wwPDB NMR Structure Validation Summary Report (i)

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PDB ID	:	2KC8
Title	:	Structure of E. coli toxin RelE (R81A/R83A) mutant in complex with antitoxin RelBc (K47-L79) peptide
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Deposited on	:	2008-12-17

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

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

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

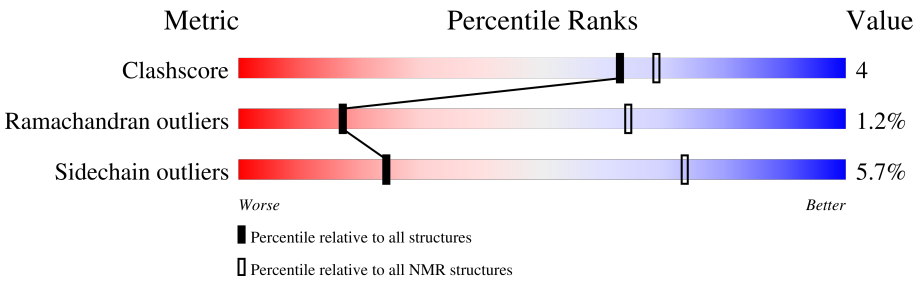
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
RCI	:	v_1n_11_5_13_A (Berjanski et al., 2005)
PANAV	:	Wang et al. (2010)
ShiftChecker	:	2.23.2
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.23.2

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 was not calculated.

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 <=5%

Mol	Chain	Length	Quality of chain
1	A	98	69% 11% 16% .
2	B	36	58% 14% 19% 8%

2 Ensemble composition and analysis (i)

This entry contains 20 models. Model 10 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 range (total)**	**Backbone RMSD (Å)**	**Medoid model**
1	A:2-A:80, B:54-B:79 (105)	0.34	10

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

3 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 2154 atoms, of which 1106 are hydrogens and 0 are deuteriums.

- Molecule 1 is a protein called Toxin relE.

Mol	Chain	Residues	Atoms						Trace
			Total	C	H	N	O	S	
1	A	95	1599	501	821	134	140	3	0

There are 5 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-2	GLY	-	expression tag	UNP P0C077
A	-1	SER	-	expression tag	UNP P0C077
A	0	HIS	-	expression tag	UNP P0C077
A	81	ALA	ARG	engineered mutation	UNP P0C077
A	83	ALA	ARG	engineered mutation	UNP P0C077

- Molecule 2 is a protein called Antitoxin RelB.

Mol	Chain	Residues	Atoms					Trace
			Total	C	H	N	O	
2	B	33	555	168	285	47	55	0

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	44	GLY	-	expression tag	UNP P0C079
B	45	SER	-	expression tag	UNP P0C079
B	46	HIS	-	expression tag	UNP P0C079

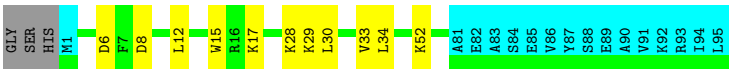
4 Residue-property plots (i)

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.

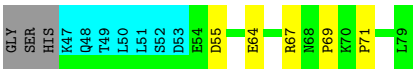
- Molecule 1: Toxin relE

Chain A: 69% 11% 16% •



- Molecule 2: Antitoxin RelB

Chain B: 58% 14% 19% 8%



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

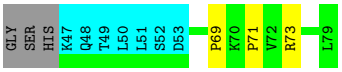
The representative model is number 10. Colouring as in section 4.1 above.

- Molecule 1: Toxin relE

Chain A: 66% 13% • 16% •

- Molecule 2: Antitoxin RelB

Chain B: 64% 8% 19% 8%



5 Refinement protocol and experimental data overview (i)

The models were refined using the following method: *torsion angle dynamics, 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
CYANA	structure calculation	2.1
CNS	structure solution	1.1
CNS	refinement	1.1

No chemical shift data was provided.

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
1	A	0.0±0.0	0.1±0.3
2	B	0.0±0.0	0.2±0.4
All	All	0	7

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	B	73	ARG	Sidechain	5
1	A	45	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	A	653	694	694	6±2
2	B	215	226	226	3±1
All	All	17360	18400	18400	159

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 4.

5 of 63 unique clashes are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Clash(Å)	Distance(Å)	Models	
				Worst	Total
2:B:64:GLU:O	2:B:67:ARG:HG2	0.61	1.94	9	11
1:A:30:LEU:O	1:A:34:LEU:HG	0.57	1.99	3	13
2:B:54:GLU:O	2:B:58:LEU:HG	0.56	2.00	11	1
1:A:12:LEU:HA	1:A:15:TRP:CZ3	0.56	2.36	9	12
1:A:29:LYS:HE2	1:A:29:LYS:N	0.56	2.16	19	1

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	79/98 (81%)	76±1 (96±1%)	3±1 (3±2%)	0±1 (0±1%)	44	80
2	B	25/36 (69%)	22±1 (88±3%)	2±1 (8±4%)	1±0 (4±2%)	5	31
All	All	2080/2680 (78%)	1960 (94%)	95 (5%)	25 (1%)	17	64

All 5 unique Ramachandran outliers are listed below. They are sorted by the frequency of occurrence in the ensemble.

Mol	Chain	Res	Type	Models (Total)
2	B	69	PRO	18
1	A	71	VAL	3
2	B	70	LYS	2
1	A	2	ALA	1
1	A	58	SER	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	A	72/87 (83%)	67±2 (93±2%)	5±2 (7±2%)	18	66
2	B	25/34 (74%)	25±0 (99±2%)	0±0 (1±2%)	68	95

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
All	All	1940/2420 (80%)	1829 (94%)	111 (6%)	24 73

5 of 21 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	17	LYS	16
1	A	28	LYS	15
1	A	8	ASP	11
1	A	33	VAL	11
1	A	6	ASP	9

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)

No chemical shift data were provided