wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2N27
BMRB ID	:	25588
Title	:	Competitive inhibition of TRPV1 calmodulin interaction by vanilloids
Authors	:	Hetenyi, A.; Nemeth, L.; Weber, E.; Szakonyi, G.; Winter, Z.; Josvay, K.; Bartus, E.; Olah, Z.; Martinek, T.A.
Deposited on	:	2015-04-29

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:

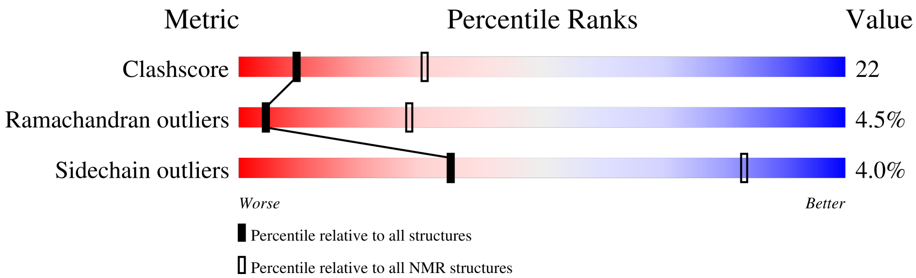
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
BMRB Restraints 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 67%.

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	148	 58% 31% 5% 6%

2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 16 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:10-A:148 (139)	0.73	16

Ill-defined regions of proteins are excluded from the global statistics.

Ligands and non-protein polymers are included in the analysis.

The models can be grouped into 2 clusters. No single-model clusters were found.

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

3 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 2316 atoms, of which 1124 are hydrogens and 0 are deuteriums.

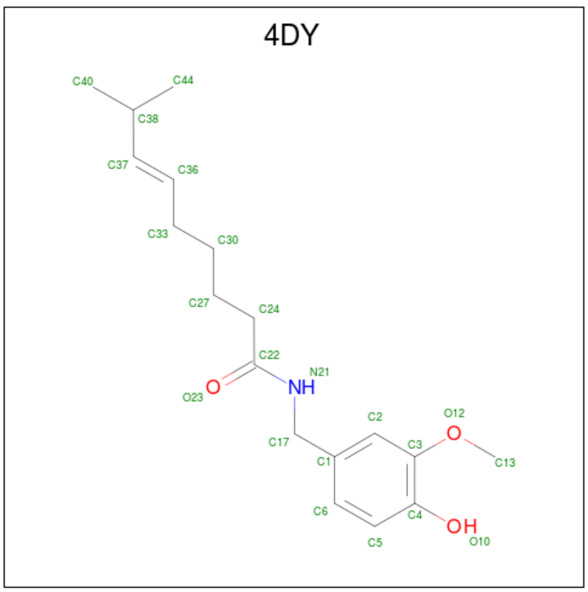
- Molecule 1 is a protein called Calmodulin.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	148	2263	714	1097	188	255	9	0

- Molecule 2 is CALCIUM ION (three-letter code: CA) (formula: Ca).

Mol	Chain	Residues	Atoms	
			Total	Ca
2	A	4	4	4

- Molecule 3 is (6E)-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide (three-letter code: 4DY) (formula: $C_{18}H_{27}NO_3$).



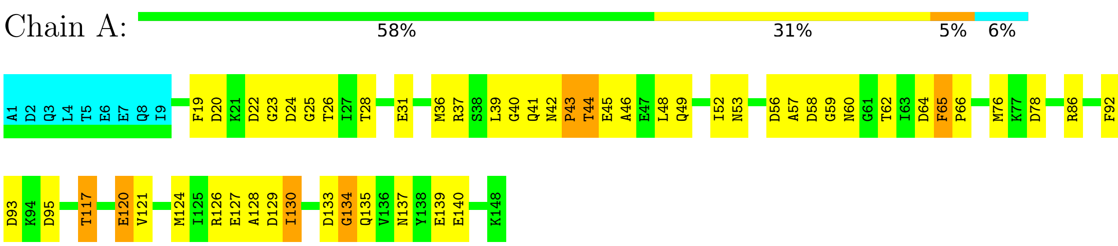
Mol	Chain	Residues	Atoms				
			Total	C	H	N	O
3	A	1	49	18	27	1	3

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.

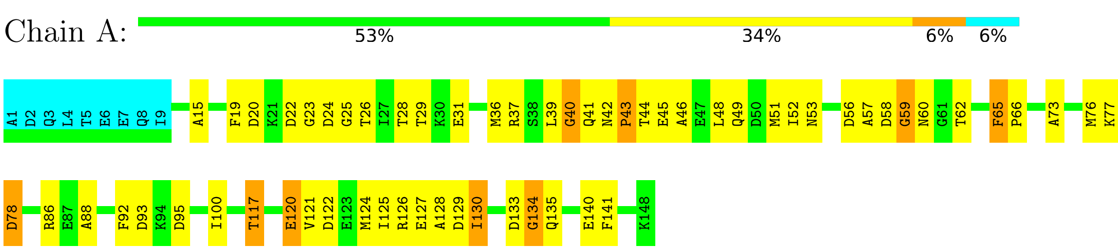
• Molecule 1: Calmodulin



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

The representative model is number 16. Colouring as in section 4.1 above.

• Molecule 1: Calmodulin



5 Refinement protocol and experimental data overview (i)

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

Of the 100 calculated structures, 20 were deposited, based on the following criterion: *back calculated data agree with experimental NOESY spectrum*.

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

Software name	Classification	Version
X-PLOR NIH	geometry optimization	
X-PLOR NIH	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	3
Total number of shifts	3290
Number of shifts mapped to atoms	3290
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	67%

6 Model quality (i)

6.1 Standard geometry (i)

Bond lengths and bond angles in the following residue types are not validated in this section: 4DY, CA

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the (average) root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	#Z>5	RMSZ	#Z>5
1	A	0.76±0.01	0±0/1106 (0.0± 0.0%)	0.82±0.03	2±1/1482 (0.1± 0.1%)
All	All	0.76	0/22120 (0.0%)	0.82	31/29640 (0.1%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	Chirality	Planarity
1	A	0.0±0.0	2.1±0.9
All	All	0	42

There are no bond-length outliers.

5 of 8 unique angle outliers are listed below. They are sorted according to the Z-score of the worst occurrence in the ensemble.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
1	A	126	ARG	NE-CZ-NH2	-8.04	116.28	120.30	10	11
1	A	37	ARG	NE-CZ-NH2	-7.27	116.67	120.30	5	9
1	A	37	ARG	CG-CD-NE	-6.18	98.83	111.80	15	2
1	A	86	ARG	CG-CD-NE	-5.44	100.37	111.80	15	2
1	A	126	ARG	CG-CD-NE	-5.33	100.62	111.80	15	2

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)
1	A	86	ARG	Sidechain	20

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Mol	Chain	Res	Type	Group	Models (Total)
1	A	37	ARG	Sidechain	13
1	A	126	ARG	Sidechain	9

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	1094	1029	1028	48±4
3	A	22	27	0	0±0
All	All	22400	21120	20543	960

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 22.

5 of 127 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:39:LEU:O	1:A:128:ALA:HB2	0.77	1.80	14	5
1:A:120:GLU:N	1:A:120:GLU:OE1	0.64	2.31	20	20
1:A:53:ASN:O	1:A:57:ALA:HB2	0.63	1.94	15	20
1:A:29:THR:HG21	1:A:49:GLN:HE22	0.59	1.56	4	1
1:A:43:PRO:O	1:A:46:ALA:N	0.59	2.34	3	20

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	138/148 (93%)	119±2 (86±2%)	13±3 (9±2%)	6±1 (5±1%)	4	28
All	All	2760/2960 (93%)	2376 (86%)	259 (9%)	125 (5%)	4	28

5 of 11 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	59	GLY	20
1	A	134	GLY	20
1	A	40	GLY	16
1	A	43	PRO	16
1	A	78	ASP	14

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	118/126 (94%)	113±0 (96±0%)	5±0 (4±0%)	35	83
All	All	2360/2520 (94%)	2265 (96%)	95 (4%)	35	83

5 of 7 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	65	PHE	20
1	A	117	THR	20
1	A	120	GLU	20
1	A	130	ILE	20
1	A	64	ASP	9

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)

Of 5 ligands modelled in this entry, 4 are monoatomic - leaving 1 for Mogul analysis.

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
3	4DY	A	205	-	22,22,22	1.66±0.01	2±0 (9±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
3	4DY	A	205	-	27,27,27	1.36±0.03	1±0 (3±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
3	4DY	A	205	-	-	0±0,16,16,16	0±0,1,1,1

All unique bond outliers are listed below. They are sorted according to the Z-score of the worst occurrence in the ensemble.

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)	Models	
								Worst	Total
3	A	205	4DY	C17-C1	5.63	1.39	1.51	13	20
3	A	205	4DY	C37-C36	5.13	1.53	1.31	11	20

All unique angle outliers are listed below.

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})	Models	
								Worst	Total
3	A	205	4DY	C13-O12-C3	5.43	109.34	117.53	12	20

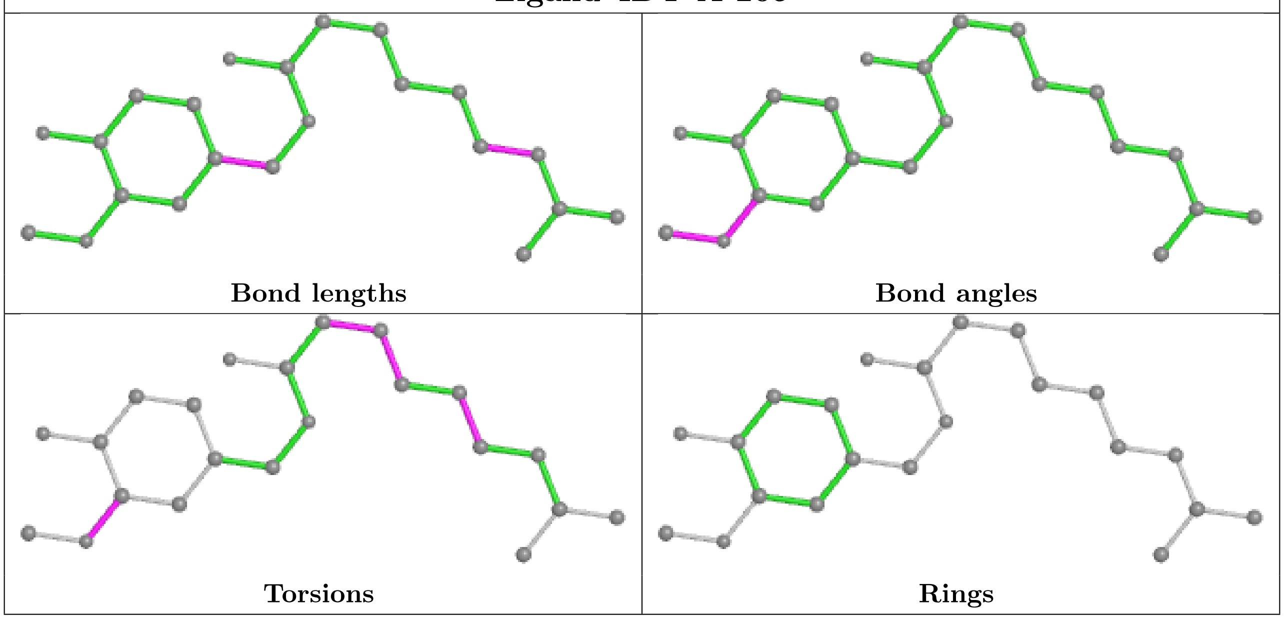
There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.

Ligand 4DY A 205



6.7 Other polymers ⓘ

There are no such molecules in this entry.

6.8 Polymer linkage issues (i)

There are no chain breaks in this entry.

7 Chemical shift validation (i)

The completeness of assignment taking into account all chemical shift lists is 67% for the well-defined parts and 67% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: *CaM*

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	1232
Number of shifts mapped to atoms	1232
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
$^{13}C_{\alpha}$	148	2.41 ± 0.10	Should be checked
$^{13}C_{\beta}$	119	2.78 ± 0.08	Should be checked
$^{13}C'$	143	2.11 ± 0.17	Should be applied
^{15}N	144	0.15 ± 0.23	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 63%, i.e. 1152 atoms were assigned a chemical shift out of a possible 1823. 0 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	681/702 (97%)	271/287 (94%)	273/278 (98%)	137/137 (100%)
Sidechain	471/1015 (46%)	322/649 (50%)	149/330 (45%)	0/36 (0%)

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	Total	^{1}H	^{13}C	^{15}N
Aromatic	0/106 (0%)	0/52 (0%)	0/52 (0%)	0/2 (0%)
Overall	1152/1823 (63%)	593/988 (60%)	422/660 (64%)	137/175 (78%)

7.1.4 Statistically unusual chemical shifts (i)

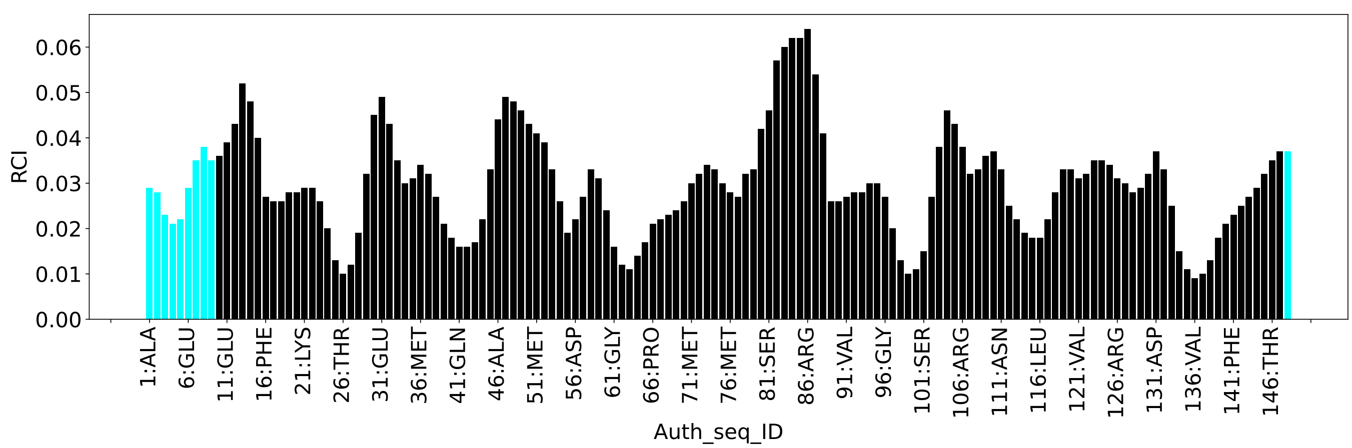
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
1	A	130	ILE	HD11	5.37	-0.72 – 2.09	16.7
1	A	130	ILE	HD12	5.37	-0.72 – 2.09	16.7
1	A	130	ILE	HD13	5.37	-0.72 – 2.09	16.7

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:



7.2 Chemical shift list 2

File name: working_cs.cif

Chemical shift list name: *CaM-CAP*

7.2.1 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	1243
Number of shifts mapped to atoms	1243
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	7

7.2.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_{\alpha}$	148	2.38 ± 0.11	Should be checked
$^{13}C_{\beta}$	127	2.83 ± 0.12	Should be checked
$^{13}C'$	143	2.16 ± 0.12	Should be applied
^{15}N	145	0.24 ± 0.20	None needed (< 0.5 ppm)

7.2.3 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 64%, i.e. 1162 atoms were assigned a chemical shift out of a possible 1823. 0 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	686/702 (98%)	276/287 (96%)	273/278 (98%)	137/137 (100%)
Sidechain	470/1015 (46%)	308/649 (47%)	161/330 (49%)	1/36 (3%)
Aromatic	6/106 (6%)	6/52 (12%)	0/52 (0%)	0/2 (0%)
Overall	1162/1823 (64%)	590/988 (60%)	434/660 (66%)	138/175 (79%)

7.2.4 Statistically unusual chemical shifts (i)

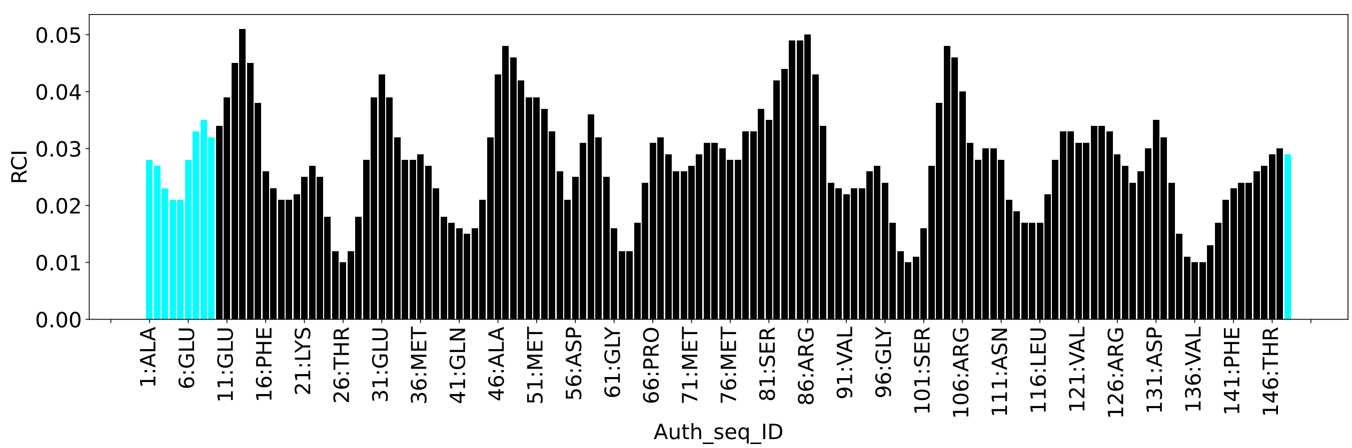
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
2	A	143	GLN	NE2	57.36	103.38 – 120.35	-32.1
2	A	130	ILE	HD11	5.61	-0.72 – 2.09	17.5
2	A	130	ILE	HD12	5.61	-0.72 – 2.09	17.5
2	A	130	ILE	HD13	5.61	-0.72 – 2.09	17.5
2	A	127	GLU	HG2	0.86	1.24 – 3.30	-6.8
2	A	12	PHE	HD1	8.99	5.51 – 8.60	6.3
2	A	12	PHE	HD2	8.99	5.52 – 8.61	6.2

7.2.5 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:



7.3 Chemical shift list 3

File name: working_cs.cif

Chemical shift list name: *CaM-RTX*

7.3.1 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	815
Number of shifts mapped to atoms	815
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	4

7.3.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction ± precision, *ppm*	Suggested action
$^{13}C_\alpha$	116	2.37 ± 0.09	Should be checked
$^{13}C_\beta$	77	2.92 ± 0.14	Should be checked
$^{13}C'$	96	1.98 ± 0.14	Should be applied
^{15}N	132	0.24 ± 0.23	None needed (< 0.5 ppm)

7.3.3 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 42%, i.e. 763 atoms were assigned a chemical shift out of a possible 1823. 0 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^{1}H	^{13}C	^{15}N
Backbone	544/702 (77%)	220/287 (77%)	200/278 (72%)	124/137 (91%)
Sidechain	219/1015 (22%)	147/649 (23%)	72/330 (22%)	0/36 (0%)
Aromatic	0/106 (0%)	0/52 (0%)	0/52 (0%)	0/2 (0%)
Overall	763/1823 (42%)	367/988 (37%)	272/660 (41%)	124/175 (71%)

7.3.4 Statistically unusual chemical shifts (i)

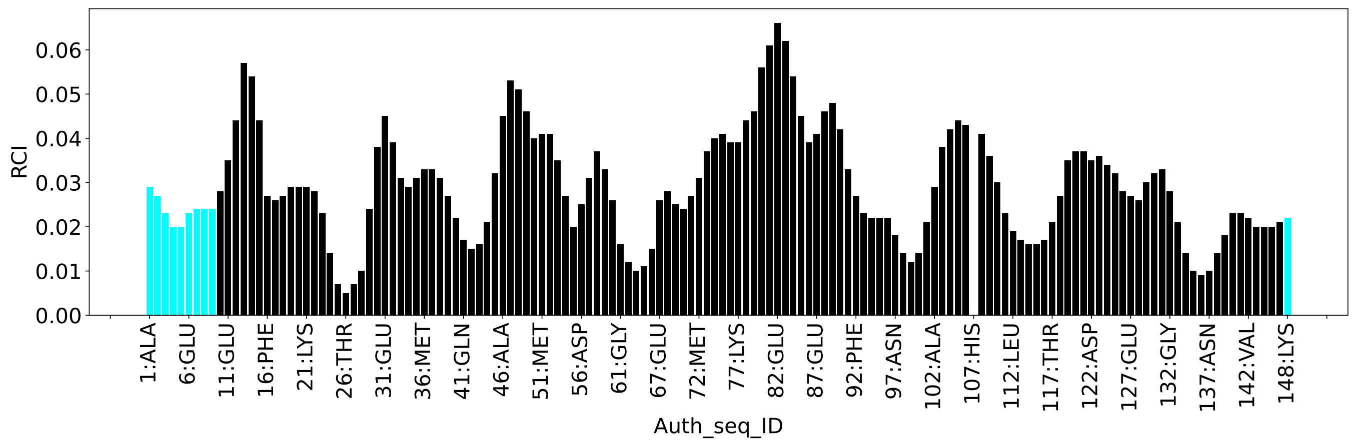
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, *ppm*	Expected range, *ppm*	Z-score
3	A	28	THR	CB	9.89	61.12 – 78.27	-34.9
3	A	26	THR	CB	10.16	61.12 – 78.27	-34.7
3	A	135	GLN	HB2	3.48	0.80 – 3.29	5.8
3	A	135	GLN	HB3	3.48	0.71 – 3.33	5.6

7.3.5 Random Coil Index (RCI) plots ⓘ

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 (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	314
Intra-residue (|i-j|=0)	82
Sequential (|i-j|=1)	90
Medium range (|i-j|>1 and |i-j|<5)	57
Long range (|i-j|≥5)	76
Inter-chain	0
Hydrogen bond restraints	9
Disulfide bond restraints	0
Total dihedral-angle restraints	205
Number of unmapped restraints	32
Number of restraints per residue	3.5
Number of long range restraints per residue[1]	0.6

[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)	11.5	0.2
0.2-0.5 (Medium)	4.2	0.44
>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)	20.2	4.1
10.0-20.0 (Medium)	None	None
>20.0 (Large)	None	None

9 Distance violation analysis (i)

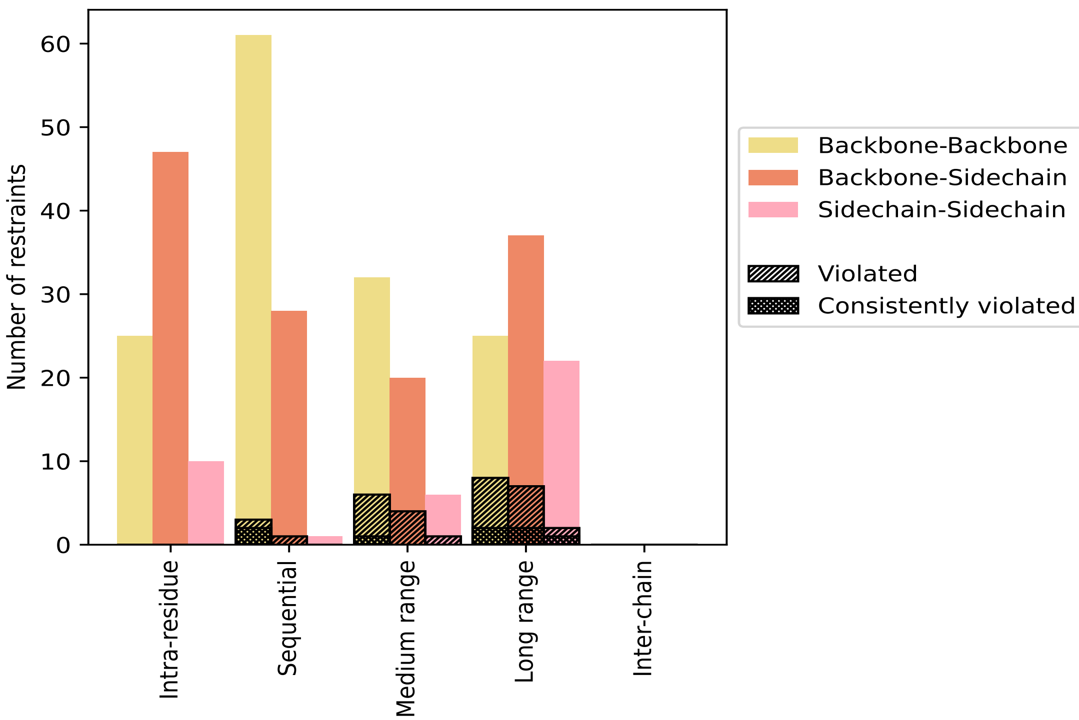
9.1 Summary of distance violations (i)

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)	82	26.1	0	0.0	0.0	0	0.0	0.0
Backbone-Backbone	25	8.0	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	47	15.0	0	0.0	0.0	0	0.0	0.0
Sidechain-Sidechain	10	3.2	0	0.0	0.0	0	0.0	0.0
Sequential (|i-j|=1)	90	28.7	4	4.4	1.3	2	2.2	0.6
Backbone-Backbone	61	19.4	3	4.9	1.0	2	3.3	0.6
Backbone-Sidechain	28	8.9	1	3.6	0.3	0	0.0	0.0
Sidechain-Sidechain	1	0.3	0	0.0	0.0	0	0.0	0.0
Medium range (|i-j|>1 & |i-j|<5)	57	18.2	10	17.5	3.2	1	1.8	0.3
Backbone-Backbone	31	9.9	5	16.1	1.6	1	3.2	0.3
Backbone-Sidechain	20	6.4	4	20.0	1.3	0	0.0	0.0
Sidechain-Sidechain	6	1.9	1	16.7	0.3	0	0.0	0.0
Long range (|i-j|≥5)	76	24.2	15	19.7	4.8	5	6.6	1.6
Backbone-Backbone	17	5.4	6	35.3	1.9	2	11.8	0.6
Backbone-Sidechain	37	11.8	7	18.9	2.2	2	5.4	0.6
Sidechain-Sidechain	22	7.0	2	9.1	0.6	1	4.5	0.3
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	9	2.9	3	33.3	1.0	0	0.0	0.0
Disulfide bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Total	314	100.0	32	10.2	10.2	8	2.5	2.5
Backbone-Backbone	143	45.5	17	11.9	5.4	5	3.5	1.6
Backbone-Sidechain	132	42.0	12	9.1	3.8	2	1.5	0.6
Sidechain-Sidechain	39	12.4	3	7.7	1.0	1	2.6	0.3

[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	2	6	7	0	15	0.19	0.4	0.08	0.16
2	0	2	5	8	0	15	0.19	0.43	0.08	0.17
3	0	3	5	8	0	16	0.18	0.41	0.08	0.15
4	0	2	3	10	0	15	0.19	0.37	0.07	0.16
5	0	2	3	14	0	19	0.18	0.38	0.08	0.17
6	0	2	6	7	0	15	0.19	0.4	0.08	0.16
7	0	3	6	9	0	18	0.18	0.38	0.07	0.15
8	0	2	5	8	0	15	0.19	0.43	0.08	0.17
9	0	2	3	14	0	19	0.18	0.38	0.08	0.16
10	0	2	2	9	0	13	0.21	0.44	0.09	0.18
11	0	2	6	7	0	15	0.19	0.4	0.08	0.16

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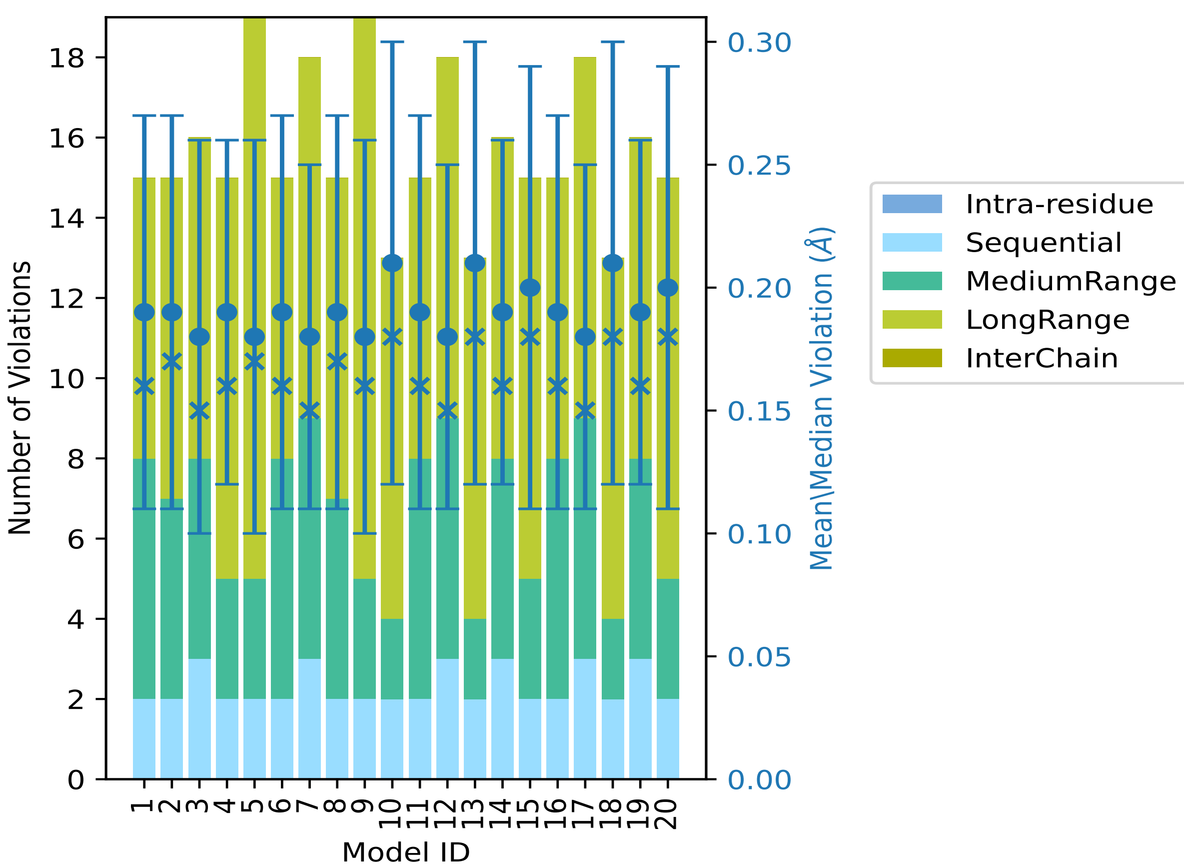
Model ID	Number of violations						Mean (Å)	Max (Å)	SD[6] (Å)	Median (Å)
	IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total				
12	0	3	6	9	0	18	0.18	0.38	0.07	0.15
13	0	2	2	9	0	13	0.21	0.44	0.09	0.18
14	0	3	5	8	0	16	0.19	0.41	0.07	0.16
15	0	2	3	10	0	15	0.2	0.43	0.09	0.18
16	0	2	6	7	0	15	0.19	0.4	0.08	0.16
17	0	3	6	9	0	18	0.18	0.38	0.07	0.15
18	0	2	2	9	0	13	0.21	0.44	0.09	0.18
19	0	3	5	8	0	16	0.19	0.41	0.07	0.16
20	0	2	3	10	0	15	0.2	0.43	0.09	0.18

[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

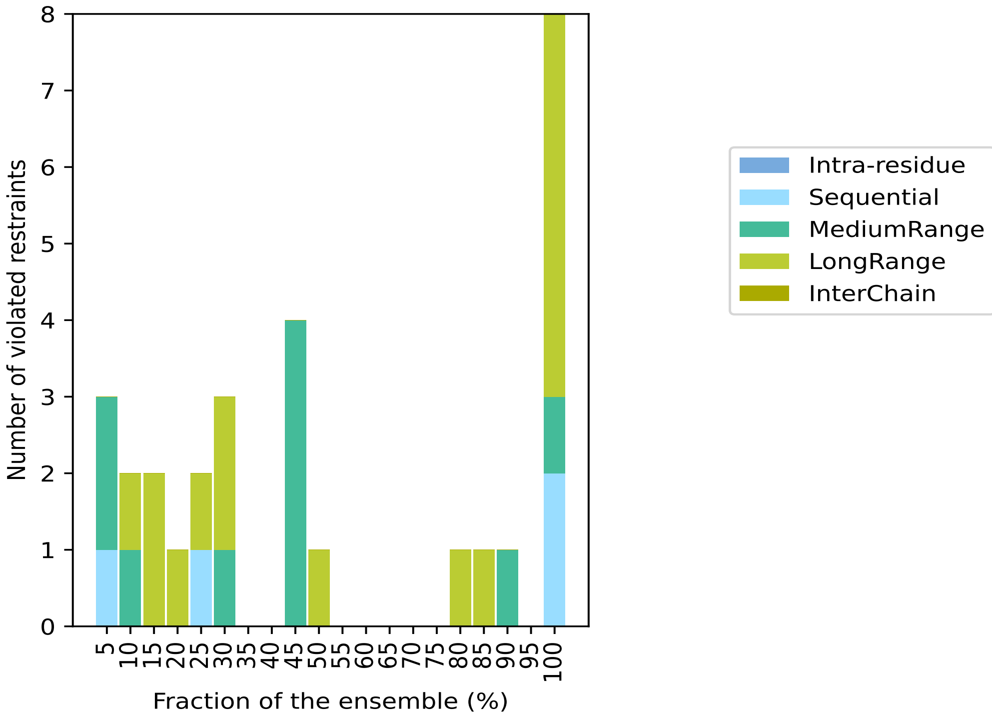
9.3 Distance violation statistics for the ensemble (i)

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, 276(IR:82, SQ:86, MR:47, LR:61, IC:0) restraints are not violated in the ensemble.

Number of violated restraints						Fraction of the ensemble	
IR[1]	SQ[2]	MR[3]	LR[4]	IC[5]	Total	Count[6]	%
0	1	2	0	0	3	1	5.0
0	0	1	1	0	2	2	10.0
0	0	0	2	0	2	3	15.0
0	0	0	1	0	1	4	20.0
0	1	0	1	0	2	5	25.0
0	0	1	2	0	3	6	30.0
0	0	0	0	0	0	7	35.0
0	0	0	0	0	0	8	40.0
0	0	4	0	0	4	9	45.0
0	0	0	1	0	1	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	1	0	1	16	80.0
0	0	0	1	0	1	17	85.0
0	0	1	0	0	1	18	90.0
0	0	0	0	0	0	19	95.0
0	2	1	5	0	8	20	100.0

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

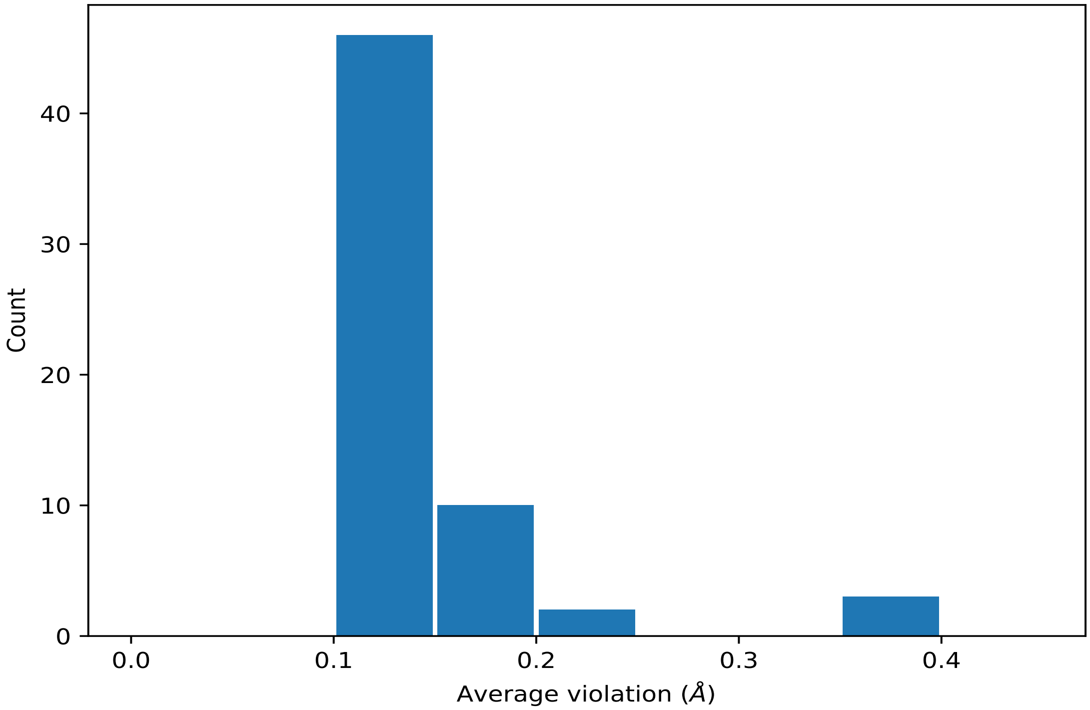
9.3.1 Bar graph : Distance violation statistics for the ensemble (i)



9.4 Most violated distance restraints in the ensemble (i)

9.4.1 Histogram : Distribution of mean distance violations (i)

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



9.4.2 Table: Most violated distance restraints (i)

The following table provides the mean and the standard deviation of the violations for the 10 worst performing restraints, sorted by number of violated models and the mean violation value. The Key (restraint list ID, restraint ID) is the unique identifier for a given restraint. Rows with same key represent combinatorial or ambiguous restraints and are counted as a single restraint.

Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,55)	1:A:120:GLU:H	1:A:43:PRO:HA	20	0.38	0.04	0.38
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB2	20	0.37	0.04	0.37
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB3	20	0.37	0.04	0.37
(1,86)	1:A:61:GLY:H	1:A:53:ASN:HA	20	0.22	0.03	0.23
(1,104)	1:A:59:GLY:H	1:A:62:THR:H	20	0.19	0.02	0.19
(1,180)	1:A:126:ARG:H	1:A:127:GLU:HA	20	0.19	0.03	0.2
(1,240)	1:A:26:THR:HG21	1:A:20:ASP:HB2	20	0.17	0.02	0.18
(1,240)	1:A:26:THR:HG21	1:A:20:ASP:HB3	20	0.17	0.02	0.18
(1,240)	1:A:26:THR:HG22	1:A:20:ASP:HB2	20	0.17	0.02	0.18
(1,240)	1:A:26:THR:HG22	1:A:20:ASP:HB3	20	0.17	0.02	0.18
(1,240)	1:A:26:THR:HG23	1:A:20:ASP:HB2	20	0.17	0.02	0.18
(1,240)	1:A:26:THR:HG23	1:A:20:ASP:HB3	20	0.17	0.02	0.18
(1,136)	1:A:94:LYS:H	1:A:95:ASP:HA	20	0.16	0.02	0.16
(1,75)	1:A:120:GLU:H	1:A:47:GLU:HB2	20	0.15	0.03	0.16
(1,75)	1:A:120:GLU:H	1:A:47:GLU:HB3	20	0.15	0.03	0.16
(1,188)	1:A:134:GLY:H	1:A:131:ASP:H	18	0.14	0.01	0.14

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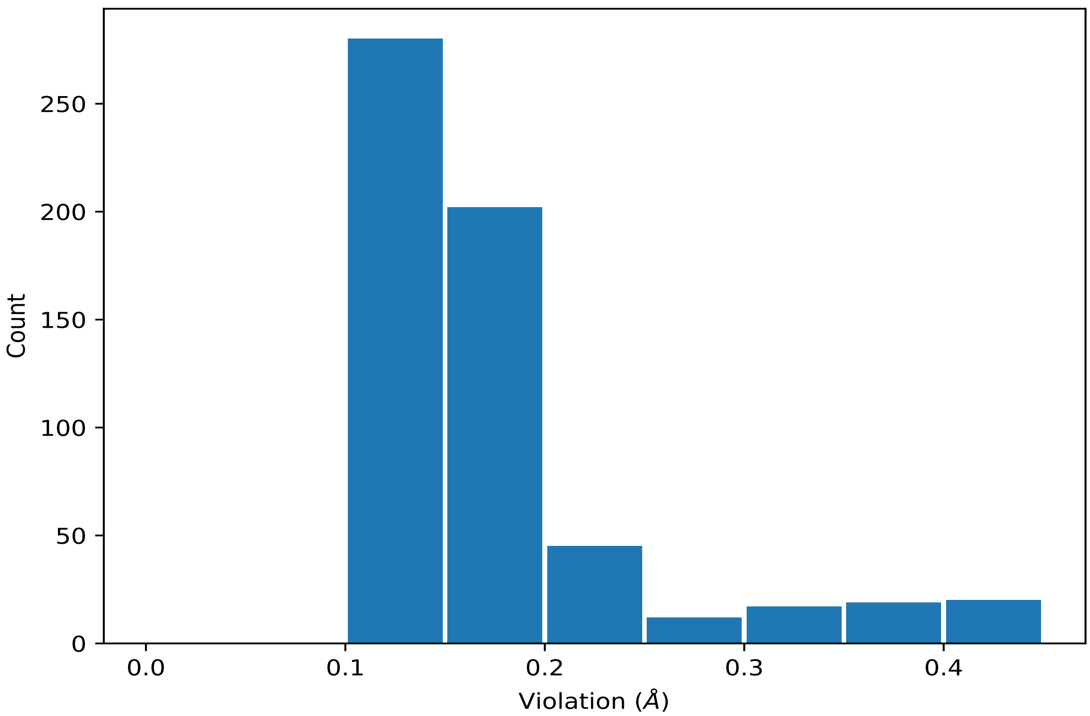
Key	Atom-1	Atom-2	Models[1]	Mean (Å)	SD[1] (Å)	Median (Å)
(1,169)	1:A:44:THR:H	1:A:117:THR:HB	17	0.15	0.02	0.15

[1]Number of violated models, [2]Standard deviation

9.5 All violated distance restraints

9.5.1 Histogram : Distribution of distance violations

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

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)
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB2	10	0.44
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB3	10	0.44
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB2	13	0.44
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB3	13	0.44

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB2	18	0.44
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB3	18	0.44
(1,55)	1:A:120:GLU:H	1:A:43:PRO:HA	2	0.43
(1,55)	1:A:120:GLU:H	1:A:43:PRO:HA	8	0.43
(1,55)	1:A:120:GLU:H	1:A:43:PRO:HA	15	0.43
(1,55)	1:A:120:GLU:H	1:A:43:PRO:HA	20	0.43
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB2	3	0.41
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB3	3	0.41
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB2	14	0.41
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB3	14	0.41
(1,79)	1:A:48:LEU:H	1:A:43:PRO:HB2	19	0.41

10 Dihedral-angle violation analysis (i)

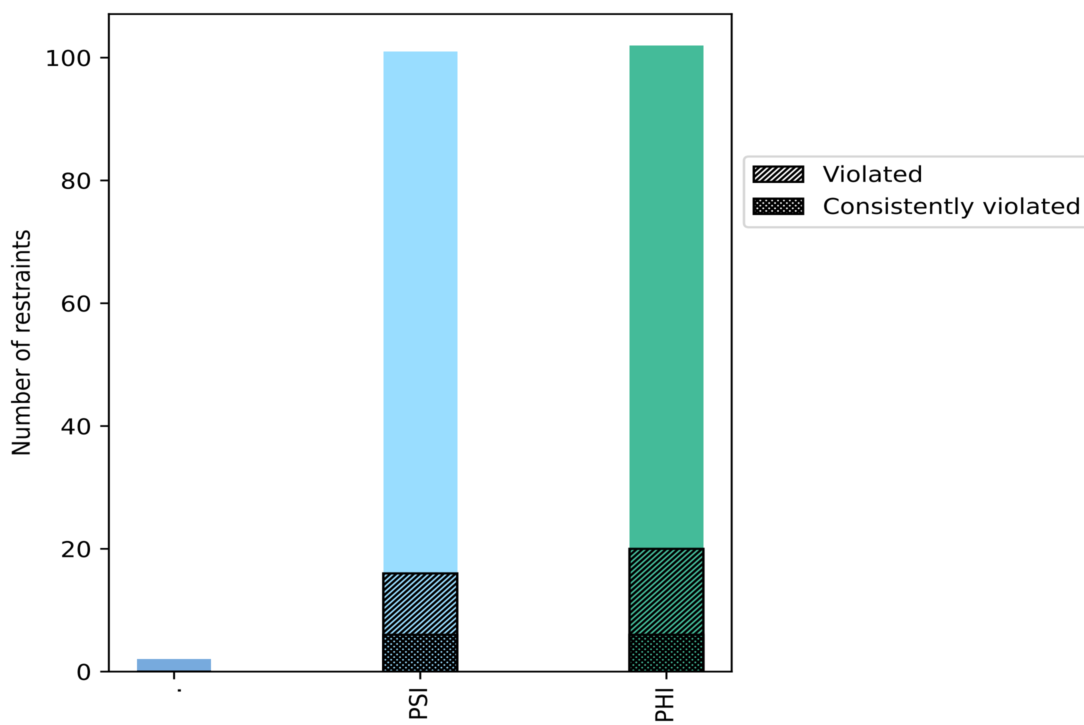
10.1 Summary of dihedral-angle violations (i)

