

Full wwPDB X-ray Structure Validation Report (i)

Aug 26, 2023 – 04:18 pm BST

PDB ID	:	8B6R
Title	:	X-ray structure of the haloalkane dehalogenase HaloTag7 labeled with a
		chloroalkane Cyanine3 fluorophore substrate
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Deposited on	:	2022-09-27
Resolution	:	1.50 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 (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	:	FAILED
Mogul	:	1.8.4, CSD as 541 be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.35
buster-report	:	1.1.7(2018)
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)	:	2.35

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure: $X\text{-}RAY \, DIFFRACTION$

The reported resolution of this entry is 1.50 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.



Motrio	Whole archive	Similar resolution
Metric	$(\# {\rm Entries})$	$(\# { m Entries}, { m resolution} { m range}({ m \AA}))$
R _{free}	130704	2936 (1.50-1.50)
RSRZ outliers	127900	2884 (1.50-1.50)

MolProbity failed to run properly - the sequence quality summary graphics cannot be shown.



8B6R

2 Entry composition (i)

There are 6 unique types of molecules in this entry. The entry contains 2734 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 Haloalkane dehalogenase.

Mol	Chain	Residues		At	oms			ZeroOcc	AltConf	Trace
1	А	293	Total 2365	C 1540	N 395	O 420	S 10	0	3	0

Chain	Residue	Modelled	Actual	Comment	Reference
А	3	GLY	-	expression tag	UNP P0A3G3
А	47	VAL	LEU	engineered mutation	UNP P0A3G3
А	58	THR	SER	engineered mutation	UNP P0A3G3
А	78	GLY	ASP	engineered mutation	UNP P0A3G3
А	87	PHE	TYR	engineered mutation	UNP P0A3G3
А	88	MET	LEU	engineered mutation	UNP P0A3G3
А	128	PHE	CYS	engineered mutation	UNP P0A3G3
А	155	THR	ALA	engineered mutation	UNP P0A3G3
А	160	LYS	GLU	engineered mutation	UNP P0A3G3
А	167	VAL	ALA	engineered mutation	UNP P0A3G3
А	172	THR	ALA	engineered mutation	UNP P0A3G3
А	175	MET	LYS	engineered mutation	UNP P0A3G3
А	176	GLY	CYS	engineered mutation	UNP P0A3G3
А	195	ASN	LYS	engineered mutation	UNP P0A3G3
А	224	GLU	ALA	engineered mutation	UNP P0A3G3
А	227	ASP	ASN	engineered mutation	UNP P0A3G3
А	257	LYS	GLU	engineered mutation	UNP P0A3G3
А	264	ALA	THR	engineered mutation	UNP P0A3G3
А	272	ASN	HIS	engineered mutation	UNP P0A3G3
А	273	LEU	TYR	engineered mutation	UNP P0A3G3
A	291	SER	PRO	engineered mutation	UNP P0A3G3
A	292	THR	ALA	engineered mutation	UNP P0A3G3
A	294	GLU	-	expression tag	UNP P0A3G3
A	295	ILE	-	expression tag	UNP P0A3G3

There are 24 discrepancies between the modelled and reference sequences:

• Molecule 2 is {N}-[2-[2-(6-chloranylhexoxy)ethoxy]ethyl]-6-[3,3-dimethyl-2-[({E})-3-(1,3,3-trimethylindol-2-ylidene)prop-1-enyl]indol-1-ium-1-yl]hexanamide (three-letter code: PJI)



(formula: $C_{40}H_{57}ClN_3O_3$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
2	А	1	Total 46	C 40	N 3	O 3	0	0

• Molecule 3 is CHLORIDE ION (three-letter code: CL) (formula: Cl).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	А	1	Total Cl 1 1	0	0

• Molecule 4 is GLYCEROL (three-letter code: GOL) (formula: $C_3H_8O_3$).





Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	1	$\begin{array}{ccc} \text{Total} \text{C} \text{O} \\ 6 3 3 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 6 & 3 & 3 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 6 & 3 & 3 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 6 & 3 & 3 \end{array}$	0	0

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

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	А	1	Total Mg 1 1	0	0

• Molecule 6 is water.

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
6	А	297	Total O 297 297	0	0

MolProbity failed to run properly - this section is therefore empty.



3 Data and refinement statistics (i)

Property	Value	Source
Space group	P 42 21 2	Depositor
Cell constants	112.56Å 112.56Å 44.33Å	Depositor
a, b, c, α , β , γ	90.00° 90.00° 90.00°	Depositor
Bosolution (Å)	41.25 - 1.50	Depositor
Resolution (A)	41.25 - 1.50	EDS
% Data completeness	99.9 (41.25-1.50)	Depositor
(in resolution range)	99.9 (41.25-1.50)	EDS
R_{merge}	0.07	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) > 1$	$2.20 (at 1.50 \text{\AA})$	Xtriage
Refinement program	PHENIX 1.19.2_4158	Depositor
P. P.	0.162 , 0.192	Depositor
n, n_{free}	0.161 , 0.189	DCC
R_{free} test set	2306 reflections $(5.00%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	14.4	Xtriage
Anisotropy	0.151	Xtriage
Bulk solvent $k_{sol}(e/A^3), B_{sol}(A^2)$	0.37, 41.9	EDS
L-test for $twinning^2$	$ < L >=0.50, < L^2>=0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.97	EDS
Total number of atoms	2734	wwPDB-VP
Average B, all atoms $(Å^2)$	16.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 5.21% of the height of the origin peak. No significant pseudotranslation is detected.

