120.648	0.3	1
1	A	138	THR	C	174.754	0.3	1
1	A	138	THR	CA	62.845	0.3	1
1	A	138	THR	CB	70.736	0.3	1
1	A	138	THR	CG2	22.497	0.3	1
1	A	138	THR	H	8.393	0.020	1
1	A	138	THR	HA	4.457	0.020	1
1	A	138	THR	HB	4.234	0.020	1
1	A	138	THR	HG21	1.27	0.020	1
1	A	138	THR	HG22	1.27	0.020	1
1	A	138	THR	HG23	1.27	0.020	1
1	A	138	THR	N	118.918	0.3	1
1	A	139	ILE	C	175.722	0.3	1
1	A	139	ILE	CA	61.705	0.3	1
1	A	139	ILE	CB	39.691	0.3	1
1	A	139	ILE	CG1	27.764	0.3	1
1	A	139	ILE	CG2	18.653	0.3	1
1	A	139	ILE	CD1	13.677	0.3	1
1	A	139	ILE	H	8.327	0.020	1
1	A	139	ILE	HA	4.297	0.020	1
1	A	139	ILE	HB	1.957	0.020	1
1	A	139	ILE	HG12	1.242	0.020	1
1	A	139	ILE	HG13	1.242	0.020	1
1	A	139	ILE	HG21	1.063	0.020	1
1	A	139	ILE	HG22	1.063	0.020	1
1	A	139	ILE	HG23	1.063	0.020	1
1	A	139	ILE	HD11	0.933	0.020	1
1	A	139	ILE	HD12	0.933	0.020	1
1	A	139	ILE	HD13	0.933	0.020	1
1	A	139	ILE	N	123.87	0.3	1
1	A	140	ASN	C	180.02	0.3	1

Continued on next page...

Continued from previous page...

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	140	ASN	CA	55.619	0.3	1
1	A	140	ASN	CB	41.535	0.3	1
1	A	140	ASN	H	8.119	0.020	1
1	A	140	ASN	HA	4.555	0.020	1
1	A	140	ASN	HB2	2.742	0.020	1
1	A	140	ASN	HB3	2.742	0.020	1
1	A	140	ASN	N	127.955	0.3	1

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	134	-0.68 ± 0.13	Should be applied
$^{13}C_{\beta}$	123	-0.78 ± 0.19	Should be applied
$^{13}C'$	131	-0.61 ± 0.19	Should be applied
^{15}N	121	0.28 ± 0.32	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 77%, i.e. 1033 atoms were assigned a chemical shift out of a possible 1341. 0 out of 8 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	503/549 (92%)	207/226 (92%)	203/218 (93%)	93/105 (89%)
Sidechain	526/669 (79%)	352/423 (83%)	165/218 (76%)	9/28 (32%)
Aromatic	4/123 (3%)	2/60 (3%)	0/59 (0%)	2/4 (50%)
Overall	1033/1341 (77%)	561/709 (79%)	368/495 (74%)	104/137 (76%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 78%, i.e. 1134 atoms were assigned a chemical shift out of a possible 1447. 0 out of 8 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	546/592 (92%)	224/243 (92%)	221/236 (94%)	101/113 (89%)
Sidechain	584/732 (80%)	391/462 (85%)	183/240 (76%)	10/30 (33%)
Aromatic	4/123 (3%)	2/60 (3%)	0/59 (0%)	2/4 (50%)
Overall	1134/1447 (78%)	617/765 (81%)	404/535 (76%)	113/147 (77%)

