



Full wwPDB NMR Structure Validation Report ⓘ

May 30, 2020 – 01:52 am BST

PDB ID : 6PX8
Title : Dg3b in Weaponisation 'on the fly': Convergent recruitment of knottin and defensin scaffolds as neurotoxins in the venom of assassin fly *Dolopus genitalis* (Diptera: Asilidae)
Authors : Agwa, A.J.; Schroeder, C.
Deposited on : 2019-07-25

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 ⓘ symbol.

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

Cyrange : Kirchner and Güntert (2011)
NmrClust : Kelley et al. (1996)
MolProbity : 4.02b-467
Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI : v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV : Wang et al. (2010)
ShiftChecker : 2.11
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.11

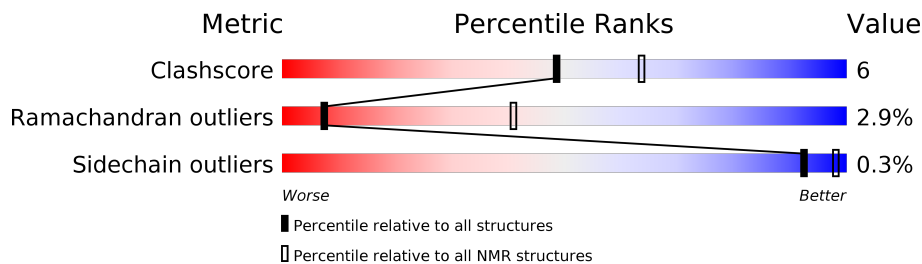
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

SOLUTION NMR

The overall completeness of chemical shifts assignment is 78%.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	NMR archive (#Entries)
Clashscore	158937	12864
Ramachandran outliers	154571	11451
Sidechain outliers	154315	11428

The table below summarises the geometric issues observed across the polymeric chains and their fit to the experimental data. The red, orange, yellow and green segments indicate the fraction of residues that contain outliers for ≥ 3 , 2, 1 and 0 types of geometric quality criteria. A cyan segment indicates the fraction of residues that are not part of the well-defined cores, and a grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions $\leq 5\%$

Mol	Chain	Length	Quality of chain
1	A	37	

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: *fewest violations*.

The following residues are included in the computation of the global validation metrics.

Well-defined (core) protein residues			
Well-defined core	Residue range (total)	Backbone RMSD (Å)	Medoid model
1	A:2-A:37 (36)	0.27	14

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 and 3 single-model clusters were found.

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

3 Entry composition

There is only 1 type of molecule in this entry. The entry contains 568 atoms, of which 283 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Venom polypeptide.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	37	568	172	283	59	48	6	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	GLY	-	expression tag	UNP A0A3G5BIB1

SEQUENCE-PLOTS INFOmissingINFO

4 Refinement protocol and experimental data overview

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

Of the 50 calculated structures, 20 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
CYANA	structure calculation	
CNS	refinement	

The following table shows chemical shift validation statistics as aggregates over all chemical shift files. Detailed validation can be found in section 6 of this report.

Chemical shift file(s)	input_cs.cif
Number of chemical shift lists	1
Total number of shifts	400
Number of shifts mapped to atoms	400
Number of unparsed shifts	0
Number of shifts with mapping errors	0
Number of shifts with mapping warnings	0
Assignment completeness (well-defined parts)	78%

No validations of the models with respect to experimental NMR restraints is performed at this time.

5 Model quality [i](#)

5.1 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
1	A	0.0±0.0	0.1±0.2
All	All	0	1

There are no bond-length outliers.

There are no bond-angle outliers.

There are no chirality outliers.

All unique planar outliers are listed below.

Mol	Chain	Res	Type	Group	Models (Total)
1	A	30	ARG	Sidechain	1

5.2 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	281	278	278	3±1
All	All	5620	5560	5560	69

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 6.

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

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:17:CYS:SG	1:A:25:GLY:HA3	0.71	2.25	11	20
1:A:2:ILE:HB	1:A:33:CYS:SG	0.66	2.31	5	11

