

wwPDB NMR Structure Validation Summary Report (i)

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:	8HEQ
:	25358
:	Solution structure of the periplasmic domain of the anti-sigma factor RsgI2
	from Clostridium thermocellum
:	Chen, C.; Feng, Y.
:	2022-11-08
	:

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

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

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

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

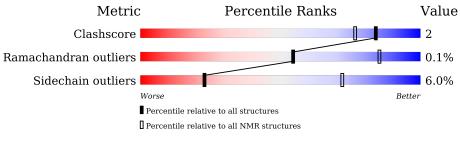
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
wwPDB-RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
wwPDB-ShiftChecker	:	v1.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.32.2

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 95%.

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	$egin{array}{c} { m Whole \ archive} \ (\#{ m Entries}) \end{array}$	${f NMR} \ {f archive} \ (\#{f Entries})$
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	А	10	100%		
2	В	159	83%	6%	11%



2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 14 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	Well-defined core Residue range (total) Backbone RMSD (Å) Medoid model						
1	A:1-A:10, B:11-B:78, B:89-	0.30	14				
	B:162 (152)						

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 4 single-model clusters were found.

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



3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2647 atoms, of which 1329 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called Anti-sigma-I factor RsgI2.

Mol	Chain	Residues	Atoms				Trace		
1	٨	10	Total	С	Η	Ν	0	\mathbf{S}	0
	А	10	164	55	79	11	18	1	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference	
A	1	MET	-	initiating methionine	UNP A3DC27	

• Molecule 2 is a protein called Anti-sigma-I factor RsgI2.

Mol	Chain	Residues	Atoms					Trace	
0	D	159	Total	С	Η	Ν	0	S	0
	D	159	2483	770	1250	214	247	2	0

There are 8 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
В	162	LEU	-	expression tag	UNP A3DC27
В	163	GLU	-	expression tag	UNP A3DC27
В	164	HIS	-	expression tag	UNP A3DC27
В	165	HIS	-	expression tag	UNP A3DC27
В	166	HIS	-	expression tag	UNP A3DC27
В	167	HIS	-	expression tag	UNP A3DC27
В	168	HIS	-	expression tag	UNP A3DC27
В	169	HIS	-	expression tag	UNP A3DC27



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: Anti-sigma-I factor RsgI2

Chain A:	100%		
There are no outlier residues in this cha	ain.		
• Molecule 2: Anti-sigma-I factor RsgI2	2		
Chain B: 83%		6%	11%
P11 K27 K27 K27 K27 K27 K27 K27 K27 K28 K28 K28 K28 K28 K28 K28 K28 K28 K28	<mark>0120</mark> 1166 11166 11166 11166 11169 11169		

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

The representative model is number 14. Colouring as in section 4.1 above.

 \bullet Molecule 1: Anti-sigma-I factor RsgI2

 Chain A:
 100%

 There are no outlier residues in this chain.

 • Molecule 2: Anti-sigma-I factor RsgI2

 Chain B:
 78%

 11%
 11%

 11%
 11%

 11%
 11%

 11%
 11%



5 Refinement protocol and experimental data overview (i)

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	refinement	
CNS	structure calculation	

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	2128
Number of shifts mapped to atoms	2128
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	95%



6 Model quality (i)

6.1 Standard geometry (i)

There are no covalent bond-length or bond-angle outliers.

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
2	В	$0.0{\pm}0.0$	0.2 ± 0.4
All	All	0	4

There are no bond-length outliers.

There are no bond-angle outliers.

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)
2	В	119	ARG	Sidechain	2
2	В	151	ARG	Sidechain	2

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	А	85	79	79	0 ± 1
2	В	1090	1134	1134	6±1
All	All	23500	24260	24260	112

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 2.

5 of 34 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
2:B:149:ASP:O	2:B:153:LEU:HG	0.55	2.02	2	7
1:A:9:ILE:HG23	2:B:64:ILE:HG23	0.54	1.80	5	1
1:A:8:ASP:OD2	2:B:131:ARG:HB2	0.53	2.04	17	1
2:B:91:ILE:O	2:B:95:ILE:HG12	0.53	2.03	19	20
2:B:69:ASN:ND2	2:B:107:VAL:HA	0.52	2.20	19	8

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	Perce	entiles
1	А	8/10 (80%)	$7{\pm}0~(88{\pm}3\%)$	$1\pm0~(11\pm4\%)$	$0{\pm}0~(1{\pm}3\%)$	29	74
2	В	141/159~(89%)	$139 \pm 1 \ (98 \pm 1\%)$	$2\pm1~(2\pm1\%)$	0±0 (0±0%)	54	85
All	All	2980/3380~(88%)	2913~(98%)	64 (2%)	$3\;(0\%)$	54	85

All 2 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
2	В	44	ASP	2
1	А	2	TYR	1

6.3.2 Protein sidechains (i)

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

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	А	9/9~(100%)	$9{\pm}1$ (96 ${\pm}6\%$)	$0\pm1~(4\pm6\%)$	32 81
2	В	122/138~(88%)	$115 \pm 1 (94 \pm 1\%)$	$7\pm1~(6\pm1\%)$	22 71
All	All	2620/2940~(89%)	2463~(94%)	157~(6%)	23 72



 $\mathbf{2}$

 $\overline{2}$

1	v			
Mol	Chain	Res	Type	Models (Total)
2	В	78	ASN	19
2	В	69	ASN	18
2	В	72	LEU	18

GLN

LYS

5 of 33 unique residues with a non-rotameric side chain are listed below. They are sorted by the frequency of occurrence in the ensemble.

6.3.3 RNA (i)

В

В

There are no RNA molecules in this entry.

120

27

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

12

12

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 95% for the well-defined parts and 93% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: assigned_chem_shift_list_1

7.1.1 Bookkeeping (i)

The following table shows the results of parsing the chemical shift list and reports the number of nuclei with statistically unusual chemical shifts.

Total number of shifts	2128
Number of shifts mapped to atoms	2128
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Number of shift outliers (ShiftChecker)	2

7.1.2 Chemical shift referencing (i)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	${\rm Correction}\pm{\rm precision},ppm$	Suggested action
$^{13}C_{\alpha}$	168	-0.31 ± 0.23	None needed (< 0.5 ppm)
$^{13}C_{\beta}$	159	0.09 ± 0.12	None needed (< 0.5 ppm)
$^{13}C'$	168	0.38 ± 0.12	None needed (< 0.5 ppm)
^{15}N	161	0.27 ± 0.33	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 95%, i.e. 1965 atoms were assigned a chemical shift out of a possible 2066. 0 out of 25 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Backbone	748/755~(99%)	302/305~(99%)	302/304~(99%)	144/146~(99%)
Sidechain	1149/1237~(93%)	785/805~(98%)	348/393~(89%)	16/39~(41%)

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	Total	$^{1}\mathrm{H}$	$^{13}\mathrm{C}$	$^{15}\mathbf{N}$
Aromatic	68/74~(92%)	34/34~(100%)	34/40~(85%)	$0/0 \ (\%)$
Overall	1965/2066~(95%)	1121/1144~(98%)	684/737~(93%)	160/185~(86%)

7.1.4 Statistically unusual chemical shifts (i)

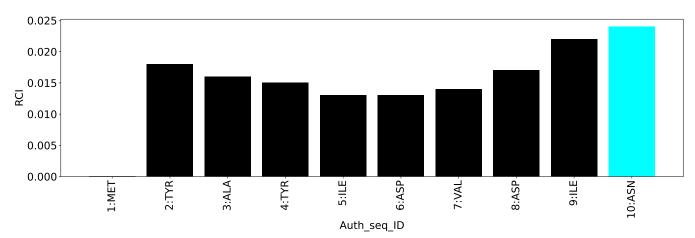
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	В	129	MET	Н	11.13	5.39 - 11.10	5.1
1	А	6	ASP	HA	6.13	3.04 - 6.12	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:



Random coil index (RCI) for chain B:



