wwPDB NMR Structure Validation Summary Report

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PDB ID	:	2NPU
BMRB ID	:	6760
Title	:	The solution structure of the rapamycin-binding domain of mTOR (FRB)
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Deposited on	:	2006-10-30

This is a wwPDB NMR Structure Validation Summary Report for a publicly released PDB entry.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/NMRValidationReportHelp
with specific help available everywhere you see the (i) symbol.

The types of validation reports are described at
http://www.wwpdb.org/validation/2017/FAQs#types.

The following versions of software and data (see references (i)) were used in the production of this report:

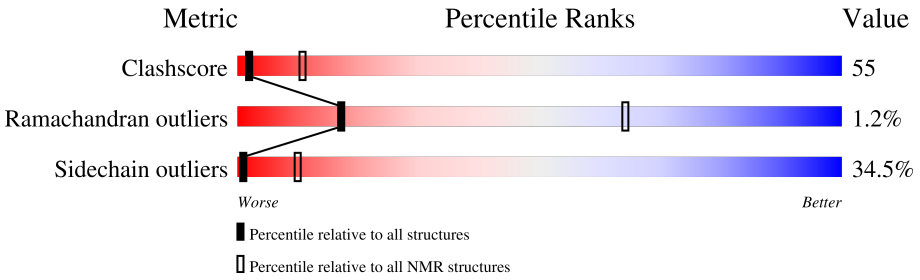
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints Analysis	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal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

1 Overall quality at a glance (i)

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

The overall completeness of chemical shifts assignment is 73%.

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	126	11% 52% 6% 10% 20%

2 Ensemble composition and analysis (i)

This entry contains 32 models. Model 7 is the overall representative, medoid model (most similar to other models). The authors have identified model 1 as representative, based on the following criterion: *closest to the average*.

The following residues are included in the computation of the global validation metrics.

Well-defined (core) protein residues			
Well-defined core	**Residue range (total)**	**Backbone RMSD (Å)**	**Medoid model**
1	A:2022-A:2038, A:2043-A:2113 (88)	0.50	7

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 5 clusters and 7 single-model clusters were found.

Cluster number	Models
1	1, 7, 9, 10, 11, 14, 16, 17, 23, 25, 28, 30
2	4, 5, 13, 18, 20
3	2, 8, 24
4	21, 26, 27
5	3, 6
Single-model clusters	12; 15; 19; 22; 29; 31; 32

3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 1690 atoms, of which 831 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called FKBP12-rapamycin complex-associated protein.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	101	1690	549	831	151	151	8	0

There are 27 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1989	MET	-	expression tag	UNP P42345
A	1990	SER	-	expression tag	UNP P42345
A	1991	TYR	-	expression tag	UNP P42345
A	1992	TYR	-	expression tag	UNP P42345
A	1993	HIS	-	expression tag	UNP P42345
A	1994	HIS	-	expression tag	UNP P42345
A	1995	HIS	-	expression tag	UNP P42345
A	1996	HIS	-	expression tag	UNP P42345
A	1997	HIS	-	expression tag	UNP P42345
A	1998	HIS	-	expression tag	UNP P42345
A	1999	ASP	-	linker	UNP P42345
A	2000	TYR	-	linker	UNP P42345
A	2001	ASP	-	linker	UNP P42345
A	2002	ILE	-	linker	UNP P42345
A	2003	PRO	-	linker	UNP P42345
A	2004	THR	-	linker	UNP P42345
A	2005	THR	-	linker	UNP P42345
A	2006	GLU	-	linker	UNP P42345
A	2007	ASN	-	linker	UNP P42345
A	2008	LEU	-	linker	UNP P42345
A	2009	TYR	-	linker	UNP P42345
A	2010	PHE	-	linker	UNP P42345
A	2011	GLN	-	linker	UNP P42345
A	2012	GLY	-	linker	UNP P42345
A	2013	ALA	-	linker	UNP P42345
A	2014	MET	-	linker	UNP P42345
A	2020	PRO	ALA	engineered mutation	UNP P42345

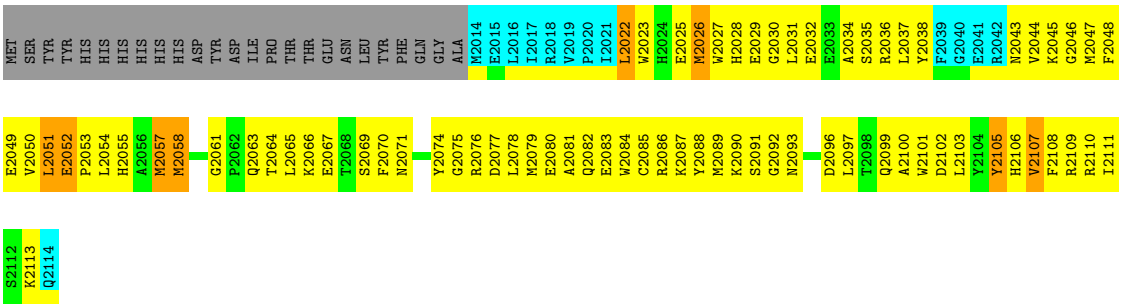
4 Residue-property plots (i)

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: FKBP12-rapamycin complex-associated protein

Chain A:

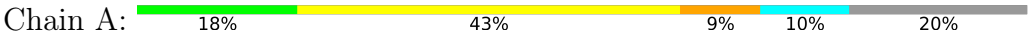


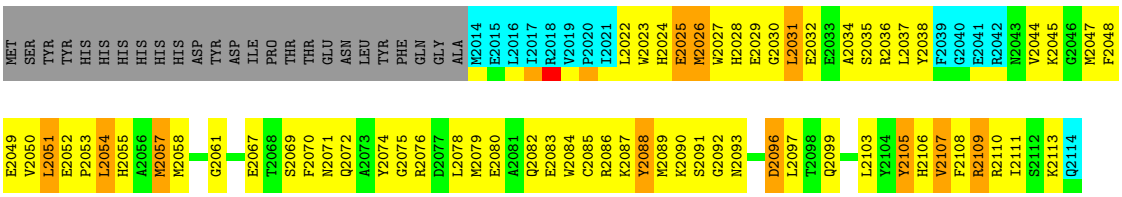
4.2 Residue scores for the representative (medoid) model from the NMR ensemble

The representative model is number 7. Colouring as in section 4.1 above.

• Molecule 1: FKBP12-rapamycin complex-associated protein

Chain A:





5 Refinement protocol and experimental data overview

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

Of the 100 calculated structures, 32 were deposited, based on the following criterion: *structures with the least restraint violations*.

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

Software name	Classification	Version
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	1267
Number of shifts mapped to atoms	1091
Number of unparsed shifts	0
Number of shifts with mapping errors	176
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	73%

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	749	717	718	81±8
All	All	23968	22944	22976	2596

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 55.

5 of 525 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:2031:LEU:HD22	1:A:2108:PHE:CG	1.05	1.87	10	18
1:A:2031:LEU:HD22	1:A:2101:TRP:CH2	1.01	1.90	16	6
1:A:2031:LEU:HD22	1:A:2108:PHE:CD2	0.99	1.92	27	17
1:A:2044:VAL:HG11	1:A:2097:LEU:HD21	0.99	1.34	11	6
1:A:2031:LEU:HD11	1:A:2108:PHE:CG	0.96	1.95	23	10

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	88/126 (70%)	76±2 (86±2%)	11±1 (12±2%)	1±1 (1±1%)	17	64
All	All	2816/4032 (70%)	2433 (86%)	348 (12%)	35 (1%)	17	64

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	2091	SER	10
1	A	2062	PRO	5
1	A	2063	GLN	5
1	A	2022	LEU	4
1	A	2043	ASN	4

6.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all NMR entries. The Analysed column shows the number of residues for which the sidechain conformation was analysed and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	78/113 (69%)	51±3 (66±4%)	27±3 (34±4%)	1	10
All	All	2496/3616 (69%)	1635 (66%)	861 (34%)	1	10

5 of 60 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	2026	MET	32
1	A	2045	LYS	32
1	A	2051	LEU	32
1	A	2057	MET	32
1	A	2083	GLU	32

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

The completeness of assignment taking into account all chemical shift lists is 73% for the well-defined parts and 74% 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	1267
Number of shifts mapped to atoms	1091
Number of unparsed shifts	0
Number of shifts with mapping errors	176
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	6

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 176) occurrences are reported below.

List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	1989	MET	CA	45.5	0.15	1
1	A	1990	SER	H	8.12	0.015	1
1	A	1990	SER	HA	4.32	0.015	1
1	A	1990	SER	HB2	3.74	0.015	2
1	A	1990	SER	HB3	3.8	0.015	2
1	A	1990	SER	CA	58.6	0.15	1
1	A	1990	SER	CB	63.9	0.15	1
1	A	1990	SER	N	115.7	0.15	1
1	A	1991	TYR	H	8.53	0.015	1
1	A	1991	TYR	HA	4.16	0.015	1
1	A	1991	TYR	CA	56.9	0.15	1
1	A	1991	TYR	CB	30.0	0.15	1
1	A	1991	TYR	N	122.5	0.15	1
1	A	1992	TYR	H	7.94	0.015	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	1992	TYR	HA	4.35	0.015	1
1	A	1992	TYR	HB2	2.71	0.015	1
1	A	1992	TYR	HB3	2.71	0.015	1
1	A	1992	TYR	CA	58.0	0.15	1
1	A	1992	TYR	CB	39.0	0.15	1
1	A	1992	TYR	N	121.6	0.15	1
1	A	1997	HIS	CA	60.9	0.15	1
1	A	1998	HIS	H	8.11	0.015	1
1	A	1998	HIS	HA	3.89	0.015	1
1	A	1998	HIS	HB2	2.9	0.015	1
1	A	1998	HIS	HB3	2.9	0.015	1
1	A	1998	HIS	CA	56.1	0.15	1
1	A	1998	HIS	CB	30.4	0.15	1
1	A	1998	HIS	N	126.5	0.15	1
1	A	1999	ASP	H	8.33	0.015	1
1	A	1999	ASP	HA	4.42	0.015	1
1	A	1999	ASP	HB2	2.49	0.015	2
1	A	1999	ASP	HB3	2.38	0.015	2
1	A	1999	ASP	CA	54.5	0.15	1
1	A	1999	ASP	CB	40.9	0.15	1
1	A	1999	ASP	N	121.5	0.15	1
1	A	2000	TYR	H	7.85	0.015	1
1	A	2000	TYR	HA	4.42	0.015	1
1	A	2000	TYR	HB2	2.95	0.015	2
1	A	2000	TYR	HB3	2.79	0.015	2
1	A	2000	TYR	HD1	6.95	0.015	1
1	A	2000	TYR	HD2	6.95	0.015	1
1	A	2000	TYR	HE1	6.64	0.015	1
1	A	2000	TYR	HE2	6.64	0.015	1
1	A	2000	TYR	CA	57.5	0.15	1
1	A	2000	TYR	CB	38.6	0.15	1
1	A	2000	TYR	N	119.1	0.15	1
1	A	2001	ASP	H	8.15	0.015	1
1	A	2001	ASP	HA	4.51	0.015	1
1	A	2001	ASP	HB2	2.53	0.015	2
1	A	2001	ASP	HB3	2.45	0.015	2
1	A	2001	ASP	CA	54.2	0.15	1
1	A	2001	ASP	CB	41.1	0.15	1
1	A	2001	ASP	N	121.6	0.15	1
1	A	2002	ILE	H	7.83	0.015	1
1	A	2002	ILE	HA	4.27	0.015	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	2002	ILE	HB	1.72	0.015	1
1	A	2002	ILE	HG12	1.36	0.015	2
1	A	2002	ILE	HG13	1.0	0.015	2
1	A	2002	ILE	HG21	0.8	0.015	1
1	A	2002	ILE	HG22	0.8	0.015	1
1	A	2002	ILE	HG23	0.8	0.015	1
1	A	2002	ILE	HD11	0.71	0.015	1
1	A	2002	ILE	HD12	0.71	0.015	1
1	A	2002	ILE	HD13	0.71	0.015	1
1	A	2002	ILE	CA	58.8	0.15	1
1	A	2002	ILE	CB	38.5	0.15	1
1	A	2002	ILE	CG1	27.0	0.15	1
1	A	2002	ILE	CG2	17.1	0.15	1
1	A	2002	ILE	CD1	12.8	0.15	1
1	A	2002	ILE	N	122.1	0.15	1
1	A	2003	PRO	HA	4.38	0.015	1
1	A	2003	PRO	HB2	2.17	0.015	2
1	A	2003	PRO	HB3	1.8	0.015	2
1	A	2003	PRO	HG2	1.92	0.015	2
1	A	2003	PRO	HG3	1.85	0.015	2
1	A	2003	PRO	HD2	3.55	0.015	2
1	A	2003	PRO	HD3	3.75	0.015	2
1	A	2003	PRO	CA	63.2	0.15	1
1	A	2003	PRO	CB	32.1	0.15	1
1	A	2003	PRO	CG	27.4	0.15	1
1	A	2003	PRO	CD	51.0	0.15	1
1	A	2004	THR	H	8.2	0.015	1
1	A	2004	THR	HA	4.29	0.015	1
1	A	2004	THR	HB	4.18	0.015	1
1	A	2004	THR	HG21	1.11	0.015	1
1	A	2004	THR	HG22	1.11	0.015	1
1	A	2004	THR	HG23	1.11	0.015	1
1	A	2004	THR	CA	61.8	0.15	1
1	A	2004	THR	CB	69.9	0.15	1
1	A	2004	THR	CG2	21.5	0.15	1
1	A	2004	THR	N	114.4	0.15	1
1	A	2005	THR	H	7.99	0.015	1
1	A	2005	THR	HA	4.19	0.015	1
1	A	2005	THR	HB	4.15	0.015	1
1	A	2005	THR	HG21	1.09	0.015	1
1	A	2005	THR	HG22	1.09	0.015	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	2005	THR	HG23	1.09	0.015	1
1	A	2005	THR	CA	62.2	0.15	1
1	A	2005	THR	CB	69.7	0.15	1
1	A	2005	THR	CG2	21.6	0.15	1
1	A	2005	THR	N	115.1	0.15	1
1	A	2006	GLU	H	8.29	0.015	1
1	A	2006	GLU	HA	4.13	0.015	1
1	A	2006	GLU	HB2	1.91	0.015	2
1	A	2006	GLU	HB3	1.81	0.015	2
1	A	2006	GLU	HG2	2.13	0.015	1
1	A	2006	GLU	HG3	2.13	0.015	1
1	A	2006	GLU	CA	56.9	0.15	1
1	A	2006	GLU	CB	30.1	0.15	1
1	A	2006	GLU	CG	36.2	0.15	1
1	A	2006	GLU	N	122.4	0.15	1
1	A	2007	ASN	H	8.24	0.015	1
1	A	2007	ASN	HA	4.53	0.015	1
1	A	2007	ASN	HB2	2.61	0.015	2
1	A	2007	ASN	HB3	2.65	0.015	2
1	A	2007	ASN	CA	53.3	0.15	1
1	A	2007	ASN	CB	38.6	0.15	1
1	A	2007	ASN	N	119.2	0.15	1
1	A	2008	LEU	H	8.0	0.015	1
1	A	2008	LEU	HA	4.09	0.015	1
1	A	2008	LEU	HB2	1.21	0.015	2
1	A	2008	LEU	HB3	1.35	0.015	2
1	A	2008	LEU	HG	1.35	0.015	1
1	A	2008	LEU	HD11	0.66	0.015	2
1	A	2008	LEU	HD12	0.66	0.015	2
1	A	2008	LEU	HD13	0.66	0.015	2
1	A	2008	LEU	HD21	0.73	0.015	2
1	A	2008	LEU	HD22	0.73	0.015	2
1	A	2008	LEU	HD23	0.73	0.015	2
1	A	2008	LEU	CA	55.5	0.15	1
1	A	2008	LEU	CB	42.2	0.15	1
1	A	2008	LEU	CG	26.7	0.15	1
1	A	2008	LEU	CD1	23.2	0.15	1
1	A	2008	LEU	CD2	24.8	0.15	1
1	A	2008	LEU	N	122.2	0.15	1
1	A	2009	TYR	H	7.94	0.015	1
1	A	2009	TYR	HA	4.39	0.015	1

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List ID	Chain	Res	Type	Atom	Shift Data		
					Value	Uncertainty	Ambiguity
1	A	2009	TYR	HB2	2.72	0.015	2
1	A	2009	TYR	HB3	2.8	0.015	2
1	A	2009	TYR	HD1	6.88	0.015	1
1	A	2009	TYR	HD2	6.88	0.015	1
1	A	2009	TYR	HE1	6.64	0.015	1
1	A	2009	TYR	HE2	6.64	0.015	1
1	A	2009	TYR	CA	58.0	0.15	1
1	A	2009	TYR	CB	38.6	0.15	1
1	A	2009	TYR	N	119.5	0.15	1
1	A	2010	PHE	H	7.94	0.015	1
1	A	2010	PHE	HA	4.44	0.015	1
1	A	2010	PHE	HB2	2.9	0.015	1
1	A	2010	PHE	HB3	2.9	0.015	1
1	A	2010	PHE	CA	57.8	0.15	1
1	A	2010	PHE	CB	39.5	0.15	1
1	A	2010	PHE	N	121.2	0.15	1
1	A	2011	GLN	H	8.16	0.015	1
1	A	2011	GLN	HA	4.12	0.015	1
1	A	2011	GLN	HB2	1.96	0.015	2
1	A	2011	GLN	HB3	1.8	0.015	2
1	A	2011	GLN	HG2	2.17	0.015	1
1	A	2011	GLN	HG3	2.17	0.015	1
1	A	2011	GLN	CA	56.1	0.15	1
1	A	2011	GLN	CB	29.2	0.15	1
1	A	2011	GLN	CG	33.7	0.15	1
1	A	2011	GLN	N	121.8	0.15	1
1	A	2012	GLY	H	7.81	0.015	1
1	A	2012	GLY	HA2	3.78	0.015	1
1	A	2012	GLY	HA3	3.78	0.015	1
1	A	2012	GLY	CA	45.2	0.15	1
1	A	2012	GLY	N	109.4	0.15	1
1	A	2013	ALA	H	8.08	0.015	1
1	A	2013	ALA	HA	4.21	0.015	1
1	A	2013	ALA	HB1	1.25	0.015	1
1	A	2013	ALA	HB2	1.25	0.015	1
1	A	2013	ALA	HB3	1.25	0.015	1
1	A	2013	ALA	CA	52.6	0.15	1
1	A	2013	ALA	CB	19.2	0.15	1
1	A	2013	ALA	N	123.5	0.15	1

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	122	-0.50 ± 0.18	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	109	0.42 ± 0.09	None needed (< 0.5 ppm)
$^{13}C'$	0	—	None (insufficient data)
^{15}N	113	0.09 ± 0.17	None needed (< 0.5 ppm)

7.1.3 Completeness of resonance assignments (i)

The following table shows the completeness of the chemical shift assignments for the well-defined regions of the structure. The overall completeness is 73%, i.e. 935 atoms were assigned a chemical shift out of a possible 1274. 0 out of 13 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	351/441 (80%)	179/179 (100%)	88/176 (50%)	84/86 (98%)
Sidechain	521/678 (77%)	357/437 (82%)	164/210 (78%)	0/31 (0%)
Aromatic	63/155 (41%)	59/75 (79%)	0/68 (0%)	4/12 (33%)
Overall	935/1274 (73%)	595/691 (86%)	252/454 (56%)	88/129 (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.

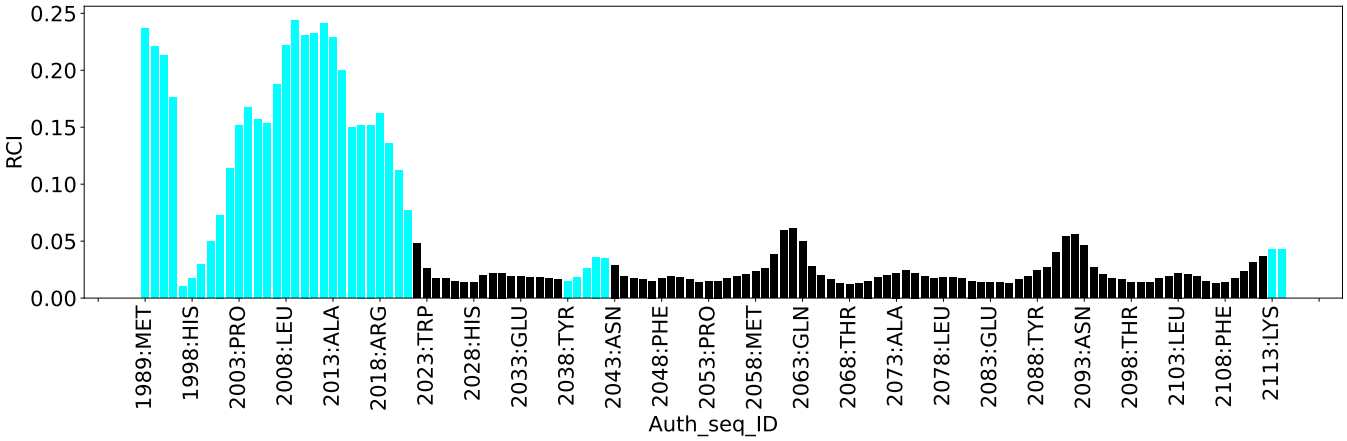
List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	2054	LEU	HB3	-0.92	-0.26 – 3.31	-6.9
1	A	2110	ARG	HD2	1.55	1.97 – 4.26	-6.8
1	A	2099	GLN	HB3	0.37	0.71 – 3.33	-6.3
1	A	2038	TYR	HD1	5.34	5.49 – 8.39	-5.5
1	A	2038	TYR	HD2	5.34	5.48 – 8.39	-5.5
1	A	2110	ARG	HD3	1.69	1.81 – 4.39	-5.5

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	1633
Intra-residue (|i-j|=0)	404
Sequential (|i-j|=1)	446
Medium range (|i-j|>1 and |i-j|<5)	374
Long range (|i-j|≥5)	311
Inter-chain	0
Hydrogen bond restraints	98
Disulfide bond restraints	0
Total dihedral-angle restraints	0
Number of unmapped restraints	86
Number of restraints per residue	13.0
Number of long range restraints per residue[1]	2.5

[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)	34.0	0.2
0.2-0.5 (Medium)	27.4	0.5
>0.5 (Large)	51.3	3.48

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)

