wwPDB X-ray Structure Validation Summary Report (i)

Jun 15, 2024 – 06:04 PM EDT

PDB ID	:	4OO8
Title	:	Crystal structure of Streptococcus pyogenes Cas9 in complex with guide RNA and target DNA
Authors	:	Nishimasu, H.; Ishitani, R.; Nureki, O.
Deposited on	:	2014-01-31
Resolution	:	2.50 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
https://www.wwpdb.org/validation/2017/XrayValidationReportHelp
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:

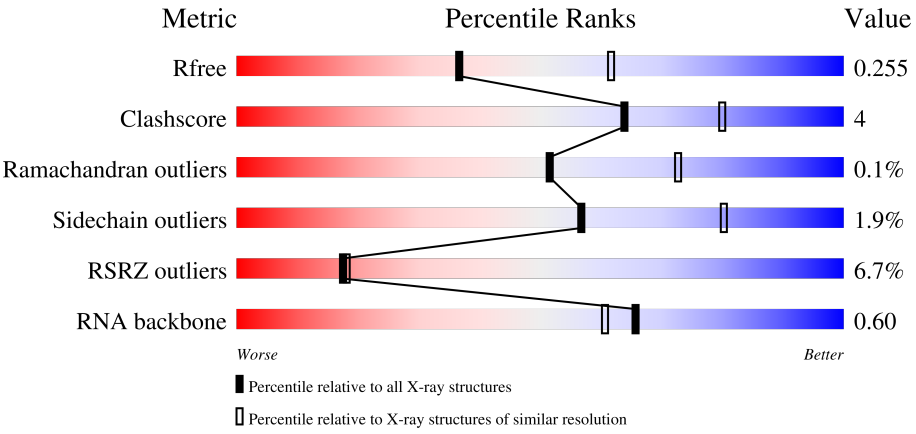
MolProbity	:	4.02b-467
Xtriage (Phenix)	:	1.13
EDS	:	2.37.1
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.37.1

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

The reported resolution of this entry is 2.50 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}	130704	4661 (2.50-2.50)
Clashscore	141614	5346 (2.50-2.50)
Ramachandran outliers	138981	5231 (2.50-2.50)
Sidechain outliers	138945	5233 (2.50-2.50)
RSRZ outliers	127900	4559 (2.50-2.50)
RNA backbone	3102	1008 (2.84-2.16)

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 of the lower bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. 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% 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	1372	8% 81% 13% • 5%
1	D	1372	5% 76% 8% 15%
2	B	98	2% 72% 24% ••
2	E	98	3% 66% 30% ••

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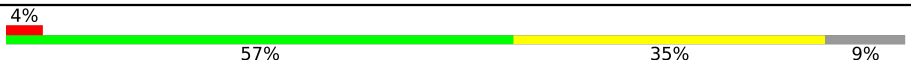
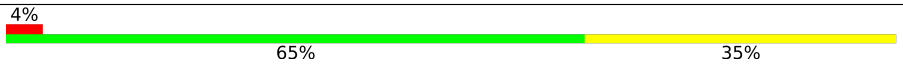
Mol	Chain	Length	Quality of chain
3	C	23	
3	F	23	

2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 24153 atoms, of which 0 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 protein called CRISPR-associated endonuclease Cas9/Csn1.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	1301	Total	C	N	O	S	0	0	0
			9999	6388	1708	1883	20			
1	D	1163	Total	C	N	O	S	0	0	0
			8998	5754	1529	1698	17			

There are 16 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-3	GLY	-	expression tag	UNP Q99ZW2
A	-2	SER	-	expression tag	UNP Q99ZW2
A	-1	GLY	-	expression tag	UNP Q99ZW2
A	0	HIS	-	expression tag	UNP Q99ZW2
A	10	ALA	ASP	engineered mutation	UNP Q99ZW2
A	80	LEU	CYS	engineered mutation	UNP Q99ZW2
A	574	GLU	CYS	engineered mutation	UNP Q99ZW2
A	840	ALA	HIS	engineered mutation	UNP Q99ZW2
D	-3	GLY	-	expression tag	UNP Q99ZW2
D	-2	SER	-	expression tag	UNP Q99ZW2
D	-1	GLY	-	expression tag	UNP Q99ZW2
D	0	HIS	-	expression tag	UNP Q99ZW2
D	10	ALA	ASP	engineered mutation	UNP Q99ZW2
D	80	LEU	CYS	engineered mutation	UNP Q99ZW2
D	574	GLU	CYS	engineered mutation	UNP Q99ZW2
D	840	ALA	HIS	engineered mutation	UNP Q99ZW2

- Molecule 2 is a RNA chain called RNA (97-MER).

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	B	97	Total	C	N	O	P	0	0	0
			2082	930	380	675	97			
2	E	97	Total	C	N	O	P	0	0	0
			2082	930	380	675	97			

- Molecule 3 is a DNA chain called DNA (5'-D(*CP*CP*AP*GP*CP*CP*AP*AP*GP*CP*GP*CP*AP*CP*CP*TP*AP*AP*TP*TP*TP*CP*C)-3').

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
3	C	21	Total	C	N	O	P	0	0	1
			404	192	72	120	20			
3	F	23	Total	C	N	O	P	0	0	1
			444	211	80	131	22			

- Molecule 4 is water.

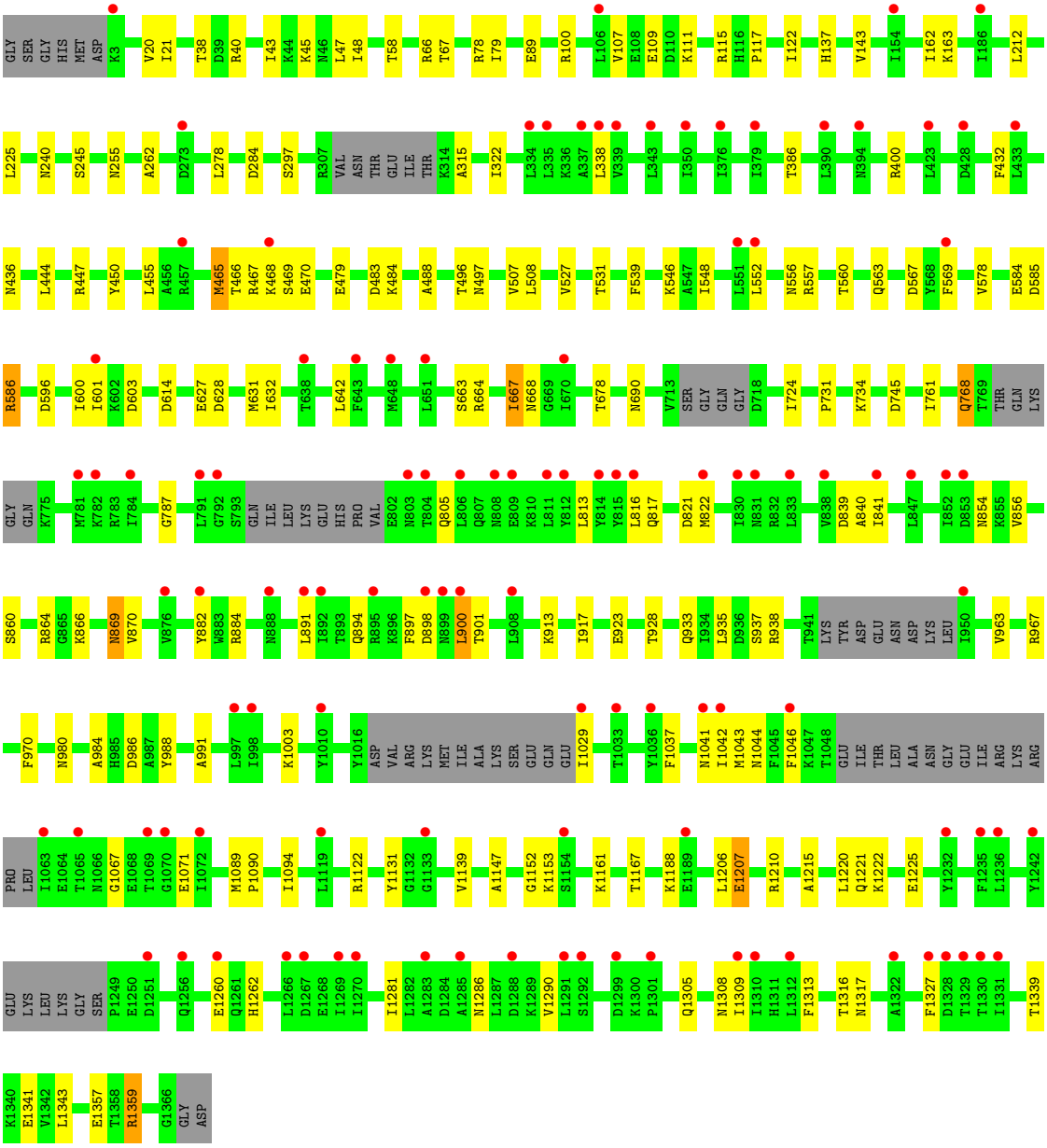
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	32	Total	O	0	0
			32	32		
4	B	28	Total	O	0	0
			28	28		
4	C	2	Total	O	0	0
			2	2		
4	D	49	Total	O	0	0
			49	49		
4	E	32	Total	O	0	0
			32	32		
4	F	1	Total	O	0	0
			1	1		

3 Residue-property plots (i)

These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and electron density. Residues are color-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. A red dot above a residue indicates a poor fit to the electron density (RSRZ > 2). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

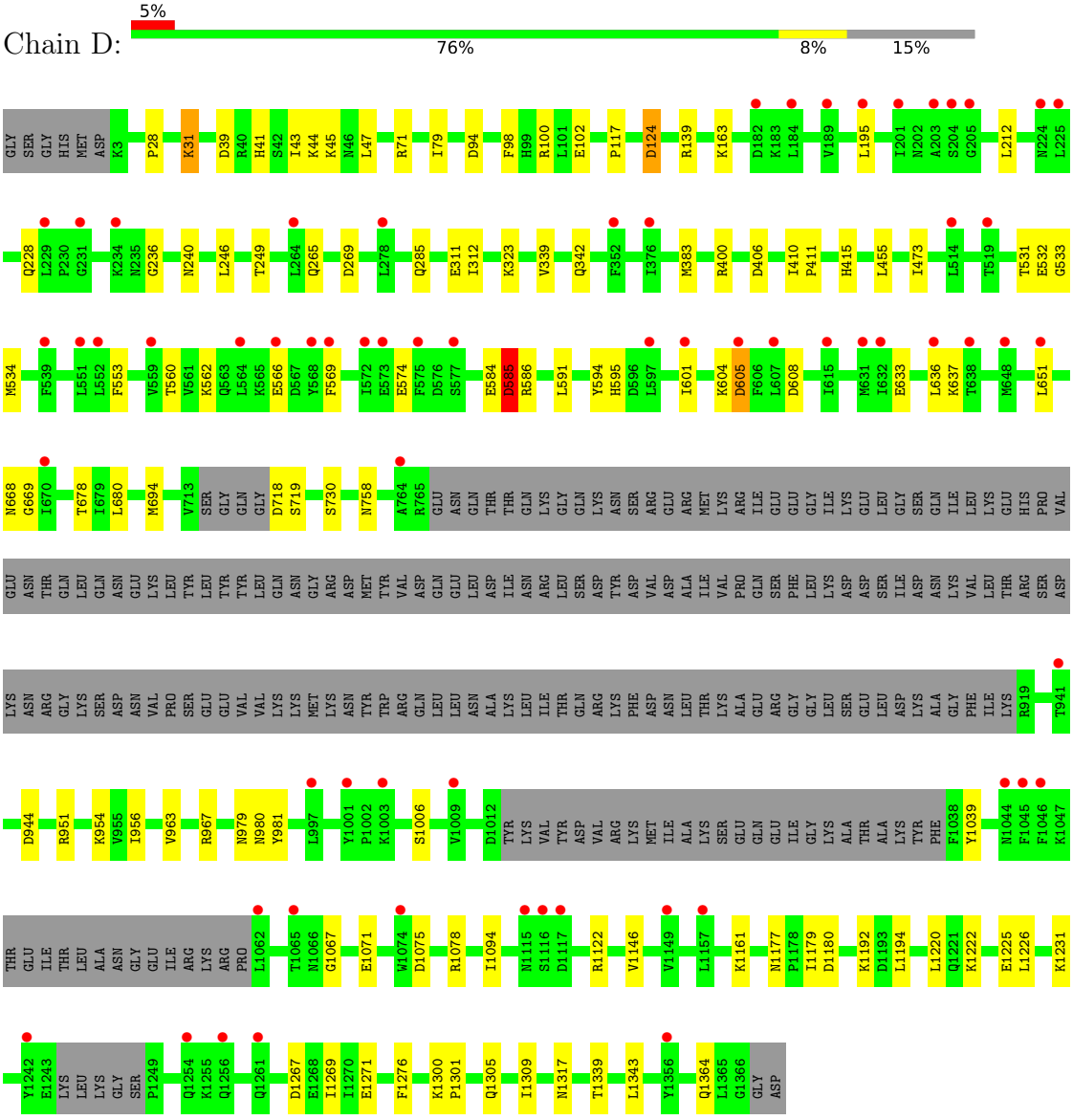
- Molecule 1: CRISPR-associated endonuclease Cas9/Csn1

Chain A:



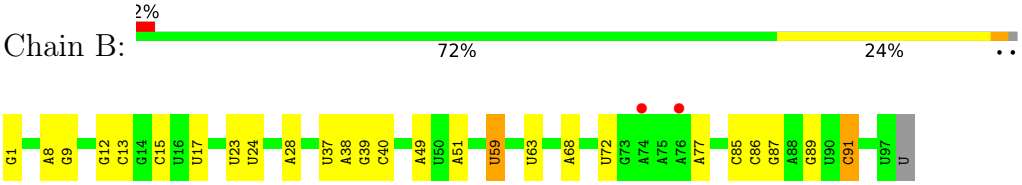
• Molecule 1: CRISPR-associated endonuclease Cas9/Csn1

Chain D:



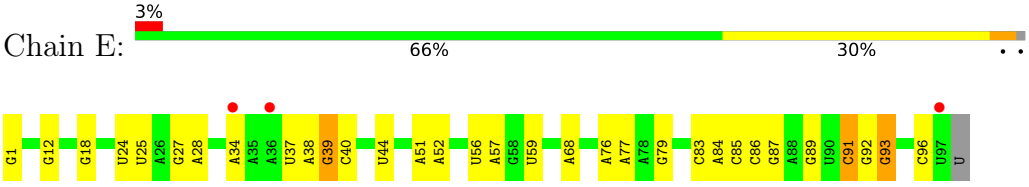
• Molecule 2: RNA (97-MER)

Chain B:



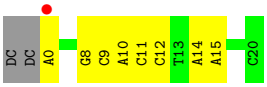
• Molecule 2: RNA (97-MER)

Chain E:



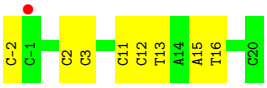
• Molecule 3: DNA (5'-D(*CP*CP*AP*GP*CP*CP*AP*AP*GP*CP*GP*CP*AP*CP*CP*TP *AP*AP*TP*TP*TP*CP*C)-3')

Chain C:



• Molecule 3: DNA (5'-D(*CP*CP*AP*GP*CP*CP*AP*AP*GP*CP*GP*CP*AP*CP*CP*TP *AP*AP*TP*TP*TP*CP*C)-3')

Chain F:



4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1	Depositor
Cell constants a, b, c, α, β, γ	76.71Å 105.69Å 126.82Å 97.68° 98.43° 100.31°	Depositor
Resolution (Å)	46.70 – 2.50 46.88 – 2.50	Depositor EDS
% Data completeness (in resolution range)	98.5 (46.70-2.50) 98.6 (46.88-2.50)	Depositor EDS
R_{merge}	0.06	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.02 (at 2.51Å)	Xtriage
Refinement program	PHENIX 1.8.3_1479	Depositor
R, R_{free}	0.222 , 0.254 0.226 , 0.255	Depositor DCC
R_{free} test set	6523 reflections (5.00%)	wwPDB-VP
Wilson B-factor (Å^2)	59.8	Xtriage
Anisotropy	0.201	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.31 , 54.8	EDS
L-test for twinning[2]	$< \|L\| > = 0.49$, $< L^2 > = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.94	EDS
Total number of atoms	24153	wwPDB-VP
Average B, all atoms (Å^2)	80.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.33% of the height of the origin peak. No significant pseudotranslation is detected.*

[1] Intensities estimated from amplitudes.

[2] Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality

5.1 Standard geometry

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	0.22	0/10177	0.36	0/13792
1	D	0.22	0/9164	0.37	0/12424
2	B	0.29	1/2332 (0.0%)	0.70	0/3633
2	E	0.30	1/2332 (0.0%)	0.70	0/3633
3	C	0.70	1/451 (0.2%)	0.86	0/693
3	F	0.70	1/496 (0.2%)	0.91	0/762
All	All	0.27	4/24952 (0.0%)	0.48	0/34937

All (4) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	E	1	G	OP3-P	-10.58	1.48	1.61
3	F	-2	DC	O3'-P	-10.56	1.48	1.61
2	B	1	G	OP3-P	-10.56	1.48	1.61
3	C	0	DA	O3'-P	-10.52	1.48	1.61

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts

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	9999	0	9573	96	1
1	D	8998	0	8657	71	1
2	B	2082	0	1043	8	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
2	E	2082	0	1043	13	0
3	C	404	0	225	5	0
3	F	444	0	247	5	0
4	A	32	0	0	2	0
4	B	28	0	0	0	0
4	C	2	0	0	0	0
4	D	49	0	0	0	0
4	E	32	0	0	0	0
4	F	1	0	0	0	0
All	All	24153	0	20788	185	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 4.

The worst 5 of 185 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)
1:D:139:ARG:HH22	1:D:415:HIS:HD2	1.29	0.78
1:D:28:PRO:HG2	1:D:47:LEU:HD12	1.73	0.69
1:D:100:ARG:NH1	1:D:117:PRO:O	2.26	0.69
1:A:967:ARG:NH1	1:A:986:ASP:OD1	2.26	0.69
1:A:21:ILE:HD12	1:A:991:ALA:HB3	1.76	0.68

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)
1:A:860:SER:OG	1:D:944:ASP:O[1_454]	2.16	0.04

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
1	A	1283/1372 (94%)	1252 (98%)	30 (2%)	1 (0%)	51 73
1	D	1151/1372 (84%)	1108 (96%)	42 (4%)	1 (0%)	51 73
All	All	2434/2744 (89%)	2360 (97%)	72 (3%)	2 (0%)	51 73

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	D	585	ASP
1	A	585	ASP

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
1	A	1013/1227 (83%)	987 (97%)	26 (3%)	46 72
1	D	921/1227 (75%)	911 (99%)	10 (1%)	73 89
All	All	1934/2454 (79%)	1898 (98%)	36 (2%)	57 80

5 of 36 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	D	228	GLN
1	D	1039	TYR
1	D	323	LYS
1	D	595	HIS
1	A	586	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 21 such sidechains are listed below:

Mol	Chain	Res	Type
1	D	354	GLN
1	D	668	ASN
1	D	1254	GLN
1	D	758	ASN

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Mol	Chain	Res	Type
1	D	426	GLN

5.3.3 RNA

Mol	Chain	Analysed	Backbone Outliers	Pucker Outliers
2	B	96/98 (97%)	15 (15%)	1 (1%)
2	E	96/98 (97%)	17 (17%)	0
All	All	192/196 (97%)	32 (16%)	1 (0%)

5 of 32 RNA backbone outliers are listed below:

Mol	Chain	Res	Type
2	B	8	A
2	B	9	G
2	B	28	A
2	B	37	U
2	B	39	G

All (1) RNA pucker outliers are listed below:

Mol	Chain	Res	Type
2	B	38	A

5.4 Non-standard residues in protein, DNA, RNA chains

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

5.5 Carbohydrates

There are no monosaccharides in this entry.

5.6 Ligand geometry

There are no ligands in this entry.

5.7 Other polymers

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

6.1 Protein, DNA and RNA chains

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, 95^{th} 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	1301/1372 (94%)	0.62	110 (8%) 10 10	36, 82, 120, 160	0
1	D	1163/1372 (84%)	0.50	65 (5%) 24 25	40, 75, 117, 166	0
2	B	97/98 (98%)	0.20	2 (2%) 63 66	40, 75, 146, 168	0
2	E	97/98 (98%)	0.15	3 (3%) 49 52	44, 77, 172, 189	0
3	C	21/23 (91%)	0.36	1 (4%) 30 32	49, 65, 102, 108	0
3	F	23/23 (100%)	0.40	1 (4%) 35 38	53, 64, 131, 152	0
All	All	2702/2986 (90%)	0.54	182 (6%) 17 18	36, 78, 121, 189	0

The worst 5 of 182 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	1330	THR	6.4
1	A	643	PHE	5.6
1	A	1312	LEU	5.3
1	D	1116	SER	5.2
1	D	552	LEU	5.2

6.2 Non-standard residues in protein, DNA, RNA chains

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

6.3 Carbohydrates

There are no monosaccharides in this entry.

6.4 Ligands

There are no ligands in this entry.

6.5 Other polymers (i)

There are no such residues in this entry.