



wwPDB X-ray Structure Validation Summary Report ⓘ

Jun 9, 2016 – 02:08 PM EDT

PDB ID : 5FW3
Title : Crystal structure of SpCas9 variant VRER bound to sgRNA and TGCG PAM target DNA
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Deposited on : 2016-02-11
Resolution : 2.70 Å(reported)

This is a wwPDB X-ray 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
<http://wwpdb.org/validation/2016/XrayValidationReportHelp>
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:

MolProbity : 4.02b-467
Mogul : unknown
Xtriage (Phenix) : 1.9-1692
EDS : rb-20027674
Percentile statistics : 20151230.v01 (using entries in the PDB archive December 30th 2015)
Refmac : 5.8.0135
CCP4 : 6.5.0
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : rb-20027674

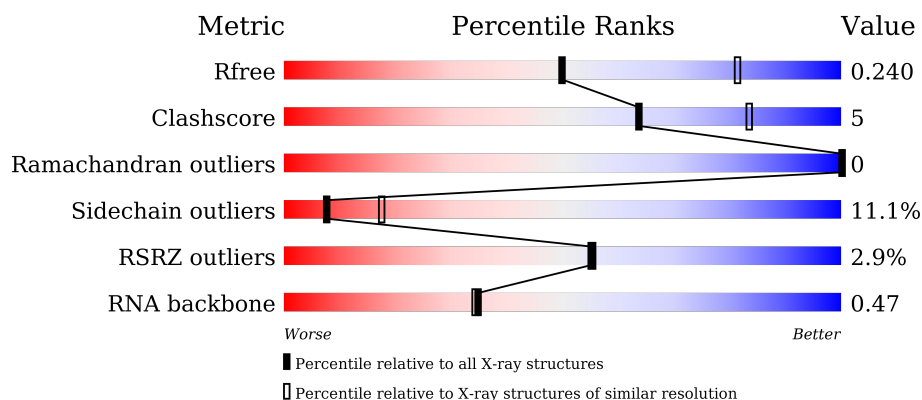
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.70 Å.

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)	Similar resolution (#Entries, resolution range(Å))
R_{free}	91344	2103 (2.70-2.70)
Clashscore	102246	2422 (2.70-2.70)
Ramachandran outliers	100387	2382 (2.70-2.70)
Sidechain outliers	100360	2382 (2.70-2.70)
RSRZ outliers	91569	2107 (2.70-2.70)
RNA backbone	2183	1069 (3.10-2.30)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments on the lower bar indicate the fraction of residues that contain outliers for ≥ 3 , 2, 1 and 0 types of geometric quality criteria. 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 $\leq 5\%$. The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	83	
2	B	1372	
3	C	28	
4	D	12	

2 Entry composition

There are 7 unique types of molecules in this entry. The entry contains 25841 atoms, of which 12327 are hydrogens and 0 are deuteriums.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a RNA chain called SGRNA.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
1	A	81	Total	C	H	N	O	P	0	0	0
			2601	778	869	318	555	81			

- Molecule 2 is a protein called CRISPR-ASSOCIATED ENDONUCLEASE CAS9/CSN1.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
2	B	1322	Total	C	H	N	O	S	0	0	0
			21835	6900	11011	1880	2022	22			

There are 10 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-3	GLY	-	EXPRESSION TAG	UNP Q99ZW2
B	-2	ALA	-	EXPRESSION TAG	UNP Q99ZW2
B	-1	ALA	-	EXPRESSION TAG	UNP Q99ZW2
B	0	SER	-	EXPRESSION TAG	UNP Q99ZW2
B	10	ALA	ASP	ENGINEERED MUTATION	UNP Q99ZW2
B	840	ALA	HIS	ENGINEERED MUTATION	UNP Q99ZW2
B	1135	VAL	ASP	ENGINEERED MUTATION	UNP Q99ZW2
B	1218	ARG	GLY	ENGINEERED MUTATION	UNP Q99ZW2
B	1335	GLU	ARG	ENGINEERED MUTATION	UNP Q99ZW2
B	1337	ARG	THR	ENGINEERED MUTATION	UNP Q99ZW2

- Molecule 3 is a DNA chain called TARGET DNA STRAND.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
3	C	28	Total	C	H	N	O	P	0	0	0
			888	275	321	97	168	27			

- Molecule 4 is a DNA chain called NON-TARGET DNA STRAND.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
4	D	11	Total	C	H	N	O	P	0	0	0
			352	109	126	44	63	10			

- Molecule 5 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

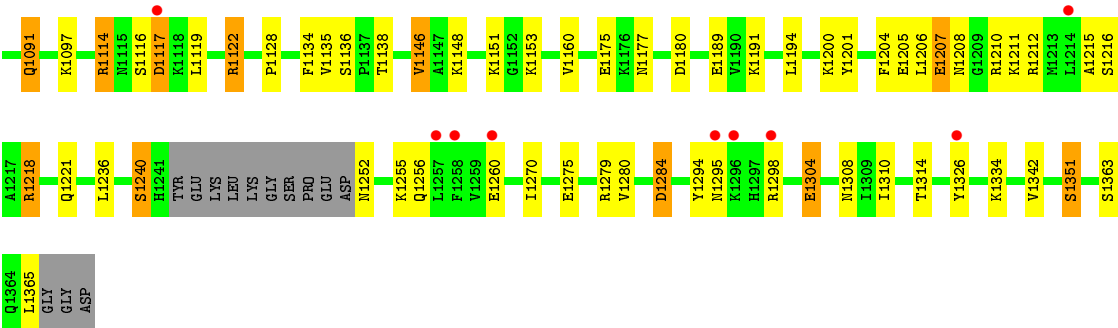
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	A	2	Total	Mg	0	0
			2	2		

- Molecule 6 is POTASSIUM ION (three-letter code: K) (formula: K).

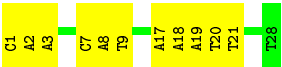
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
6	B	9	Total	K	0	0
			9	9		
6	A	2	Total	K	0	0
			2	2		

- Molecule 7 is water.

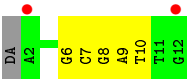
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
7	A	54	Total	O	0	0
			54	54		
7	B	93	Total	O	0	0
			93	93		
7	C	5	Total	O	0	0
			5	5		



● Molecule 3: TARGET DNA STRAND



● Molecule 4: NON-TARGET DNA STRAND



4 Data and refinement statistics

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants a, b, c, α , β , γ	177.52Å 67.80Å 187.70Å 90.00° 111.15° 90.00°	Depositor
Resolution (Å)	47.98 – 2.70 47.98 – 2.70	Depositor EDS
% Data completeness (in resolution range)	99.9 (47.98-2.70) 100.0 (47.98-2.70)	Depositor EDS
R_{merge}	0.13	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	2.04 (at 2.69Å)	Xtriage
Refinement program	PHENIX (PHENIX.REFINE)	Depositor
R, R_{free}	0.214 , 0.242 0.214 , 0.240	Depositor DCC
R_{free} test set	2883 reflections (5.00%)	DCC
Wilson B-factor (Å ²)	49.2	Xtriage
Anisotropy	0.630	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.35 , 39.1	EDS
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.92	EDS
Total number of atoms	25841	wwPDB-VP
Average B, all atoms (Å ²)	60.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.29% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical values of $\langle |L| \rangle$, $\langle L^2 \rangle$ for acentric reflections are 0.5, 0.375 respectively for untwinned datasets, and 0.333, 0.2 for perfectly twinned datasets.

5 Model quality [i](#)

5.1 Standard geometry [i](#)

Bond lengths and bond angles in the following residue types are not validated in this section: K, MG

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 root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	# Z >5	RMSZ	# Z >5
1	A	0.35	0/1942	0.88	0/3023
2	B	0.27	0/11012	0.47	0/14793
3	C	0.59	0/634	1.01	0/976
4	D	0.76	0/254	0.99	0/391
All	All	0.32	0/13842	0.60	0/19183

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

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 the 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 within the asymmetric unit, whereas Symm-Clashes lists symmetry related clashes.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	1732	869	869	28	0
2	B	10824	11011	11010	100	1
3	C	567	321	321	9	0
4	D	226	126	126	8	0
5	A	2	0	0	0	0
6	A	2	0	0	0	0
6	B	9	0	0	0	0
7	A	54	0	0	3	0
7	B	93	0	0	7	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
7	C	5	0	0	0	0
All	All	13514	12327	12326	134	1

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 5.

The worst 5 of 134 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:B:557:ARG:NH2	2:B:596:ASP:OD1	2.03	0.91
2:B:1207:GLU:OE2	2:B:1210:ARG:NH1	2.07	0.88
2:B:1236:LEU:O	2:B:1240:SER:OG	1.94	0.86
2:B:1114:ARG:NH1	4:D:9:DA:OP1	2.11	0.83
1:A:20:A:OP2	2:B:403:ARG:NH1	2.12	0.82

All (1) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:B:571:LYS:NZ	2:B:825:ASP:OD2 4_758	2.19	0.01

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 X-ray entries followed by that with respect to entries of similar resolution.

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	
2	B	1310/1372 (96%)	1254 (96%)	56 (4%)	0	100	100

There are no Ramachandran outliers to report.

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 X-ray entries followed by that with respect to entries of similar resolution.

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
2	B	1188/1227 (97%)	1056 (89%)	132 (11%)	8 17

5 of 132 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	630	GLU
2	B	782	LYS
2	B	1206	LEU
2	B	637	LYS
2	B	709	GLN

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
2	B	497	ASN

5.3.3 RNA [i](#)

Mol	Chain	Analysed	Backbone Outliers	Pucker Outliers
1	A	80/83 (96%)	22 (27%)	4 (5%)

5 of 22 RNA backbone outliers are listed below:

Mol	Chain	Res	Type
1	A	9	A
1	A	20	A
1	A	24	U
1	A	28	A
1	A	29	G

All (4) RNA pucker outliers are listed below:

Mol	Chain	Res	Type
1	A	8	A
1	A	27	G
1	A	28	A
1	A	42	A

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](#)

Of 13 ligands modelled in this entry, 13 are monoatomic - leaving 0 for Mogul analysis.

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

5.7 Other polymers [i](#)

There are no such residues in this entry.

5.8 Polymer linkage issues [i](#)

There are no chain breaks in this entry.

6 Fit of model and data [i](#)

6.1 Protein, DNA and RNA chains [i](#)

In the following table, the column labelled ‘#RSRZ> 2’ contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95th percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled ‘Q< 0.9’ lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2	OWAB(Å ²)	Q<0.9
1	A	81/83 (97%)	-0.15	0 100 100	23, 45, 114, 147	0
2	B	1322/1372 (96%)	0.27	40 (3%) 54 54	20, 52, 92, 144	0
3	C	28/28 (100%)	-0.15	0 100 100	30, 47, 75, 105	0
4	D	11/12 (91%)	0.68	2 (18%) 2 1	38, 69, 112, 116	0
All	All	1442/1495 (96%)	0.24	42 (2%) 55 55	20, 52, 93, 147	0

The worst 5 of 42 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	B	1053	ALA	5.3
2	B	178	ASN	4.9
2	B	197	GLU	3.6
2	B	721	HIS	3.1
2	B	242	ILE	3.0

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

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

6.3 Carbohydrates [i](#)

There are no carbohydrates in this entry.

6.4 Ligands [i](#)

In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors

of atoms in the group. The column labelled 'Q< 0.9' lists the number of atoms with occupancy less than 0.9.

Mol	Type	Chain	Res	Atoms	RSCC	RSR	LLDF	B-factors(\AA^2)	Q<0.9
5	MG	A	1084	1/1	0.94	0.17	-0.71	32,32,32,32	0
6	K	B	2368	1/1	0.97	0.17	-0.86	41,41,41,41	0
6	K	B	2371	1/1	0.64	0.13	-1.49	60,60,60,60	0
6	K	B	2367	1/1	0.94	0.11	-1.81	53,53,53,53	0
6	K	B	2370	1/1	0.98	0.12	-1.90	59,59,59,59	0
6	K	B	2372	1/1	0.91	0.08	-2.05	61,61,61,61	0
6	K	B	2369	1/1	0.97	0.08	-2.09	45,45,45,45	0
6	K	B	2366	1/1	0.96	0.15	-2.15	46,46,46,46	0
6	K	B	2374	1/1	0.87	0.12	-	61,61,61,61	0
6	K	B	2373	1/1	0.91	0.10	-	62,62,62,62	0
5	MG	A	1083	1/1	0.99	0.14	-	45,45,45,45	0
6	K	A	1182	1/1	0.97	0.10	-	60,60,60,60	0
6	K	A	1183	1/1	0.75	0.20	-	59,59,59,59	0

6.5 Other polymers [i](#)

There are no such residues in this entry.