7.1.4 Statistically unusual chemical shifts (i)

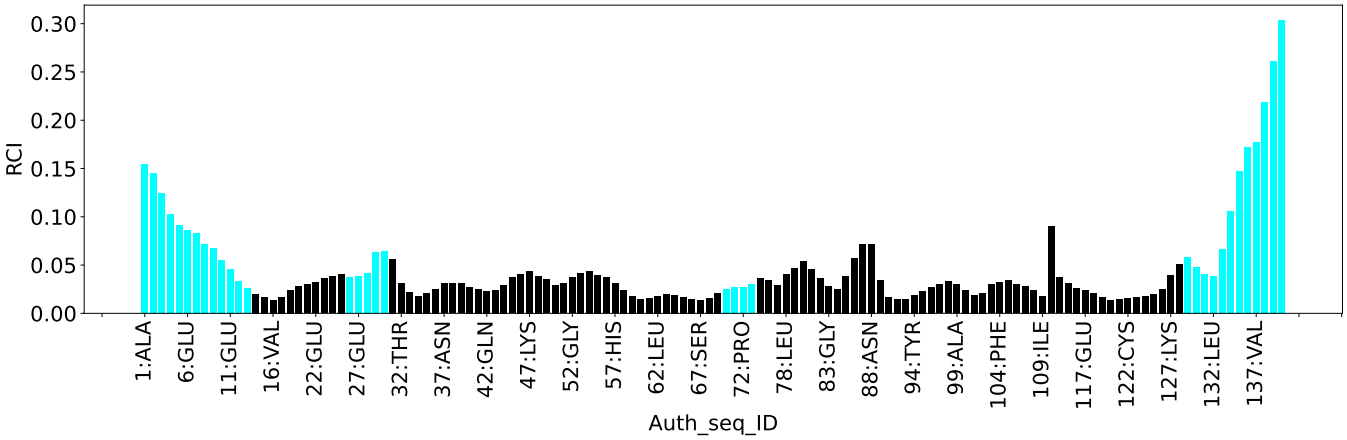
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	80	VAL	HG11	-1.28	-0.48 – 2.12	-8.1
1	A	80	VAL	HG12	-1.28	-0.48 – 2.12	-8.1
1	A	80	VAL	HG13	-1.28	-0.48 – 2.12	-8.1
1	A	84	VAL	CB	43.05	23.86 – 41.50	5.9

7.1.5 Random Coil Index (RCI) plots (i)

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

Random coil index (RCI) for chain A:



8 NMR restraints analysis

8.1 Conformationally restricting restraints

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

Description	Value
Total distance restraints	38
Intra-residue (|i-j|=0)	0
Sequential (|i-j|=1)	0
Medium range (|i-j|>1 and |i-j|<5)	1
Long range (|i-j|≥5)	37
Inter-chain	0
Hydrogen bond restraints	0
Disulfide bond restraints	0
Total dihedral-angle restraints	0
Number of unmapped restraints	0
Number of restraints per residue	0.3
Number of long range restraints per residue[1]	0.3

[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)	0.6	0.15
0.2-0.5 (Medium)	2.9	0.46
>0.5 (Large)	0.7	1.67

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)

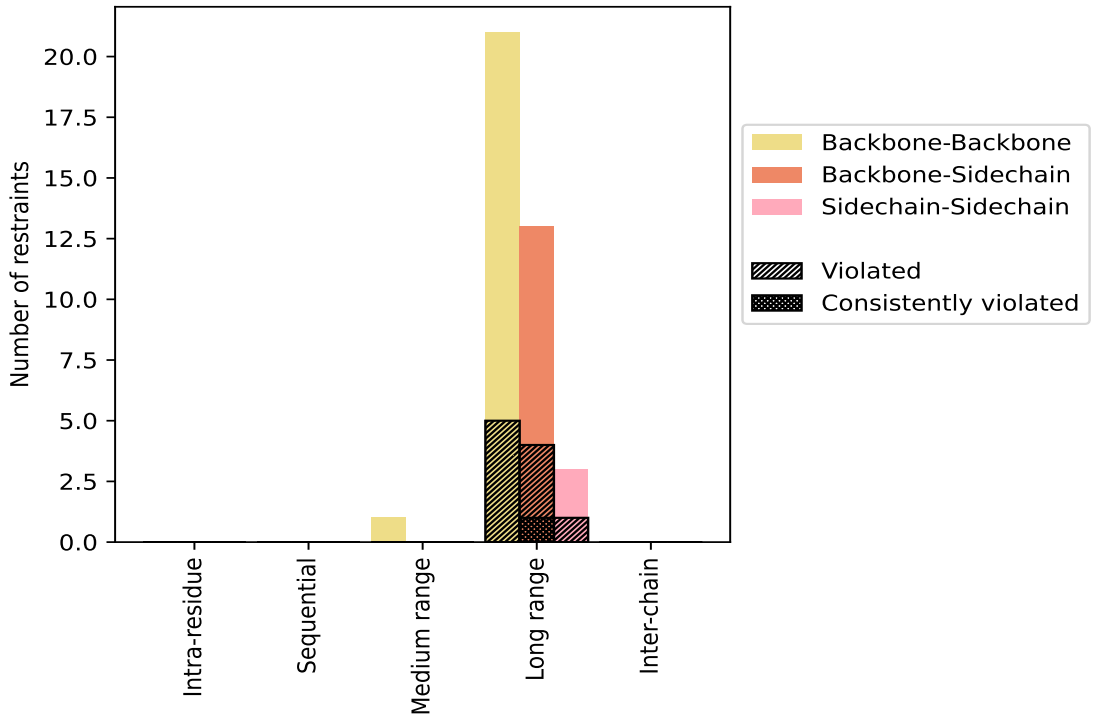
9.1 Summary of distance violations (i)

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)	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
Sequential (|i-j|=1)	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
Medium range (|i-j|>1 & |i-j|<5)	1	2.6	0	0.0	0.0	0	0.0	0.0
Backbone-Backbone	1	2.6	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
Long range (|i-j|≥5)	37	97.4	10	27.0	26.3	1	2.7	2.6
Backbone-Backbone	21	55.3	5	23.8	13.2	0	0.0	0.0
Backbone-Sidechain	13	34.2	4	30.8	10.5	1	7.7	2.6
Sidechain-Sidechain	3	7.9	1	33.3	2.6	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	38	100.0	10	26.3	26.3	1	2.6	2.6
Backbone-Backbone	22	57.9	5	22.7	13.2	0	0.0	0.0
Backbone-Sidechain	13	34.2	4	30.8	10.5	1	7.7	2.6
Sidechain-Sidechain	3	7.9	1	33.3	2.6	0	0.0	0.0

