Full wwPDB X-ray Structure Validation Report (i)

Jan 13, 2024 – 08:47 pm GMT

PDB ID	:	6Y2T
Title	:	Streptavidin wildtype with a biotC4-1 cofactor - an artificial iron hydroxylase
Authors	:	Serrano-Plana, J.; Rumo, C.; Rebelein, J.G.; Peterson, R.L.; Barnet, M.; Ward, T.R.
Deposited on	:	2020-02-17
Resolution	:	1.55 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 (i)) were used in the production of this report:

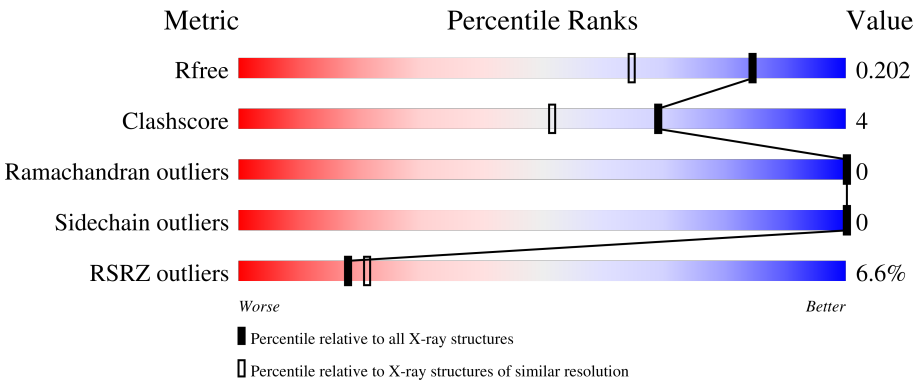
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

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 1.55 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	1483 (1.56-1.56)
Clashscore	141614	1529 (1.56-1.56)
Ramachandran outliers	138981	1498 (1.56-1.56)
Sidechain outliers	138945	1495 (1.56-1.56)
RSRZ outliers	127900	1465 (1.56-1.56)

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	AAA	159	5% 72% • 24%

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
2	O6T	AAA	203	X	-	-	-

2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 2020 atoms, of which 890 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.

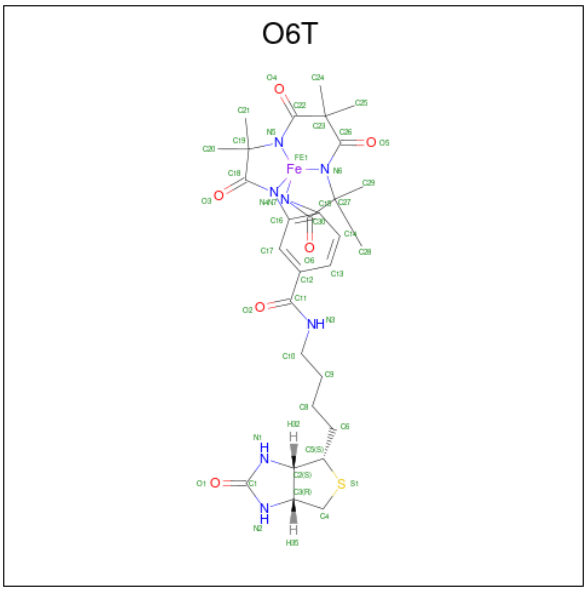
- Molecule 1 is a protein called Streptavidin.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	H	N	O			
1	AAA	121	1803	581	874	160	188	61	3	0

There are 14 discrepancies between the modelled and reference sequences:

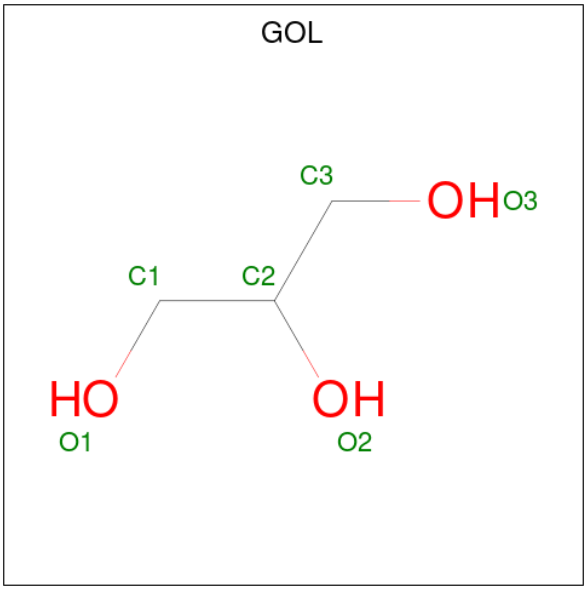
Chain	Residue	Modelled	Actual	Comment	Reference
AAA	1	MET	-	initiating methionine	UNP P22629
AAA	2	ALA	-	expression tag	UNP P22629
AAA	3	SER	-	expression tag	UNP P22629
AAA	4	MET	-	expression tag	UNP P22629
AAA	5	THR	-	expression tag	UNP P22629
AAA	6	GLY	-	expression tag	UNP P22629
AAA	7	GLY	-	expression tag	UNP P22629
AAA	8	GLN	-	expression tag	UNP P22629
AAA	9	GLN	-	expression tag	UNP P22629
AAA	10	MET	-	expression tag	UNP P22629
AAA	11	GLY	-	expression tag	UNP P22629
AAA	12	ARG	-	expression tag	UNP P22629
AAA	13	ASP	-	expression tag	UNP P22629
AAA	14	GLN	-	expression tag	UNP P22629

- Molecule 2 is biotC4-1 cofactor (three-letter code: O6T) (formula: $C_{29}H_{37}FeN_7O_6S$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms						ZeroOcc	AltConf
			Total	C	Fe	N	O	S		
2	AAA	1	44	29	1	7	6	1	0	0
			Total	C	Fe	N	O	S		
2	AAA	1	44	29	1	7	6	1	0	0

- Molecule 3 is GLYCEROL (three-letter code: GOL) (formula: $C_3H_8O_3$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	H	O		
3	AAA	1	14	3	8	3	2	0
			Total	C	H	O		
3	AAA	1	14	3	8	3	2	0

- Molecule 4 is water.

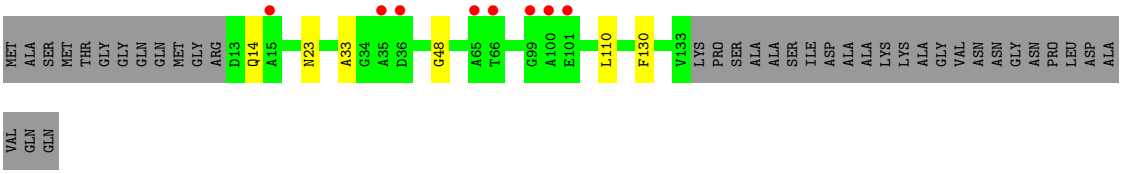
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
4	AAA	101	101	101	0	0

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

Chain AAA:



4 Data and refinement statistics (i)

Property	Value	Source
Space group	I 41 2 2	Depositor
Cell constants a, b, c, α, β, γ	57.71Å 57.71Å 184.13Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	46.08 – 1.55 46.03 – 1.55	Depositor EDS
% Data completeness (in resolution range)	99.9 (46.08-1.55) 100.0 (46.03-1.55)	Depositor EDS
R_{merge}	0.07	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.60 (at 1.55Å)	Xtriage
Refinement program	REFMAC 5.8.0258	Depositor
R, R_{free}	0.169 , 0.200 0.179 , 0.202	Depositor DCC
R_{free} test set	1161 reflections (5.01%)	wwPDB-VP
Wilson B-factor (Å^2)	22.2	Xtriage
Anisotropy	0.113	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.42 , 45.0	EDS
L-test for twinning[2]	$< \|L\| > = 0.50$, $< L^2 > = 0.34$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.97	EDS
Total number of atoms	2020	wwPDB-VP
Average B, all atoms (Å^2)	26.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 8.12% 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 (i)

5.1 Standard geometry (i)

Bond lengths and bond angles in the following residue types are not validated in this section: GOL, O6T

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	AAA	0.76	0/956	0.94	0/1310

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	AAA	929	874	869	6	0
2	AAA	88	0	0	1	0
3	AAA	12	16	16	3	0
4	AAA	101	0	0	0	0
All	All	1130	890	885	7	0

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.

