

wwPDB NMR Structure Validation Summary Report (i)

Jun 3, 2023 – 01:12 PM EDT

PDB ID : 6WA1 BMRB ID : 30737

Title : Dimeric form of the trans-stabilized Hemolysin II C-terminal domain

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Deposited on : 2020-03-24

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 (1)) were used in the production of this report:

MolProbity: 4.02b-467

Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)

wwPDB-RCI : v 1n 11 5 13 A (Berjanski et al., 2005)

PANAV : Wang et al. (2010)

 $\begin{array}{ccc} wwPDB\text{-}ShiftChecker &:& v1.2\\ BMRB \ Restraints \ Analysis &:& v1.2 \end{array}$

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

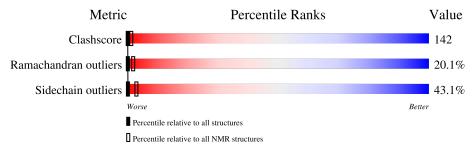
Validation Pipeline (wwPDB-VP) : 2.33

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 17%.

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	94	50%	37% • 10%			
1	В	94	• 49%	38% • 10%			



2 Ensemble composition and analysis (i)

This entry contains 25 models. Model 1 is the overall representative, medoid model (most similar to other models).

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:94, B:10-B:94 (170)	2.20	1		

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

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

The models can be grouped into 4 clusters. No single-model clusters were found.

Cluster number	Models
1	[4, 5, 6, 7, 15, 19, 20, 22, 24]
2	3, 8, 9, 12, 14, 16, 17, 23
3	1, 2, 10, 11
4	13, 18, 21, 25



3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 2930 atoms, of which 1464 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called Hemolysin II.

Mol	Chain	Residues		${f Atoms}$					Trace
1	Λ	94	Total	С	Н	N	О	S	0
1	Α	94	1465	454	732	126	150	3	U
1	D	0.4	Total	С	Н	N	О	S	0
	B 94	1465	454	732	126	150	3	0	

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	87	MET	PRO	engineered mutation	UNP Q81AN8
В	87	MET	PRO	engineered mutation	UNP Q81AN8

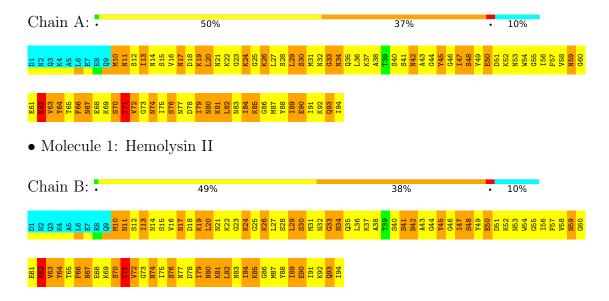


4 Residue-property plots (i)

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.

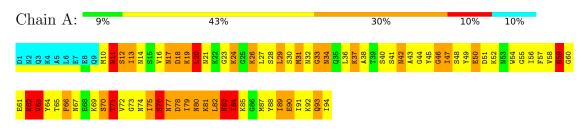




4.2 Residue scores for the representative (medoid) model from the NMR ensemble

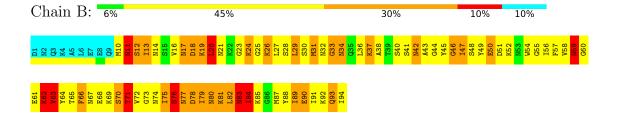
The representative model is number 1. Colouring as in section 4.1 above.

• Molecule 1: Hemolysin II



• Molecule 1: Hemolysin II







Refinement protocol and experimental data overview (i) 5



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

Of the 100 calculated structures, 25 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
TALOS	geometry optimization	TALOS-N
X-PLOR NIH	structure calculation	
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	1
Total number of shifts	424
Number of shifts mapped to atoms	424
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	17%



6 Model quality (i)

6.1 Standard geometry (i)

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	Е	ond lengths	Bond angles		
IVIOI	Chain	RMSZ	#Z>5	RMSZ	#Z>5	
1	A	0.67 ± 0.01	$0\pm0/666$ ($0.0\pm~0.0\%$)	0.94 ± 0.01	$1\pm1/889$ ($0.1\pm$ 0.1%)	
1	В	0.67 ± 0.01	$0\pm0/666$ ($0.0\pm$ 0.0%)	0.94 ± 0.01	1±1/889 (0.1± 0.1%)	
All	All	0.67	0/33300 (0.0%)	0.94	40/44450 (0.1%)	

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	Mol Chain		Chain Res Type		\mathbf{z}	$Observed(^o)$	$Ideal(^{o})$	Models	
IVIOI	Chain	nes	Type	Atoms		Observed()	ideai()	Worst	Total
1	В	64	TYR	CB-CG-CD2	-5.86	117.49	121.00	13	7
1	A	64	TYR	CB-CG-CD1	-5.85	117.49	121.00	16	6
1	В	64	TYR	CB-CG-CD1	-5.79	117.52	121.00	16	6
1	A	64	TYR	CB-CG-CD2	-5.79	117.53	121.00	13	7
1	A	66	PHE	CB-CG-CD2	-5.58	116.90	120.80	15	4

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	659	663	663	195 ± 16
1	В	659	663	663	196 ± 16
All	All	32950	33150	33150	9384

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 142.

5 of 2307 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
Atom-1	Atom-2	Clash(A) Distance(A)		Worst	Total
1:A:17:ASN:HD21	1:A:29:LEU:HD23	1.11	1.02	10	17
1:A:29:LEU:HD22	1:A:29:LEU:H	1.11	1.04	10	25
1:B:29:LEU:H	1:B:29:LEU:HD22	1.09	1.07	22	25
1:B:17:ASN:HD21	1:B:29:LEU:HD23	1.08	1.02	10	17
1:B:17:ASN:ND2	1:B:29:LEU:HD23	1.04	1.67	10	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	84/94 (89%)	49±3 (59±3%)	18±2 (21±3%)	17±3 (20±3%)	0 2		
1	В	84/94 (89%)	49±3 (59±3%)	18±2 (21±3%)	17±3 (20±3%)	0 2		
All	All	4200/4700 (89%)	2473 (59%)	882 (21%)	845 (20%)	0 2		

5 of 72 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	62	LYS	25
1	A	76	SER	25
1	A	79	ILE	25
1	A	84	ILE	25
1	В	62	LYS	25

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	74/82~(90%)	42±3 (57±4%)	32±3 (43±4%)	0 3
1	В	74/82 (90%)	42±3 (57±4%)	32±3 (43±4%)	0 3
All	All	3700/4100 (90%)	2104 (57%)	1596 (43%)	0 3

5 of 122 unique residues with a non-rotameric side chain are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
1	A	19	LYS	25
1	A	29	LEU	25
1	A	81	LYS	25
1	A	89	ILE	25
1	A	93	GLN	25

6.3.3 RNA (i)

There are no RNA molecules in this entry.

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

There are no non-standard protein/DNA/RNA residues in this entry.

6.5 Carbohydrates (i)

There are no monosaccharides in this entry.

6.6 Ligand geometry (i)

There are no ligands in this entry.

6.7 Other polymers (i)

There are no such molecules in this entry.

6.8 Polymer linkage issues (i)

There are no chain breaks in this entry.



7 Chemical shift validation (i)

The completeness of assignment taking into account all chemical shift lists is 17% for the well-defined parts and 17% for the entire structure.

7.1 Chemical shift list 1

File name: working cs.cif

Chemical shift list name: pdb_deposit_nmrstar.star

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	424
Number of shifts mapped to atoms	424
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	0

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
$^{13}\mathrm{C}_{\alpha}$	93	-0.62 ± 0.15	Should be applied
$^{13}C_{\beta}$	83	-0.32 ± 0.16	None needed (< 0.5 ppm)
¹³ C′	65	-0.27 ± 0.23	None needed ($< 0.5 \text{ ppm}$)
^{15}N	91	-0.16 ± 0.60	None needed ($< 0.5 \text{ ppm}$)

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 17%, i.e. 388 atoms were assigned a chemical shift out of a possible 2258. 0 out of 18 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathbf{H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Backbone	313/868 (36%)	84/358 (23%)	146/340 (43%)	83/170 (49%)
Sidechain	75/1254~(6%)	0/806 (0%)	75/394 (19%)	0/54 (0%)



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	Total	$^{1}\mathbf{H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Aromatic	0/136 (0%)	0/64 (0%)	0/70 (0%)	0/2~(0%)
Overall	388/2258 (17%)	84/1228 (7%)	221/804 (27%)	83/226 (37%)

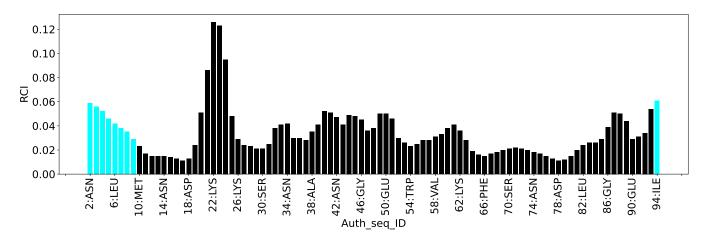
7.1.4 Statistically unusual chemical shifts (i)

There are no statistically unusual chemical shifts.

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	3014
Intra-residue (i-j =0)	1358
Sequential (i-j =1)	812
Medium range ($ i-j >1$ and $ i-j <5$)	320
Long range (i-j ≥5)	418
Inter-chain	0
Hydrogen bond restraints	106
Disulfide bond restraints	0
Total dihedral-angle restraints	140
Number of unmapped restraints	0
Number of restraints per residue	16.8
Number of long range restraints per residue ¹	2.5

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)	113.4	0.2
0.2-0.5 (Medium)	74.1	0.5
>0.5 (Large)	30.6	28.48



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

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

Bins $(^{\circ})$	Average number of violations per model	$\mathbf{Max} \ (^{\circ})$
1.0-10.0 (Small)	17.2	5.2
10.0-20.0 (Medium)	None	None
>20.0 (Large)	None	None



9 Distance violation analysis (i)

9.1 Summary of distance violations (i)

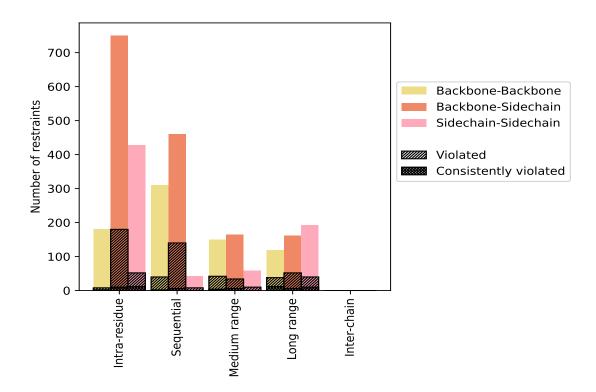
The following table shows the summary of distance violations in different restraint categories based on the sequence separation of the atoms involved. Each category is further sub-divided into three sub-categories based on the atoms involved. Violations less than 0.1 Å are not included in the statistics.

Dordensinda dom o	Count	% ¹	Vi	olated	3	Consis	stently	$\overline{ m Violated^4}$
Restraints type	Count	Count /0		$\%^2$	$\frac{1}{\%}$	Count	$\%^2$	$\%^1$
Intra-residue (i-j =0)	1358	45.1	240	17.7	8.0	24	1.8	0.8
Backbone-Backbone	180	6.0	8	4.4	0.3	2	1.1	0.1
Backbone-Sidechain	750	24.9	180	24.0	6.0	10	1.3	0.3
Sidechain-Sidechain	428	14.2	52	12.1	1.7	12	2.8	0.4
Sequential (i-j =1)	812	26.9	188	23.2	6.2	8	1.0	0.3
Backbone-Backbone	310	10.3	40	12.9	1.3	2	0.6	0.1
Backbone-Sidechain	460	15.3	140	30.4	4.6	6	1.3	0.2
Sidechain-Sidechain	42	1.4	8	19.0	0.3	0	0.0	0.0
Medium range ($ i-j >1 \& i-j <5$)	320	10.6	70	21.9	2.3	6	1.9	0.2
Backbone-Backbone	98	3.3	26	26.5	0.9	0	0.0	0.0
Backbone-Sidechain	164	5.4	34	20.7	1.1	6	3.7	0.2
Sidechain-Sidechain	58	1.9	10	17.2	0.3	0	0.0	0.0
Long range ($ i-j \ge 5$)	418	13.9	110	26.3	3.6	16	3.8	0.5
Backbone-Backbone	64	2.1	18	28.1	0.6	0	0.0	0.0
Backbone-Sidechain	162	5.4	52	32.1	1.7	6	3.7	0.2
Sidechain-Sidechain	192	6.4	40	20.8	1.3	10	5.2	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	106	3.5	36	34.0	1.2	16	15.1	0.5
Disulfide bond	0	0.0	0	0.0	0.0	0	0.0	0.0
Total	3014	100.0	644	21.4	21.4	70	2.3	2.3
Backbone-Backbone	758	25.1	128	16.9	4.2	20	2.6	0.7
Backbone-Sidechain	1536	51.0	406	26.4	13.5	28	1.8	0.9
Sidechain-Sidechain	720	23.9	110	15.3	3.6	22	3.1	0.7

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.

Madal ID		Nun	nber o	f viola	ations	5	Mean (Å)	M (Å)	${ m SD}^6$ (Å)	Madian (8)
Model ID	IR^1	SQ^2	$ m MR^3$	LR^4	IC^5	Total	Mean (A)	Max (Å)	$SD^*(A)$	Median (Å)
1	82	60	32	52	0	226	1.86	25.67	5.3	0.19
2	76	50	26	54	0	206	1.87	24.09	5.12	0.21
3	78	58	24	48	0	208	2.13	28.32	5.97	0.2
4	84	48	32	52	0	216	2.08	28.38	5.87	0.2
5	86	58	34	58	0	236	1.76	26.43	5.21	0.19
6	70	50	22	58	0	200	2.13	26.91	5.77	0.21
7	80	52	28	50	0	210	1.88	24.43	5.23	0.18
8	80	50	30	48	0	208	1.94	25.34	5.34	0.2
9	76	54	40	52	0	222	2.02	28.39	5.82	0.21
10	78	50	38	44	0	210	1.82	23.85	5.03	0.21
11	74	50	30	46	0	200	1.84	23.16	4.93	0.19



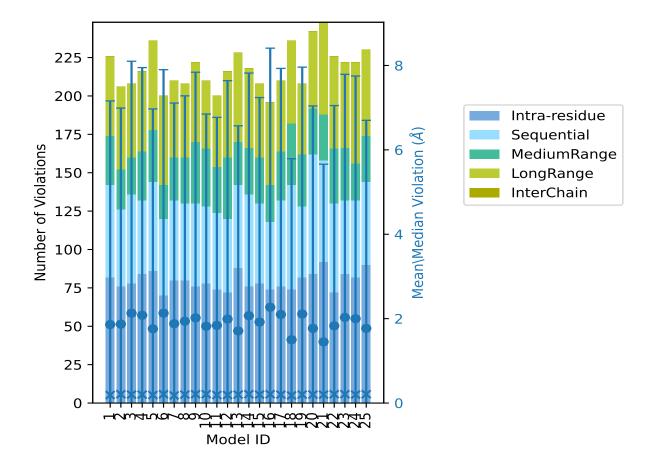
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Model ID		Nun	nber o	f viola	ations	3	Mean (Å)	Max (Å)	${ m SD}^6$ (Å)	Median (Å)
Model 1D	IR^1	SQ^2	MR^3	LR^4	IC^5	Total	Mean (A)	Max (A)	SD (A)	Median (A)
12	72	48	40	56	0	216	1.99	27.17	5.65	0.19
13	88	54	28	58	0	228	1.71	23.67	4.86	0.2
14	76	60	30	52	0	218	2.07	27.28	5.75	0.21
15	78	52	30	48	0	208	1.92	24.88	5.32	0.2
16	74	44	24	54	0	196	2.27	28.48	6.14	0.21
17	76	56	32	46	0	210	2.1	27.86	5.83	0.2
18	74	68	40	54	0	236	1.5	21.53	4.29	0.18
19	82	46	34	46	0	208	2.11	27.55	5.85	0.2
20	84	78	30	50	0	242	1.77	26.81	5.27	0.2
21	92	66	30	60	0	248	1.45	21.46	4.21	0.2
22	72	58	36	60	0	226	1.83	25.51	5.22	0.21
23	84	48	34	56	0	222	2.03	28.04	5.76	0.21
24	82	50	24	66	0	222	2.0	27.82	5.75	0.2
25	90	54	30	56	0	230	1.77	23.93	4.93	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, 2300(IR:1118, SQ:624, MR:250, LR:308, IC:0) restraints are not violated in the ensemble.

Nu	ımber	of vio	lated	restr	aints	Fraction of the ensemble		
IR^1	SQ^2	MR^3	LR^4	$ IC^5 $	Total	Count ⁶	%	
48	38	12	30	0	128	1	4.0	
36	20	10	8	0	74	2	8.0	
22	18	6	8	0	54	3	12.0	
14	22	4	8	0	48	4	16.0	
10	8	0	8	0	26	5	20.0	
20	8	6	6	0	40	6	24.0	



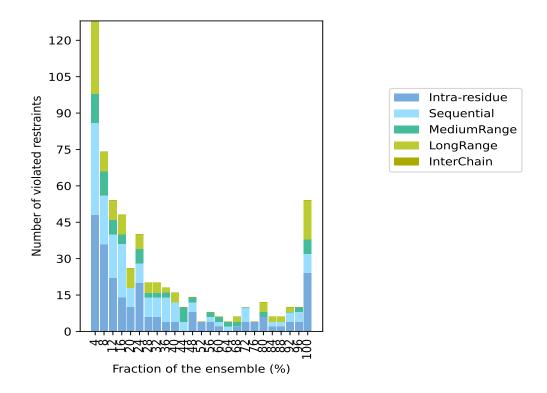
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Nu	mber	of vio	lated	restra	aints	Fraction	n of the ensemble
IR^1	SQ^2	$ m MR^3$	LR^4	IC^5	Total	Count ⁶	%
6	8	2	4	0	20	7	28.0
6	8	2	4	0	20	8	32.0
4	10	2	2	0	18	9	36.0
4	8	0	4	0	16	10	40.0
0	4	6	0	0	10	11	44.0
8	4	2	0	0	14	12	48.0
4	0	0	0	0	4	13	52.0
4	2	2	0	0	8	14	56.0
2	2	2	0	0	6	15	60.0
0	2	2	0	0	4	16	64.0
2	0	2	2	0	6	17	68.0
4	6	0	0	0	10	18	72.0
4	0	0	0	0	4	19	76.0
6	0	2	4	0	12	20	80.0
2	2	0	2	0	6	21	84.0
2	2	0	2	0	6	22	88.0
4	4	0	2	0	10	23	92.0
4	4	2	0	0	10	24	96.0
24	8	6	16	0	54	25	100.0

 $^{^1{\}rm Intra-residue}$ restraints, $^2{\rm Sequential}$ restraints, $^3{\rm Medium}$ range restraints, $^4{\rm Long}$ range restraints, $^5{\rm Inter-chain}$ restraints, 6 Number of models with violations



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

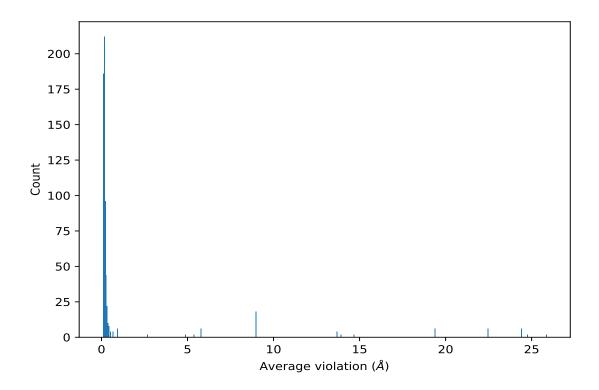


9.4 Most violated distance restraints in the ensemble (i)

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)
(1,1)	1:B:94:ILE:H	1:B:53:ASN:O	25	25.86	2.15	26.43
(1,2)	1:B:94:ILE:H	1:B:53:ASN:O	25	25.86	2.15	26.43
(1,3)	1:B:53:ASN:O	1:B:94:ILE:N	25	24.77	2.2	25.12
(1,4)	1:B:53:ASN:O	1:B:94:ILE:N	25	24.77	2.2	25.12
(2,1)	1:B:94:ILE:HD11	1:B:54:TRP:HZ2	25	24.41	1.42	24.61
(2,1)	1:B:94:ILE:HD12	1:B:54:TRP:HZ2	25	24.41	1.42	24.61
(2,1)	1:B:94:ILE:HD13	1:B:54:TRP:HZ2	25	24.41	1.42	24.61
(2,2)	1:B:94:ILE:HD11	1:B:54:TRP:HZ2	25	24.41	1.42	24.61
(2,2)	1:B:94:ILE:HD12	1:B:54:TRP:HZ2	25	24.41	1.42	24.61
(2,2)	1:B:94:ILE:HD13	1:B:54:TRP:HZ2	25	24.41	1.42	24.61
(2,15)	1:B:94:ILE:HG21	1:B:54:TRP:HZ2	25	22.46	1.43	22.67
(2,15)	1:B:94:ILE:HG22	1:B:54:TRP:HZ2	25	22.46	1.43	22.67
(2,15)	1:B:94:ILE:HG23	1:B:54:TRP:HZ2	25	22.46	1.43	22.67
(2,16)	1:B:94:ILE:HG21	1:B:54:TRP:HZ2	25	22.46	1.43	22.67
(2,16)	1:B:94:ILE:HG22	1:B:54:TRP:HZ2	25	22.46	1.43	22.67
(2,16)	1:B:94:ILE:HG23	1:B:54:TRP:HZ2	25	22.46	1.43	22.67



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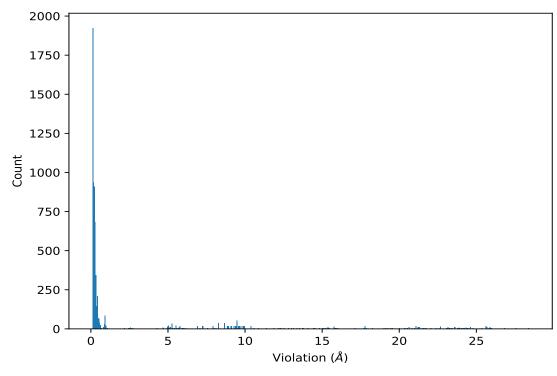
Key	Atom-1	Atom-2	\mathbf{Models}^1	Mean (Å)	\mathbf{SD}^1 (Å)	Median (Å)
(2,17)	1:B:94:ILE:HG21	1:B:54:TRP:HA	25	19.35	1.73	19.56
(2,17)	1:B:94:ILE:HG22	1:B:54:TRP:HA	25	19.35	1.73	19.56
(2,17)	1:B:94:ILE:HG23	1:B:54:TRP:HA	25	19.35	1.73	19.56
(2,18)	1:B:94:ILE:HG21	1:B:54:TRP:HA	25	19.35	1.73	19.56

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)
(1,2)	1:B:94:ILE:H	1:B:53:ASN:O	16	28.48



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Key	Atom-1	Atom-2	Model ID	Violation (Å)
(1,1)	1:B:94:ILE:H	1:B:53:ASN:O	16	28.48
(1,2)	1:B:94:ILE:H	1:B:53:ASN:O	9	28.39
(1,1)	1:B:94:ILE:H	1:B:53:ASN:O	9	28.39
(1,2)	1:B:94:ILE:H	1:B:53:ASN:O	4	28.38
(1,1)	1:B:94:ILE:H	1:B:53:ASN:O	4	28.38
(1,2)	1:B:94:ILE:H	1:B:53:ASN:O	3	28.32
(1,1)	1:B:94:ILE:H	1:B:53:ASN:O	3	28.32
(1,2)	1:B:94:ILE:H	1:B:53:ASN:O	23	28.04
(1,1)	1:B:94:ILE:H	1:B:53:ASN:O	23	28.04



10 Dihedral-angle violation analysis (i)

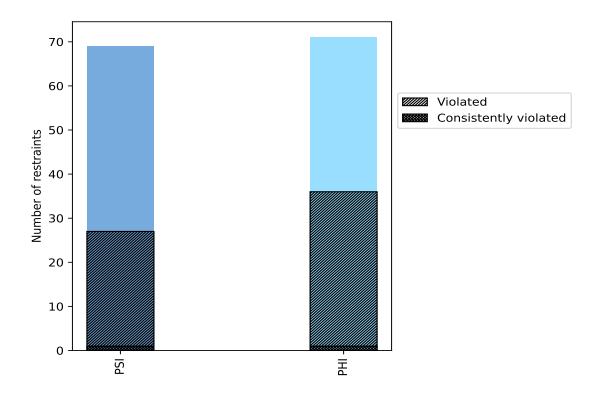
10.1 Summary of dihedral-angle violations (i)

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	Count	$\%^{1}$	${f Violated}^3$			Consistently Violated ⁴		
Angle type	Count	70	Count	$\%^2$	$\%^1$	Count	$\%^2$	% ¹
PSI	69	49.3	27	39.1	19.3	1	1.4	0.7
PHI	71	50.7	36	50.7	25.7	1	1.4	0.7
Total	140	100.0	63	45.0	45.0	2	1.4	1.4

 $^{^1}$ 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



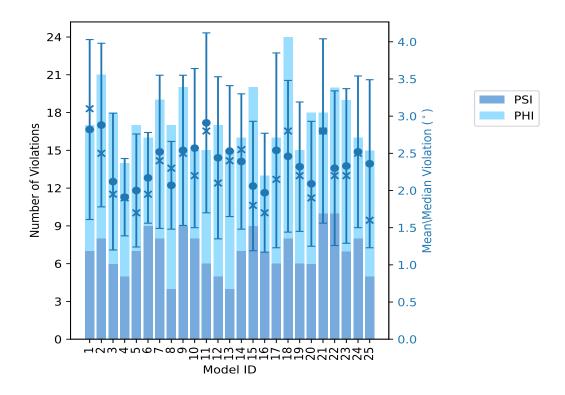
10.2 Dihedral-angle violation statistics for each model (i)

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.

Madalin	Nun	nber o	of violations	N /L (0)	N I (0)	CD (0)	N (- 1: (0)
Model ID	PSI	PHI	Total	$ Mean (^{\circ}) $	$\mathbf{Max} \ (^{\circ})$	\mathbf{SD} (°)	\mid Median (°) \mid
1	7	10	17	2.82	4.6	1.21	3.1
2	8	13	21	2.88	4.7	1.1	2.5
3	6	12	18	2.12	4.7	0.92	1.95
4	5	9	14	1.91	2.7	0.52	1.9
5	7	10	17	2.0	3.9	0.76	1.7
6	9	7	16	2.17	3.5	0.61	1.95
7	8	11	19	2.52	4.4	1.03	2.4
8	4	13	17	2.07	3.1	0.59	2.3
9	9	11	20	2.54	5.0	1.01	2.5
10	8	7	15	2.57	5.2	1.07	2.2
11	6	9	15	2.91	4.9	1.21	2.8
12	5	12	17	2.44	4.3	1.09	2.1
13	4	11	15	2.53	4.4	0.88	2.4
14	7	9	16	2.39	3.7	0.91	2.55
15	9	11	20	2.06	4.7	0.87	1.8
16	7	6	13	1.97	3.3	0.8	1.7
17	6	10	16	2.54	5.0	1.31	2.15
18	8	16	24	2.46	4.0	1.02	2.8
19	6	9	15	2.32	4.1	0.87	2.2
20	6	12	18	2.09	4.3	0.84	1.9
21	10	8	18	2.8	4.9	1.24	2.8
22	10	10	20	2.3	4.2	1.04	2.2
23	7	12	19	2.33	4.8	1.04	2.2
24	8	8	16	2.52	4.4	1.02	2.5
25	5	10	15	2.36	4.5	1.13	1.6



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	of violated restraints	Fractio	n of the ensemble
PSI	PHI	Total	Count ¹	%
8	5	13	1	4.0
3	8	11	2	8.0
2	1	3	3	12.0
0	4	4	4	16.0
1	1	2	5	20.0
3	2	5	6	24.0
0	4	4	7	28.0
1	1	2	8	32.0
0	1	1	9	36.0
2	0	2	10	40.0
2	1	3	11	44.0

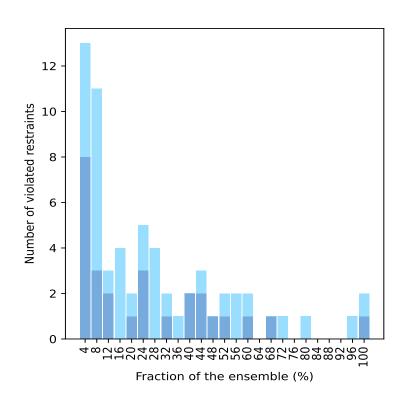


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Nun	nber o	f violated restraints	Fraction	n of the ensemble
PSI	PHI	Total	Count ¹	%
1	0	1	12	48.0
1	1	2	13	52.0
0	2	2	14	56.0
1	1	2	15	60.0
0	0	0	16	64.0
1	0	1	17	68.0
0	1	1	18	72.0
0	0	0	19	76.0
0	1	1	20	80.0
0	0	0	21	84.0
0	0	0	22	88.0
0	0	0	23	92.0
0	1	1	24	96.0
1	1	2	25	100.0

¹ Number of models with violations

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



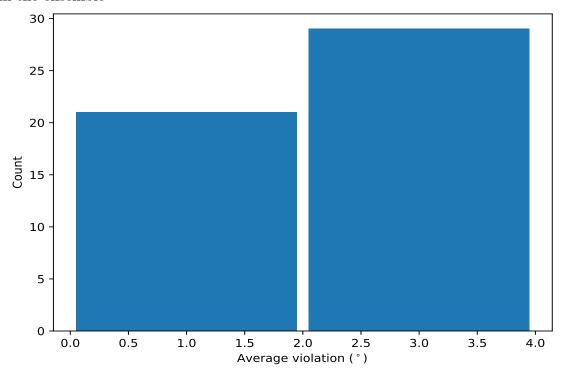




10.4 Most violated dihedral-angle restraints in the ensemble (i)

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	\mathbf{Models}^1	Mean	\mathbf{SD}^2	Median
(1,52)	1:A:38:ALA:N	1:A:38:ALA:CA	1:A:38:ALA:C	1:A:39:THR:N	25	3.14	0.82	3.1
(1,51)	1:A:37:LYS:C	1:A:38:ALA:N	1:A:38:ALA:CA	1:A:38:ALA:C	25	3.04	0.86	3.2
(1,110)	1:A:75:ILE:C	1:A:76:SER:N	1:A:76:SER:CA	1:A:76:SER:C	24	2.43	0.94	2.2
(1,104)	1:A:72:VAL:C	1:A:73:GLY:N	1:A:73:GLY:CA	1:A:73:GLY:C	20	2.62	0.49	2.5
(1,43)	1:A:31:MET:C	1:A:32:ASN:N	1:A:32:ASN:CA	1:A:32:ASN:C	18	2.06	0.84	1.9
(1,134)	1:A:90:GLU:N	1:A:90:GLU:CA	1:A:90:GLU:C	1:A:91:ILE:N	17	2.49	0.77	2.3
(1,128)	1:A:86:GLY:C	1:A:87:MET:N	1:A:87:MET:CA	1:A:87:MET:C	15	2.23	1.06	2.4
(1,64)	1:A:47:ILE:N	1:A:47:ILE:CA	1:A:47:ILE:C	1:A:48:SER:N	15	2.17	0.85	2.2
(1,61)	1:A:44:GLY:C	1:A:45:TYR:N	1:A:45:TYR:CA	1:A:45:TYR:C	14	3.87	0.9	4.15
(1,133)	1:A:89:ILE:C	1:A:90:GLU:N	1:A:90:GLU:CA	1:A:90:GLU:C	14	2.37	0.77	2.35

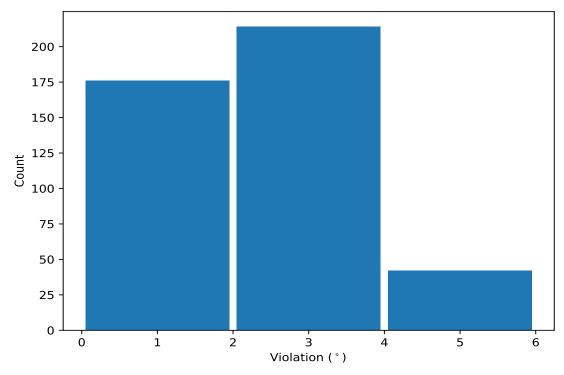
¹ Number of violated models, ²Standard deviation, All angle values are in degree (°)



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 (°)
(1,101)	1:A:70:SER:N	1:A:70:SER:CA	1:A:70:SER:C	1:A:71:THR:N	10	5.2
(1,101)	1:A:70:SER:N	1:A:70:SER:CA	1:A:70:SER:C	1:A:71:THR:N	10	5.2
(1,61)	1:A:44:GLY:C	1:A:45:TYR:N	1:A:45:TYR:CA	1:A:45:TYR:C	9	5.0
(1,127)	1:A:85:LYS:N	1:A:85:LYS:CA	1:A:85:LYS:C	1:A:86:GLY:N	17	5.0
(1,61)	1:A:44:GLY:C	1:A:45:TYR:N	1:A:45:TYR:CA	1:A:45:TYR:C	11	4.9
(1,58)	1:A:43:ALA:N	1:A:43:ALA:CA	1:A:43:ALA:C	1:A:44:GLY:N	11	4.9
(1,52)	1:A:38:ALA:N	1:A:38:ALA:CA	1:A:38:ALA:C	1:A:39:THR:N	21	4.9
(1,59)	1:A:43:ALA:C	1:A:44:GLY:N	1:A:44:GLY:CA	1:A:44:GLY:C	21	4.8
(1,58)	1:A:43:ALA:N	1:A:43:ALA:CA	1:A:43:ALA:C	1:A:44:GLY:N	21	4.8
(1,110)	1:A:75:ILE:C	1:A:76:SER:N	1:A:76:SER:CA	1:A:76:SER:C	23	4.8
(1,62)	1:A:45:TYR:N	1:A:45:TYR:CA	1:A:45:TYR:C	1:A:46:GLY:N	15	4.7