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]
.	2	1.0	0	0.0	0.0	0	0.0	0.0
PSI	101	49.3	16	15.8	7.8	6	5.9	2.9
PHI	102	49.8	20	19.6	9.8	6	5.9	2.9
Total	205	100.0	36	17.6	17.6	12	5.9	5.9

[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)



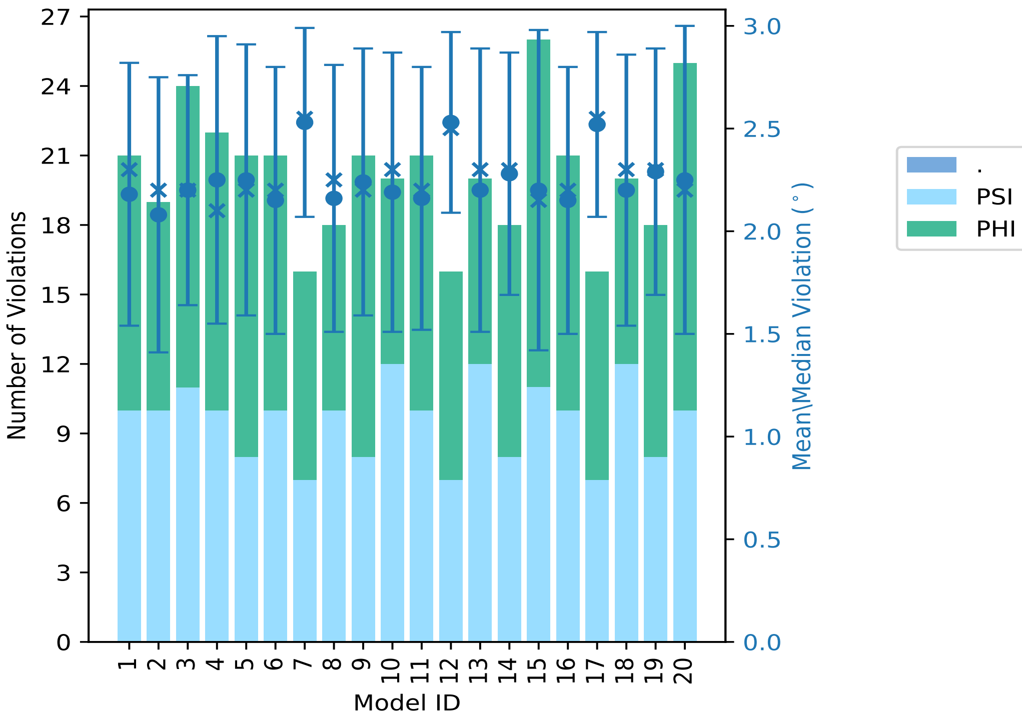
Violated and consistently violated restraints are shown using different hatch patterns in their respective categories

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 (°)
	.	PSI	PHI	Total				
1	0	10	11	21	2.18	3.6	0.64	2.3
2	0	10	9	19	2.08	3.3	0.67	2.2
3	0	11	13	24	2.2	3.2	0.56	2.2
4	0	10	12	22	2.25	3.9	0.7	2.1
5	0	8	13	21	2.25	4.1	0.66	2.2
6	0	10	11	21	2.15	3.6	0.65	2.2
7	0	7	9	16	2.53	3.4	0.46	2.55
8	0	10	8	18	2.16	3.3	0.65	2.25
9	0	8	13	21	2.24	4.1	0.65	2.2
10	0	12	8	20	2.19	3.4	0.68	2.3
11	0	10	11	21	2.16	3.6	0.64	2.2
12	0	7	9	16	2.53	3.4	0.44	2.5
13	0	12	8	20	2.2	3.4	0.69	2.3
14	0	8	10	18	2.28	3.7	0.59	2.3
15	0	11	15	26	2.2	3.9	0.78	2.15
16	0	10	11	21	2.15	3.6	0.65	2.2
17	0	7	9	16	2.52	3.4	0.45	2.55
18	0	12	8	20	2.2	3.3	0.66	2.3
19	0	8	10	18	2.29	3.7	0.6	2.3
20	0	10	15	25	2.25	3.9	0.75	2.2

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	
.	PSI	PHI	Total	Count[1]	%
0	2	1	3	1	5.0
0	0	4	4	2	10.0
0	0	0	0	3	15.0
0	0	1	1	4	20.0
0	2	1	3	5	25.0
0	1	0	1	6	30.0
0	1	2	3	7	35.0
0	0	0	0	8	40.0
0	2	1	3	9	45.0
0	0	1	1	10	50.0
0	0	0	0	11	55.0

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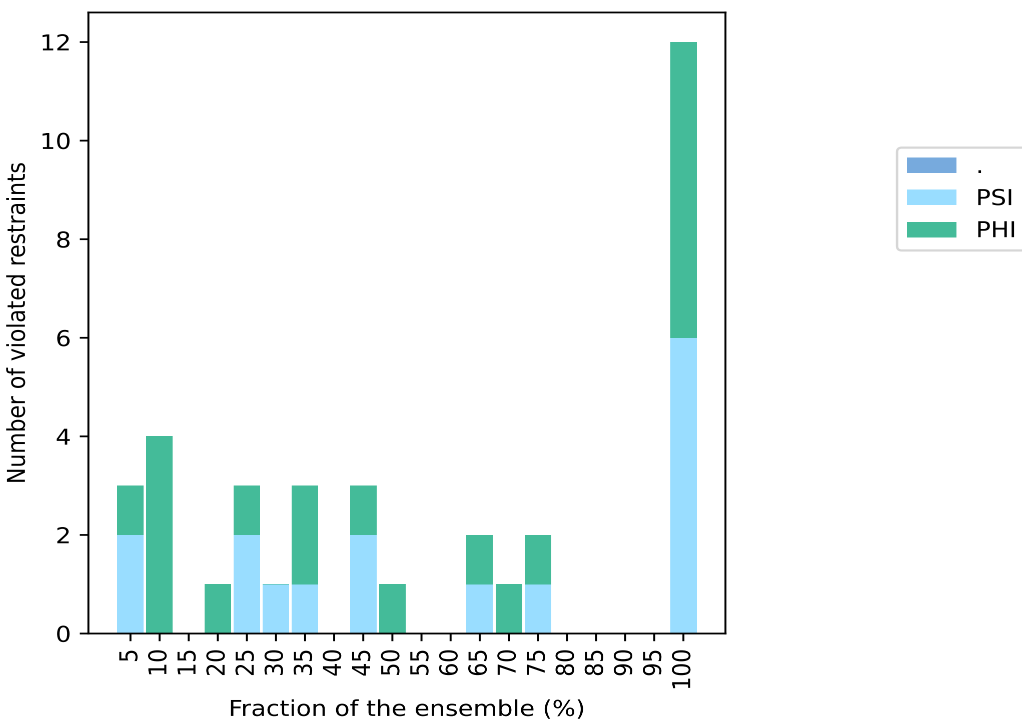
Number of violated restraints				Fraction of the ensemble	
.	PSI	PHI	Total	Count[1]	%
0	0	0	0	12	60.0
0	1	1	2	13	65.0
0	0	1	1	14	70.0
0	1	1	2	15	75.0
0	0	0	0	16	80.0
0	0	0	0	17	85.0
0	0	0	0	18	90.0
0	0	0	0	19	95.0
0	6	6	12	20	100.0

