

wwPDB NMR Structure Validation Summary Report (i)

Jun 6, 2023 – 07:42 pm BST

PDB ID : 709U BMRB ID : 34618

Title: Solution structure of oxidized cytochrome c552 from Thioalkalivibrio para-

doxus

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Deposited on : 2021-04-17

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

We welcome your comments at validation@mail.wwpdb.org
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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:

 $Mol Probity \quad : \quad 4.02b\text{--}467$

Mogul : 1.8.4, 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)

Ideal geometry (proteins) : Engh & Huber (2001) Ideal geometry (DNA, RNA) : Parkinson et al. (1996)

Validation Pipeline (wwPDB-VP) : 2.33

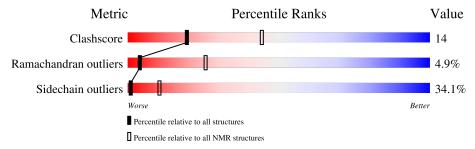


1 Overall quality at a glance (i)

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

The overall completeness of chemical shifts assignment is 81%.

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 $(\# \mathrm{Entries})$	$egin{array}{c} { m NMR \ archive} \ (\#{ m Entries}) \end{array}$	
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	153	34%	25%	6%	35%



2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 12 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 ran	ige (total)	Backbone RMSD (Å)	Medoid model		
1	A:26-A:99,	A:104-A:128	0.39	12		
	(99)					

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 3 clusters and 3 single-model clusters were found.

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



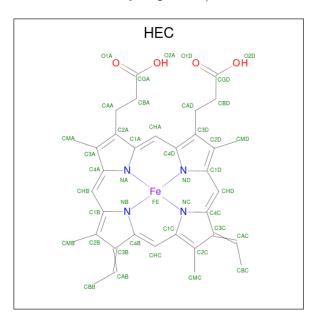
3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2292 atoms, of which 1069 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called Cytochrome c552.

Mol	Chain	Residues	Atoms			Trace			
1	Λ	153	Total	С	Н	N	О	S	0
1	A	199	2217	715	1037	235	225	5	U

• Molecule 2 is HEME C (three-letter code: HEC) (formula: $C_{34}H_{34}FeN_4O_4$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms					
2	Λ	1	Total	С	Fe	Н	N	О
2	A	1	75	34	1	32	4	4

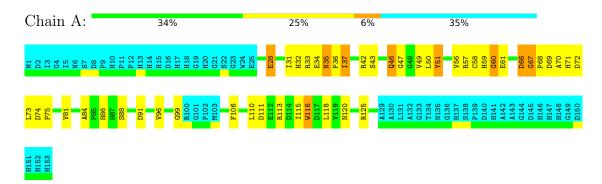


4 Residue-property plots (i)

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.

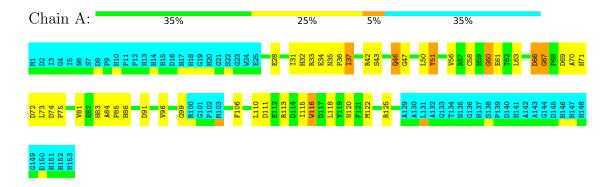
• Molecule 1: Cytochrome c552



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

The representative model is number 12. Colouring as in section 4.1 above.

• Molecule 1: Cytochrome c552





Refinement protocol and experimental data overview (i) 5



The models were refined using the following method: simulated annealing.

Of the 100 calculated structures, 20 were deposited, based on the following criterion: structures with the lowest energy.

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

Software name	Classification	Version
CNS	structure calculation	1.21
X-PLOR NIH	refinement	3.2

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	1313
Number of shifts mapped to atoms	1313
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	81%



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: HEC

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	770	705	705	19±2
2	A	43	32	30	4±1
All	All	16260	14740	14700	433

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 14.

5 of 77 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	$Distance(\mathring{A})$	Models	
Atom-1	Atom-2	Clash(A)	Distance(A)	Worst	Total
1:A:47:GLY:HA2	1:A:50:LEU:HD12	0.74	1.59	16	7
2:A:201:HEC:HMB1	2:A:201:HEC:HBB3	0.73	1.60	7	20
1:A:51:TYR:CE1	2:A:201:HEC:HMB3	0.69	2.21	8	9
1:A:35:ASN:ND2	1:A:37:ILE:HD11	0.69	2.03	9	5
1:A:58:CYS:SG	1:A:73:LEU:HD13	0.67	2.29	8	12



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	99/153~(65%)	83±1 (84±1%)	11±2 (11±2%)	5±1 (5±1%)	4 26
All	All	1980/3060 (65%)	1668 (84%)	215 (11%)	97 (5%)	4 26

5 of 8 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	60	GLY	20
1	A	67	GLY	20
1	A	75	PRO	20
1	A	99	GLY	12
1	A	68	PRO	11

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	80/120 (67%)	53±2 (66±3%)	27±2 (34±3%)	1 10		
All	All	1600/2400~(67%)	1054 (66%)	546 (34%)	1 10		

5 of 44 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	32	HIS	20
1	A	33	ARG	20
1	A	35	ASN	20
1	A	42	ARG	20
1	A	43	SER	20



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)

1 ligand is modelled in this entry.

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

Mal	Tuno	Chain	Res	Link	Bond lengths		
Mol	туре	Chain			Counts	RMSZ	#Z>2
2	HEC	A	201	1	32,50,50	1.53 ± 0.07	4±0 (12±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.

Mal	Tuno	Chain	Peg	Link		Bond ang	les
MIOI	туре	Chain	nes	LIIIK	Counts	RMSZ	#Z>2
2	HEC	A	201	1	24,82,82	0.98 ± 0.03	$0\pm0 \ (0\pm0\%)$

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
ſ	2	HEC	A	201	1	-	$0\pm0,10,54,54$	-

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

Mol Chain		n Ros	Tuno	Atoms	\mathbf{z}	Observed(Å)	Ideal(Å)	Models	
IVIOI	Chain	nes	туре	Atoms		Observed(A)	Ideal(A)	Worst	Total
2	A	201	HEC	C2B-C3B	5.47	1.35	1.40	12	20
2	A	201	HEC	C3C-C2C	4.52	1.36	1.40	4	20
2	A	201	HEC	O2D-CGD	2.52	1.22	1.30	17	20
2	A	201	HEC	O2A-CGA	2.50	1.22	1.30	7	20

There are no bond-angle outliers.

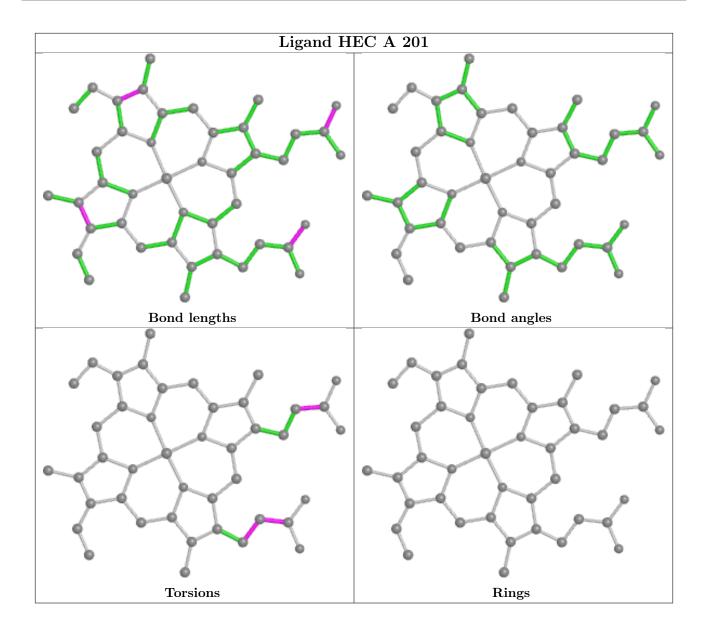
There are no chirality outliers.

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.





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 81% 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: assigned_chem_shift_list

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	1313
Number of shifts mapped to atoms	1313
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	29

The following errors were found when reading this chemical shift list.

• Chemical shift has been reported more than once. All 1 occurrences are reported below.

List ID	Chain	Roc	Type	Atom	Atom Shift Data Value Uncertainty Amb			
LIST ID	Chain	rtes	Type	Atom	Value	Uncertainty	Ambiguity	
1	A	44	LEU	HD13	0.663	0.005	2	

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction \pm precision, ppm	Suggested action
$^{13}\mathrm{C}_{\alpha}$	124	-0.72 ± 0.38	None needed (imprecise)
$^{13}C_{\beta}$	105	0.31 ± 0.27	None needed ($< 0.5 \text{ ppm}$)
¹³ C′	113	-0.72 ± 0.47	None needed (imprecise)
^{15}N	113	0.85 ± 0.31	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 81%, i.e. 1045 atoms were assigned a chemical shift out of a possible 1294. 0 out of 15 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Backbone	471/487 (97%)	196/199 (98%)	186/198 (94%)	89/90 (99%)
Sidechain	564/708 (80%)	384/456 (84%)	176/221 (80%)	4/31 (13%)
Aromatic	10/99 (10%)	7/52 (13%)	2/39 (5%)	1/8 (12%)
Overall	1045/1294 (81%)	587/707 (83%)	364/458 (79%)	94/129 (73%)

7.1.4 Statistically unusual chemical shifts (i)

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

List Id	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	A	80	LEU	HG	5.96	-0.13 - 3.16	13.5
1	A	96	VAL	HG21	-2.25	-0.58 - 2.19	-11.0
1	A	96	VAL	HG22	-2.25	-0.58 - 2.19	-11.0
1	A	96	VAL	HG23	-2.25	-0.58 - 2.19	-11.0
1	A	99	GLY	HA2	7.41	2.15 - 5.77	9.5
1	A	80	LEU	HD21	3.32	-0.65 - 2.13	9.3
1	A	80	LEU	HD22	3.32	-0.65 - 2.13	9.3
1	A	80	LEU	HD23	3.32	-0.65 - 2.13	9.3
1	A	77	VAL	HG11	-1.51	-0.48 - 2.12	-9.0
1	A	77	VAL	HG12	-1.51	-0.48 - 2.12	-9.0
1	A	77	VAL	HG13	-1.51	-0.48 - 2.12	-9.0
1	A	99	GLY	HA3	6.83	2.08 - 5.71	8.1
1	A	96	VAL	HG11	-1.22	-0.48 - 2.12	-7.9
1	A	96	VAL	HG12	-1.22	-0.48 - 2.12	-7.9
1	A	96	VAL	HG13	-1.22	-0.48 - 2.12	-7.9
1	A	104	PRO	HG3	3.97	0.33 - 3.48	6.5
1	A	35	ASN	HA	2.38	2.91 - 6.40	-6.5
1	A	118	LEU	HG	-0.56	-0.13 - 3.16	-6.3
1	A	104	PRO	HG2	3.81	0.41 - 3.45	6.2
1	A	80	LEU	HD11	2.39	-0.61 - 2.12	6.0
1	A	80	LEU	HD12	2.39	-0.61 - 2.12	6.0
1	A	80	LEU	HD13	2.39	-0.61 - 2.12	6.0
1	A	100	ARG	HD2	1.77	1.97 - 4.26	-5.9
1	A	36	PRO	HD3	1.47	1.76 - 5.48	-5.8

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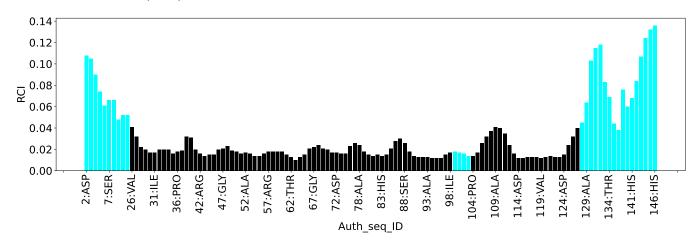
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List Id	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	A	80	LEU	HB2	3.55	-0.07 - 3.30	5.8
1	A	55	CYS	HB3	0.38	0.69 - 5.10	-5.7
1	A	105	GLY	HA3	5.95	2.08 - 5.71	5.7
1	A	102	PRO	HG2	0.22	0.41 - 3.45	-5.6
1	A	118	LEU	HB3	-0.27	-0.26 - 3.31	-5.0

7.1.5 Random Coil Index (RCI) plots (i)

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

Random coil index (RCI) for chain A:





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	1907
Intra-residue ($ i-j =0$)	735
Sequential ($ i-j =1$)	622
Medium range ($ i-j >1$ and $ i-j <5$)	321
Long range (i-j ≥5)	229
Inter-chain	0
Hydrogen bond restraints	0
Disulfide bond restraints	0
Total dihedral-angle restraints	246
Number of unmapped restraints	0
Number of restraints per residue	14.1
Number of long range restraints per residue ¹	1.5

¹Long 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)	140.3	0.2
0.2-0.5 (Medium)	120.0	0.5
>0.5 (Large)	5.2	1.32



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 $(^{\circ})$	Average number of violations per model	$\mathbf{Max} \ (^{\circ})$
1.0-10.0 (Small)	69.0	10.0
10.0-20.0 (Medium)	1.1	15.7
>20.0 (Large)	None	None



9 Distance violation analysis (i)

9.1 Summary of distance violations (i)

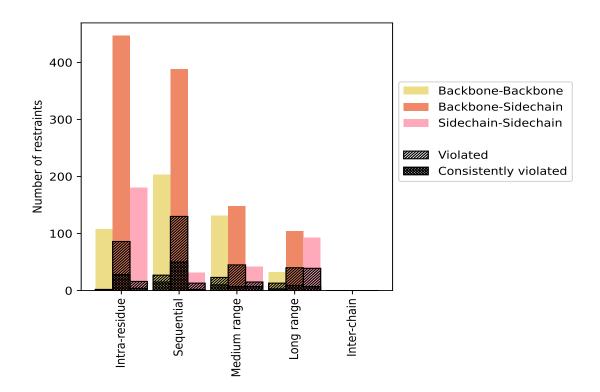
The following table shows the summary of distance violations in different restraint categories based on the sequence separation of the atoms involved. Each category is further sub-divided into three sub-categories based on the atoms involved. Violations less than 0.1 Å are not included in the statistics.

Doodnointe tour	C	% ¹	Vi	olated	3	Consis	tently	$\mathbf{Violated}^4$
Restraints type	Count	70	Count	$\%^2$	$\%^{1}$	Count	$\%^2$	$\%^1$
Intra-residue (i-j =0)	735	38.5	104	14.1	5.5	33	4.5	1.7
Backbone-Backbone	108	5.7	2	1.9	0.1	1	0.9	0.1
Backbone-Sidechain	447	23.4	86	19.2	4.5	28	6.3	1.5
Sidechain-Sidechain	180	9.4	16	8.9	0.8	4	2.2	0.2
Sequential (i-j =1)	622	32.6	170	27.3	8.9	67	10.8	3.5
Backbone-Backbone	203	10.6	27	13.3	1.4	15	7.4	0.8
Backbone-Sidechain	388	20.3	130	33.5	6.8	50	12.9	2.6
Sidechain-Sidechain	31	1.6	13	41.9	0.7	2	6.5	0.1
Medium range ($ i-j >1 & i-j <5$)	321	16.8	83	25.9	4.4	24	7.5	1.3
Backbone-Backbone	131	6.9	23	17.6	1.2	10	7.6	0.5
Backbone-Sidechain	148	7.8	45	30.4	2.4	7	4.7	0.4
Sidechain-Sidechain	42	2.2	15	35.7	0.8	7	16.7	0.4
Long range ($ i-j \ge 5$)	229	12.0	92	40.2	4.8	19	8.3	1.0
Backbone-Backbone	32	1.7	13	40.6	0.7	3	9.4	0.2
Backbone-Sidechain	104	5.5	40	38.5	2.1	9	8.7	0.5
Sidechain-Sidechain	93	4.9	39	41.9	2.0	7	7.5	0.4
Inter-chain	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Backbone	0	0.0	0	0.0	0.0	0	0.0	0.0
Backbone-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Sidechain-Sidechain	0	0.0	0	0.0	0.0	0	0.0	0.0
Hydrogen bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Disulfide bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Total	1907	100.0	449	23.5	23.5	143	7.5	7.5
Backbone-Backbone	474	24.9	65	13.7	3.4	29	6.1	1.5
Backbone-Sidechain	1087	57.0	301	27.7	15.8	94	8.6	4.9
Sidechain-Sidechain	346	18.1	83	24.0	4.4	20	5.8	1.0

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		Nun	nber o	f viola	ations	3	Mean (Å)	Morr (Å)	${ m SD}^6$ (Å)	Modian (Å)
Model ID	IR^1	SQ^2	MR^3	LR^4	IC^5	Total	Mean (A)	Max (Å)	$SD^*(A)$	Median (Å)
1	61	99	43	49	0	252	0.22	1.01	0.11	0.2
2	59	103	45	49	0	256	0.22	1.3	0.12	0.2
3	63	104	47	51	0	265	0.22	1.28	0.11	0.19
4	60	102	46	47	0	255	0.23	1.29	0.12	0.2
5	60	108	42	49	0	259	0.23	1.3	0.12	0.2
6	62	108	46	53	0	269	0.22	1.27	0.12	0.2
7	64	106	45	55	0	270	0.22	0.9	0.11	0.19
8	57	106	47	52	0	262	0.23	1.28	0.12	0.2
9	63	104	47	51	0	265	0.22	1.27	0.11	0.2
10	64	106	53	58	0	281	0.22	1.01	0.1	0.2
11	61	104	47	54	0	266	0.23	1.32	0.13	0.2

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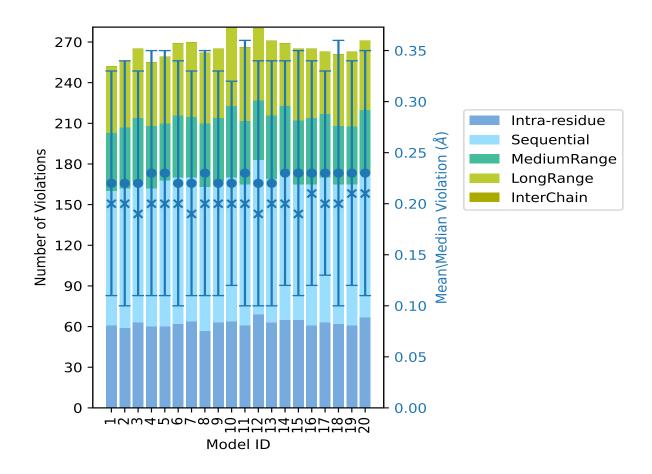


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Model ID		Nun	nber o	f viola	ations	3	Mean (Å)	Max (Å)	SD^6 (Å)	Median (Å)
Model 1D	IR^1	SQ^2	MR^3	LR^4	IC^5	Total	Mean (A)	Max (A)	SD (A)	Median (A)
12	69	114	44	53	0	280	0.22	1.29	0.12	0.19
13	63	106	47	55	0	271	0.22	1.28	0.12	0.2
14	65	110	48	46	0	269	0.23	1.02	0.11	0.2
15	65	100	47	53	0	265	0.23	1.28	0.12	0.19
16	61	104	49	51	0	265	0.23	1.05	0.11	0.21
17	63	107	47	46	0	263	0.23	0.99	0.1	0.2
18	62	103	43	53	0	261	0.23	1.28	0.13	0.2
19	61	104	43	55	0	263	0.23	1.03	0.11	0.21
20	67	106	47	51	0	271	0.23	1.32	0.12	0.21

 $^{^1}$ 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)

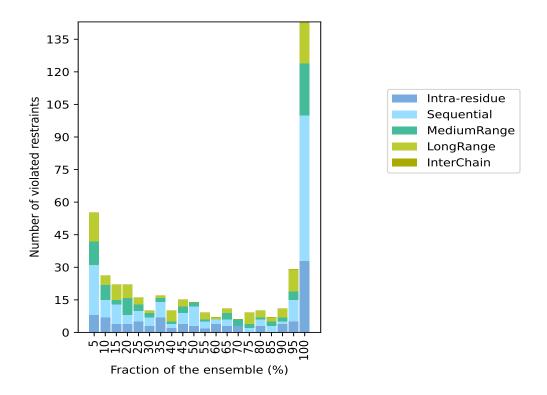
Violation analysis may find that some restraints are violated in few models and some are violated in most of models. The following table provides this information as number of violated restraints for a given fraction of the ensemble. In total, 1458(IR:631, SQ:452, MR:238, LR:137, IC:0) restraints are not violated in the ensemble.

Nu	$\overline{\mathbf{mber}}$	of vio	lated	restra	aints	Fraction	n of the ensemble
IR^1	SQ^2	MR^3	LR^4	IC^5	Total	Count ⁶	%
8	23	11	13	0	55	1	5.0
7	8	7	4	0	26	2	10.0
4	9	2	7	0	22	3	15.0
4	4	8	6	0	22	4	20.0
5	5	3	3	0	16	5	25.0
3	4	2	1	0	10	6	30.0
7	7	2	1	0	17	7	35.0
2	2	1	5	0	10	8	40.0
4	5	3	3	0	15	9	45.0
3	9	2	0	0	14	10	50.0
2	3	1	3	0	9	11	55.0
4	2	0	1	0	7	12	60.0
3	3	3	2	0	11	13	65.0
3	0	3	0	0	6	14	70.0
0	2	2	5	0	9	15	75.0
3	3	1	3	0	10	16	80.0
0	3	2	2	0	7	17	85.0
4	1	2	4	0	11	18	90.0
5	10	4	10	0	29	19	95.0
33	67	24	19	0	143	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)

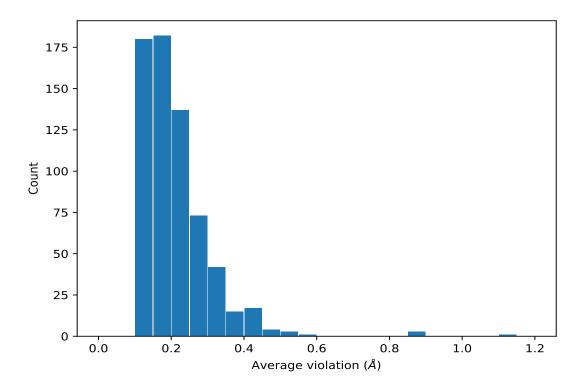


9.4 Most violated distance restraints in the ensemble (i)

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	\mathbf{Models}^1	Mean (Å)	SD^1 (Å)	Median (Å)
(1,1570)	1:A:47:GLY:HA3	1:A:114:ASP:HA	20	0.58	0.13	0.57
(1,1034)	1:A:124:ASP:H	1:A:125:ARG:HB3	20	0.51	0.03	0.51
(1,979)	1:A:119:VAL:H	1:A:120:ASN:HB3	20	0.49	0.03	0.49
(1,1863)	1:A:102:PRO:HA	1:A:102:PRO:HD2	20	0.45	0.09	0.39
(1,211)	1:A:43:SER:H	1:A:44:LEU:HG	20	0.44	0.04	0.44
(1,115)	1:A:33:ARG:H	1:A:90:GLY:HA2	20	0.43	0.04	0.43
(1,153)	1:A:35:ASN:HD22	1:A:34:GLU:HG3	20	0.41	0.02	0.41
(1,1897)	2:A:201:HEC:HBB1	1:A:110:LEU:HD11	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB1	1:A:110:LEU:HD12	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB1	1:A:110:LEU:HD13	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB2	1:A:110:LEU:HD11	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB2	1:A:110:LEU:HD12	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB2	1:A:110:LEU:HD13	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB3	1:A:110:LEU:HD11	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB3	1:A:110:LEU:HD12	20	0.4	0.11	0.39
(1,1897)	2:A:201:HEC:HBB3	1:A:110:LEU:HD13	20	0.4	0.11	0.39

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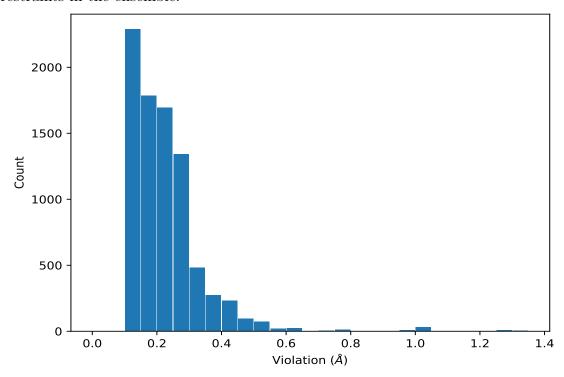
Key	Atom-1	Atom-2	$Models^1$	Mean (Å)	\mathbf{SD}^1 (Å)	Median (Å)
(1,68)	1:A:30:GLU:H	1:A:31:ILE:HG12	20	0.39	0.13	0.36
(1,1015)	1:A:122:MET:H	1:A:123:ARG:HB3	20	0.37	0.03	0.37

¹Number of violated models, ²Standard deviation

9.5 All violated distance restraints (i)

9.5.1 Histogram: Distribution of distance violations (i)

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



9.5.2 Table : All distance violations (i)

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

Key	Atom-1	Atom-2	Model ID	Violation (Å)	
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	11	1.32	
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	20	1.32	
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	2	1.3	

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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	5	1.3
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	4	1.29
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	12	1.29
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	3	1.28
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	8	1.28
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	13	1.28
(1,1569)	1:A:47:GLY:HA3	1:A:50:LEU:HG	15	1.28



10 Dihedral-angle violation analysis (i)

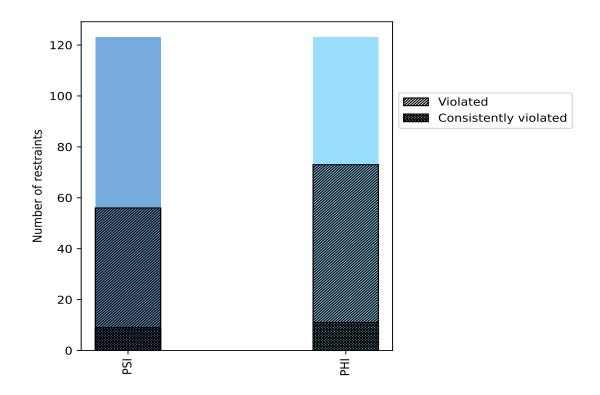
10.1 Summary of dihedral-angle violations (i)

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 true	- t Ct		Vie	olated	3	Consis	tentl	${f y}$ Violated 4
Angle type	Count	$\%^1$	Count	$\%^2$	$\%^1$	Count	$\%^2$	$\%^1$
PSI	123	50.0	56	45.5	22.8	9	7.3	3.7
PHI	123	50.0	73	59.3	29.7	11	8.9	4.5
Total	246	100.0	129	52.4	52.4	20	8.1	8.1

 $^{^1}$ 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



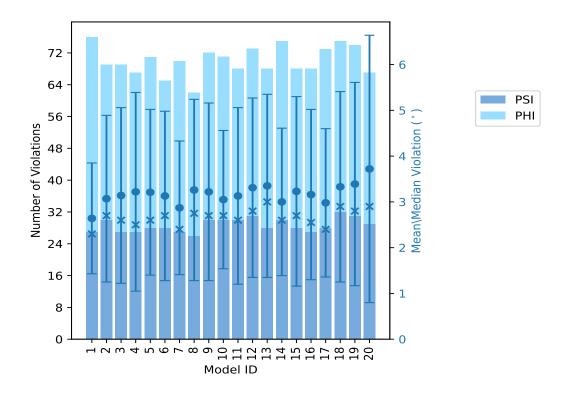
10.2 Dihedral-angle violation statistics for each model (i)

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			Moon (°)	Morr (°)	SD (°)	Modian (°)	
Model 1D	PSI	PSI PHI Total		$ $ Mean $(^{\circ})$	$\mathbf{Max} (^{\circ})$	\mathbf{SD} (°)	\mid Median (°) \mid	
1	27	49	76	2.64	7.6	1.21	2.3	
2	30	39	69	3.07	11.1	1.82	2.7	
3	27	42	69	3.14	11.4	1.92	2.6	
4	27	40	67	3.22	12.9	2.17	2.5	
5	28	43	71	3.21	10.9	1.81	2.6	
6	28	37	65	3.13	12.1	1.85	2.7	
7	27	43	70	2.87	6.9	1.46	2.4	
8	26	36	62	3.26	12.0	1.98	2.75	
9	30	42	72	3.22	12.5	1.94	2.7	
10	30	41	71	3.05	8.9	1.51	2.7	
11	30	38	68	3.13	12.2	1.93	2.6	
12	31	42	73	3.31	11.8	1.96	2.8	
13	28	40	68	3.35	12.2	2.0	3.0	
14	30	45	75	3.0	10.6	1.61	2.6	
15	28	40	68	3.23	13.2	2.07	2.7	
16	27	41	68	3.16	9.1	1.86	2.55	
17	28	45	73	2.98	10.0	1.62	2.4	
18	32	43	75	3.33	13.1	2.08	2.9	
19	31	43	74	3.39	11.8	2.22	2.8	
20	29	38	67	3.72	15.7	2.92	2.9	



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.

Nun	nber o	f violated restraints	Fraction of the ensemble			
PSI	PHI	Total	Count ¹	%		
6	10	16	1	5.0		
4	5	9	2	10.0		
7	1	8	3	15.0		
5	2	7	4	20.0		
1	4	5	5	25.0		
1	2	3	6	30.0		
2	3	5	7	35.0		
1	2	3	8	40.0		
1	2	3	9	45.0		
1	3	4	10	50.0		
0	0	0	11	55.0		

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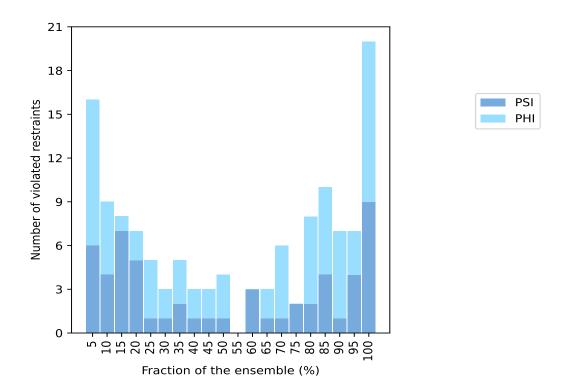


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Nun	nber o	of violated restraints	Fraction of the ensemble			
PSI	PHI	Total	Count ¹	%		
3	0	3	12	60.0		
1	2	3	13	65.0		
1	5	6	14	70.0		
2	0	2	15	75.0		
2	6	8	16	80.0		
4	6	10	17	85.0		
1	6	7	18	90.0		
4	3	7	19	95.0		
9	11	20	20	100.0		

¹ Number of models with violations

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



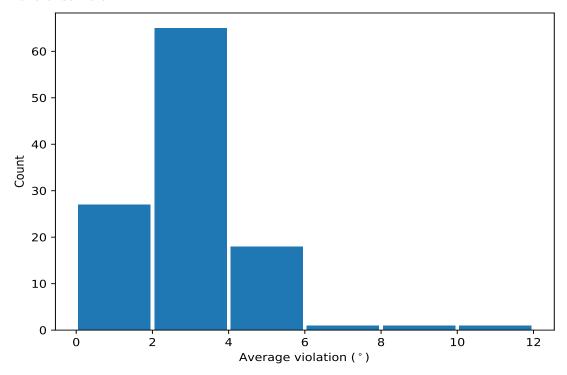
10.4 Most violated dihedral-angle restraints in the ensemble (i)

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	${f Models}^1$	Mean	\mathbf{SD}^2	Median
(1,35)	1:A:36:PRO:N	1:A:36:PRO:CA	1:A:36:PRO:C	1:A:37:ILE:N	20	8.91	1.57	8.4
(1,202)	1:A:121:PHE:C	1:A:122:MET:N	1:A:122:MET:CA	1:A:122:MET:C	20	5.82	1.03	5.95
(1,124)	1:A:79:ASP:C	1:A:80:LEU:N	1:A:80:LEU:CA	1:A:80:LEU:C	20	5.47	1.19	5.3
(1,53)	1:A:45:ASP:N	1:A:45:ASP:CA	1:A:45:ASP:C	1:A:46:GLN:N	20	4.88	0.8	5.35
(1,33)	1:A:35:ASN:N	1:A:35:ASN:CA	1:A:35:ASN:C	1:A:36:PRO:N	20	4.78	2.79	4.25
(1,116)	1:A:75:PRO:C	1:A:76:PRO:N	1:A:76:PRO:CA	1:A:76:PRO:C	20	4.62	0.59	4.6
(1,52)	1:A:43:SER:C	1:A:44:LEU:N	1:A:44:LEU:CA	1:A:44:LEU:C	20	4.59	0.99	4.8
(1,97)	1:A:67:GLY:N	1:A:67:GLY:CA	1:A:67:GLY:C	1:A:68:PRO:N	20	4.38	1.14	4.5
(1,100)	1:A:67:GLY:C	1:A:68:PRO:N	1:A:68:PRO:CA	1:A:68:PRO:C	20	4.35	0.63	4.4
(1,180)	1:A:110:LEU:C	1:A:111:ASP:N	1:A:111:ASP:CA	1:A:111:ASP:C	20	4.26	0.67	4.2

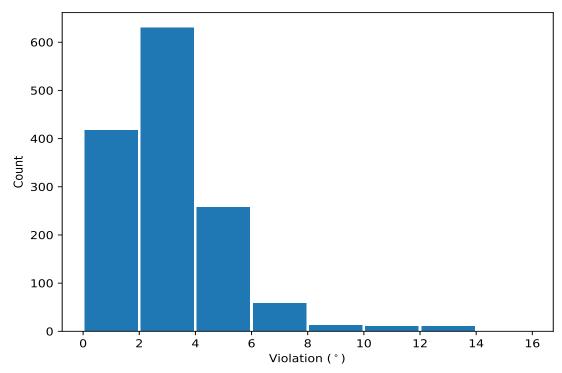
¹ Number of violated models, ²Standard deviation, All angle values are in degree (°)



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 violations for 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 (°)
(1,33)	1:A:35:ASN:N	1:A:35:ASN:CA	1:A:35:ASN:C	1:A:36:PRO:N	20	15.7
(1,35)	1:A:36:PRO:N	1:A:36:PRO:CA	1:A:36:PRO:C	1:A:37:ILE:N	20	13.7
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	15	13.2
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	18	13.1
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	4	12.9
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	20	12.8
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	9	12.5
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	11	12.2
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	13	12.2
(1,60)	1:A:47:GLY:C	1:A:48:GLY:N	1:A:48:GLY:CA	1:A:48:GLY:C	6	12.1