[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

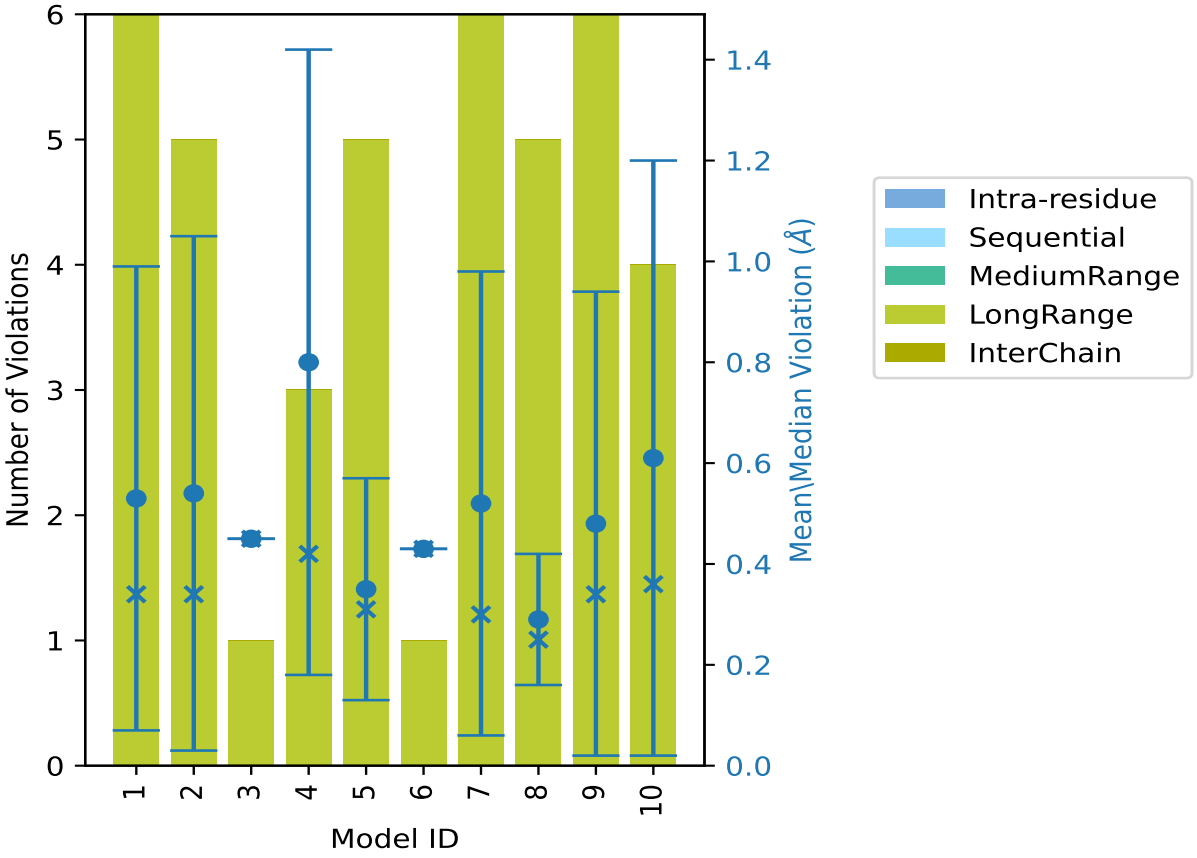
The following table provides the distance violation statistics for each model in the ensemble. Violations less than 0.1 Å are not included in the statistics.

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	0	0	0	6	0	6	0.53	1.56	0.46	0.34
2	0	0	0	5	0	5	0.54	1.55	0.51	0.34
3	0	0	0	1	0	1	0.45	0.45	0.0	0.45
4	0	0	0	3	0	3	0.8	1.67	0.62	0.42
5	0	0	0	5	0	5	0.35	0.75	0.22	0.31
6	0	0	0	1	0	1	0.43	0.43	0.0	0.43
7	0	0	0	6	0	6	0.52	1.53	0.46	0.3
8	0	0	0	5	0	5	0.29	0.46	0.13	0.25
9	0	0	0	6	0	6	0.48	1.49	0.46	0.34
10	0	0	0	4	0	4	0.61	1.62	0.59	0.36

[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

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, 28(IR:0, SQ:0, MR:1, LR:27, 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]	%
0	0	0	2	0	2	1	10.0
0	0	0	1	0	1	2	20.0
0	0	0	3	0	3	3	30.0
0	0	0	1	0	1	4	40.0

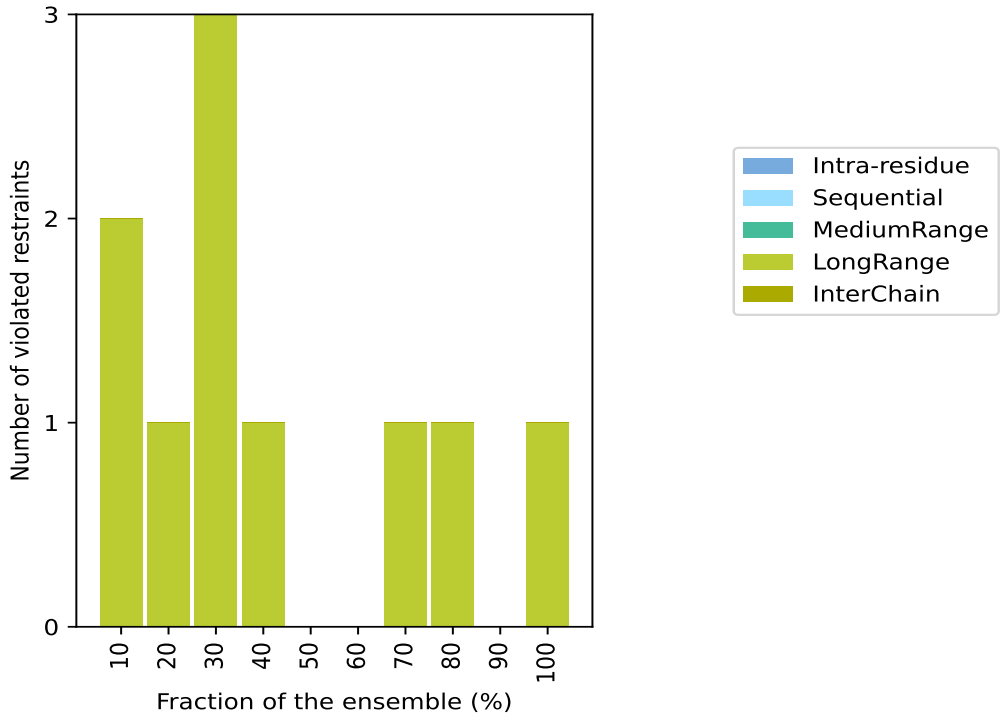
Continued on next page...

Continued from previous page...

Number of violated restraints						Fraction of the ensemble	
IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total	Count[6]	%
0	0	0	0	0	0	5	50.0
0	0	0	0	0	0	6	60.0
0	0	0	1	0	1	7	70.0
0	0	0	1	0	1	8	80.0
0	0	0	0	0	0	9	90.0
0	0	0	1	0	1	10	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

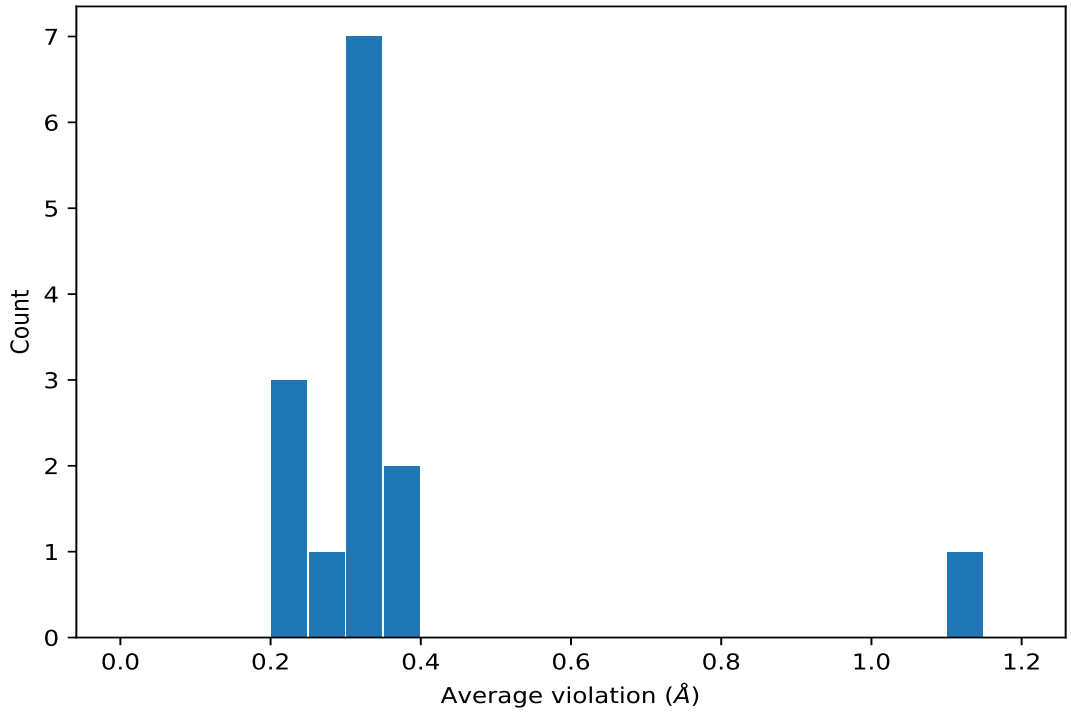
9.3.1 Bar graph : Distance violation statistics for the ensemble