[1] Number of models with violations

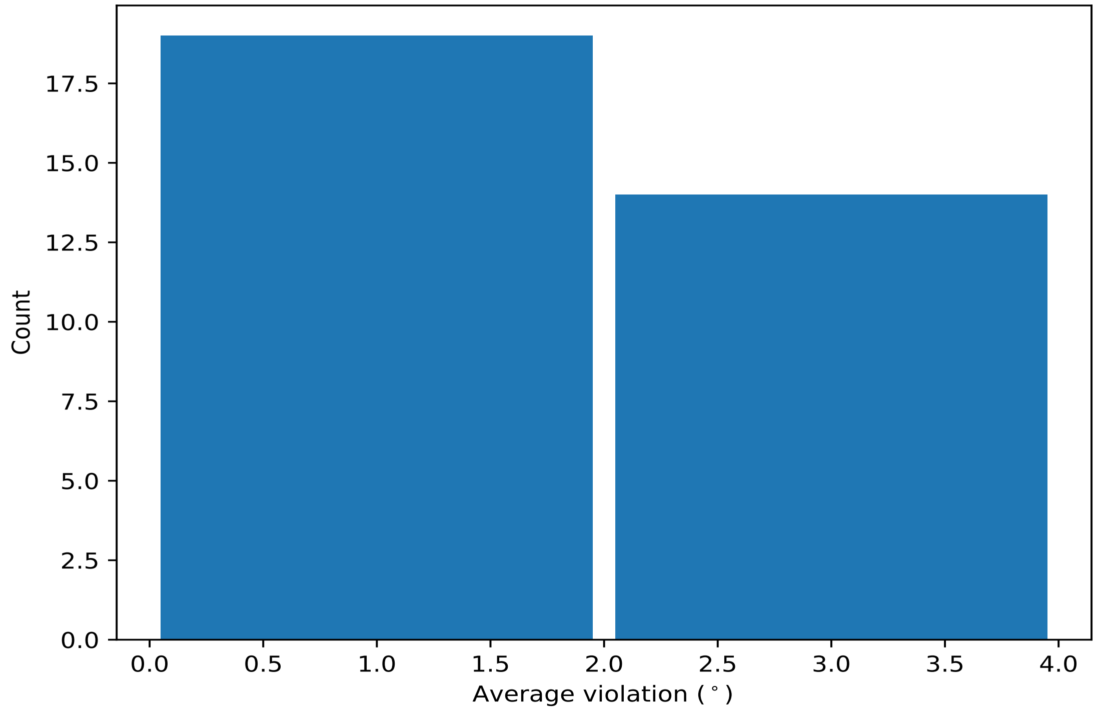
10.3.1 Bar graph : Dihedral-angle Violation statistics for the ensemble (i)



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 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.

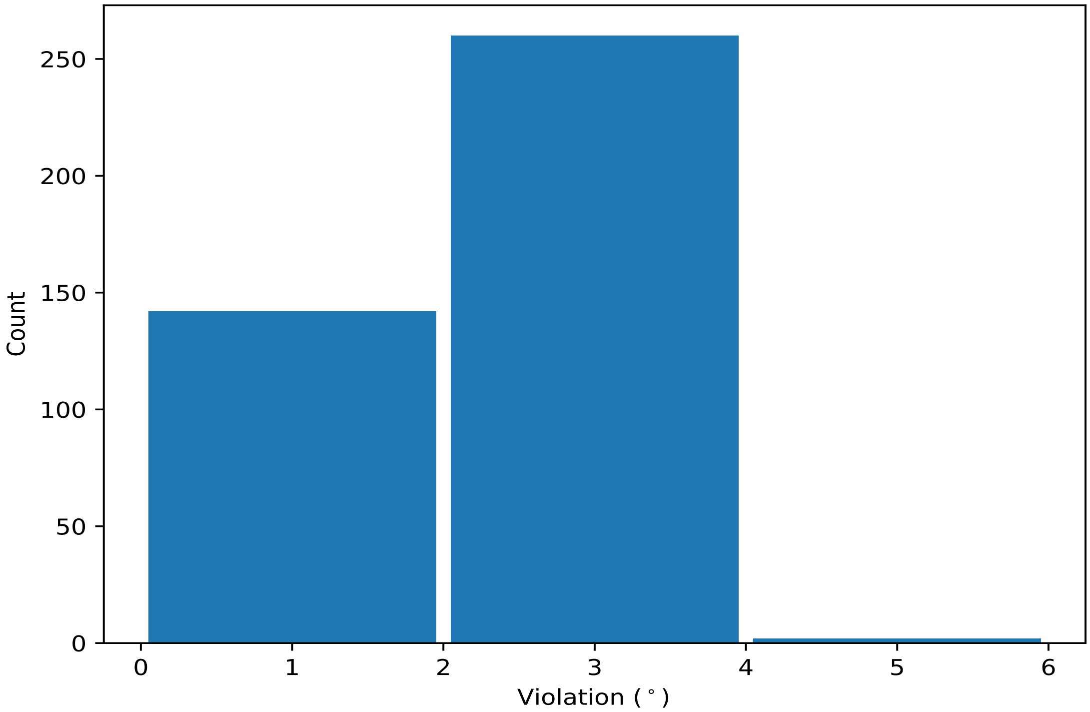
Key	Atom-1	Atom-2	Atom-3	Atom-4	Models[1]	Mean	SD[2]	Median
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	20	3.59	0.27	3.6
(1,153)	1:A:117:THR:C	1:A:118:ASP:N	1:A:118:ASP:CA	1:A:118:ASP:C	20	2.97	0.39	3.1
(1,197)	1:A:56:ASP:C	1:A:57:ALA:N	1:A:57:ALA:CA	1:A:57:ALA:C	20	2.93	0.17	2.9
(1,36)	1:A:56:ASP:N	1:A:56:ASP:CA	1:A:56:ASP:C	1:A:57:ALA:N	20	2.9	0.33	2.8
(1,177)	1:A:24:ASP:C	1:A:25:GLY:N	1:A:25:GLY:CA	1:A:25:GLY:C	20	2.82	0.13	2.75
(1,169)	1:A:25:GLY:N	1:A:25:GLY:CA	1:A:25:GLY:C	1:A:26:THR:N	20	2.67	0.22	2.7
(1,178)	1:A:25:GLY:C	1:A:26:THR:N	1:A:26:THR:CA	1:A:26:THR:C	20	2.48	0.21	2.6
(1,201)	1:A:117:THR:N	1:A:117:THR:CA	1:A:117:THR:C	1:A:118:ASP:N	20	2.35	0.26	2.35
(1,181)	1:A:97:ASN:C	1:A:98:GLY:N	1:A:98:GLY:CA	1:A:98:GLY:C	20	2.28	0.17	2.3
(1,192)	1:A:24:ASP:N	1:A:24:ASP:CA	1:A:24:ASP:C	1:A:25:GLY:N	20	2.18	0.21	2.2

[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 provides the list of violationsfor 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.

Key	Atom-1	Atom-2	Atom-3	Atom-4	Model ID	Violation (°)
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	5	4.1
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	9	4.1
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	4	3.9
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	15	3.9
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	20	3.9
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	14	3.7
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	19	3.7
(1,153)	1:A:117:THR:C	1:A:118:ASP:N	1:A:118:ASP:CA	1:A:118:ASP:C	15	3.7
(1,153)	1:A:117:THR:C	1:A:118:ASP:N	1:A:118:ASP:CA	1:A:118:ASP:C	20	3.7
(1,155)	1:A:119:GLU:C	1:A:120:GLU:N	1:A:120:GLU:CA	1:A:120:GLU:C	1	3.6