

Full wwPDB NMR Structure Validation Report (i)

Jun 3, 2023 – 10:02 PM EDT

PDB ID : 7M1E BMRB ID : 30879

Title : Structural and functional studies about scorpine showed the presence of block-

ing channel and cytolytic activities as well as two different structural domains

Authors: del Rio, J.F.; Lopez, A.E.; Titaux, G.

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This is a Full wwPDB NMR Structure Validation 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)

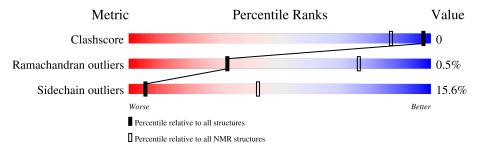
Validation Pipeline (wwPDB-VP) : 2.33

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 52%.

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	NMR archive
Metric	$(\# \mathrm{Entries})$	$(\# { m Entries})$
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				
		2.2					
1	A	28	54%	14%	32%		



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	Well-defined core Residue range (total) Backbone RMSD (Å) Medoid model						
1	A:2-A:20 (19)	0.60	12				

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

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

The models can be grouped into 5 clusters and 1 single-model cluster was found.

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



3 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 448 atoms, of which 230 are hydrogens and 0 are deuteriums.

• Molecule 1 is a protein called Scorpine.

Mol	Chain	Residues	Atoms					Trace	
1	Λ	20	Total	С	Н	N	О	S	0
1	$\begin{array}{c c} 1 & A \end{array}$	28	448	136	230	39	41	2	U



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: Scorpine



4.2 Scores per residue for each member of the ensemble

Colouring as in section 4.1 above.

4.2.1 Score per residue for model 1

• Molecule 1: Scorpine

Chain A: 61% 7% 32%

4.2.2 Score per residue for model 2

• Molecule 1: Scorpine

Chain A: 61% 7% 32%





4.2.3 Score per residue for model 3

• Molecule 1: Scorpine

Chain A: 57% 11% 32%



4.2.4 Score per residue for model 4

• Molecule 1: Scorpine

Chain A: 54% 14% 32%



4.2.5 Score per residue for model 5

• Molecule 1: Scorpine

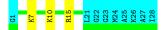
Chain A: 54% 14% 32%



4.2.6 Score per residue for model 6

• Molecule 1: Scorpine

Chain A: 57% 11% 32%



4.2.7 Score per residue for model 7

• Molecule 1: Scorpine

Chain A: 50% 18% 32%





4.2.8 Score per residue for model 8

• Molecule 1: Scorpine

Chain A: 54% 14% 32%



4.2.9 Score per residue for model 9

• Molecule 1: Scorpine

Chain A: 54% 14% 32%



4.2.10 Score per residue for model 10

• Molecule 1: Scorpine

Chain A: 61% 7% 32%



4.2.11 Score per residue for model 11

• Molecule 1: Scorpine

Chain A: 50% 14% · 32%



4.2.12 Score per residue for model 12 (medoid)

• Molecule 1: Scorpine

Chain A: 57% 11% 32%





4.2.13 Score per residue for model 13

• Molecule 1: Scorpine

Chain A: 54% 11% · 32%



4.2.14 Score per residue for model 14

• Molecule 1: Scorpine

Chain A: 54% 11% · 32%



4.2.15 Score per residue for model 15

• Molecule 1: Scorpine

Chain A: 46% 21% 32%



4.2.16 Score per residue for model 16

• Molecule 1: Scorpine

Chain A: 57% 11% 32%



4.2.17 Score per residue for model 17

• Molecule 1: Scorpine

Chain A: 54% 11% · 32%





4.2.18 Score per residue for model 18

• Molecule 1: Scorpine

Chain A: 54% 14% 32%



4.2.19 Score per residue for model 19

• Molecule 1: Scorpine

Chain A: 54% 14% 32%



4.2.20 Score per residue for model 20

• Molecule 1: Scorpine

Chain A: 57% 11% 32%



Refinement protocol and experimental data overview (i) 5



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

Of the 500 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
Amber	refinement	16
CYANA	structure calculation	2.1

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	202
Number of shifts mapped to atoms	202
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	52%



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 Bond		Sond lengths	Bond angles		
IVIOI	Chain	RMSZ	RMSZ $\#Z>5$		#Z>5	
1	A	0.63 ± 0.02	$0\pm0/164~(~0.0\pm~0.0\%)$	1.15 ± 0.06	$1\pm1/219~(~0.4\pm~0.2\%)$	
All	All	0.63	0/3280 (0.0%)	1.15	16/4380 (0.4%)	

There are no bond-length outliers.

All 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	Atoma	7	$Observed(^o)$	$Ideal(^{o})$	Mod	dels	
MIOI	Chain	nes	Туре	Atoms	Z Observed(*)		Z Observed(*) Ideal(*)		Worst	Total
1	A	15	ARG	NE-CZ-NH1	8.85	124.73	120.30	11	15	
1	A	4	ASN	N-CA-CB	-5.43	100.83	110.60	7	1	

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	162	165	165	0±0
All	All	3240	3300	3300	3

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 0.

All unique clashes are listed below, sorted by their clash magnitude.



Atom-1	Atom-2	Clack(Å)	$\operatorname{Distance}(\mathring{\mathrm{A}})$	Models		
Atom-1	Atom-2	Clash(A)	Distance(A)	Worst	Total	
1:A:4:ASN:ND2	1:A:4:ASN:H	0.46	2.08	11	3	

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	ntiles
1	A	19/28 (68%)	18±1 (95±4%)	1±1 (4±4%)	0±0 (1±2%)	32	76
All	All	380/560 (68%)	361 (95%)	17 (4%)	2 (1%)	32	76

All 1 unique Ramachandran outliers are listed below.

\mathbf{Mol}	Chain	Res	Type	Models (Total)
1	A	3	ILE	2

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	18/22 (82%)	15±1 (84±5%)	$3\pm1 \ (16\pm5\%)$	5 43
All	All	360/440 (82%)	304 (84%)	56 (16%)	5 43

All 6 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	7	LYS	18
1	A	10	LYS	17
1	A	4	ASN	16
1	A	2	TRP	2
1	A	15	ARG	2

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Mol	Chain	Res	Type	Models (Total)
1	A	16	MET	1

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 52% for the well-defined parts and 52% for the entire structure.

7.1 Chemical shift list 1

File name: working_cs.cif

Chemical shift list name: nterminal.str

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	202		
Number of shifts mapped to atoms			
Number of unparsed shifts	0		
Number of shifts with mapping errors	0		
Number of shifts with mapping warnings	0		
Number of shift outliers (ShiftChecker)	1		

7.1.2 Chemical shift referencing (i)

No chemical shift referencing corrections were calculated (not enough data).

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 52%, i.e. 146 atoms were assigned a chemical shift out of a possible 282. 0 out of 1 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	$^{1}\mathrm{H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Backbone	39/96 (41%)	39/39 (100%)	0/38 (0%)	0/19 (0%)
Sidechain	106/174~(61%)	106/110~(96%)	0/55~(0%)	0/9~(0%)
Aromatic	1/12 (8%)	1/6 (17%)	0/5 (0%)	0/1 (0%)
Overall	$146/282 \ (52\%)$	$146/155 \ (94\%)$	0/98 (0%)	0/29~(0%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 52%, i.e. 202 atoms were assigned a chemical shift out of a possible 387. 0 out of 2 assigned methyl groups (LEU and VAL) were assigned stereospecifically.



	Total	$^{1}\mathrm{H}$	$^{13}\mathbf{C}$	$^{15}{ m N}$
Backbone	59/144 (41%)	59/60 (98%)	0/56 (0%)	0/28~(0%)
Sidechain	142/231 (61%)	142/149 (95%)	0/72~(0%)	0/10 (0%)
Aromatic	1/12 (8%)	1/6 (17%)	0/5 (0%)	0/1 (0%)
Overall	202/387 (52%)	202/215 (94%)	0/133 (0%)	0/39 (0%)

7.1.4 Statistically unusual chemical shifts (i)

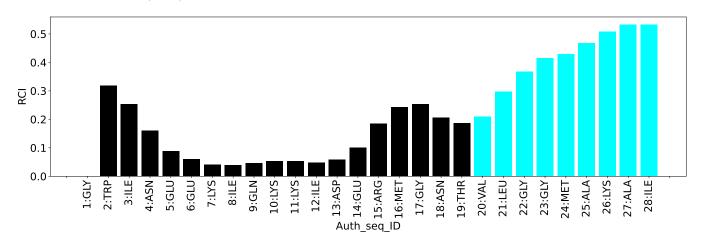
The following table lists the statistically unusual chemical shifts. These are statistical measures, and large deviations from the mean do not necessarily imply incorrect assignments. Molecules containing paramagnetic centres or hemes are expected to give rise to anomalous chemical shifts.

List Id	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	A	15	ARG	HB2	3.23	0.52 - 3.08	5.6

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)

No restraints data found



9 Distance violation analysis (i)

No distance restraints data found



10 Dihedral-angle violation analysis (i)

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

