Full wwPDB NMR Structure Validation Report (i)

Jun 3, 2023 – 11:19 AM EDT

PDB ID	:	2JRH
BMRB ID	:	15332
Title	:	Solution structure of human MEKK3 PB1 domain cis isomer
Authors	:	Qi, H.; Jiahai, Z.; Jihui, W.; Yunyu, S.
Deposited on	:	2007-06-26

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:

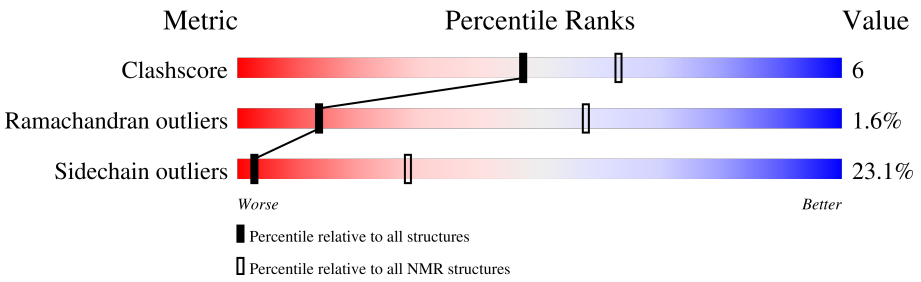
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal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

1 Overall quality at a glance (i)

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

The overall completeness of chemical shifts assignment is 86%.

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	94	62% 23% 14% •

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: *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:3-A:82 (80)	0.86	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 4 clusters and 4 single-model clusters were found.

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

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 1554 atoms, of which 776 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Mitogen-activated protein kinase kinase kinase 3.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	93	1554	484	776	147	145	2	0

There are 9 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	0	MET	-	cloning artifact	UNP Q99759
A	86	LEU	-	cloning artifact	UNP Q99759
A	87	GLU	-	cloning artifact	UNP Q99759
A	88	HIS	-	cloning artifact	UNP Q99759
A	89	HIS	-	cloning artifact	UNP Q99759
A	90	HIS	-	cloning artifact	UNP Q99759
A	91	HIS	-	cloning artifact	UNP Q99759
A	92	HIS	-	cloning artifact	UNP Q99759
A	93	HIS	-	cloning artifact	UNP Q99759

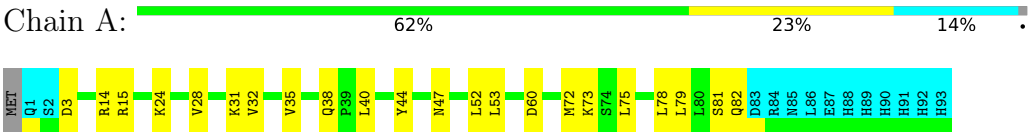
4 Residue-property plots (i)

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: Mitogen-activated protein kinase kinase kinase 3

Chain A:



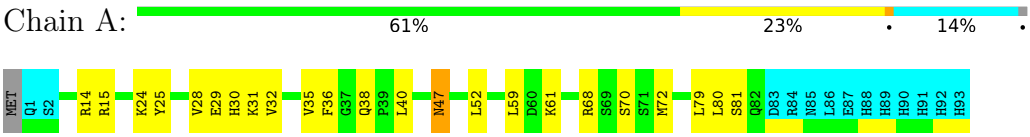
4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Mitogen-activated protein kinase kinase kinase 3

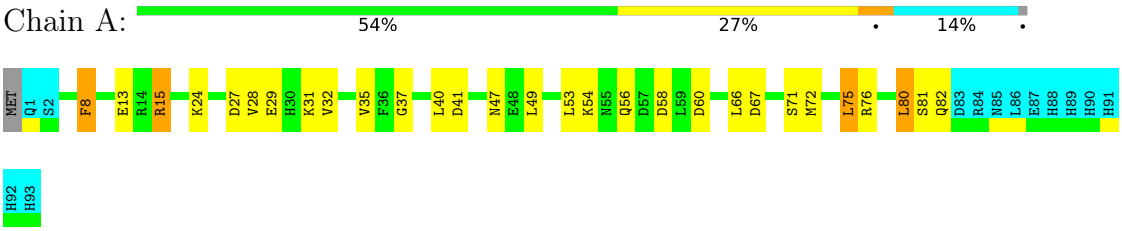
Chain A:



4.2.2 Score per residue for model 2

• Molecule 1: Mitogen-activated protein kinase kinase kinase 3

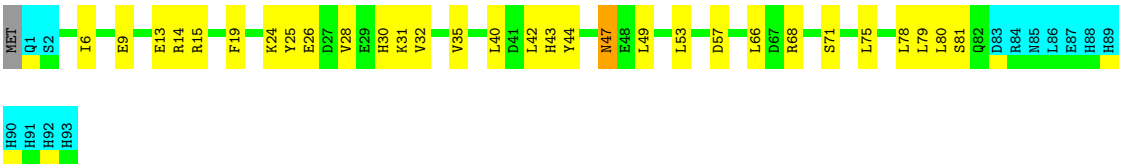
Chain A:



4.2.3 Score per residue for model 3

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

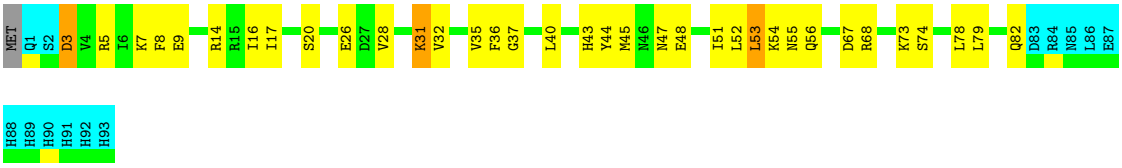
Chain A: 53% 31% • 14% •



4.2.4 Score per residue for model 4

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

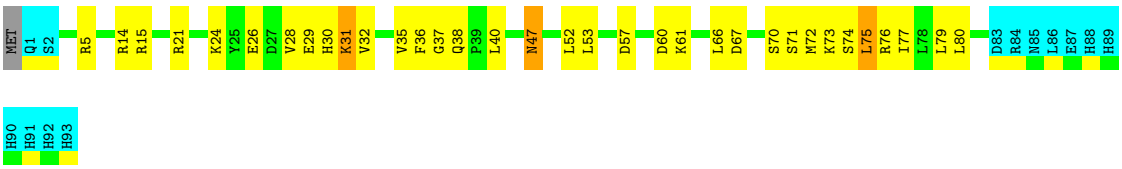
Chain A: 48% 34% • 14% •



4.2.5 Score per residue for model 5

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

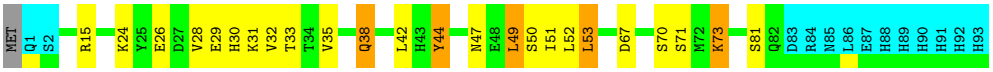
Chain A: 49% 33% • 14% •



4.2.6 Score per residue for model 6

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

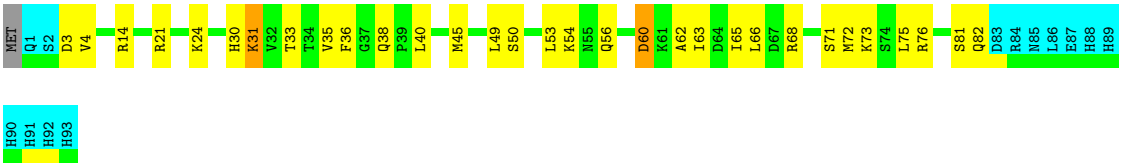
Chain A: 60% 20% 5% 14% •



4.2.7 Score per residue for model 7

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

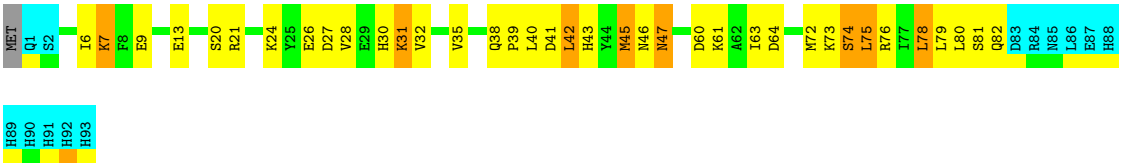
Chain A: 52% 31% • 14% •



4.2.8 Score per residue for model 8

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

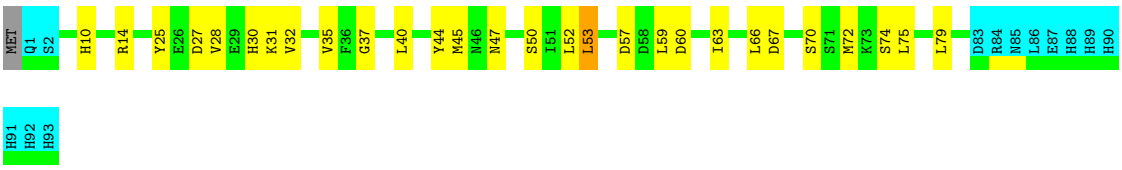
Chain A: 46% 31% 9% 14% •



4.2.9 Score per residue for model 9

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

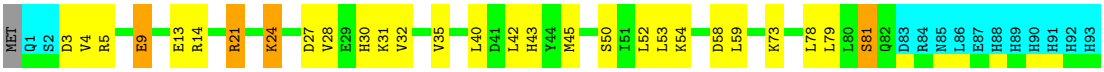
Chain A: 55% 29% • 14% •



4.2.10 Score per residue for model 10

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

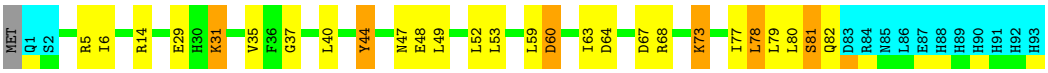
Chain A: 55% 26% • 14% •



4.2.11 Score per residue for model 11

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

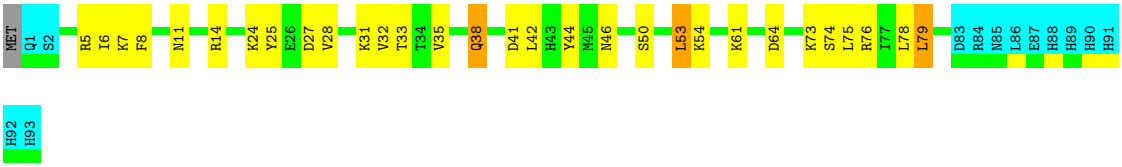
Chain A: 56% 22% 6% 14%



4.2.12 Score per residue for model 12

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

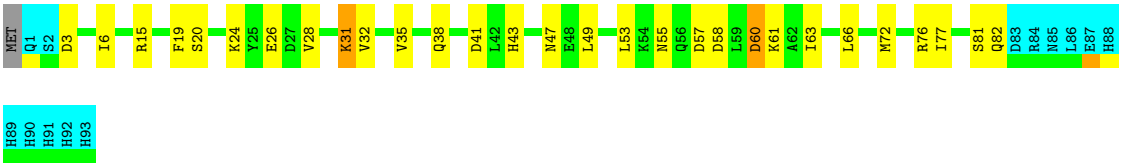
Chain A: 53% 29% 14%



4.2.13 Score per residue for model 13 (medoid)

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

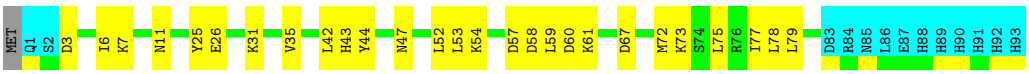
Chain A: 54% 29% 14%



4.2.14 Score per residue for model 14

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

Chain A: 56% 29% 14%



4.2.15 Score per residue for model 15

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

Chain A: 53% 29% • 14% •

MET Q1 S2 D3 I6 E9 E13 R14 R15 F19 V23 K24 V28 E29 H30 K31 V32 V35 F36 L40 D41 L42 H43 Y44 N47 L52 L53 K54 N55 Q56 L66 M72 K73 S74 L75 S81 Q82 D83 R84 N85 L86 E87 H88 H89 H90 H91 H92

H93

4.2.16 Score per residue for model 16

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

Chain A: 60% 23% • 14% •

MET Q1 S2 D3 V4 R5 E13 R14 R15 S20 R21 D27 K31 V32 T33 T34 V35 Q38 P39 L40 H43 Y44 N47 S50 D57 D58 A62 S70 S71 S81 Q82 D83 R84 N85 L86 E87 H88 H89 H90 H91 H92 H93

4.2.17 Score per residue for model 17

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

Chain A: 56% 26% • 14% •

MET Q1 S2 D3 V4 R5 I6 K7 F8 E9 R14 S20 K24 V28 E29 H30 K31 V32 T33 T34 V35 Q38 H43 Y44 M45 L52 L53 K54 N55 D58 L59 D60 M72 L78 L79 Q82 D83 R84 N85 L86 E87 H88 H89 H90 H91 H92 H93

4.2.18 Score per residue for model 18

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

Chain A: 53% 28% • 14% •

MET Q1 S2 R5 I6 R15 F19 K24 V28 K31 V35 F36 G37 Q38 P39 L40 Y44 N47 E48 L49 S50 I51 L52 L53 D60 K61 A62 I63 D64 I65 L66 D67 M72 K73 S74 I77 S81 Q82 D83 R84 N85 L86 E87 H88 H89 H90 H91

H92 H93

4.2.19 Score per residue for model 19

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

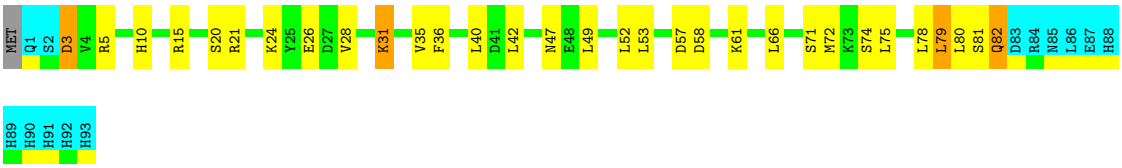
Chain A: 59% 22% • 14% •

MET Q1 S2 E9 E13 R14 R15 I16 I17 A18 F19 K24 Y25 E26 D27 V28 K31 V32 V35 Q38 P39 L40 D41 N47 E48 L49 K54 N55 Q56 D60 K61 A62 I63 L78 S81 Q82 D83 R84 N85 L86 E87 H88 H89 H90 H91 H92 H93

4.2.20 Score per residue for model 20

- Molecule 1: Mitogen-activated protein kinase kinase kinase 3

Chain A:



5 Refinement protocol and experimental data overview (i)

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

Of the 200 calculated structures, 20 were deposited, based on the following criterion: *structures with the lowest energy.*

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

Software name	**Classification**	**Version**
CNS	structure solution	1.1
MOLMOL	structure solution	
NMRPipe	structure solution	
CNS	refinement	1.1

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	1060
Number of shifts mapped to atoms	1060
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	86%

6 Model quality (i)

6.1 Standard geometry (i)

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 (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	658	675	672	9±2
All	All	13160	13500	13440	171

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 6.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:31:LYS:O	1:A:35:VAL:HG23	0.71	1.86	17	20
1:A:6:ILE:HG23	1:A:77:ILE:HD12	0.68	1.66	11	1
1:A:60:ASP:HA	1:A:63:ILE:HD12	0.67	1.67	13	6
1:A:40:LEU:HD23	1:A:79:LEU:HD13	0.66	1.65	8	1
1:A:44:TYR:HB2	1:A:53:LEU:HD21	0.66	1.67	18	2
1:A:44:TYR:HB3	1:A:53:LEU:HD21	0.65	1.69	4	1
1:A:19:PHE:CE2	1:A:28:VAL:HG22	0.64	2.27	15	2
1:A:37:GLY:O	1:A:40:LEU:HD12	0.62	1.94	4	6
1:A:17:ILE:HD13	1:A:18:ALA:N	0.62	2.08	19	1
1:A:44:TYR:HB2	1:A:53:LEU:HD11	0.61	1.72	15	4
1:A:28:VAL:O	1:A:32:VAL:HG23	0.60	1.96	13	14
1:A:62:ALA:HA	1:A:65:ILE:HD12	0.60	1.74	18	2
1:A:44:TYR:HB3	1:A:53:LEU:HD11	0.59	1.74	17	1
1:A:44:TYR:CB	1:A:53:LEU:HD21	0.58	2.28	4	1

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:59:LEU:C	1:A:59:LEU:HD13	0.58	2.19	10	1
1:A:33:THR:HG23	1:A:38:GLN:HB3	0.58	1.75	12	3
1:A:66:LEU:HD11	1:A:75:LEU:HG	0.57	1.76	9	2
1:A:36:PHE:CE2	1:A:79:LEU:HD21	0.57	2.35	4	3
1:A:66:LEU:HD13	1:A:66:LEU:C	0.56	2.21	3	1
1:A:40:LEU:CD2	1:A:79:LEU:HD13	0.56	2.31	8	1
1:A:44:TYR:CZ	1:A:62:ALA:HB1	0.55	2.37	16	1
1:A:9:GLU:HB3	1:A:78:LEU:HD12	0.55	1.77	3	2
1:A:17:ILE:HD12	1:A:19:PHE:CD2	0.55	2.37	19	1
1:A:4:VAL:HG21	1:A:21:ARG:NH2	0.55	2.16	10	1
1:A:66:LEU:HD12	1:A:72:MET:HB3	0.54	1.80	2	1
1:A:40:LEU:HD13	1:A:79:LEU:HD13	0.54	1.78	10	1
1:A:24:LYS:O	1:A:28:VAL:HG23	0.54	2.03	8	10
1:A:40:LEU:HD23	1:A:81:SER:HA	0.53	1.79	11	8
1:A:33:THR:HG23	1:A:38:GLN:HG3	0.53	1.80	6	1
1:A:8:PHE:CZ	1:A:32:VAL:HG21	0.53	2.37	4	1
1:A:79:LEU:O	1:A:79:LEU:HD12	0.53	2.04	8	3
1:A:6:ILE:HG12	1:A:75:LEU:HD11	0.53	1.80	8	1
1:A:53:LEU:HD22	1:A:53:LEU:N	0.52	2.20	11	3
1:A:8:PHE:CD1	1:A:79:LEU:HD23	0.51	2.40	17	1
1:A:66:LEU:HD13	1:A:75:LEU:HD12	0.51	1.82	7	1
1:A:45:MET:HB2	1:A:78:LEU:HD21	0.51	1.81	8	1
1:A:66:LEU:HD22	1:A:75:LEU:HG	0.51	1.81	2	1
1:A:10:HIS:HB2	1:A:79:LEU:HD11	0.51	1.82	9	1
1:A:25:TYR:HB2	1:A:59:LEU:HD22	0.51	1.81	1	1
1:A:52:LEU:HD23	1:A:53:LEU:N	0.50	2.20	6	3
1:A:36:PHE:HB2	1:A:40:LEU:HD11	0.50	1.83	15	3
1:A:6:ILE:CG2	1:A:77:ILE:HD12	0.49	2.35	11	4
1:A:44:TYR:CE2	1:A:62:ALA:HB1	0.49	2.43	16	1
1:A:33:THR:HG22	1:A:38:GLN:HG3	0.49	1.85	16	1
1:A:9:GLU:HB2	1:A:78:LEU:HD12	0.48	1.84	19	2
1:A:44:TYR:CD2	1:A:53:LEU:HD21	0.48	2.44	6	1
1:A:66:LEU:HD13	1:A:75:LEU:CD1	0.47	2.38	7	1
1:A:9:GLU:CB	1:A:78:LEU:HD12	0.47	2.40	19	1
1:A:32:VAL:HG11	1:A:42:LEU:HD21	0.46	1.87	8	1
1:A:74:SER:C	1:A:75:LEU:HD22	0.46	2.31	15	1
1:A:36:PHE:CE2	1:A:79:LEU:HD11	0.45	2.46	20	1
1:A:6:ILE:HD12	1:A:19:PHE:CD1	0.45	2.46	15	2
1:A:17:ILE:HD12	1:A:19:PHE:CE2	0.45	2.46	19	1
1:A:53:LEU:HD13	1:A:58:ASP:CB	0.45	2.42	13	1
1:A:52:LEU:HD23	1:A:54:LYS:HE2	0.45	1.89	14	1

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:53:LEU:CD2	1:A:59:LEU:HD12	0.45	2.42	14	1
1:A:53:LEU:HD13	1:A:59:LEU:HD13	0.45	1.89	9	1
1:A:25:TYR:OH	1:A:42:LEU:HD12	0.44	2.11	3	1
1:A:6:ILE:HD13	1:A:19:PHE:HD2	0.44	1.71	18	1
1:A:19:PHE:CZ	1:A:28:VAL:HG13	0.44	2.46	15	1
1:A:4:VAL:HG23	1:A:21:ARG:HG3	0.44	1.89	7	1
1:A:42:LEU:HB3	1:A:53:LEU:HD12	0.43	1.90	6	1
1:A:66:LEU:HD22	1:A:75:LEU:HD21	0.43	1.89	15	1
1:A:78:LEU:HD23	1:A:80:LEU:HD11	0.43	1.91	11	1
1:A:66:LEU:HD22	1:A:75:LEU:CD2	0.42	2.43	15	1
1:A:6:ILE:N	1:A:6:ILE:HD12	0.42	2.29	18	2
1:A:4:VAL:HG21	1:A:21:ARG:CZ	0.42	2.44	10	1
1:A:39:PRO:C	1:A:40:LEU:HD12	0.42	2.34	8	1
1:A:63:ILE:HA	1:A:66:LEU:HD12	0.42	1.92	9	1
1:A:25:TYR:CD2	1:A:59:LEU:HD22	0.42	2.50	9	1
1:A:8:PHE:CE2	1:A:15:ARG:HB3	0.42	2.50	2	1
1:A:32:VAL:HG11	1:A:42:LEU:CD2	0.41	2.45	8	1
1:A:25:TYR:CZ	1:A:42:LEU:HD12	0.41	2.50	12	1
1:A:44:TYR:CB	1:A:53:LEU:HD11	0.41	2.45	12	1
1:A:31:LYS:HE3	1:A:35:VAL:HG21	0.41	1.93	7	1
1:A:49:LEU:HD22	1:A:51:ILE:HD11	0.41	1.91	6	1
1:A:80:LEU:N	1:A:80:LEU:HD12	0.41	2.31	2	2
1:A:7:LYS:CD	1:A:74:SER:HB3	0.41	2.46	8	1
1:A:23:VAL:O	1:A:23:VAL:HG13	0.41	2.16	15	1
1:A:53:LEU:HD23	1:A:58:ASP:HB3	0.41	1.93	20	1
1:A:16:ILE:O	1:A:17:ILE:HD13	0.40	2.16	4	1
1:A:8:PHE:HB3	1:A:79:LEU:HD21	0.40	1.91	12	1
1:A:42:LEU:HD23	1:A:79:LEU:HB3	0.40	1.94	3	1
1:A:25:TYR:CD1	1:A:59:LEU:HD13	0.40	2.51	14	1
1:A:75:LEU:HD23	1:A:77:ILE:HD11	0.40	1.92	5	1
1:A:33:THR:HG22	1:A:38:GLN:CG	0.40	2.47	16	1

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	80/94 (85%)	76±1 (96±2%)	2±1 (3±2%)	1±1 (2±1%)	13 57
All	All	1600/1880 (85%)	1528 (96%)	46 (3%)	26 (2%)	13 57

All 5 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	47	ASN	15
1	A	3	ASP	7
1	A	73	LYS	2
1	A	71	SER	1
1	A	72	MET	1

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	76/90 (84%)	58±4 (77±5%)	18±4 (23±5%)	3 28
All	All	1520/1800 (84%)	1169 (77%)	351 (23%)	3 28

All 58 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	14	ARG	11
1	A	15	ARG	11
1	A	82	GLN	11
1	A	30	HIS	10
1	A	72	MET	10
1	A	73	LYS	10
1	A	49	LEU	9
1	A	53	LEU	9
1	A	81	SER	9
1	A	26	GLU	9
1	A	43	HIS	9
1	A	5	ARG	9
1	A	38	GLN	8

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Mol	Chain	Res	Type	Models (Total)
1	A	52	LEU	8
1	A	61	LYS	8
1	A	60	ASP	8
1	A	67	ASP	8
1	A	24	LYS	8
1	A	31	LYS	8
1	A	74	SER	8
1	A	78	LEU	8
1	A	47	ASN	7
1	A	13	GLU	7
1	A	54	LYS	7
1	A	75	LEU	7
1	A	57	ASP	7
1	A	50	SER	7
1	A	27	ASP	6
1	A	41	ASP	6
1	A	71	SER	6
1	A	76	ARG	6
1	A	9	GLU	6
1	A	20	SER	6
1	A	45	MET	6
1	A	29	GLU	5
1	A	68	ARG	5
1	A	70	SER	5
1	A	80	LEU	5
1	A	56	GLN	5
1	A	58	ASP	5
1	A	3	ASP	5
1	A	7	LYS	5
1	A	21	ARG	5
1	A	48	GLU	4
1	A	42	LEU	4
1	A	64	ASP	4
1	A	55	ASN	3
1	A	44	TYR	3
1	A	79	LEU	3
1	A	46	ASN	2
1	A	11	ASN	2
1	A	66	LEU	2
1	A	8	PHE	1
1	A	51	ILE	1
1	A	59	LEU	1

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Mol	Chain	Res	Type	Models (Total)
1	A	33	THR	1
1	A	17	ILE	1
1	A	10	HIS	1

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 86% 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: *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	1060
Number of shifts mapped to atoms	1060
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	3

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	85	-0.24 ± 0.08	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	82	0.25 ± 0.09	None needed (< 0.5 ppm)
$^{13}C'$	78	0.04 ± 0.10	None needed (< 0.5 ppm)
^{15}N	82	-0.78 ± 0.36	Should be applied

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 86%, i.e. 1006 atoms were assigned a chemical shift out of a possible 1168. 0 out of 16 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	392/398 (98%)	160/160 (100%)	154/160 (96%)	78/78 (100%)
Sidechain	592/698 (85%)	406/451 (90%)	177/216 (82%)	9/31 (29%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	22/72 (31%)	22/35 (63%)	0/31 (0%)	0/6 (0%)
Overall	1006/1168 (86%)	588/646 (91%)	331/407 (81%)	87/115 (76%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 78%, i.e. 1058 atoms were assigned a chemical shift out of a possible 1361. 0 out of 17 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	414/463 (89%)	169/186 (91%)	163/186 (88%)	82/91 (90%)
Sidechain	622/778 (80%)	426/501 (85%)	185/241 (77%)	11/36 (31%)
Aromatic	22/120 (18%)	22/59 (37%)	0/43 (0%)	0/18 (0%)
Overall	1058/1361 (78%)	617/746 (83%)	348/470 (74%)	93/145 (64%)

7.1.4 Statistically unusual chemical shifts (i)

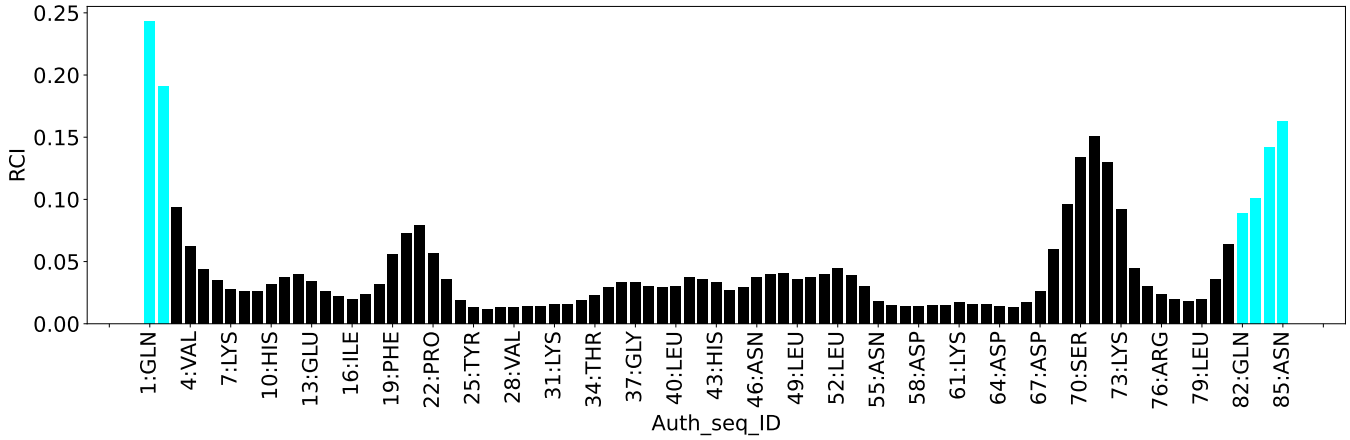
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	21	ARG	NE	111.66	76.53 – 92.65	16.8
1	A	15	ARG	NE	111.53	76.53 – 92.65	16.7
1	A	6	ILE	CG1	12.38	19.24 – 36.26	-9.0

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:


RCI
0.25
0.20
0.15
0.10
0.05
0.00
1:GLN
4:VAL
7:LYS
10:HIS
13:GLU
16:ILE
19:PHE
22:PRO
25:TYR
28:VAL
31:LYS
34:THR
37:GLY
40:LEU
43:HIS
46:ASN
49:LEU
52:LEU
55:ASN
58:ASP
61:LYS
64:ASP
67:ASP
70:SER
73:LYS
76:ARG
79:LEU
82:GLN
85:ASN
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	1184
Intra-residue (|i-j|=0)	382
Sequential (|i-j|=1)	325
Medium range (|i-j|>1 and |i-j|<5)	203
Long range (|i-j|≥5)	246
Inter-chain	0
Hydrogen bond restraints	28
Disulfide bond restraints	0
Total dihedral-angle restraints	112
Number of unmapped restraints	0
Number of restraints per residue	13.8
Number of long range restraints per residue[1]	2.7

[1]Long range hydrogen bonds and disulfide bonds are counted as long range restraints while calculating the number of long range restraints per residue

8.2 Residual restraint violations (i)

This section provides the overview of the restraint violations analysis. The violations are binned as small, medium and large violations based on its absolute value. Average number of violations per model is calculated by dividing the total number of violations in each bin by the size of the ensemble.

8.2.1 Average number of distance violations per model (i)

Distance violations less than 0.1 Å are not included in the calculation.

Bins (Å)	Average number of violations per model	Max (Å)
0.1-0.2 (Small)	1.1	0.18
0.2-0.5 (Medium)	None	None
>0.5 (Large)	None	None

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

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

Bins (°)	Average number of violations per model	Max (°)
1.0-10.0 (Small)	0.7	1.7
10.0-20.0 (Medium)	None	None
>20.0 (Large)	None	None

9 Distance violation analysis (i)

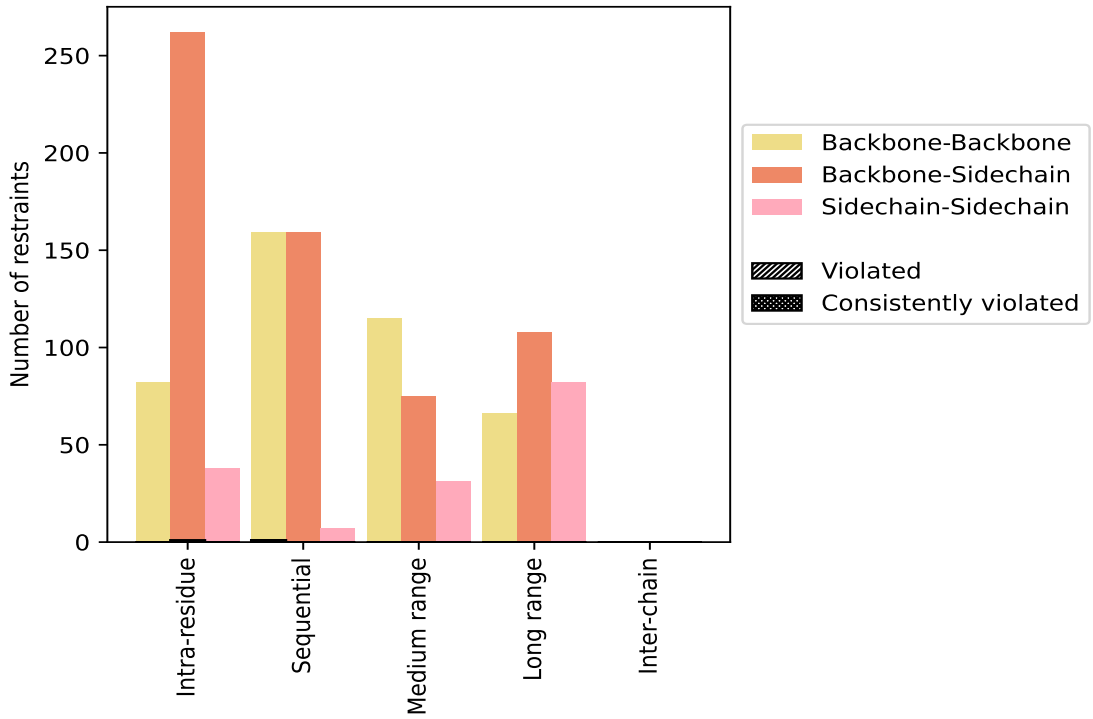
9.1 Summary of distance violations (i)

The following table shows the summary of distance violations in different restraint categories based on the sequence separation of the atoms involved. Each category is further sub-divided into three sub-categories based on the atoms involved. Violations less than 0.1 Å are not included in the statistics.

Restraints type	Count	%[1]	Violated[3]			Consistently Violated[4]		
			Count	%[2]	%[1]	Count	%[2]	%[1]
Intra-residue (|i-j|=0)	382	32.3	1	0.3	0.1	0	0.0	0.0
Backbone-Backbone	82	6.9	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	262	22.1	1	0.4	0.1	0	0.0	0.0
Sidechain-Sidechain	38	3.2	0	0.0	0.0	0	0.0	0.0
Sequential (|i-j|=1)	325	27.4	1	0.3	0.1	1	0.3	0.1
Backbone-Backbone	159	13.4	1	0.6	0.1	1	0.6	0.1
Backbone-Sidechain	159	13.4	0	0.0	0.0	0	0.0	0.0
Sidechain-Sidechain	7	0.6	0	0.0	0.0	0	0.0	0.0
Medium range (|i-j|>1 & |i-j|<5)	203	17.1	0	0.0	0.0	0	0.0	0.0
Backbone-Backbone	97	8.2	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	75	6.3	0	0.0	0.0	0	0.0	0.0
Sidechain-Sidechain	31	2.6	0	0.0	0.0	0	0.0	0.0
Long range (|i-j|≥5)	246	20.8	0	0.0	0.0	0	0.0	0.0
Backbone-Backbone	56	4.7	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	108	9.1	0	0.0	0.0	0	0.0	0.0
Sidechain-Sidechain	82	6.9	0	0.0	0.0	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	28	2.4	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	1184	100.0	2	0.2	0.2	1	0.1	0.1
Backbone-Backbone	422	35.6	1	0.2	0.1	1	0.2	0.1
Backbone-Sidechain	604	51.0	1	0.2	0.1	0	0.0	0.0
Sidechain-Sidechain	158	13.3	0	0.0	0.0	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 (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	0	1	0	0	0	1	0.18	0.18	0.0	0.18
2	0	1	0	0	0	1	0.17	0.17	0.0	0.17
3	0	1	0	0	0	1	0.16	0.16	0.0	0.16
4	0	1	0	0	0	1	0.16	0.16	0.0	0.16
5	0	1	0	0	0	1	0.17	0.17	0.0	0.17
6	0	1	0	0	0	1	0.17	0.17	0.0	0.17
7	0	1	0	0	0	1	0.17	0.17	0.0	0.17
8	0	1	0	0	0	1	0.17	0.17	0.0	0.17
9	0	1	0	0	0	1	0.18	0.18	0.0	0.18
10	0	1	0	0	0	1	0.18	0.18	0.0	0.18
11	0	1	0	0	0	1	0.16	0.16	0.0	0.16

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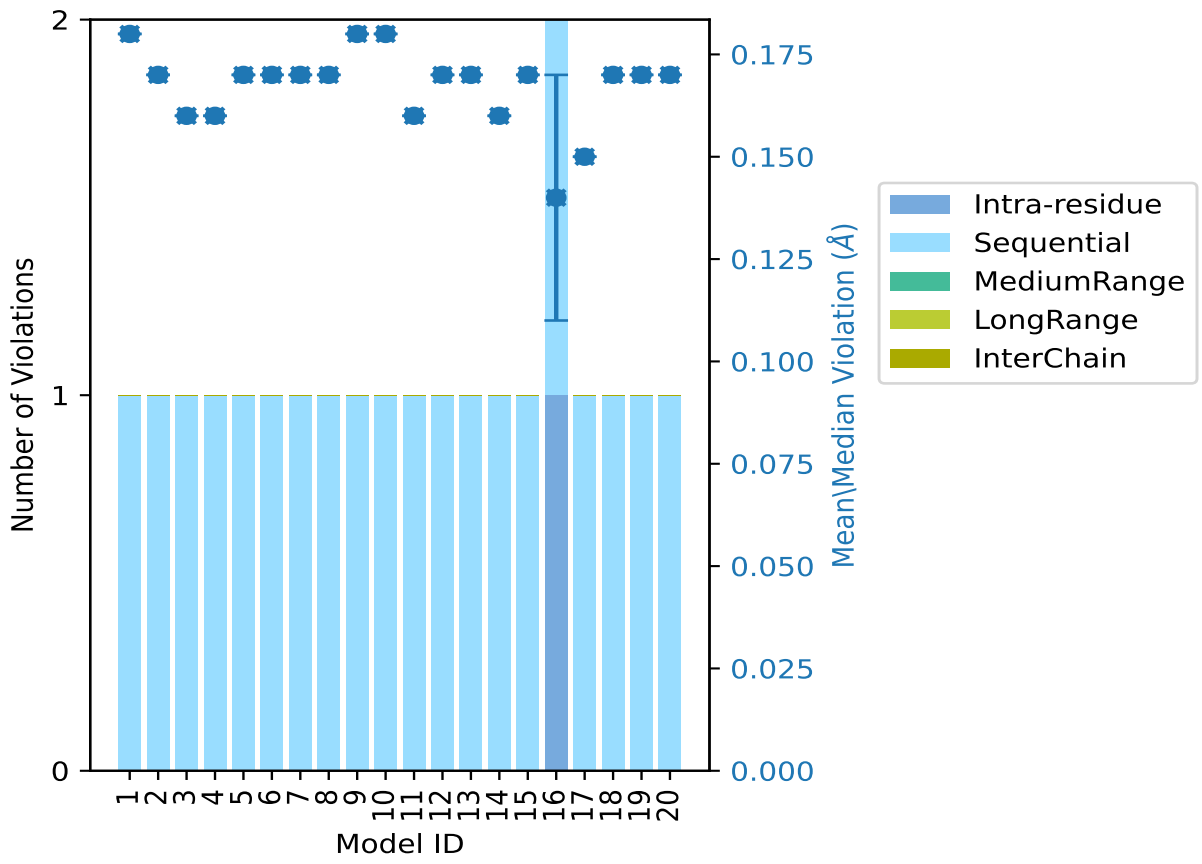
Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
12	0	1	0	0	0	1	0.17	0.17	0.0	0.17
13	0	1	0	0	0	1	0.17	0.17	0.0	0.17
14	0	1	0	0	0	1	0.16	0.16	0.0	0.16
15	0	1	0	0	0	1	0.17	0.17	0.0	0.17
16	1	1	0	0	0	2	0.14	0.17	0.03	0.14
17	0	1	0	0	0	1	0.15	0.15	0.0	0.15
18	0	1	0	0	0	1	0.17	0.17	0.0	0.17
19	0	1	0	0	0	1	0.17	0.17	0.0	0.17
20	0	1	0	0	0	1	0.17	0.17	0.0	0.17

[1]Intra-residue restraints, [2]Sequential restraints, [3]Medium range restraints, [4]Long range restraints, [5]Inter-chain restraints, [6]Standard deviation

9.2.1 Bar graph : Distance Violation statistics for each model (i)



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

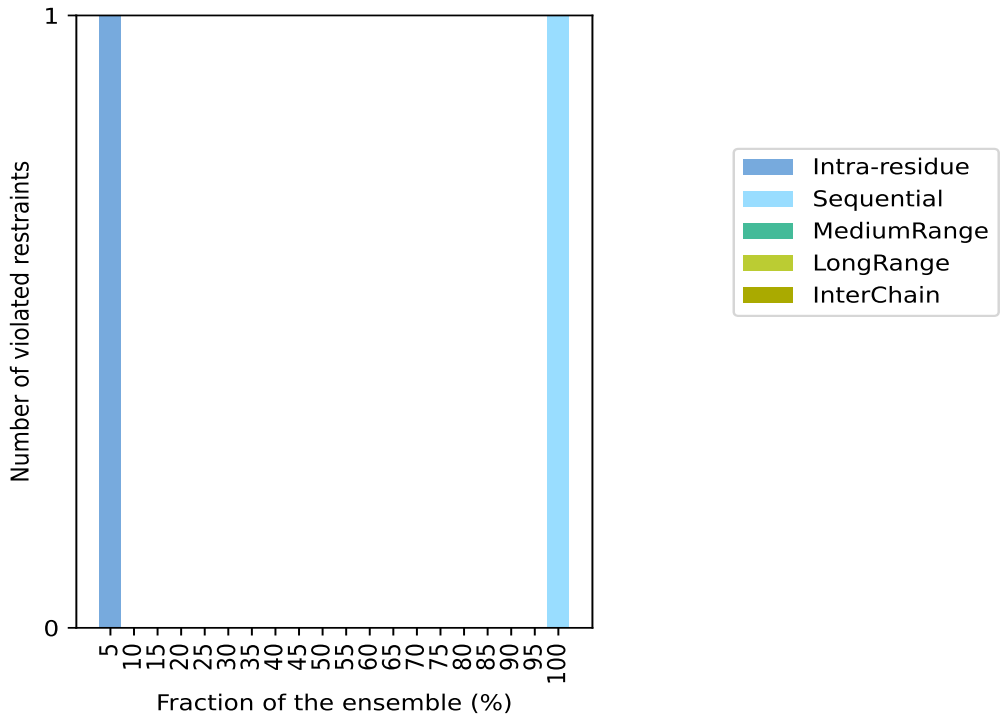
9.3 Distance violation statistics for the ensemble (i)

Violation analysis may find that some restraints are violated in few models and some are violated in most of models. The following table provides this information as number of violated restraints for a given fraction of the ensemble. In total, 1154(IR:381, SQ:324, MR:203, LR:246, 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]	%
1	0	0	0	0	1	1	5.0
0	0	0	0	0	0	2	10.0
0	0	0	0	0	0	3	15.0
0	0	0	0	0	0	4	20.0
0	0	0	0	0	0	5	25.0
0	0	0	0	0	0	6	30.0
0	0	0	0	0	0	7	35.0
0	0	0	0	0	0	8	40.0
0	0	0	0	0	0	9	45.0
0	0	0	0	0	0	10	50.0
0	0	0	0	0	0	11	55.0
0	0	0	0	0	0	12	60.0
0	0	0	0	0	0	13	65.0
0	0	0	0	0	0	14	70.0
0	0	0	0	0	0	15	75.0
0	0	0	0	0	0	16	80.0
0	0	0	0	0	0	17	85.0
0	0	0	0	0	0	18	90.0
0	0	0	0	0	0	19	95.0
0	1	0	0	0	1	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

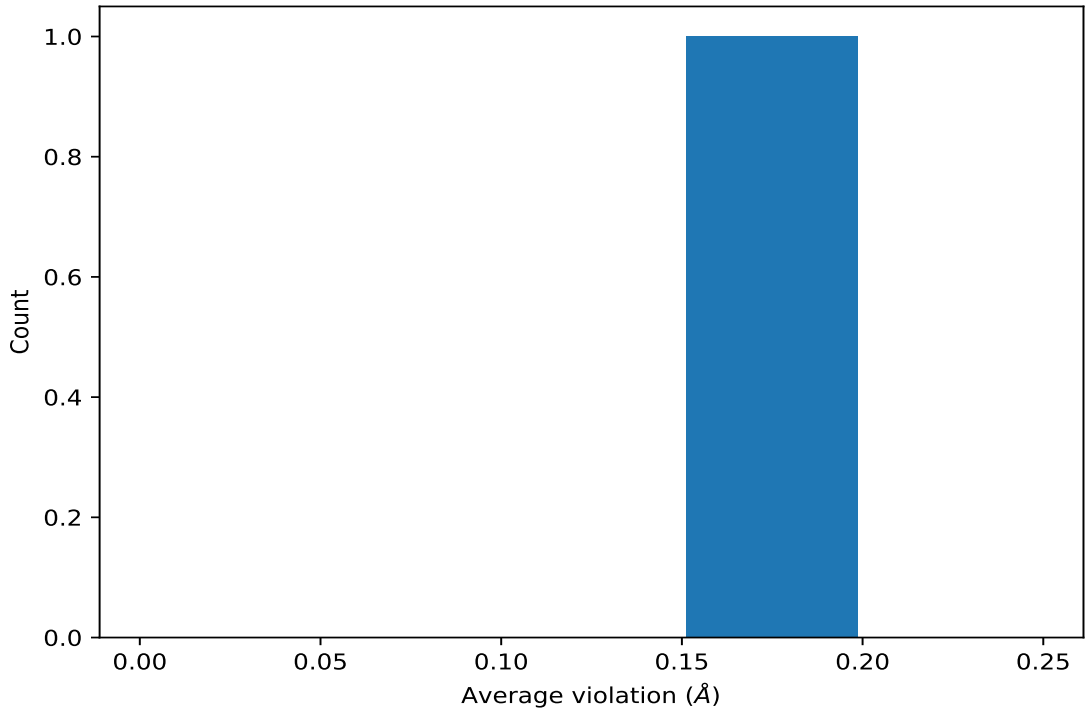
9.3.1 Bar graph : Distance violation statistics for the ensemble (i)



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 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.

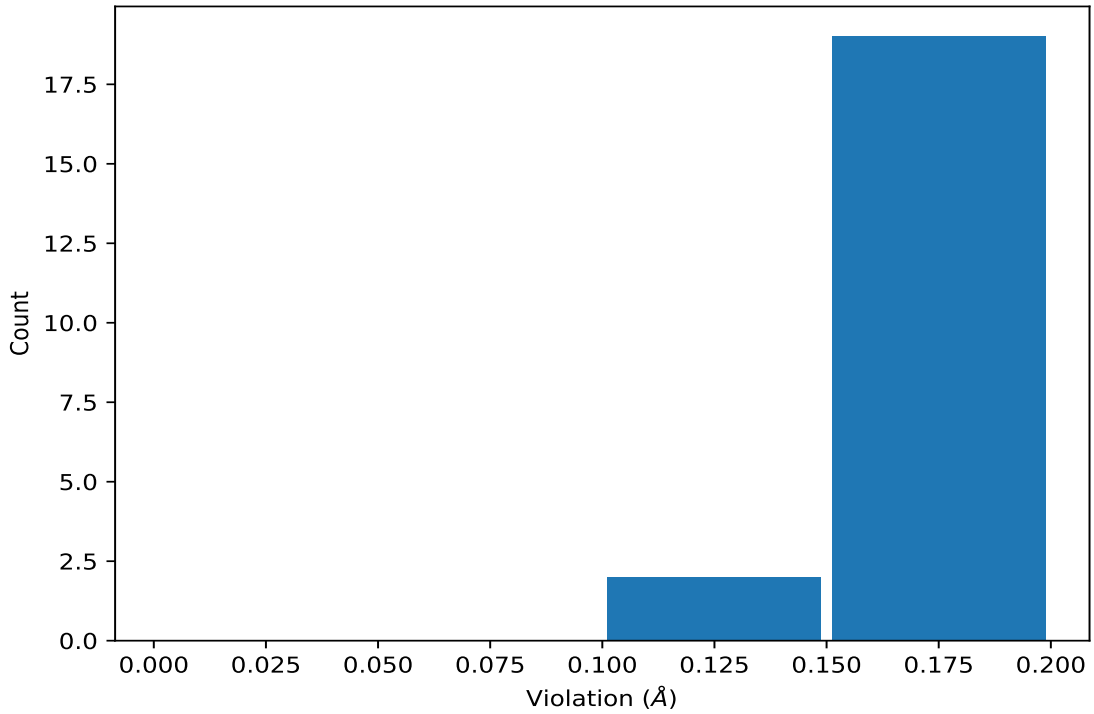
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	20	0.17	0.01	0.17

[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)
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	1	0.18
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	9	0.18
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	10	0.18
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	2	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	5	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	6	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	7	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	8	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	12	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	13	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	15	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	16	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	18	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	19	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	20	0.17
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	3	0.16

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	4	0.16
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	11	0.16
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	14	0.16
(1,1129)	1:A:82:GLN:HA	1:A:83:ASP:H	17	0.15
(1,514)	1:A:33:THR:HB	1:A:33:THR:H	16	0.11

10 Dihedral-angle violation analysis (i)

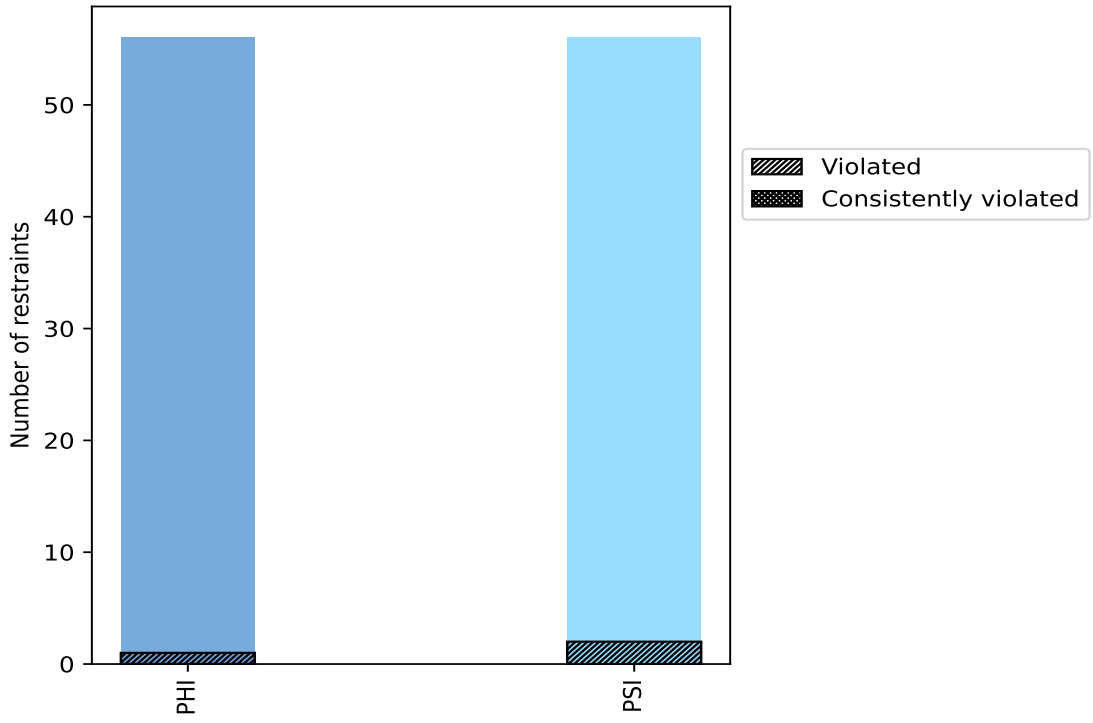
10.1 Summary of dihedral-angle violations (i)

The following table provides the summary of dihedral-angle violations in different dihedral-angle types. Violations less than 1° are not included in the calculation.

Angle type	Count	%[1]	Violated[3]			Consistently Violated[4]		
			Count	%[2]	%[1]	Count	%[2]	%[1]
PHI	56	50.0	1	1.8	0.9	0	0.0	0.0
PSI	56	50.0	2	3.6	1.8	0	0.0	0.0
Total	112	100.0	3	2.7	2.7	0	0.0	0.0

[1] percentage calculated with respect to total number of dihedral-angle restraints, [2] percentage calculated with respect to number of restraints in a particular dihedral-angle type, [3] violated in at least one model, [4] violated in all the models

10.1.1 Bar chart : Distribution of dihedral-angles and violations (i)



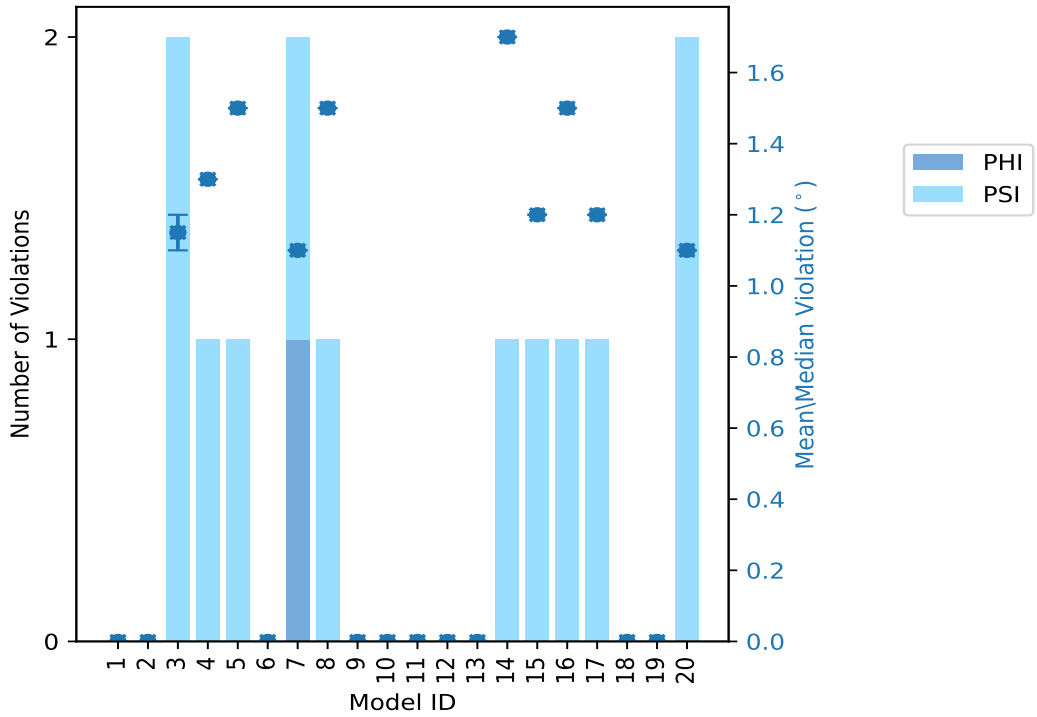
Violated and consistently violated restraints are shown using different hatch patterns in their respective categories

10.2 Dihedral-angle violation statistics for each model (i)

The following table provides the dihedral-angle violation statistics for each model in the ensemble. Violations less than 1° are not included in the statistics.

Model ID	Number of violations			Mean (°)	Max (°)	SD (°)	Median (°)
	PHI	PSI	Total				
1	0	0	0	0.0	0.0	0.0	0.0
2	0	0	0	0.0	0.0	0.0	0.0
3	0	2	2	1.15	1.2	0.05	1.15
4	0	1	1	1.3	1.3	0.0	1.3
5	0	1	1	1.5	1.5	0.0	1.5
6	0	0	0	0.0	0.0	0.0	0.0
7	1	1	2	1.1	1.1	0.0	1.1
8	0	1	1	1.5	1.5	0.0	1.5
9	0	0	0	0.0	0.0	0.0	0.0
10	0	0	0	0.0	0.0	0.0	0.0
11	0	0	0	0.0	0.0	0.0	0.0
12	0	0	0	0.0	0.0	0.0	0.0
13	0	0	0	0.0	0.0	0.0	0.0
14	0	1	1	1.7	1.7	0.0	1.7
15	0	1	1	1.2	1.2	0.0	1.2
16	0	1	1	1.5	1.5	0.0	1.5
17	0	1	1	1.2	1.2	0.0	1.2
18	0	0	0	0.0	0.0	0.0	0.0
19	0	0	0	0.0	0.0	0.0	0.0
20	0	2	2	1.1	1.1	0.0	1.1

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



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

10.3 Dihedral-angle violation statistics for the ensemble (i)

Violation analysis may find that some restraints are violated in very few models and some are violated in most of models. The following table provides this information as number of violated restraints for a given fraction of ensemble.

Number of violated restraints			Fraction of the ensemble	
PHI	PSI	Total	Count[1]	%
1	0	1	1	5.0
0	1	1	2	10.0
0	0	0	3	15.0
0	0	0	4	20.0
0	0	0	5	25.0
0	0	0	6	30.0
0	0	0	7	35.0
0	0	0	8	40.0
0	0	0	9	45.0
0	1	1	10	50.0
0	0	0	11	55.0

