wwPDB NMR Structure Validation Summary Report

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PDB ID	:	2M5B
BMRB ID	:	19045
Title	:	The NMR structure of the BID-BAK complex
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Deposited on	:	2013-02-19

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:

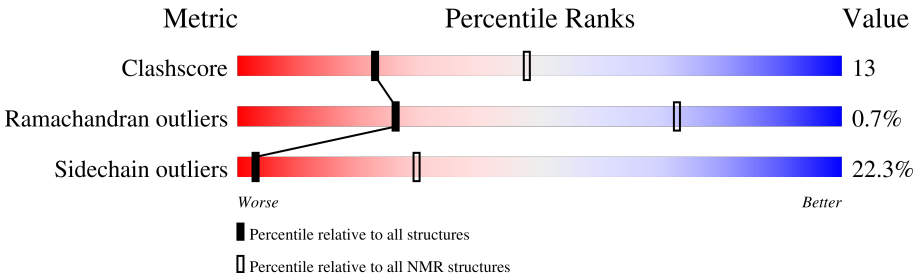
Cyrange	:	Kirchner and Güntert (2011)
NmrClust	:	Kelley et al. (1996)
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
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)	:	2.36

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 80%.

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	169	53% 30% · 15%
2	B	23	43% 30% · 22%

2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 13 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *fewest violations*.

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:22-A:45, A:66-A:184, B:81-B:91, B:93-B:95, B:98-B:101 (161)	0.62	13

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 and 1 single-model cluster was found.

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

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2783 atoms, of which 1268 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Bcl-2 homologous antagonist/killer.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	169	2440	850	1103	234	248	5	0

- Molecule 2 is a protein called human_BID_BH3_SAHB.

Mol	Chain	Residues	Atoms					Trace
			Total	C	H	N	O	
2	B	23	343	111	165	35	32	1

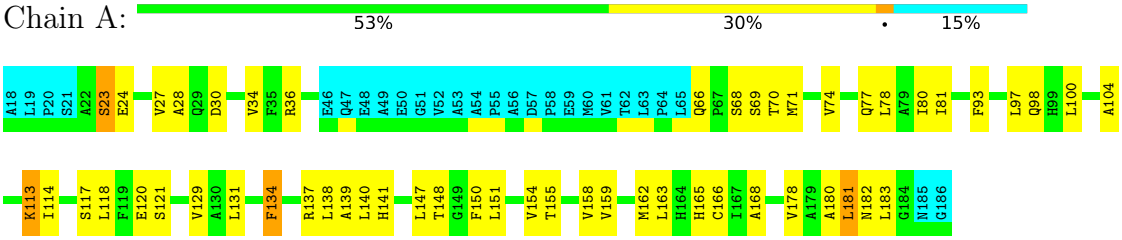
4 Residue-property plots

4.1 Average score per residue in the NMR ensemble

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

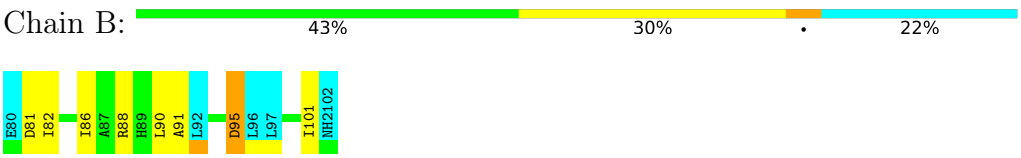
• Molecule 1: Bcl-2 homologous antagonist/killer

Chain A:



• Molecule 2: human_BID_BH3_SAHB

Chain B:

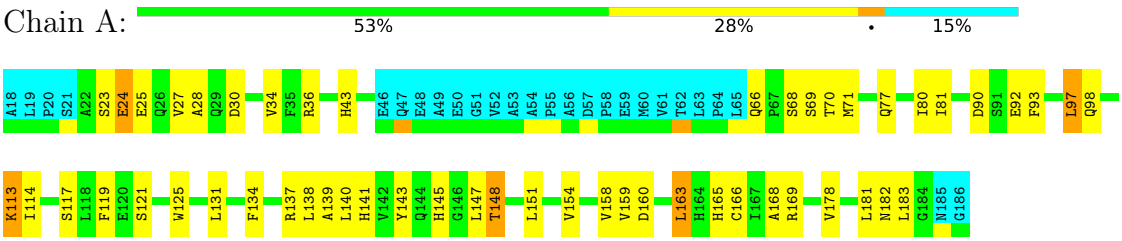


4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 13. Colouring as in section 4.1 above.

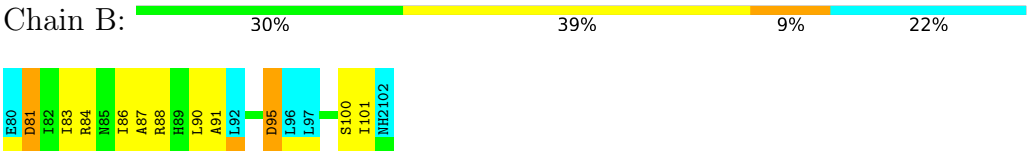
• Molecule 1: Bcl-2 homologous antagonist/killer

Chain A:



• Molecule 2: human_BID_BH3_SAHB

Chain B:


30%
39%
9%
22%
E80
D81
I82
I83
R84
N85
I86
A87
R88
H89
L90
A91
L92
D95
L96
L97
S100
I101
NH2102

5 Refinement protocol and experimental data overview

The models were refined using the following method: *torsion angle dynamics, simulated annealing, molecular dynamics.*

Of the 100 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
TopSpin	structure solution	
TopSpin	structure solution	
TopSpin	structure solution	
CYANA	refinement	

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

Chemical shift file(s)	working_cs.cif
Number of chemical shift lists	1
Total number of shifts	2096
Number of shifts mapped to atoms	1895
Number of unparsed shifts	0
Number of shifts with mapping errors	201
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	80%

6 Model quality

6.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: NH2, NLE, MK8

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

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
2	B	0.0±0.0	2.0±0.0
All	All	0	40

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

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

Mol	Chain	Res	Type	Group	Models (Total)
2	B	91	ALA	Peptide	20
2	B	95	ASP	Peptide	20

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	1156	960	1110	31±5
2	B	142	126	146	8±3
All	All	25960	21720	25120	641

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 13.