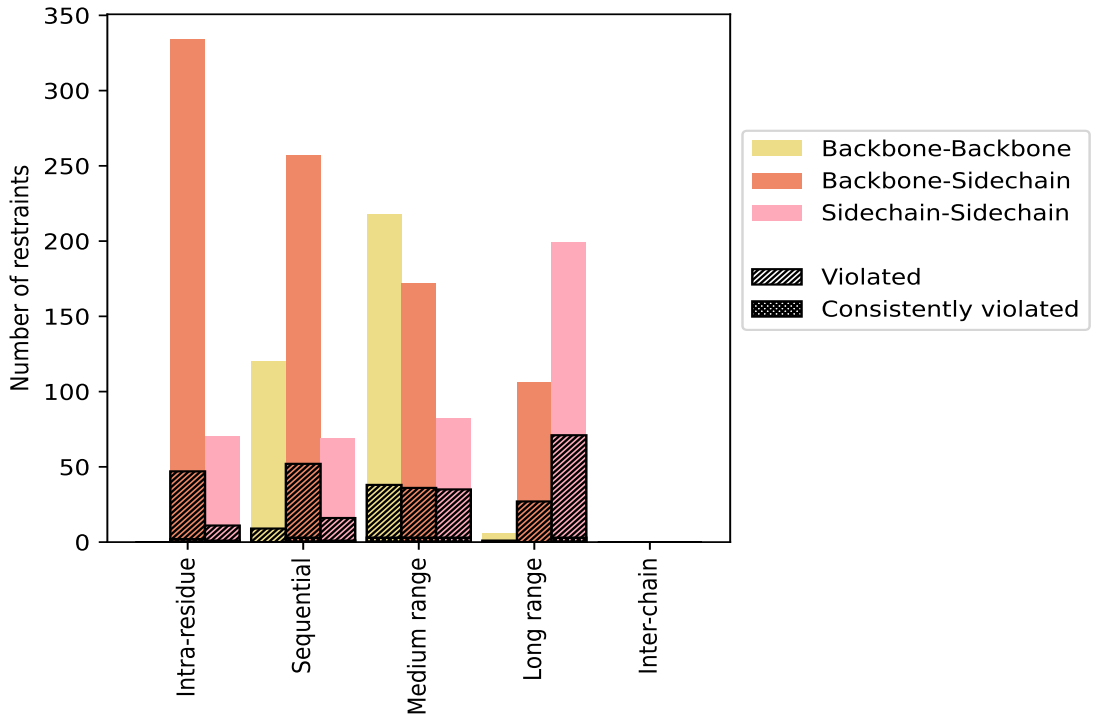
9.1 Summary of distance violations (i)

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)	404	24.7	58	14.4	3.6	3	0.7	0.2
Backbone-Backbone	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	334	20.5	47	14.1	2.9	2	0.6	0.1
Sidechain-Sidechain	70	4.3	11	15.7	0.7	1	1.4	0.1
Sequential (|i-j|=1)	446	27.3	77	17.3	4.7	4	0.9	0.2
Backbone-Backbone	120	7.3	9	7.5	0.6	0	0.0	0.0
Backbone-Sidechain	257	15.7	52	20.2	3.2	3	1.2	0.2
Sidechain-Sidechain	69	4.2	16	23.2	1.0	1	1.4	0.1
Medium range (|i-j|>1 & |i-j|<5)	374	22.9	80	21.4	4.9	6	1.6	0.4
Backbone-Backbone	120	7.3	9	7.5	0.6	0	0.0	0.0
Backbone-Sidechain	172	10.5	36	20.9	2.2	3	1.7	0.2
Sidechain-Sidechain	82	5.0	35	42.7	2.1	3	3.7	0.2
Long range (|i-j|≥5)	311	19.0	99	31.8	6.1	3	1.0	0.2
Backbone-Backbone	6	0.4	1	16.7	0.1	0	0.0	0.0
Backbone-Sidechain	106	6.5	27	25.5	1.7	0	0.0	0.0
Sidechain-Sidechain	199	12.2	71	35.7	4.3	3	1.5	0.2
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	98	6.0	29	29.6	1.8	3	3.1	0.2
Disulfide bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Total	1633	100.0	343	21.0	21.0	19	1.2	1.2
Backbone-Backbone	344	21.1	48	14.0	2.9	3	0.9	0.2
Backbone-Sidechain	869	53.2	162	18.6	9.9	8	0.9	0.5
Sidechain-Sidechain	420	25.7	133	31.7	8.1	8	1.9	0.5

[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	12	21	30	36	0	99	0.73	2.74	0.59	0.6
2	17	24	35	38	0	114	0.66	2.46	0.58	0.4
3	18	19	36	32	0	105	0.64	2.73	0.59	0.39
4	18	18	31	21	0	88	0.67	3.24	0.62	0.48
5	12	17	34	32	0	95	0.7	3.27	0.65	0.5
6	15	15	31	21	0	82	0.57	1.96	0.49	0.37
7	13	19	35	22	0	89	0.62	2.13	0.56	0.39
8	20	28	47	33	0	128	0.64	2.76	0.58	0.47
9	19	18	29	24	0	90	0.66	2.84	0.57	0.48
10	18	19	38	35	0	110	0.68	2.98	0.63	0.46
11	21	26	44	38	0	129	0.69	3.29	0.62	0.49

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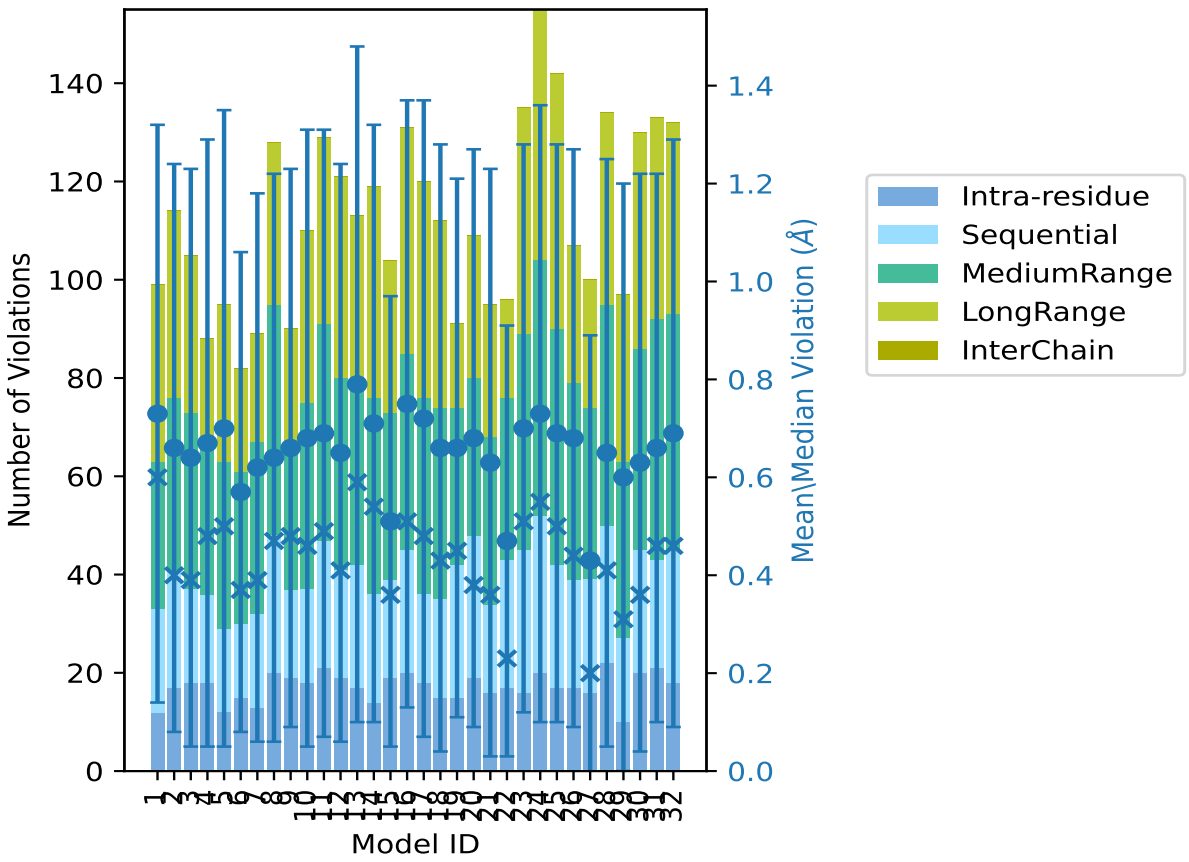
Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
12	19	22	39	41	0	121	0.65	3.22	0.59	0.41
13	17	25	36	35	0	113	0.79	3.37	0.69	0.59
14	14	22	40	43	0	119	0.71	2.62	0.61	0.54
15	19	20	34	31	0	104	0.51	1.98	0.46	0.36
16	20	25	40	46	0	131	0.75	2.6	0.62	0.51
17	18	18	40	44	0	120	0.72	3.48	0.65	0.48
18	15	20	39	38	0	112	0.66	3.29	0.62	0.43
19	15	27	32	17	0	91	0.66	2.49	0.55	0.45
20	19	29	32	29	0	109	0.68	2.6	0.59	0.38
21	16	18	34	27	0	95	0.63	2.73	0.6	0.36
22	17	26	33	20	0	96	0.47	1.65	0.44	0.23
23	16	29	44	46	0	135	0.7	2.68	0.58	0.51
24	20	32	52	51	0	155	0.73	3.27	0.63	0.55
25	17	25	48	52	0	142	0.69	3.3	0.59	0.5
26	17	22	40	28	0	107	0.68	2.83	0.59	0.44
27	16	23	35	26	0	100	0.43	2.0	0.46	0.2
28	22	28	45	39	0	134	0.65	3.05	0.6	0.41
29	10	17	36	34	0	97	0.6	2.81	0.6	0.31
30	20	25	41	44	0	130	0.63	2.61	0.59	0.36
31	21	22	49	41	0	133	0.66	2.49	0.56	0.46
32	18	27	48	39	0	132	0.69	2.6	0.6	0.46

[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, 1221(IR:346, SQ:369, MR:294, LR:212, IC:0) restraints are not violated in the ensemble.

Number of violated restraints						Fraction of the ensemble	
IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total	Count[6]	%
11	11	15	8	0	45	1	3.1
6	10	4	5	0	25	2	6.2
2	6	3	4	0	15	3	9.4
3	3	3	5	0	14	4	12.5
4	5	2	8	0	19	5	15.6
1	2	4	3	0	10	6	18.8

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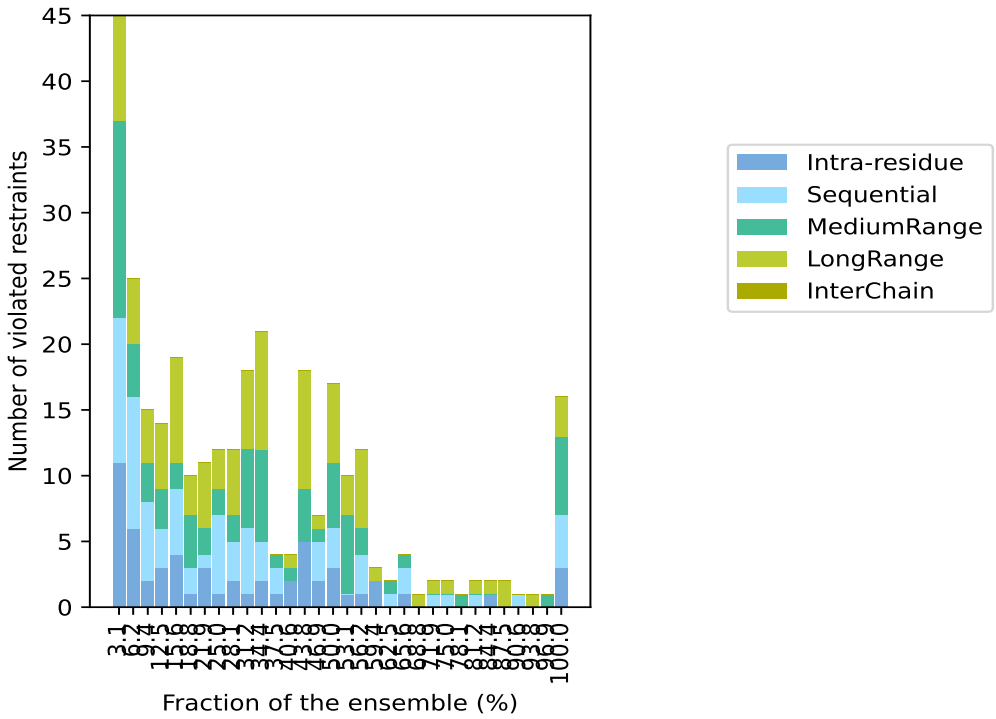
Number of violated restraints						Fraction of the ensemble	
IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total	Count[6]	%
3	1	2	5	0	11	7	21.9
1	6	2	3	0	12	8	25.0
2	3	2	5	0	12	9	28.1
1	5	6	6	0	18	10	31.2
2	3	7	9	0	21	11	34.4
1	2	1	0	0	4	12	37.5
2	0	1	1	0	4	13	40.6
5	0	4	9	0	18	14	43.8
2	3	1	1	0	7	15	46.9
3	3	5	6	0	17	16	50.0
1	0	6	3	0	10	17	53.1
1	3	2	6	0	12	18	56.2
2	0	0	1	0	3	19	59.4
0	1	1	0	0	2	20	62.5
1	2	1	0	0	4	21	65.6
0	0	0	1	0	1	22	68.8
0	1	0	1	0	2	23	71.9
0	1	0	1	0	2	24	75.0
0	0	1	0	0	1	25	78.1
0	1	0	1	0	2	26	81.2
1	0	0	1	0	2	27	84.4
0	0	0	2	0	2	28	87.5
0	1	0	0	0	1	29	90.6
0	0	0	1	0	1	30	93.8
0	0	1	0	0	1	31	96.9
3	4	6	3	0	16	32	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

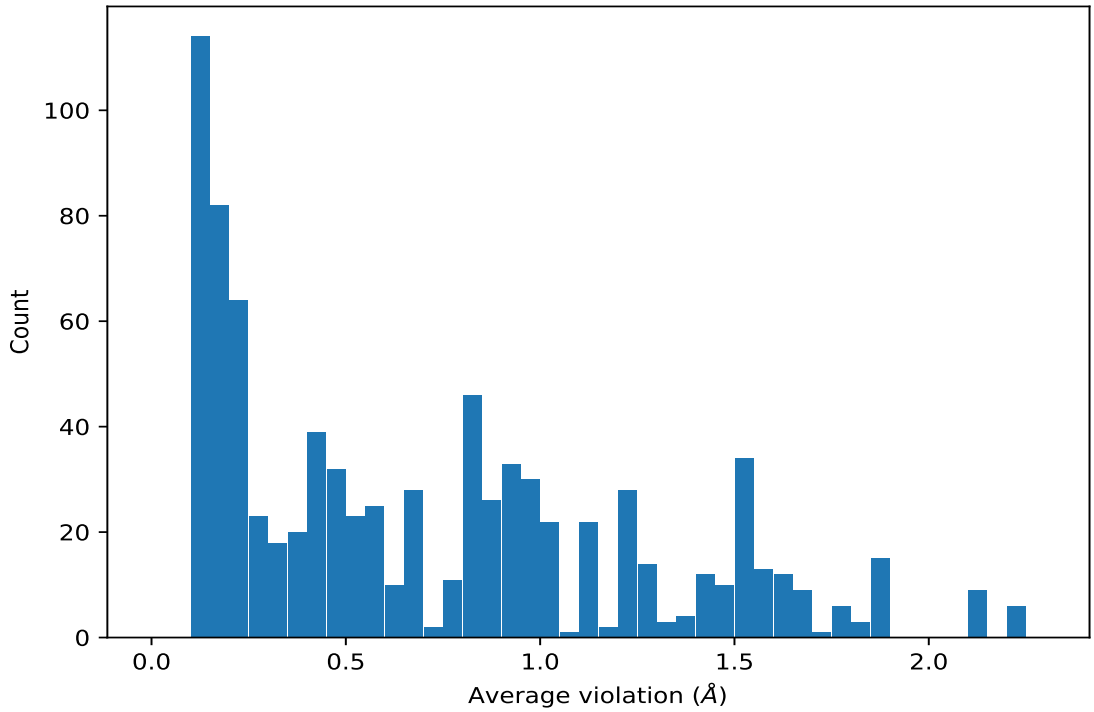
9.3.1 Bar graph : Distance violation statistics for the ensemble



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)
(2,1077)	1:A:2088:TYR:H	1:A:2089:MET:HB3	32	1.48	0.04	1.5
(2,522)	1:A:2097:LEU:HD11	1:A:2101:TRP:HB3	32	1.47	0.95	0.97
(2,522)	1:A:2097:LEU:HD12	1:A:2101:TRP:HB3	32	1.47	0.95	0.97
(2,522)	1:A:2097:LEU:HD13	1:A:2101:TRP:HB3	32	1.47	0.95	0.97
(2,527)	1:A:2024:HIS:HB2	1:A:2111:ILE:HG21	32	1.32	0.06	1.32
(2,527)	1:A:2024:HIS:HB2	1:A:2111:ILE:HG22	32	1.32	0.06	1.32
(2,527)	1:A:2024:HIS:HB2	1:A:2111:ILE:HG23	32	1.32	0.06	1.32
(2,528)	1:A:2024:HIS:HB2	1:A:2111:ILE:HD11	32	1.26	0.08	1.27
(2,528)	1:A:2024:HIS:HB2	1:A:2111:ILE:HD12	32	1.26	0.08	1.27
(2,528)	1:A:2024:HIS:HB2	1:A:2111:ILE:HD13	32	1.26	0.08	1.27
(2,131)	1:A:2023:TRP:HE1	1:A:2058:MET:HG3	32	1.15	0.36	1.2
(2,479)	1:A:2054:LEU:HA	1:A:2057:MET:HG3	32	0.95	0.07	0.96
(2,1116)	1:A:2051:LEU:HD21	1:A:2053:PRO:HD2	32	0.9	0.62	1.16
(2,1116)	1:A:2051:LEU:HD22	1:A:2053:PRO:HD2	32	0.9	0.62	1.16
(2,1116)	1:A:2051:LEU:HD23	1:A:2053:PRO:HD2	32	0.9	0.62	1.16
(2,482)	1:A:2089:MET:HB2	1:A:2089:MET:HE1	32	0.68	0.04	0.69