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.



¹Intensities estimated from amplitudes.

4 Model quality (i)

4.1 Standard geometry (i)

MolProbity failed to run properly - this section is therefore empty.

4.2 Too-close contacts (i)

MolProbity failed to run properly - this section is therefore empty.

4.3 Torsion angles (i)

4.3.1 Protein backbone (i)

MolProbity failed to run properly - this section is therefore empty.

4.3.2 Protein sidechains (i)

MolProbity failed to run properly - this section is therefore empty.

4.3.3 RNA (i)

MolProbity failed to run properly - this section is therefore empty.

4.4 Non-standard residues in protein, DNA, RNA chains (i)

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

4.5 Carbohydrates (i)

There are no monosaccharides in this entry.

4.6 Ligand geometry (i)

Of 7 ligands modelled in this entry, 2 are monoatomic - leaving 5 for Mogul analysis.

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



Mal	Tune	Chain	Deg Link		Bo	ond leng	ths	Bond angles				
	Type	Type	Type	Unain	nes	LIIIK	Counts	RMSZ	# Z >2	Counts	RMSZ	# Z > 2
2	PJI	А	301	1	49,49,50	0.36	0	67,67,68	0.55	0		
4	GOL	А	307	-	$5,\!5,\!5$	0.97	1 (20%)	$5,\!5,\!5$	0.97	0		
4	GOL	А	305	-	$5,\!5,\!5$	1.03	0	$5,\!5,\!5$	0.86	0		
4	GOL	А	303	-	$5,\!5,\!5$	1.40	2 (40%)	5,5,5	0.92	0		
4	GOL	А	304	-	5,5,5	0.46	0	5,5,5	1.12	0		

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).

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	PJI	А	301	1	-	5/27/67/68	0/4/4/4
4	GOL	А	307	-	-	0/4/4/4	-
4	GOL	А	305	-	-	0/4/4/4	-
4	GOL	А	303	-	-	0/4/4/4	-
4	GOL	А	304	-	-	0/4/4/4	-

All (3) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	$\operatorname{Ideal}(\operatorname{\AA})$
4	А	303	GOL	O2-C2	-2.04	1.37	1.43
4	А	307	GOL	C1-C2	2.04	1.60	1.51
4	А	303	GOL	C1-C2	2.00	1.60	1.51

There are no bond angle outliers.

There are no chirality outliers.

All (5) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	А	301	PJI	N1-C16-C17-C18
2	А	301	PJI	C17-C18-C19-C20
2	А	301	PJI	C18-C19-C20-N2
2	А	301	PJI	C18-C19-C20-C28
2	А	301	PJI	C31-C16-C17-C18

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 then 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 sufficient the outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.



4.7 Other polymers (i)

There are no such residues in this entry.



4.8 Polymer linkage issues (i)

There are no chain breaks in this entry.



5 Fit of model and data (i)

5.1 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, 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	$\mathbf{ed} \langle \mathbf{RSRZ} \rangle \#\mathbf{RSRZ} \rangle 2$		$OWAB(Å^2)$	Q<0.9
1	А	293/293~(100%)	-0.01	6 (2%) 65 70	9, 13, 25, 45	0

All (6) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	А	295	ILE	5.2
1	А	155	THR	3.9
1	А	3	GLY	3.8
1	А	31	ASP	3.2
1	А	154	THR	2.8
1	А	156	ASP	2.0

5.2 Non-standard residues in protein, DNA, RNA chains (i)

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

5.3 Carbohydrates (i)

There are no monosaccharides in this entry.

5.4 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, 95^{th} 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	$\mathbf{B} ext{-factors}(\mathrm{\AA}^2)$	Q < 0.9
2	PJI	А	301	46/47	0.84	0.17	11,20,39,49	0

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Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å ²)	Q<0.9
4	GOL	А	307	6/6	0.89	0.12	19,22,27,31	0
4	GOL	А	303	6/6	0.90	0.10	22,25,25,29	0
4	GOL	А	305	6/6	0.92	0.09	20,23,25,26	0
4	GOL	А	304	6/6	0.94	0.11	13,16,22,35	0
5	MG	А	306	1/1	0.99	0.14	18,18,18,18	0
3	CL	А	302	1/1	1.00	0.07	10,10,10,10	0

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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.



5.5 Other polymers (i)

There are no such residues in this entry.