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Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
1:A:26:TRP:CE3	1:A:37:ARG:HG2	0.58	2.32	2	3
1:A:27:CYS:HA	1:A:32:VAL:O	0.56	2.00	18	5
1:A:27:CYS:SG	1:A:31:LYS:HA	0.50	2.47	13	1
1:A:16:HIS:NE2	1:A:20:LYS:HD2	0.48	2.23	3	5
1:A:6:LEU:O	1:A:10:GLU:HB2	0.46	2.11	9	2
1:A:10:GLU:O	1:A:14:VAL:HG23	0.46	2.10	19	11
1:A:26:TRP:CZ3	1:A:37:ARG:HG2	0.45	2.46	9	1
1:A:25:GLY:HA2	1:A:34:ASN:O	0.45	2.11	10	1
1:A:14:VAL:HG22	1:A:25:GLY:O	0.43	2.13	13	3
1:A:23:ARG:HB2	1:A:37:ARG:OXT	0.42	2.15	10	1
1:A:26:TRP:CH2	1:A:37:ARG:HG2	0.42	2.50	20	1
1:A:14:VAL:O	1:A:18:LEU:HG	0.42	2.15	18	1
1:A:16:HIS:O	1:A:20:LYS:HG3	0.41	2.15	8	1
1:A:16:HIS:NE2	1:A:20:LYS:HE3	0.41	2.31	12	1
1:A:16:HIS:NE2	1:A:20:LYS:HE2	0.40	2.32	4	1

5.3 Torsion angles [i](#)

5.3.1 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	35/37 (95%)	33±1 (94±2%)	1±1 (3±2%)	1±0 (3±0%)	7	41
All	All	700/740 (95%)	661 (94%)	19 (3%)	20 (3%)	7	41

All 1 unique Ramachandran outliers are listed below.

Mol	Chain	Res	Type	Models (Total)
1	A	36	ARG	20

5.3.2 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	31/31 (100%)	31±0 (100±1%)	0±0 (0±1%)	92	98
All	All	620/620 (100%)	618 (100%)	2 (0%)	92	98

All 2 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	30	ARG	1
1	A	23	ARG	1

5.3.3 RNA [i](#)

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains [i](#)

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

5.5 Carbohydrates [i](#)

There are no carbohydrates in this entry.

5.6 Ligand geometry [i](#)

There are no ligands in this entry.

5.7 Other polymers [i](#)

There are no such molecules in this entry.

5.8 Polymer linkage issues [i](#)

There are no chain breaks in this entry.

6 Chemical shift validation [i](#)

The completeness of assignment taking into account all chemical shift lists is 78% for the well-defined parts and 77% for the entire structure.

6.1 Chemical shift list 1

File name: input_cs.cif

Chemical shift list name: *starch_output*

6.1.1 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	400
Number of shifts mapped to atoms	400
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

6.1.2 Chemical shift referencing [i](#)

The following table shows the suggested chemical shift referencing corrections.

Nucleus	# values	Correction \pm precision, ppm	Suggested action
$^{13}\text{C}_\alpha$	37	-0.12 ± 0.23	None needed (< 0.5 ppm)
$^{13}\text{C}_\beta$	32	0.29 ± 0.18	None needed (< 0.5 ppm)
$^{13}\text{C}'$	0	—	None (insufficient data)
^{15}N	36	0.47 ± 0.72	None needed (< 0.5 ppm)

6.1.3 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 78%, i.e. 352 atoms were assigned a chemical shift out of a possible 452. 6 out of 6 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	^1H	^{13}C	^{15}N
Backbone	144/180 (80%)	72/72 (100%)	36/72 (50%)	36/36 (100%)
Sidechain	195/244 (80%)	123/144 (85%)	70/81 (86%)	2/19 (11%)

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	Total	¹ H	¹³ C	¹⁵ N
Aromatic	13/28 (46%)	12/15 (80%)	0/11 (0%)	1/2 (50%)
Overall	352/452 (78%)	207/231 (90%)	106/164 (65%)	39/57 (68%)

The following table shows the completeness of the chemical shift assignments for the full structure. The overall completeness is 77%, i.e. 354 atoms were assigned a chemical shift out of a possible 457. 6 out of 6 assigned methyl groups (LEU and VAL) were assigned stereospecifically.

	Total	¹ H	¹³ C	¹⁵ N
Backbone	146/185 (79%)	73/74 (99%)	37/74 (50%)	36/37 (97%)
Sidechain	195/244 (80%)	123/144 (85%)	70/81 (86%)	2/19 (11%)
Aromatic	13/28 (46%)	12/15 (80%)	0/11 (0%)	1/2 (50%)
Overall	354/457 (77%)	208/233 (89%)	107/166 (64%)	39/58 (67%)

6.1.4 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.

Mol	Chain	Res	Type	Atom	Shift, ppm	Expected range, ppm	Z-score
1	A	34	ASN	HB3	1.09	4.41 – 1.11	-5.1

6.1.5 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.

Random coil index (RCI) for chain A:

