



Full wwPDB X-ray Structure Validation Report ⓘ

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PDB ID : 6FH8 / pdb_00006fh8
Title : E. coli surface display of streptavidin for directed evolution of an allylic dehalocase
Authors : Heinisch, T.; Schwizer, F.; Garabedian, B.; Csibra, E.; Jeschek, M.; Pinheiro Bernhades, V.; Marliere, P.; Panke, S.; Ward, T.R.
Deposited on : 2018-01-12
Resolution : 1.64 Å(reported)

This is a Full wwPDB X-ray 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/XrayValidationReportHelp>

with specific help available everywhere you see the ⓘ 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 ⓘ](#)) were used in the production of this report:

MolProbity	:	4-5-2 with Phenix2.0
Mogul	:	2022.3.0, CSD as543be (2022)
Xtriage (Phenix)	:	2.0
EDS	:	3.0
Buster-report	:	wwPDB partial adaption of 1.1.7 (2018)
Percentile statistics	:	20250101.v01 (using entries in the PDB archive January 1st 2025)
CCP4	:	9.0.010 (Gargrove)
Density-Fitness	:	1.0.12
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.49

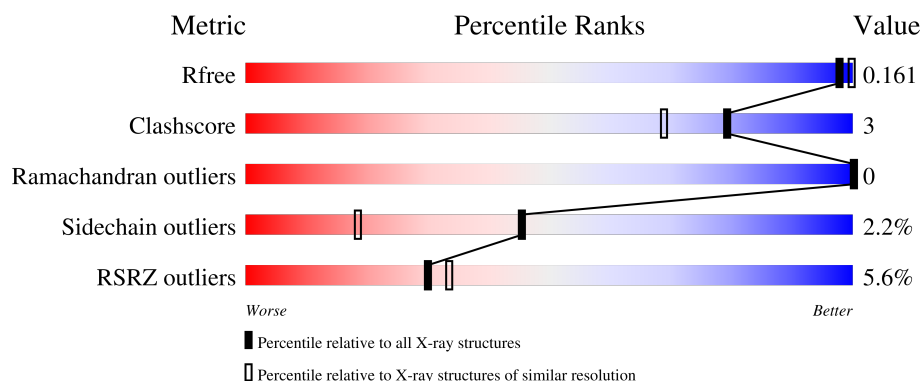
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 1.64 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}	180053	1141 (1.64-1.64)
Clashscore	190562	1171 (1.64-1.64)
Ramachandran outliers	187476	1151 (1.64-1.64)
Sidechain outliers	187428	1150 (1.64-1.64)
RSRZ outliers	180081	1141 (1.64-1.64)

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 $\leq 5\%$. 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	159	

2 Entry composition

There are 3 unique types of molecules in this entry. The entry contains 1065 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.

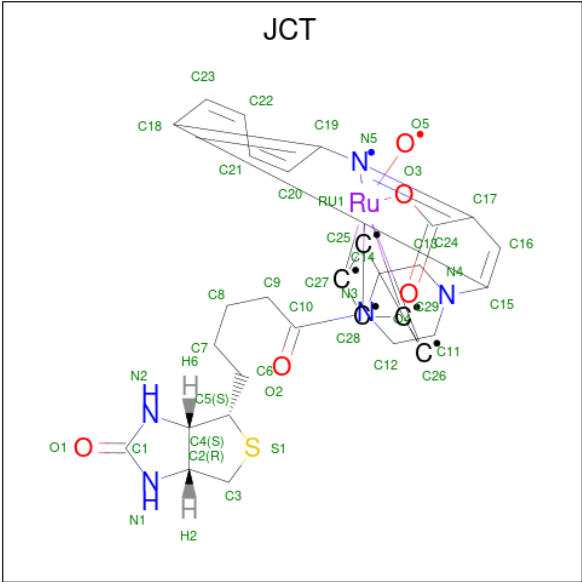
- Molecule 1 is a protein called Streptavidin.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	125	Total	C	N	O	S	0	1	0
			939	586	163	188	2			

There are 15 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	MET	-	initiating methionine	UNP P22629
A	2	ALA	-	expression tag	UNP P22629
A	3	SER	-	expression tag	UNP P22629
A	4	MET	-	expression tag	UNP P22629
A	5	THR	-	expression tag	UNP P22629
A	6	GLY	-	expression tag	UNP P22629
A	7	GLY	-	expression tag	UNP P22629
A	8	GLN	-	expression tag	UNP P22629
A	9	GLN	-	expression tag	UNP P22629
A	10	MET	-	expression tag	UNP P22629
A	11	GLY	-	expression tag	UNP P22629
A	12	ARG	-	expression tag	UNP P22629
A	13	ASP	-	expression tag	UNP P22629
A	112	MET	SER	engineered mutation	UNP P22629
A	121	ALA	LYS	engineered mutation	UNP P22629

- Molecule 2 is biotinylated ruthenium cyclopentadienide (CCD ID: JCT) (formula: $C_{29}H_{28}N_5O_5RuS$).



Mol	Chain	Residues	Atoms						ZeroOcc	AltConf
2	A	1	Total	C	N	O	Ru	S	12	1
			47	34	5	6	1	1		

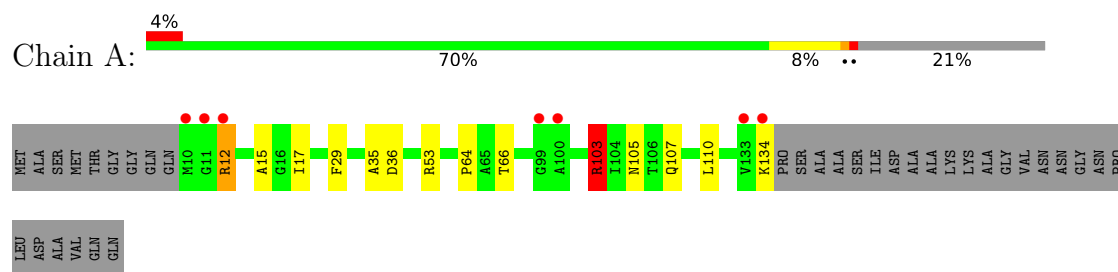
- Molecule 3 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	A	79	Total	O	0	0
			79	79		

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: Streptavidin



4 Data and refinement statistics

Property	Value	Source
Space group	I 41 2 2	Depositor
Cell constants a, b, c, α , β , γ	57.72Å 57.72Å 183.62Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	45.80 – 1.64 45.80 – 1.64	Depositor EDS
% Data completeness (in resolution range)	98.1 (45.80-1.64) 98.6 (45.80-1.64)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.09 (at 1.63Å)	Xtriage
Refinement program	REFMAC 5.8.0158	Depositor
R, R_{free}	0.120 , 0.159 0.121 , 0.161	Depositor DCC
R_{free} test set	952 reflections (4.82%)	wwPDB-VP
Wilson B-factor (Å ²)	21.2	Xtriage
Anisotropy	0.046	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.39 , 54.7	EDS
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.98	EDS
Total number of atoms	1065	wwPDB-VP
Average B, all atoms (Å ²)	28.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 7.72% 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, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality

5.1 Standard geometry

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

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	1.62	6/964 (0.6%)	1.30	7/1317 (0.5%)

All (6) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	66	THR	C-O	-11.35	1.09	1.24
1	A	29	PHE	CA-CB	6.63	1.63	1.53
1	A	36	ASP	CB-CG	6.12	1.67	1.52
1	A	29	PHE	N-CA	5.59	1.52	1.46
1	A	12	ARG	N-CA	5.58	1.53	1.46
1	A	15	ALA	C-O	5.41	1.30	1.24

All (7) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	29	PHE	N-CA-CB	-5.90	101.45	110.65
1	A	103	ARG	NE-CZ-NH1	-5.62	115.89	121.50
1	A	17	ILE	N-CA-C	5.28	116.75	111.00
1	A	35	ALA	CA-C-N	5.12	131.59	122.06
1	A	35	ALA	C-N-CA	5.12	131.59	122.06
1	A	12	ARG	N-CA-CB	5.05	117.55	110.12
1	A	103	ARG	CG-CD-NE	-5.04	100.92	112.00

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	939	0	883	5	1
2	A	47	0	0	0	0
3	A	79	0	0	1	2
All	All	1065	0	883	5	2

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 3.

All (5) 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:A:103:ARG:NH1	1:A:105:ASN:HD21	2.09	0.50
1:A:53:ARG:NH2	3:A:301:HOH:O	2.45	0.49
1:A:12:ARG:NH1	1:A:64:PRO:O	2.44	0.42

All (2) 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)
3:A:367:HOH:O	3:A:367:HOH:O[8_665]	1.86	0.34
1:A:107:GLN:OE1	3:A:367:HOH:O[8_665]	2.17	0.03

5.3 Torsion angles

5.3.1 Protein backbone

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	124/159 (78%)	120 (97%)	4 (3%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2 Protein sidechains ⓘ

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	92/114 (81%)	90 (98%)	2 (2%)	45	18

All (2) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	103	ARG
1	A	134	LYS

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

Mol	Chain	Res	Type
1	A	107	GLN

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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 oligosaccharides in this entry.

5.6 Ligand geometry

2 ligands are modelled in this entry.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. 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 $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\# Z > 2$	Counts	RMSZ	$\# Z > 2$
2	JCT	A	201[B]	-	36,51,51	3.71	18 (50%)	47,100,100	2.84	21 (44%)
2	JCT	A	201[A]	-	36,51,51	3.71	18 (50%)	47,100,100	2.84	21 (44%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	JCT	A	201[B]	-	-	1/15/175/175	0/12/11/11
2	JCT	A	201[A]	-	-	1/15/175/175	0/12/11/11

All (36) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	201[A]	JCT	C3-S1	-12.59	1.46	1.81
2	A	201[B]	JCT	C3-S1	-12.59	1.46	1.81
2	A	201[A]	JCT	O4-C24	8.91	1.39	1.21
2	A	201[B]	JCT	O4-C24	8.91	1.39	1.21
2	A	201[A]	JCT	C3-C2	5.82	1.65	1.53
2	A	201[B]	JCT	C3-C2	5.82	1.65	1.53
2	A	201[A]	JCT	C1-N1	5.15	1.44	1.35
2	A	201[B]	JCT	C1-N1	5.15	1.44	1.35
2	A	201[A]	JCT	C23-C18	-4.45	1.33	1.39
2	A	201[B]	JCT	C23-C18	-4.45	1.33	1.39
2	A	201[A]	JCT	C14-N3	-4.30	1.39	1.47
2	A	201[B]	JCT	C14-N3	-4.30	1.39	1.47
2	A	201[A]	JCT	C22-C23	4.10	1.45	1.38
2	A	201[B]	JCT	C22-C23	4.10	1.45	1.38
2	A	201[A]	JCT	C20-C19	3.86	1.46	1.39
2	A	201[B]	JCT	C20-C19	3.86	1.46	1.39

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	201[A]	JCT	O2-C10	-3.61	1.15	1.23
2	A	201[B]	JCT	O2-C10	-3.61	1.15	1.23
2	A	201[A]	JCT	C1-N2	3.60	1.41	1.35
2	A	201[B]	JCT	C1-N2	3.60	1.41	1.35
2	A	201[A]	JCT	C21-C20	3.56	1.45	1.38
2	A	201[B]	JCT	C21-C20	3.56	1.45	1.38
2	A	201[A]	JCT	C9-C10	3.49	1.58	1.51
2	A	201[B]	JCT	C9-C10	3.49	1.58	1.51
2	A	201[A]	JCT	C16-C15	3.34	1.43	1.37
2	A	201[B]	JCT	C16-C15	3.34	1.43	1.37
2	A	201[A]	JCT	C15-N4	3.29	1.47	1.37
2	A	201[B]	JCT	C15-N4	3.29	1.47	1.37
2	A	201[A]	JCT	C2-C4	3.03	1.64	1.55
2	A	201[B]	JCT	C2-C4	3.03	1.64	1.55
2	A	201[A]	JCT	C17-C24	2.94	1.55	1.49
2	A	201[B]	JCT	C17-C24	2.94	1.55	1.49
2	A	201[A]	JCT	C18-C15	2.86	1.51	1.46
2	A	201[B]	JCT	C18-C15	2.86	1.51	1.46
2	A	201[A]	JCT	C4-N2	-2.35	1.41	1.45
2	A	201[B]	JCT	C4-N2	-2.35	1.41	1.45

All (42) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	A	201[A]	JCT	C5-C4-N2	6.81	120.55	113.34
2	A	201[B]	JCT	C5-C4-N2	6.81	120.55	113.34
2	A	201[A]	JCT	C3-C2-C4	-6.39	98.94	109.06
2	A	201[B]	JCT	C3-C2-C4	-6.39	98.94	109.06
2	A	201[A]	JCT	C2-C3-S1	6.37	114.84	106.06
2	A	201[B]	JCT	C2-C3-S1	6.37	114.84	106.06
2	A	201[A]	JCT	C19-C18-C15	-6.14	114.70	123.36
2	A	201[B]	JCT	C19-C18-C15	-6.14	114.70	123.36
2	A	201[A]	JCT	C4-C5-S1	-5.36	98.95	105.03
2	A	201[B]	JCT	C4-C5-S1	-5.36	98.95	105.03
2	A	201[A]	JCT	C16-C17-N5	-4.58	119.96	124.84
2	A	201[B]	JCT	C16-C17-N5	-4.58	119.96	124.84
2	A	201[A]	JCT	C3-C2-N1	3.73	117.99	113.18
2	A	201[B]	JCT	C3-C2-N1	3.73	117.99	113.18
2	A	201[A]	JCT	C22-C23-C18	3.68	126.45	119.80
2	A	201[B]	JCT	C22-C23-C18	3.68	126.45	119.80
2	A	201[A]	JCT	O2-C10-N3	-3.47	115.94	122.12
2	A	201[B]	JCT	O2-C10-N3	-3.47	115.94	122.12

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	A	201[A]	JCT	C22-C21-C20	-3.31	116.16	120.24
2	A	201[B]	JCT	C22-C21-C20	-3.31	116.16	120.24
2	A	201[A]	JCT	C8-C9-C10	-3.07	104.72	112.66
2	A	201[B]	JCT	C8-C9-C10	-3.07	104.72	112.66
2	A	201[A]	JCT	C16-C15-N4	-3.04	117.26	122.75
2	A	201[B]	JCT	C16-C15-N4	-3.04	117.26	122.75
2	A	201[A]	JCT	C3-S1-C5	2.99	96.20	89.98
2	A	201[B]	JCT	C3-S1-C5	2.99	96.20	89.98
2	A	201[A]	JCT	C21-C20-C19	2.83	123.25	118.60
2	A	201[B]	JCT	C21-C20-C19	2.83	123.25	118.60
2	A	201[A]	JCT	C12-C11-N4	-2.73	104.99	110.42
2	A	201[B]	JCT	C12-C11-N4	-2.73	104.99	110.42
2	A	201[A]	JCT	C21-C22-C23	-2.71	116.89	120.24
2	A	201[B]	JCT	C21-C22-C23	-2.71	116.89	120.24
2	A	201[A]	JCT	O1-C1-N1	-2.68	122.03	125.89
2	A	201[B]	JCT	O1-C1-N1	-2.68	122.03	125.89
2	A	201[A]	JCT	O2-C10-C9	2.66	128.44	121.26
2	A	201[B]	JCT	O2-C10-C9	2.66	128.44	121.26
2	A	201[A]	JCT	C13-C14-N3	2.27	114.93	110.42
2	A	201[B]	JCT	C13-C14-N3	2.27	114.93	110.42
2	A	201[A]	JCT	C23-C18-C15	2.24	126.31	121.17
2	A	201[B]	JCT	C23-C18-C15	2.24	126.31	121.17
2	A	201[A]	JCT	C2-C4-N2	2.16	105.12	102.68
2	A	201[B]	JCT	C2-C4-N2	2.16	105.12	102.68

There are no chirality outliers.

All (2) torsion outliers are listed below:

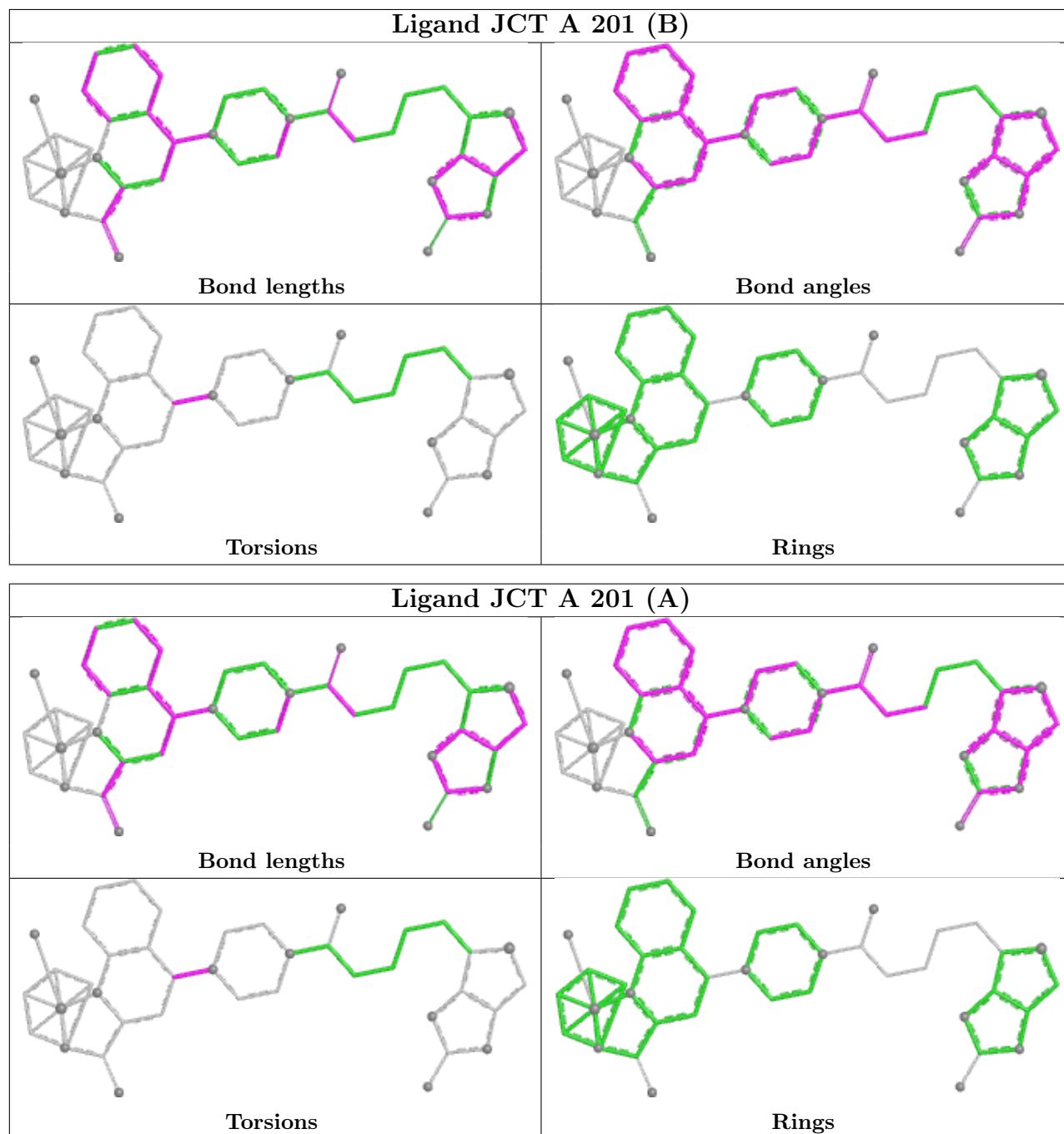
Mol	Chain	Res	Type	Atoms
2	A	201[A]	JCT	C16-C15-N4-C11
2	A	201[B]	JCT	C16-C15-N4-C11

There are no ring outliers.

No monomer is involved in short contacts.

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less than 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring

in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.



5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

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	125/159 (78%)	-0.19	7 (5%) 30 33	10, 22, 46, 70	4 (3%)

All (7) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	10	MET	3.0
1	A	134	LYS	3.0
1	A	12	ARG	2.6
1	A	100	ALA	2.5
1	A	99	GLY	2.4
1	A	11	GLY	2.2
1	A	133	VAL	2.1

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 oligosaccharides 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. 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	B-factors(Å ²)	Q<0.9
2	JCT	A	201[A]	41/41	0.95	0.10	14,30,106,131	6

Continued on next page...

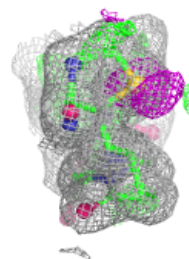
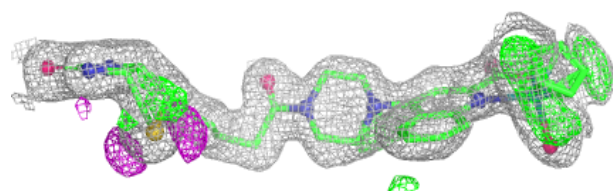
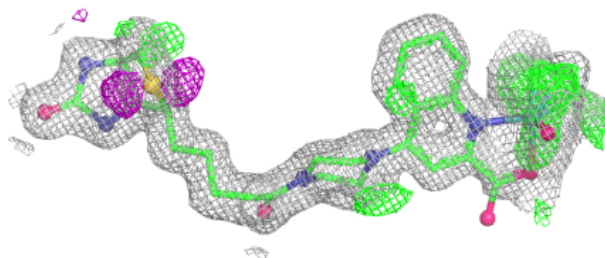
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Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors($\text{\AA}^2$)	Q<0.9
2	JCT	A	201[B]	41/41	0.95	0.10	14,30,106,131	6

The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation to approximate a three-dimensional view.

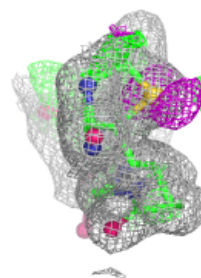
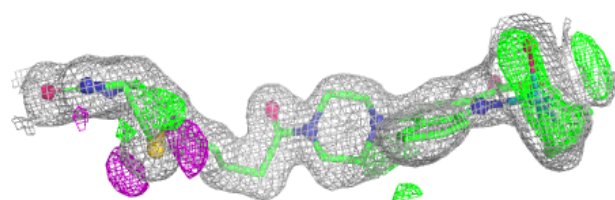
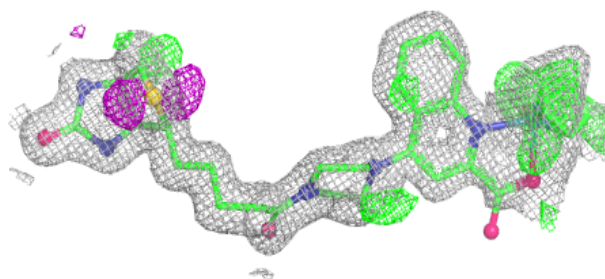
Electron density around JCT A 201 (A):

2mF_o-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)



Electron density around JCT A 201 (B):

$2mF_o-DF_c$ (at 0.7 rmsd) in gray
 mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)



6.5 Other polymers [i](#)

There are no such residues in this entry.