5 of 207 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:114:ILE:HD12	2:B:86:ILE:HG21	0.98	1.33	6	10
1:A:70:THR:HG21	1:A:178:VAL:HG13	0.95	1.35	17	8
1:A:183:LEU:HD22	2:B:101:ILE:HD12	0.94	1.32	2	8
1:A:104:ALA:HB2	1:A:147:LEU:HD13	0.94	1.39	6	1
1:A:97:LEU:HD11	1:A:138:LEU:HD12	0.85	1.44	3	3

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	143/169 (85%)	132±2 (92±1%)	10±2 (7±1%)	1±1 (1±1%)	24	71
2	B	18/23 (78%)	15±1 (84±5%)	3±1 (16±5%)	0±0 (0±0%)	100	100
All	All	3220/3840 (84%)	2933 (91%)	264 (8%)	23 (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)
1	A	149	GLY	4
1	A	166	CYS	4
1	A	146	GLY	4
1	A	184	GLY	3
1	A	148	THR	3

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	118/137 (86%)	92±3 (78±3%)	26±3 (22±3%)	3	30
2	B	15/16 (94%)	11±1 (73±8%)	4±1 (27±8%)	2	21

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
All	All	2660/3060 (87%)	2066 (78%)	594 (22%)	3 29

5 of 76 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	134	PHE	20
1	A	151	LEU	20
1	A	165	HIS	20
1	A	23	SER	17
2	B	88	ARG	17

6.3.3 RNA (i)

There are no RNA molecules in this entry.

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

3 non-standard protein/DNA/RNA residues are modelled in this entry.

In the following table, the Counts columns list the number of bonds for which Mogul statistics could be retrieved, the number of bonds that are observed in the model and the number of bonds that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length is the number of standard deviations the observed value is removed from the expected value. A bond length with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond lengths.

Mol	Type	Chain	Res	Link	Bond lengths		
					Counts	RMSZ	#Z>2
2	MK8	B	96	2	5,8,9	1.10±0.02	0±0 (0±0%)
2	MK8	B	92	2	5,8,9	1.11±0.02	0±0 (0±0%)
2	NLE	B	97	2	6,7,8	0.65±0.01	0±0 (0±0%)

In the following table, the Counts columns list the number of angles for which Mogul statistics could be retrieved, the number of angles that are observed in the model and the number of angles that are defined in the chemical component dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond angle is the number of standard deviations the observed value is removed from the expected value. A bond angle with $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the average root-mean-square of all Z scores of the bond angles.

Mol	Type	Chain	Res	Link	Bond angles		
					Counts	RMSZ	#Z>2
2	MK8	B	96	2	4,10,12	0.50±0.04	0±0 (0±0%)
2	MK8	B	92	2	4,10,12	1.10±0.03	1±0 (15±12%)
2	NLE	B	97	2	2,7,9	0.70±0.00	0±0 (0±0%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the chemical component dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	MK8	B	92	2	-	0±0,6,8,11	-
2	NLE	B	97	2	-	0±0,5,6,8	-
2	MK8	B	96	2	-	0±0,6,8,11	-

There are no bond-length outliers.

All unique angle outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
2	B	92	MK8	CB1-CA-C	2.17	100.82	108.99	10	12

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

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

The completeness of assignment taking into account all chemical shift lists is 80% for the well-defined parts and 80% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *assigned_chem_shift_list_1*

7.1.1 Bookkeeping

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

Total number of shifts	2096
Number of shifts mapped to atoms	1895
Number of unparsed shifts	0
Number of shifts with mapping errors	201
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	10

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 201) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	19	LEU	HB2	1.482	0.020	1
1	A	20	PRO	HB2	2.182	0.020	2
1	A	20	PRO	HG2	1.915	0.020	1
1	A	20	PRO	HD2	3.754	0.020	2
1	A	21	SER	HB2	3.801	0.020	1
1	A	23	SER	HB2	4.088	0.020	2
1	A	24	GLU	HB2	1.861	0.020	1
1	A	24	GLU	HG2	1.956	0.020	2
1	A	25	GLU	HB2	1.89	0.020	1
1	A	25	GLU	HG2	2.23	0.020	1
1	A	26	GLN	HB2	2.152	0.020	1
1	A	26	GLN	HG2	2.423	0.020	1
1	A	29	GLN	HB2	2.178	0.020	1
1	A	29	GLN	HG2	2.426	0.020	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	30	ASP	HB2	3.295	0.020	2
1	A	32	GLU	HB2	2.059	0.020	1
1	A	32	GLU	HG2	2.237	0.020	2
1	A	33	GLU	HB2	1.983	0.020	1
1	A	33	GLU	HG2	2.02	0.020	1
1	A	35	PHE	HB2	2.964	0.020	1
1	A	36	ARG	HB2	1.798	0.020	2
1	A	36	ARG	HG2	1.588	0.020	1
1	A	36	ARG	HD2	3.072	0.020	1
1	A	37	SER	HB2	4.032	0.020	1
1	A	38	TYR	HB2	3.233	0.020	2
1	A	40	PHE	HB2	2.903	0.020	1
1	A	41	TYR	HB2	2.881	0.020	2
1	A	42	ARG	HB2	0.675	0.020	1
1	A	42	ARG	HG2	0.794	0.020	1
1	A	42	ARG	HD2	2.643	0.020	1
1	A	43	HIS	HB2	3.415	0.020	2
1	A	44	GLN	HB2	1.85	0.020	2
1	A	44	GLN	HG2	1.938	0.020	2
1	A	45	GLN	HB2	2.064	0.020	1
1	A	45	GLN	HG2	2.35	0.020	1
1	A	46	GLU	HB2	1.924	0.020	1
1	A	46	GLU	HG2	2.45	0.020	2
1	A	47	GLN	HB2	1.882	0.020	1
1	A	47	GLN	HG2	1.919	0.020	1
1	A	48	GLU	HB2	1.966	0.020	1
1	A	48	GLU	HG2	2.286	0.020	2
1	A	50	GLU	HB2	1.89	0.020	1
1	A	50	GLU	HG2	2.233	0.020	1
1	A	55	PRO	HB2	2.177	0.020	2
1	A	55	PRO	HG2	1.941	0.020	1
1	A	55	PRO	HD2	3.705	0.020	2
1	A	57	ASP	HB2	2.881	0.020	2
1	A	58	PRO	HB2	2.255	0.020	1
1	A	58	PRO	HG2	1.925	0.020	1
1	A	58	PRO	HD2	3.643	0.020	2
1	A	60	MET	HB2	1.626	0.020	2
1	A	60	MET	HG2	2.129	0.020	1
1	A	63	LEU	HB2	1.208	0.020	1
1	A	64	PRO	HB2	1.959	0.020	2
1	A	64	PRO	HG2	1.82	0.020	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	64	PRO	HD2	3.603	0.020	2
1	A	65	LEU	HB2	1.283	0.020	2
1	A	66	GLN	HB2	1.978	0.020	2
1	A	66	GLN	HG2	2.264	0.020	2
1	A	67	PRO	HB2	2.246	0.020	2
1	A	67	PRO	HG2	1.937	0.020	2
1	A	67	PRO	HD2	3.703	0.020	2
1	A	68	SER	HB2	3.876	0.020	2
1	A	69	SER	HB2	4.174	0.020	2
1	A	71	MET	HB2	1.91	0.020	2
1	A	71	MET	HG2	2.965	0.020	2
1	A	73	GLN	HB2	2.273	0.020	2
1	A	73	GLN	HG2	2.429	0.020	1
1	A	76	ARG	HB2	1.5	0.020	2
1	A	76	ARG	HG2	0.303	0.020	2
1	A	76	ARG	HD2	2.595	0.020	2
1	A	77	GLN	HB2	2.027	0.020	1
1	A	77	GLN	HG2	2.276	0.020	1
1	A	78	LEU	HB2	2.053	0.020	2
1	A	80	ILE	HG12	1.498	0.020	2
1	A	81	ILE	HG12	1.473	0.020	2
1	A	83	ASP	HB2	2.799	0.020	2
1	A	84	ASP	HB2	2.611	0.020	1
1	A	85	ILE	HG12	1.519	0.020	2
1	A	86	ASN	HB2	2.784	0.020	1
1	A	87	ARG	HB2	1.766	0.020	1
1	A	87	ARG	HG2	1.745	0.020	2
1	A	87	ARG	HD2	3.103	0.020	1
1	A	88	ARG	HB2	1.62	0.020	2
1	A	88	ARG	HG2	1.203	0.020	2
1	A	88	ARG	HD2	2.938	0.020	2
1	A	89	TYR	HB2	3.274	0.020	2
1	A	90	ASP	HB2	2.999	0.020	2
1	A	91	SER	HB2	3.883	0.020	1
1	A	92	GLU	HB2	1.835	0.020	1
1	A	92	GLU	HG2	2.183	0.020	1
1	A	93	PHE	HB2	3.226	0.020	2
1	A	94	GLN	HB2	2.188	0.020	1
1	A	94	GLN	HG2	2.463	0.020	2
1	A	96	MET	HB2	1.676	0.020	1
1	A	96	MET	HG2	2.679	0.020	2