All (7) 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:AAA:203:O6T:C9	2:AAA:203:O6T:O2	2.40	0.69
1:AAA:48:GLY:H	3:AAA:204:GOL:H31	1.60	0.66
1:AAA:48:GLY:H	3:AAA:204:GOL:C3	2.25	0.49
1:AAA:48:GLY:N	3:AAA:204:GOL:H31	2.28	0.45
1:AAA:23:ASN:HB3	1:AAA:130:PHE:CE2	2.55	0.42
1:AAA:14:GLN:HG3	1:AAA:33:ALA:O	2.20	0.41

There are no symmetry-related clashes.

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	AAA	123/159 (77%)	120 (98%)	3 (2%)	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
1	AAA	93/115 (81%)	93 (100%)	0	100 100

There are no protein residues with a non-rotameric sidechain to report.

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

5.3.3 RNA (i)

There are no RNA molecules in this entry.

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 monosaccharides in this entry.

5.6 Ligand geometry (i)

4 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	O6T	AAA	203	-	46,50,50	1.48	6 (13%)	66,84,84	2.23	19 (28%)
2	O6T	AAA	201	-	46,50,50	1.39	7 (15%)	66,84,84	1.82	18 (27%)
3	GOL	AAA	204	-	5,5,5	0.11	0	5,5,5	0.26	0
3	GOL	AAA	202	-	5,5,5	0.09	0	5,5,5	0.25	0

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	O6T	AAA	203	-	1/1/17/20	6/12/117/117	0/7/7/7
2	O6T	AAA	201	-	-	3/12/117/117	0/7/7/7
3	GOL	AAA	204	-	-	2/4/4/4	-
3	GOL	AAA	202	-	-	1/4/4/4	-

All (13) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	AAA	203	O6T	C11-N3	6.36	1.47	1.33
2	AAA	201	O6T	C11-N3	3.48	1.41	1.33
2	AAA	201	O6T	C19-C18	-2.88	1.49	1.53
2	AAA	203	O6T	O5-C26	2.87	1.26	1.22
2	AAA	201	O6T	C27-N6	-2.79	1.46	1.49
2	AAA	203	O6T	C10-N3	2.75	1.52	1.46
2	AAA	203	O6T	C5-S1	-2.59	1.78	1.82
2	AAA	201	O6T	C4-C3	2.51	1.57	1.53
2	AAA	201	O6T	C5-S1	-2.37	1.78	1.82
2	AAA	201	O6T	FE1-N6	-2.21	1.87	1.95
2	AAA	203	O6T	C12-C11	2.20	1.54	1.50
2	AAA	201	O6T	C19-N5	-2.15	1.46	1.49
2	AAA	203	O6T	C4-S1	-2.12	1.74	1.81

All (37) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	AAA	203	O6T	C5-C2-N1	-10.79	103.46	113.13
2	AAA	203	O6T	C4-C3-C2	5.49	113.43	108.66
2	AAA	203	O6T	C2-C5-S1	4.92	109.90	105.20
2	AAA	201	O6T	C28-C27-C29	4.28	120.31	111.37
2	AAA	203	O6T	C25-C23-C24	-4.17	103.27	109.31
2	AAA	201	O6T	C10-N3-C11	4.08	131.38	122.08
2	AAA	201	O6T	C16-N4-C18	-4.04	120.34	126.14
2	AAA	201	O6T	C28-C27-N6	-3.96	106.84	111.76
2	AAA	201	O6T	C15-N7-C30	-3.67	120.87	126.14
2	AAA	201	O6T	C4-C3-N2	-3.47	108.62	113.03
2	AAA	201	O6T	C9-C10-N3	3.25	121.49	112.21
2	AAA	203	O6T	C4-S1-C5	3.23	96.53	89.89
2	AAA	201	O6T	O2-C11-C12	-3.18	115.27	120.94
2	AAA	201	O6T	O3-C18-N4	-3.17	121.74	125.97
2	AAA	201	O6T	C27-N6-C26	3.16	120.89	118.69
2	AAA	203	O6T	O4-C22-C23	-3.00	113.71	121.18
2	AAA	203	O6T	C6-C5-C2	2.96	123.34	114.73
2	AAA	203	O6T	C10-N3-C11	-2.84	115.59	122.08
2	AAA	201	O6T	O6-C30-N7	-2.69	122.38	125.97
2	AAA	203	O6T	C29-C27-N6	-2.67	108.45	111.76
2	AAA	203	O6T	C4-C3-N2	-2.63	109.69	113.03
2	AAA	203	O6T	C25-C23-C26	2.54	112.96	108.20
2	AAA	201	O6T	N2-C1-N1	2.49	111.10	108.76
2	AAA	203	O6T	C28-C27-C30	-2.47	103.13	107.79
2	AAA	201	O6T	C12-C11-N3	2.44	122.34	117.09