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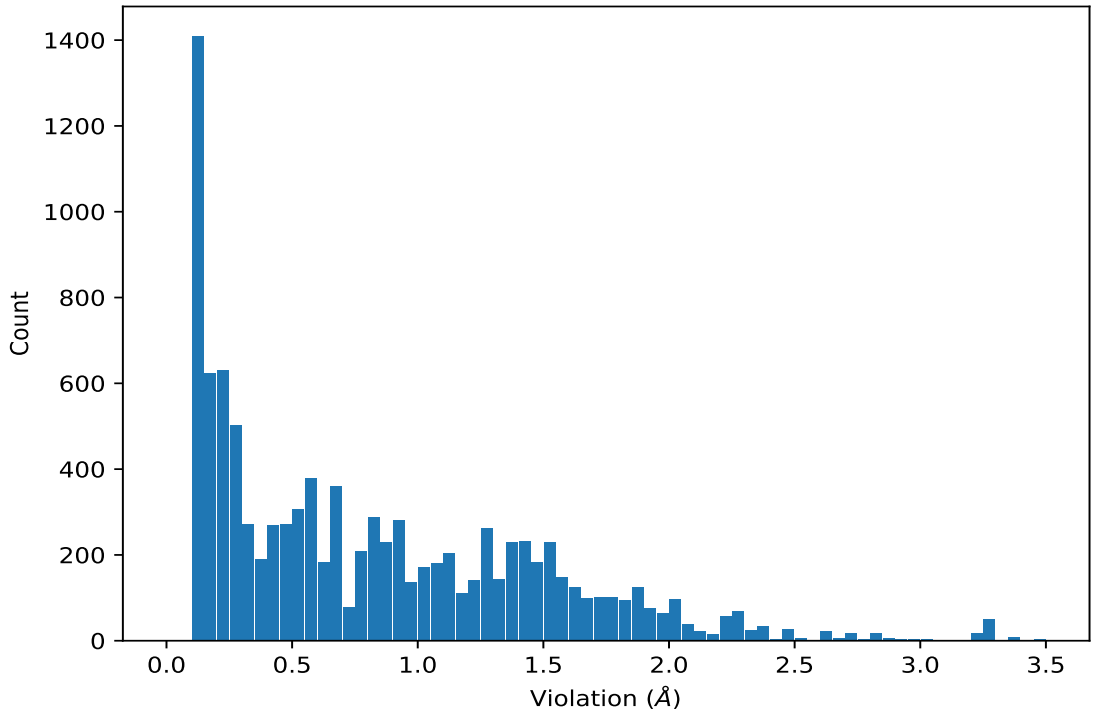
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(2,482)	1:A:2089:MET:HB2	1:A:2089:MET:HE2	32	0.68	0.04	0.69
(2,482)	1:A:2089:MET:HB2	1:A:2089:MET:HE3	32	0.68	0.04	0.69
(2,767)	1:A:2077:ASP:HB3	1:A:2079:MET:H	32	0.64	0.14	0.64
(2,1136)	1:A:2047:MET:HB3	1:A:2051:LEU:HD11	32	0.62	0.46	0.43

[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)
(2,419)	1:A:2019:VAL:HG21	1:A:2021:ILE:HG12	17	3.48

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(2,419)	1:A:2019:VAL:HG22	1:A:2021:ILE:HG12	17	3.48
(2,419)	1:A:2019:VAL:HG23	1:A:2021:ILE:HG12	17	3.48
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD11	13	3.37
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD12	13	3.37
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD13	13	3.37
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD11	13	3.37
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD12	13	3.37
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD13	13	3.37
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD11	13	3.37
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD12	13	3.37
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD13	13	3.37
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD11	25	3.3
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD12	25	3.3
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD13	25	3.3
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD11	25	3.3
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD12	25	3.3
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD13	25	3.3
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD11	25	3.3
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD12	25	3.3
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD13	25	3.3
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD11	11	3.29
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD12	11	3.29
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD13	11	3.29
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD11	11	3.29
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD12	11	3.29
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD13	11	3.29
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD11	11	3.29
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD12	11	3.29
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD13	11	3.29
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD11	18	3.29
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD12	18	3.29
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD13	18	3.29
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD11	18	3.29
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD12	18	3.29
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD13	18	3.29
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD11	18	3.29
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD12	18	3.29
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD13	18	3.29
(2,419)	1:A:2019:VAL:HG21	1:A:2021:ILE:HG12	24	3.27
(2,419)	1:A:2019:VAL:HG22	1:A:2021:ILE:HG12	24	3.27
(2,419)	1:A:2019:VAL:HG23	1:A:2021:ILE:HG12	24	3.27
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD11	5	3.27

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD12	5	3.27
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD13	5	3.27
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD11	5	3.27
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD12	5	3.27
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD13	5	3.27
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD11	5	3.27
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD12	5	3.27
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD13	5	3.27
(2,419)	1:A:2019:VAL:HG21	1:A:2021:ILE:HG12	13	3.25
(2,419)	1:A:2019:VAL:HG22	1:A:2021:ILE:HG12	13	3.25
(2,419)	1:A:2019:VAL:HG23	1:A:2021:ILE:HG12	13	3.25
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD11	24	3.25
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD12	24	3.25
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD13	24	3.25
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD11	24	3.25
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD12	24	3.25
(2,340)	1:A:2051:LEU:HD22	1:A:2054:LEU:HD13	24	3.25
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD11	24	3.25
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD12	24	3.25
(2,340)	1:A:2051:LEU:HD23	1:A:2054:LEU:HD13	24	3.25
(2,340)	1:A:2051:LEU:HD21	1:A:2054:LEU:HD11	4	3.24

10 Dihedral-angle violation analysis (i)

Dihedral angle analysis failed due to data error in the dihedral angle restraints, possibly missing target value