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	97	LEU	HB2	1.55	0.020	2
1	A	98	GLN	HB2	2.229	0.020	2
1	A	98	GLN	HG2	2.467	0.020	1
1	A	99	HIS	HB2	3.16	0.020	1
1	A	100	LEU	HB2	1.671	0.020	2
1	A	101	GLN	HB2	2.053	0.020	1
1	A	101	GLN	HG2	2.243	0.020	1
1	A	102	PRO	HB2	1.834	0.020	2
1	A	102	PRO	HG2	1.456	0.020	1
1	A	102	PRO	HD2	3.337	0.020	1
1	A	105	GLU	HB2	1.881	0.020	1
1	A	105	GLU	HG2	2.263	0.020	2
1	A	106	ASN	HB2	2.255	0.020	1
1	A	108	TYR	HB2	2.67	0.020	2
1	A	109	GLU	HB2	1.981	0.020	2
1	A	109	GLU	HG2	2.212	0.020	1
1	A	110	TYR	HB2	3.022	0.020	2
1	A	111	PHE	HB2	2.951	0.020	1
1	A	113	LYS	HB2	1.771	0.020	1
1	A	113	LYS	HG2	1.333	0.020	1
1	A	113	LYS	HD2	1.624	0.020	2
1	A	113	LYS	HE2	2.874	0.020	1
1	A	114	ILE	HG12	1.732	0.020	2
1	A	117	SER	HB2	3.661	0.020	2
1	A	118	LEU	HB2	1.688	0.020	2
1	A	119	PHE	HB2	3.283	0.020	2
1	A	120	GLU	HB2	2.139	0.020	2
1	A	120	GLU	HG2	2.273	0.020	1
1	A	121	SER	HB2	4.097	0.020	2
1	A	123	ILE	HG12	1.303	0.020	2
1	A	124	ASN	HB2	3.318	0.020	2
1	A	125	TRP	HB2	3.404	0.020	2
1	A	127	ARG	HB2	1.326	0.020	1
1	A	127	ARG	HG2	1.013	0.020	1
1	A	127	ARG	HD2	2.66	0.020	2
1	A	131	LEU	HB2	2.589	0.020	2
1	A	132	LEU	HB2	1.495	0.020	2
1	A	134	PHE	HB2	3.372	0.020	1
1	A	136	TYR	HB2	3.654	0.020	2
1	A	137	ARG	HB2	1.959	0.020	2
1	A	137	ARG	HG2	1.742	0.020	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	137	ARG	HD2	3.159	0.020	2
1	A	138	LEU	HB2	1.87	0.020	2
1	A	140	LEU	HB2	1.553	0.020	2
1	A	141	HIS	HB2	3.193	0.020	2
1	A	143	TYR	HB2	3.174	0.020	1
1	A	144	GLN	HB2	2.082	0.020	2
1	A	144	GLN	HG2	2.658	0.020	2
1	A	145	HIS	HB2	3.463	0.020	2
1	A	147	LEU	HB2	1.457	0.020	2
1	A	150	PHE	HB2	2.906	0.020	2
1	A	151	LEU	HB2	1.571	0.020	1
1	A	153	GLN	HB2	0.837	0.020	2
1	A	153	GLN	HG2	1.859	0.020	2
1	A	156	ARG	HB2	1.838	0.020	2
1	A	156	ARG	HG2	1.626	0.020	2
1	A	156	ARG	HD2	3.066	0.020	1
1	A	157	PHE	HB2	2.681	0.020	2
1	A	160	ASP	HB2	2.946	0.020	2
1	A	161	PHE	HB2	3.68	0.020	2
1	A	162	MET	HB2	2.455	0.020	2
1	A	162	MET	HG2	3.19	0.020	2
1	A	163	LEU	HB2	1.377	0.020	1
1	A	164	HIS	HB2	3.109	0.020	2
1	A	165	HIS	HB2	2.764	0.020	2
1	A	166	CYS	HB2	3.035	0.020	2
1	A	167	ILE	HG12	0.821	0.020	2
1	A	169	ARG	HB2	1.759	0.020	2
1	A	169	ARG	HG2	1.426	0.020	1
1	A	169	ARG	HD2	3.098	0.020	1
1	A	170	TRP	HB2	3.232	0.020	2
1	A	171	ILE	HG12	1.58	0.020	2
1	A	173	GLN	HB2	1.923	0.020	2
1	A	173	GLN	HG2	2.254	0.020	1
1	A	174	ARG	HB2	1.928	0.020	2
1	A	174	ARG	HG2	0.953	0.020	2
1	A	174	ARG	HD2	2.122	0.020	2
1	A	177	TRP	HB2	3.292	0.020	2
1	A	181	LEU	HB2	1.682	0.020	2
1	A	182	ASN	HB2	2.897	0.020	2
1	A	183	LEU	HB2	1.531	0.020	2
1	A	185	ASN	HB2	2.774	0.020	2

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	B	80	GLU	HB2	1.928	0.020	2
1	B	80	GLU	HG2	2.204	0.020	1
1	B	81	ASP	HB2	2.629	0.020	2
1	B	82	ILE	HG12	1.355	0.020	2
1	B	83	ILE	HG12	1.417	0.020	2
1	B	84	ARG	HB2	1.781	0.020	1
1	B	84	ARG	HG2	1.693	0.020	2
1	B	84	ARG	HD2	3.112	0.020	1
1	B	85	ASN	HB2	2.859	0.020	2
1	B	86	ILE	HG12	0.819	0.020	2
1	B	88	ARG	HB2	1.855	0.020	2
1	B	88	ARG	HG2	1.575	0.020	1
1	B	88	ARG	HD2	3.122	0.020	1
1	B	89	HIS	HB2	3.136	0.020	2
1	B	90	LEU	HB2	1.511	0.020	2
1	B	92	MK8	HB2	2.044	0.020	2
1	B	95	ASP	HB2	2.754	0.020	2
1	B	98	ASP	HB2	2.97	0.020	2
1	B	99	ARG	HB2	2.01	0.020	2
1	B	99	ARG	HG2	1.831	0.020	2
1	B	99	ARG	HD2	3.209	0.020	1
1	B	100	SER	HB2	4.081	0.020	1
1	B	101	ILE	HG12	1.684	0.020	2

7.1.2 Chemical shift referencing

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	168	-0.31 ± 0.05	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	154	0.39 ± 0.08	None needed (< 0.5 ppm)
$^{13}C'$	0	—	None (insufficient data)
^{15}N	155	0.76 ± 0.27	Should be applied

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 80%, i.e. 1797 atoms were assigned a chemical shift out of a possible 2255. 0 out of 26 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	611/815 (75%)	333/334 (100%)	143/322 (44%)	135/159 (85%)
Sidechain	1012/1202 (84%)	728/783 (93%)	267/361 (74%)	17/58 (29%)
Aromatic	174/238 (73%)	103/119 (87%)	68/109 (62%)	3/10 (30%)
Overall	1797/2255 (80%)	1164/1236 (94%)	478/792 (60%)	155/227 (68%)

7.1.4 Statistically unusual chemical shifts (i)

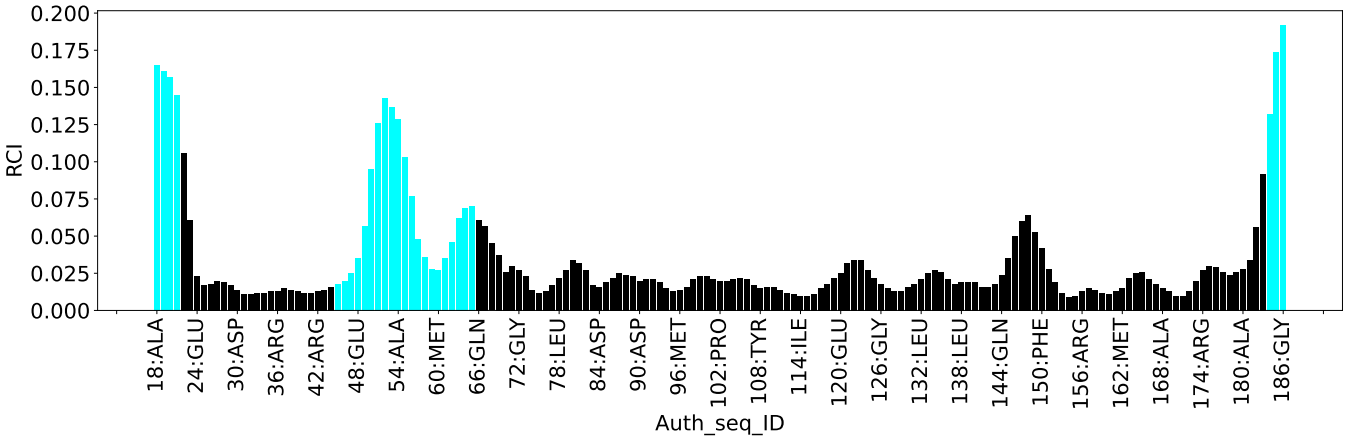
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	127	ARG	NE	117.95	76.53 – 92.65	20.7
1	A	115	ALA	HB1	-0.20	0.14 – 2.58	-6.4
1	A	115	ALA	HB2	-0.20	0.14 – 2.58	-6.4
1	A	115	ALA	HB3	-0.20	0.14 – 2.58	-6.4
1	A	174	ARG	HD3	1.46	1.81 – 4.39	-6.4
1	A	123	ILE	HG21	-0.83	-0.56 – 2.11	-6.0
1	A	123	ILE	HG22	-0.83	-0.56 – 2.11	-6.0
1	A	123	ILE	HG23	-0.83	-0.56 – 2.11	-6.0
1	A	153	GLN	HB3	0.64	0.71 – 3.33	-5.2
1	A	76	ARG	HG3	0.15	0.15 – 2.94	-5.0

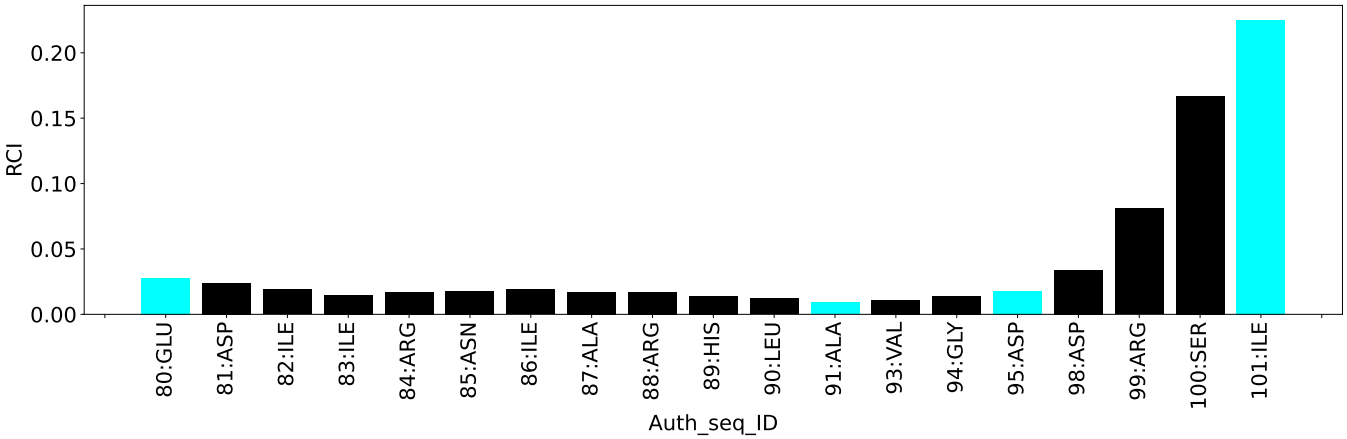
7.1.5 Random Coil Index (RCI) plots (i)

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:



Random coil index (RCI) for chain B:



8 NMR restraints analysis

8.1 Conformationally restricting restraints

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

Description	Value
Total distance restraints	3311
Intra-residue (|i-j|=0)	1108
Sequential (|i-j|=1)	695
Medium range (|i-j|>1 and |i-j|<5)	622
Long range (|i-j|≥5)	535
Inter-chain	188
Hydrogen bond restraints	163
Disulfide bond restraints	0
Total dihedral-angle restraints	0
Number of unmapped restraints	991
Number of restraints per residue	17.2
Number of long range restraints per residue[1]	2.8

[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)	58.8	0.2
0.2-0.5 (Medium)	103.1	0.5
>0.5 (Large)	148.0	3.73

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)

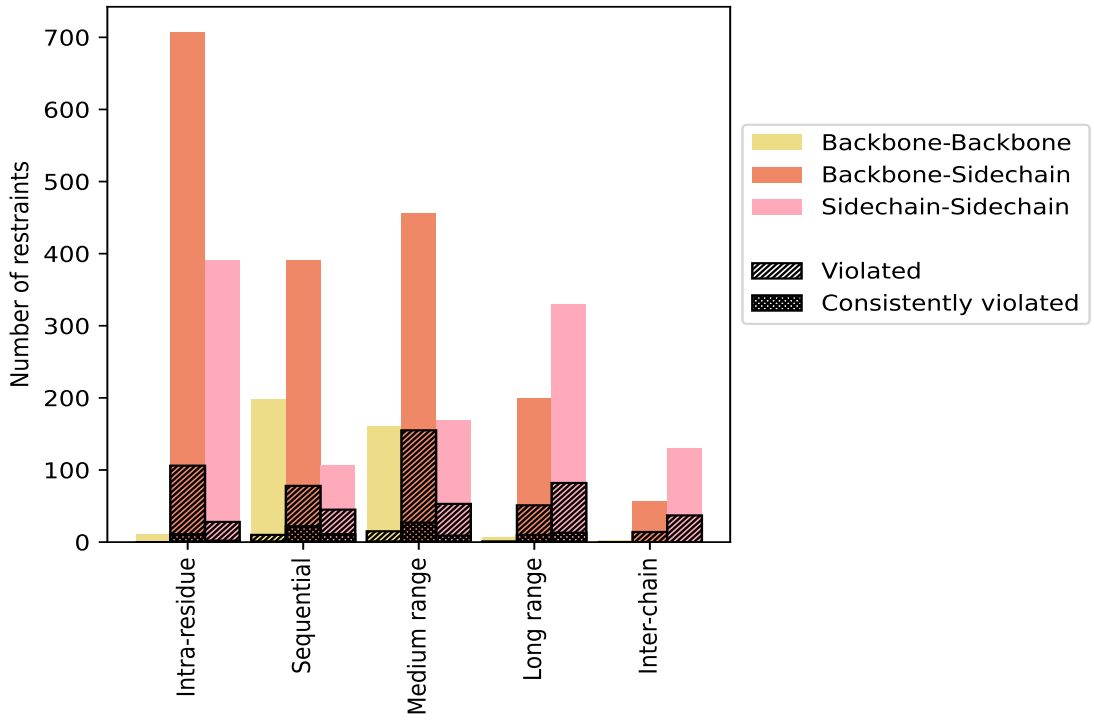
9.1 Summary of distance violations (i)

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)	1108	33.5	134	12.1	4.0	13	1.2	0.4
Backbone-Backbone	11	0.3	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	707	21.4	106	15.0	3.2	11	1.6	0.3
Sidechain-Sidechain	390	11.8	28	7.2	0.8	2	0.5	0.1
Sequential (|i-j|=1)	695	21.0	133	19.1	4.0	34	4.9	1.0
Backbone-Backbone	198	6.0	10	5.1	0.3	1	0.5	0.0
Backbone-Sidechain	391	11.8	78	19.9	2.4	22	5.6	0.7
Sidechain-Sidechain	106	3.2	45	42.5	1.4	11	10.4	0.3
Medium range (|i-j|>1 & |i-j|<5)	622	18.8	137	22.0	4.1	20	3.2	0.6
Backbone-Backbone	160	4.8	15	9.4	0.5	1	0.6	0.0
Backbone-Sidechain	293	8.8	69	23.5	2.1	10	3.4	0.3
Sidechain-Sidechain	169	5.1	53	31.4	1.6	9	5.3	0.3
Long range (|i-j|≥5)	535	16.2	134	25.0	4.0	23	4.3	0.7
Backbone-Backbone	7	0.2	1	14.3	0.0	0	0.0	0.0
Backbone-Sidechain	199	6.0	51	25.6	1.5	10	5.0	0.3
Sidechain-Sidechain	329	9.9	82	24.9	2.5	13	4.0	0.4
Inter-chain	188	5.7	51	27.1	1.5	0	0.0	0.0
Backbone-Backbone	2	0.1	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	56	1.7	14	25.0	0.4	0	0.0	0.0
Sidechain-Sidechain	130	3.9	37	28.5	1.1	0	0.0	0.0
Hydrogen bond	163	4.9	86	52.8	2.6	17	10.4	0.5
Disulfide bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Total	3311	100.0	675	20.4	20.4	107	3.2	3.2
Backbone-Backbone	378	11.4	26	6.9	0.8	2	0.5	0.1
Backbone-Sidechain	1809	54.6	404	22.3	12.2	70	3.9	2.1
Sidechain-Sidechain	1124	33.9	245	21.8	7.4	35	3.1	1.1

[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	64	86	100	58	20	328	0.59	3.1	0.5	0.42
2	60	73	101	61	11	306	0.61	3.73	0.54	0.43
3	69	78	101	67	16	331	0.66	3.62	0.53	0.45
4	66	81	108	60	16	331	0.64	2.33	0.51	0.49
5	56	76	101	51	17	301	0.64	2.32	0.5	0.47
6	55	72	95	63	21	306	0.64	2.4	0.53	0.46
7	61	78	94	58	12	303	0.63	2.79	0.51	0.48
8	54	79	88	52	18	291	0.61	2.42	0.51	0.43
9	67	75	96	65	17	320	0.65	2.93	0.54	0.47
10	60	76	111	67	16	330	0.65	2.83	0.53	0.5
11	65	83	91	57	16	312	0.6	2.69	0.49	0.42

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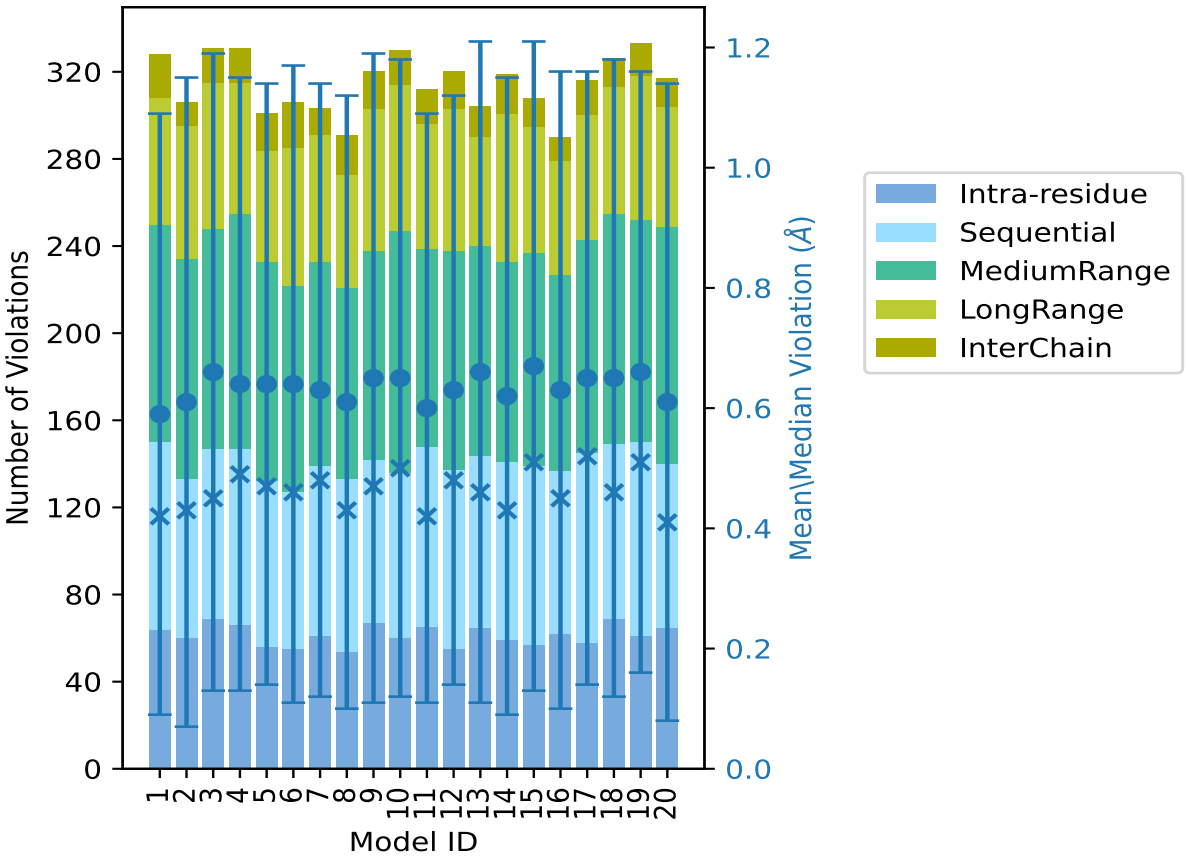
Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
12	55	82	101	65	17	320	0.63	2.93	0.49	0.48
13	65	79	96	50	14	304	0.66	2.95	0.55	0.46
14	59	82	92	68	18	319	0.62	3.27	0.53	0.43
15	57	82	98	58	13	308	0.67	3.01	0.54	0.51
16	62	75	90	52	11	290	0.63	3.03	0.53	0.45
17	58	87	98	57	16	316	0.65	2.72	0.51	0.52
18	69	80	106	58	13	326	0.65	3.0	0.53	0.46
19	61	89	102	66	15	333	0.66	2.36	0.5	0.51
20	65	75	109	55	13	317	0.61	3.35	0.53	0.41

[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

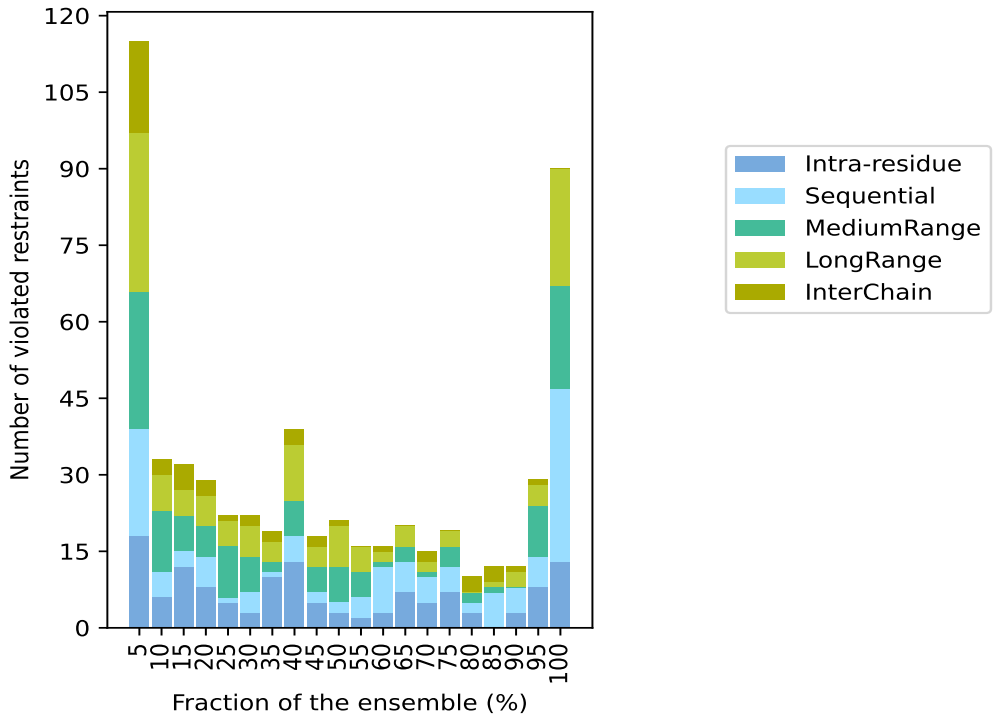
9.3 Distance violation statistics for the ensemble (i)

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, 2559(IR:974, SQ:562, MR:485, LR:401, IC:137) 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]	%
18	21	27	31	18	115	1	5.0
6	5	12	7	3	33	2	10.0
12	3	7	5	5	32	3	15.0
8	6	6	6	3	29	4	20.0
5	1	10	5	1	22	5	25.0
3	4	7	6	2	22	6	30.0
10	1	2	4	2	19	7	35.0
13	5	7	11	3	39	8	40.0
5	2	5	4	2	18	9	45.0
3	2	7	8	1	21	10	50.0
2	4	5	5	0	16	11	55.0
3	9	1	2	1	16	12	60.0
7	6	3	4	0	20	13	65.0
5	5	1	2	2	15	14	70.0
7	5	4	3	0	19	15	75.0
3	2	2	0	3	10	16	80.0
0	7	1	1	3	12	17	85.0
3	5	0	3	1	12	18	90.0
8	6	10	4	1	29	19	95.0
13	34	20	23	0	90	20	100.0

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

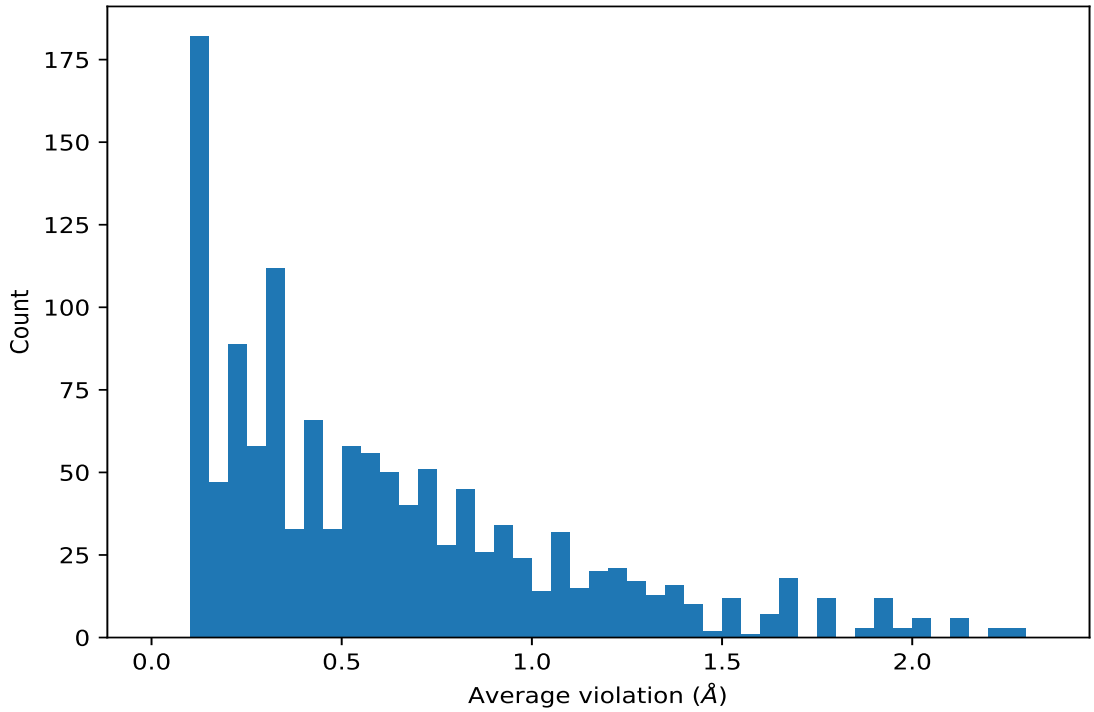
9.3.1 Bar graph : Distance violation statistics for the ensemble



9.4 Most violated distance restraints in the ensemble

9.4.1 Histogram : Distribution of mean distance violations

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



9.4.2 Table: Most violated distance restraints (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)
(1,1364)	1:34:A:VAL:HG21	1:132:A:LEU:HB3	20	2.3	0.06	2.32
(1,1364)	1:34:A:VAL:HG22	1:132:A:LEU:HB3	20	2.3	0.06	2.32
(1,1364)	1:34:A:VAL:HG23	1:132:A:LEU:HB3	20	2.3	0.06	2.32
(1,2616)	1:27:A:VAL:HG21	1:177:A:TRP:HZ2	20	2.14	0.1	2.16
(1,2616)	1:27:A:VAL:HG22	1:177:A:TRP:HZ2	20	2.14	0.1	2.16
(1,2616)	1:27:A:VAL:HG23	1:177:A:TRP:HZ2	20	2.14	0.1	2.16
(1,1932)	1:71:A:MET:H	1:74:A:VAL:HG11	20	2.0	0.26	2.09
(1,1932)	1:71:A:MET:H	1:74:A:VAL:HG12	20	2.0	0.26	2.09
(1,1932)	1:71:A:MET:H	1:74:A:VAL:HG13	20	2.0	0.26	2.09
(1,347)	1:34:A:VAL:HG11	1:71:A:MET:H	20	1.96	0.19	2.01
(1,347)	1:34:A:VAL:HG12	1:71:A:MET:H	20	1.96	0.19	2.01
(1,347)	1:34:A:VAL:HG13	1:71:A:MET:H	20	1.96	0.19	2.01
(1,1957)	1:74:A:VAL:HG11	1:70:A:THR:HG21	20	1.95	0.31	2.06
(1,1957)	1:74:A:VAL:HG11	1:70:A:THR:HG22	20	1.95	0.31	2.06
(1,1957)	1:74:A:VAL:HG11	1:70:A:THR:HG23	20	1.95	0.31	2.06
(1,1957)	1:74:A:VAL:HG12	1:70:A:THR:HG21	20	1.95	0.31	2.06

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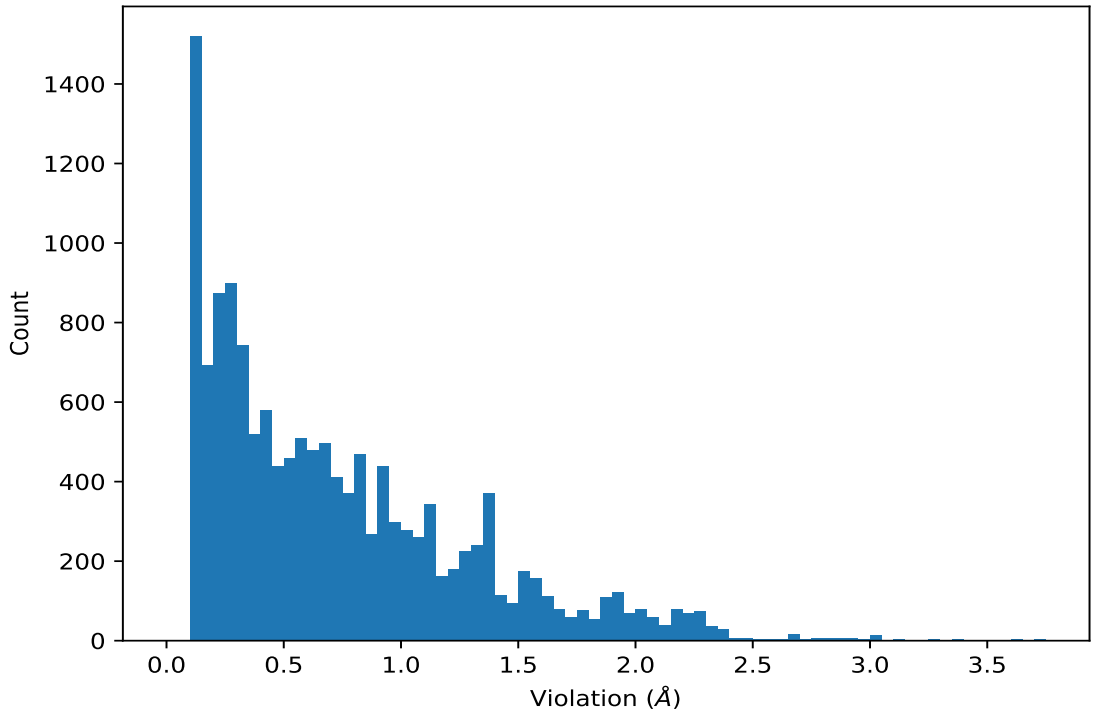
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,1957)	1:74:A:VAL:HG12	1:70:A:THR:HG22	20	1.95	0.31	2.06
(1,1957)	1:74:A:VAL:HG12	1:70:A:THR:HG23	20	1.95	0.31	2.06
(1,1957)	1:74:A:VAL:HG13	1:70:A:THR:HG21	20	1.95	0.31	2.06
(1,1957)	1:74:A:VAL:HG13	1:70:A:THR:HG22	20	1.95	0.31	2.06
(1,1957)	1:74:A:VAL:HG13	1:70:A:THR:HG23	20	1.95	0.31	2.06
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD21	20	1.89	1.11	2.45
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD22	20	1.89	1.11	2.45
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD23	20	1.89	1.11	2.45
(1,1882)	1:101:A:GLN:HB3	1:100:A:LEU:HD11	20	1.79	0.72	2.24
(1,1882)	1:101:A:GLN:HB3	1:100:A:LEU:HD12	20	1.79	0.72	2.24
(1,1882)	1:101:A:GLN:HB3	1:100:A:LEU:HD13	20	1.79	0.72	2.24
(1,2085)	1:34:A:VAL:HG11	1:71:A:MET:HE1	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG11	1:71:A:MET:HE2	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG11	1:71:A:MET:HE3	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG12	1:71:A:MET:HE1	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG12	1:71:A:MET:HE2	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG12	1:71:A:MET:HE3	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG13	1:71:A:MET:HE1	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG13	1:71:A:MET:HE2	20	1.77	0.2	1.82
(1,2085)	1:34:A:VAL:HG13	1:71:A:MET:HE3	20	1.77	0.2	1.82
(1,1126)	1:153:A:GLN:HG3	1:152:A:GLY:HA2	20	1.6	0.47	1.88
(1,1999)	1:177:A:TRP:HD1	1:34:A:VAL:HG11	20	1.6	0.09	1.65

[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)
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG21	2	3.73
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG22	2	3.73
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG23	2	3.73
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG21	3	3.62
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG22	3	3.62
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG23	3	3.62
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD21	20	3.35
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD22	20	3.35
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD23	20	3.35
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG21	14	3.27
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG22	14	3.27
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG23	14	3.27
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD21	1	3.1
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD22	1	3.1
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD23	1	3.1
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG21	20	3.04
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG22	20	3.04

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,1983)	1:36:A:ARG:HG3	1:39:A:VAL:HG23	20	3.04
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD21	16	3.03
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD22	16	3.03
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD23	16	3.03
(1,1740)	1:63:A:LEU:HD11	1:64:A:PRO:HG3	15	3.01
(1,1740)	1:63:A:LEU:HD12	1:64:A:PRO:HG3	15	3.01
(1,1740)	1:63:A:LEU:HD13	1:64:A:PRO:HG3	15	3.01
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD21	14	3.0
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD22	14	3.0
(1,1854)	1:141:A:HIS:HB3	1:97:A:LEU:HD23	14	3.0
(1,1740)	1:63:A:LEU:HD11	1:64:A:PRO:HG3	18	3.0

10 Dihedral-angle violation analysis (i)

No dihedral-angle restraints found