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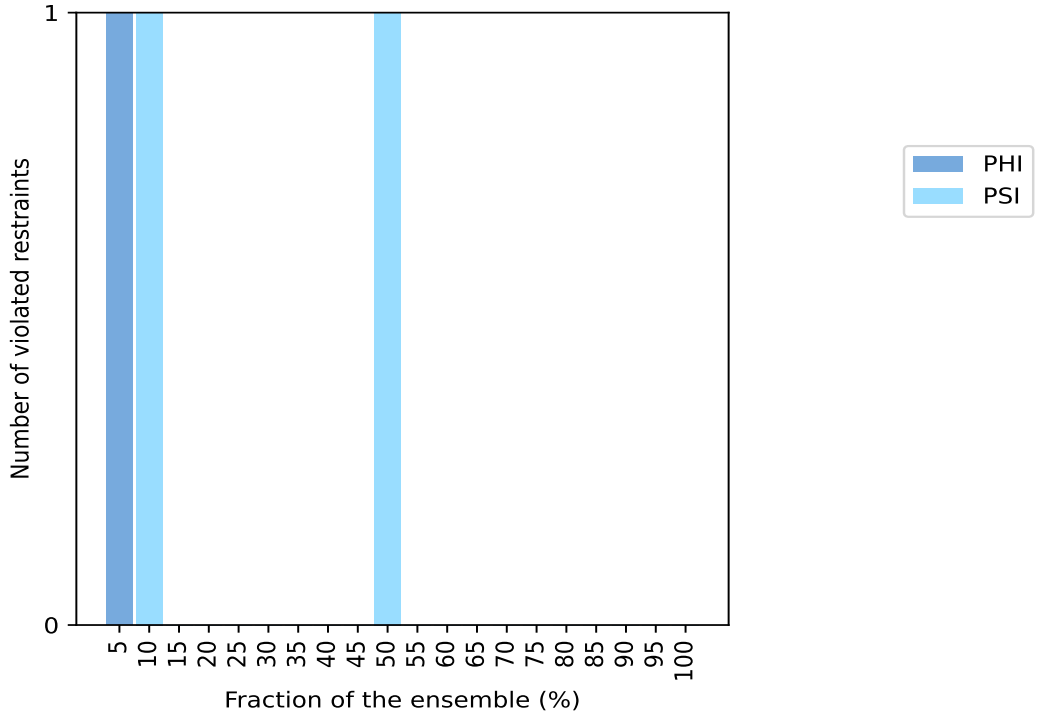
Number of violated restraints			Fraction of the ensemble	
PHI	PSI	Total	Count[1]	%
0	0	0	12	60.0
0	0	0	13	65.0
0	0	0	14	70.0
0	0	0	15	75.0
0	0	0	16	80.0
0	0	0	17	85.0
0	0	0	18	90.0
0	0	0	19	95.0
0	0	0	20	100.0

[1] Number of models with violations

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

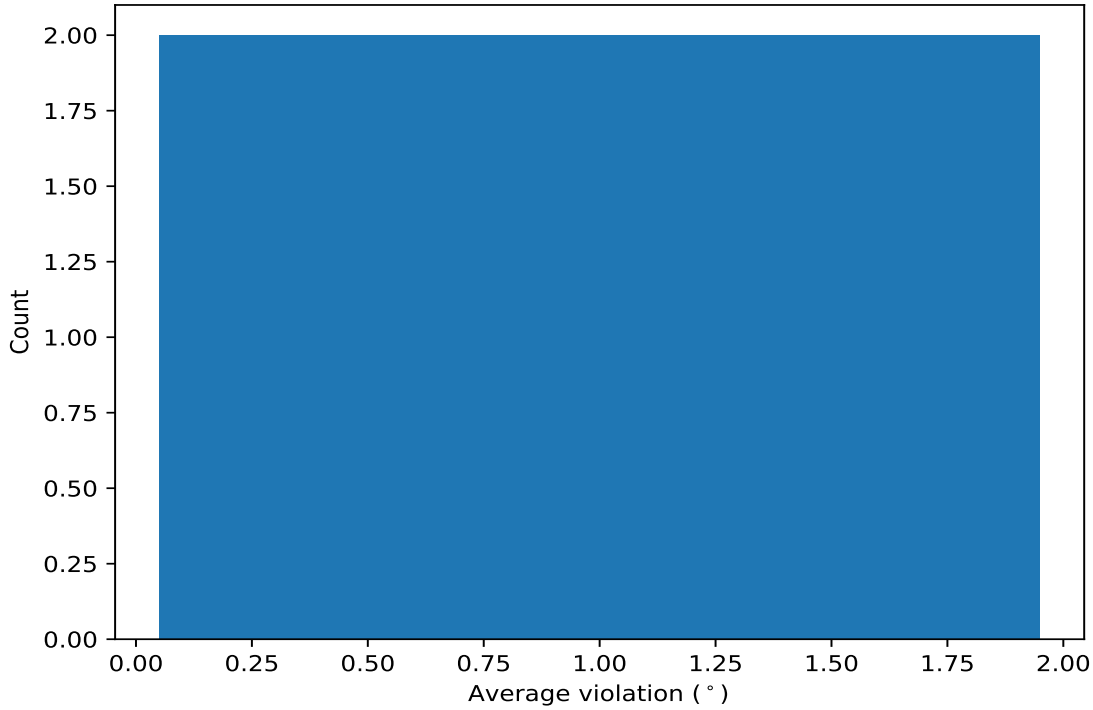


10.4 Most violated dihedral-angle restraints in the ensemble (i)

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

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

in the ensemble



10.4.2 Table: Most violated dihedral-angle restraints (i)

The following table provides the mean and the standard deviation of the 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.

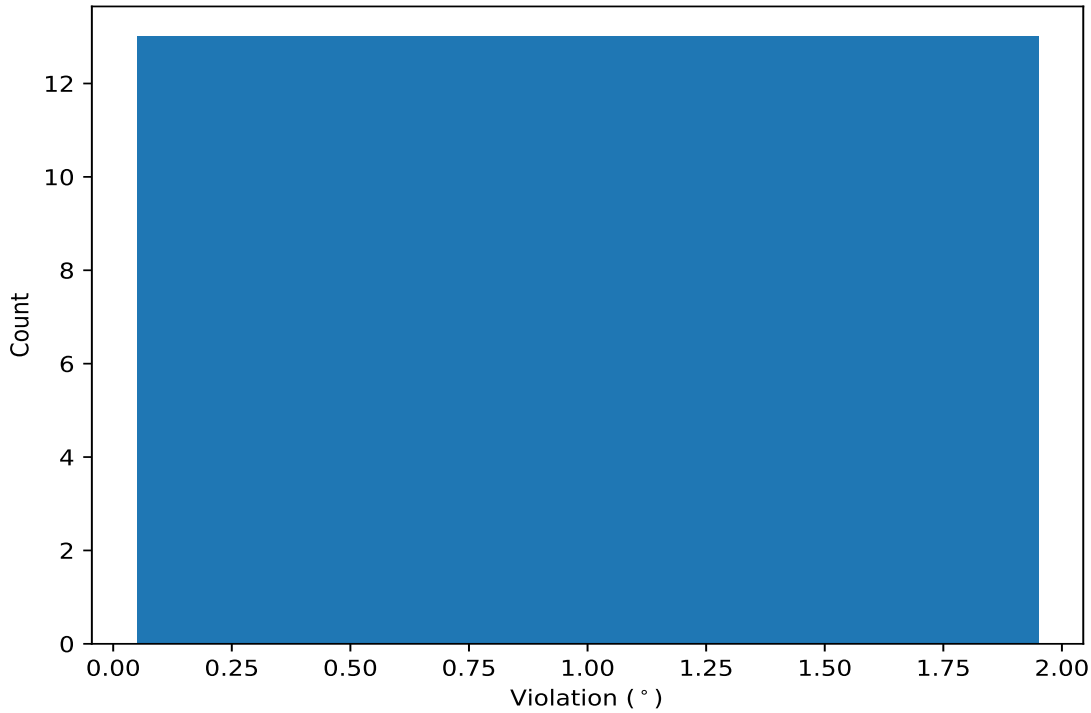
Key	Atom-1	Atom-2	Atom-3	Atom-4	Models[1]	Mean	SD[2]	Median
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	10	1.33	0.2	1.25
(1,92)	1:A:66:LEU:N	1:A:66:LEU:CA	1:A:66:LEU:C	1:A:67:ASP:N	2	1.1	0.0	1.1

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

10.5 All violated dihedral-angle restraints (i)

10.5.1 Histogram : Distribution of violations (i)

The following histogram shows the distribution of the absolute value of the violation for all violated restraints in the ensemble.



10.5.2 Table: All violated dihedral-angle restraints (i)

The following table 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.

Key	Atom-1	Atom-2	Atom-3	Atom-4	Model ID	Violation (°)
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	14	1.7
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	5	1.5
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	8	1.5
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	16	1.5
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	4	1.3
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	3	1.2
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	15	1.2
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	17	1.2
(1,92)	1:A:66:LEU:N	1:A:66:LEU:CA	1:A:66:LEU:C	1:A:67:ASP:N	3	1.1
(1,92)	1:A:66:LEU:N	1:A:66:LEU:CA	1:A:66:LEU:C	1:A:67:ASP:N	20	1.1
(1,45)	1:A:33:THR:C	1:A:34:THR:N	1:A:34:THR:CA	1:A:34:THR:C	7	1.1
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	7	1.1
(1,112)	1:A:82:GLN:N	1:A:82:GLN:CA	1:A:82:GLN:C	1:A:83:ASP:N	20	1.1