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 (i)

The following table provides the mean and the standard deviation of the violation for each restraint sorted by number of violated models and the mean 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.

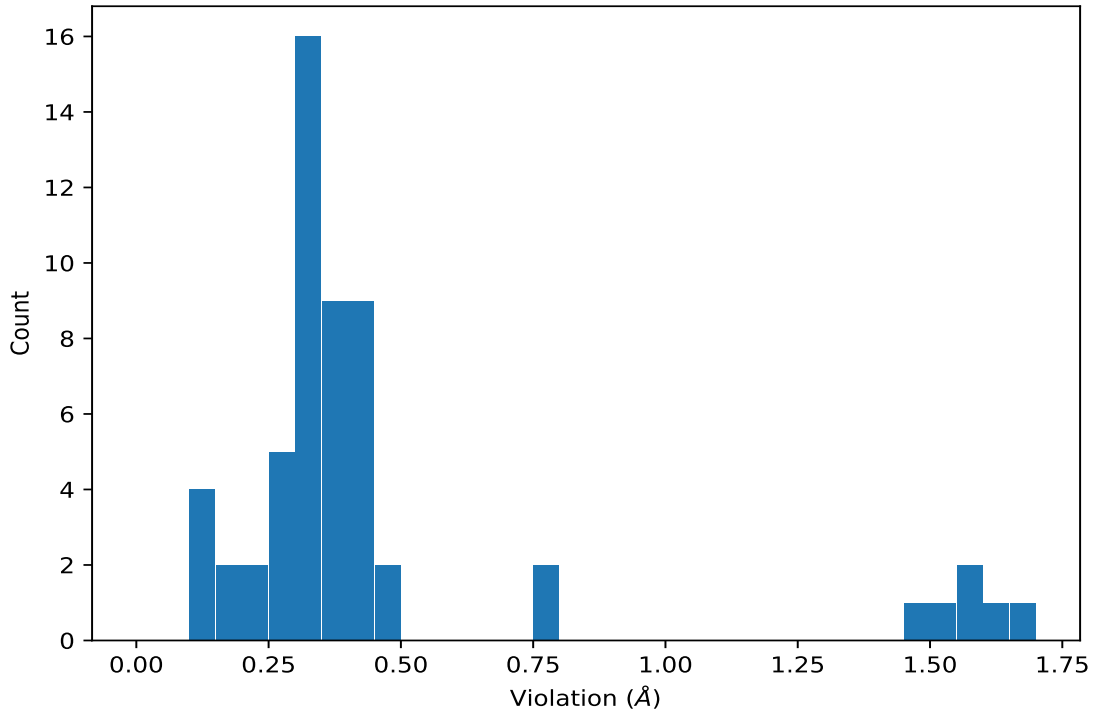
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	10	1.11	0.57	1.51
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	8	0.33	0.05	0.32
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	7	0.28	0.1	0.3
(1,31)	1:A:48:GLY:HA2	1:A:25:THR:HB	4	0.38	0.04	0.38
(1,31)	1:A:48:GLY:HA3	1:A:25:THR:HB	4	0.38	0.04	0.38
(1,6)	1:A:31:GLY:H	1:A:48:GLY:H	3	0.24	0.06	0.28
(1,16)	1:A:48:GLY:H	1:A:31:GLY:H	3	0.24	0.06	0.28
(1,22)	1:A:60:THR:H	1:A:34:ALA:H	3	0.23	0.16	0.12
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG21	2	0.32	0.02	0.32
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG22	2	0.32	0.02	0.32
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG23	2	0.32	0.02	0.32
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG21	2	0.32	0.02	0.32
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG22	2	0.32	0.02	0.32
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG23	2	0.32	0.02	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 lists the absolute value of the violation for each restraint in the ensemble sorted by its 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)
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	4	1.67
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	10	1.62
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	1	1.56
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	2	1.55
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	7	1.53
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	9	1.49
(1,19)	1:A:112:ASN:HB2	1:A:42:GLN:HA	5	0.75
(1,19)	1:A:112:ASN:HB3	1:A:42:GLN:HA	5	0.75
(1,22)	1:A:60:THR:H	1:A:34:ALA:H	8	0.46
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	3	0.45

Continued on next page...

Continued from previous page...

Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	6	0.43
(1,31)	1:A:48:GLY:HA2	1:A:25:THR:HB	4	0.42
(1,31)	1:A:48:GLY:HA3	1:A:25:THR:HB	4	0.42
(1,31)	1:A:48:GLY:HA2	1:A:25:THR:HB	10	0.41
(1,31)	1:A:48:GLY:HA3	1:A:25:THR:HB	10	0.41
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	7	0.41
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	8	0.41
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	9	0.4
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	1	0.4
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	2	0.38
(1,12)	1:A:122:CYS:H	1:A:101:CYS:HB2	5	0.38
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG21	1	0.35
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG22	1	0.35
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG23	1	0.35
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG21	1	0.35
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG22	1	0.35
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG23	1	0.35
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	9	0.35
(1,31)	1:A:48:GLY:HA2	1:A:25:THR:HB	2	0.34
(1,31)	1:A:48:GLY:HA3	1:A:25:THR:HB	2	0.34
(1,31)	1:A:48:GLY:HA2	1:A:25:THR:HB	9	0.33
(1,31)	1:A:48:GLY:HA3	1:A:25:THR:HB	9	0.33
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	1	0.33
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	2	0.33
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	5	0.31
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG21	7	0.3
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG22	7	0.3
(1,32)	1:A:28:SER:HB2	1:A:49:THR:HG23	7	0.3
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG21	7	0.3
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG22	7	0.3
(1,32)	1:A:28:SER:HB3	1:A:49:THR:HG23	7	0.3
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	7	0.3
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	4	0.3
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	10	0.3
(1,6)	1:A:31:GLY:H	1:A:48:GLY:H	1	0.28
(1,6)	1:A:31:GLY:H	1:A:48:GLY:H	7	0.28
(1,16)	1:A:48:GLY:H	1:A:31:GLY:H	1	0.28
(1,16)	1:A:48:GLY:H	1:A:31:GLY:H	7	0.28
(1,28)	1:A:24:VAL:HB	1:A:52:GLY:H	8	0.25
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	5	0.22
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	8	0.22
(1,6)	1:A:31:GLY:H	1:A:48:GLY:H	9	0.15

Continued on next page...

Continued from previous page...

Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,16)	1:A:48:GLY:H	1:A:31:GLY:H	9	0.15
(1,4)	1:A:33:HIS:H	1:A:48:GLY:H	8	0.12
(1,22)	1:A:60:THR:H	1:A:34:ALA:H	2	0.12
(1,29)	1:A:22:GLU:HA	1:A:54:GLY:H	10	0.11
(1,22)	1:A:60:THR:H	1:A:34:ALA:H	5	0.11

10 Dihedral-angle violation analysis (i)

No dihedral-angle restraints found