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	AAA	203	O6T	C14-C15-C16	-2.42	117.33	120.92
2	AAA	201	O6T	C4-C3-C2	-2.40	106.58	108.66
2	AAA	203	O6T	C15-N7-C30	2.40	129.59	126.14
2	AAA	201	O6T	C29-C27-N6	-2.36	108.83	111.76
2	AAA	201	O6T	C20-C19-N5	2.29	114.61	111.76
2	AAA	201	O6T	C19-N5-C22	2.29	120.28	118.69
2	AAA	201	O6T	C14-C13-C12	2.20	123.33	120.78
2	AAA	203	O6T	O6-C30-N7	-2.18	123.05	125.97
2	AAA	203	O6T	C17-C16-N4	2.12	130.03	125.52
2	AAA	203	O6T	O5-C26-C23	-2.06	116.04	121.18
2	AAA	203	O6T	C27-N6-C26	2.05	120.11	118.69
2	AAA	203	O6T	C19-N5-C22	2.02	120.09	118.69

All (1) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
2	AAA	203	O6T	C5

All (12) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	AAA	203	O6T	S1-C5-C6-C8
3	AAA	204	GOL	C1-C2-C3-O3
3	AAA	204	GOL	O2-C2-C3-O3
2	AAA	203	O6T	C9-C10-N3-C11
2	AAA	203	O6T	C12-C11-N3-C10
2	AAA	203	O6T	O2-C11-N3-C10
2	AAA	201	O6T	C9-C10-N3-C11
2	AAA	203	O6T	N3-C10-C9-C8
2	AAA	201	O6T	N3-C11-C12-C13
2	AAA	201	O6T	N3-C11-C12-C17
2	AAA	203	O6T	C6-C8-C9-C10
3	AAA	202	GOL	O1-C1-C2-O2

There are no ring outliers.

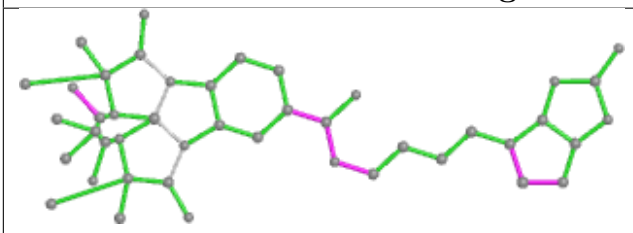
2 monomers are involved in 4 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	AAA	203	O6T	1	0
3	AAA	204	GOL	3	0

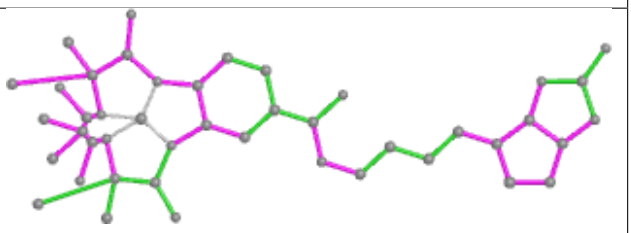
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths,

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 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.

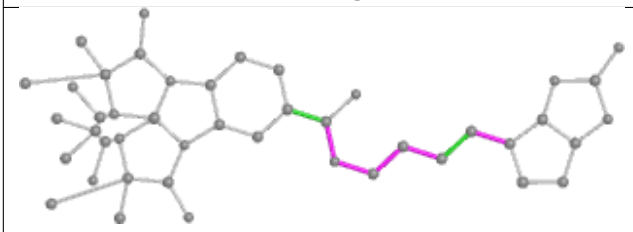
Ligand O6T AAA 203



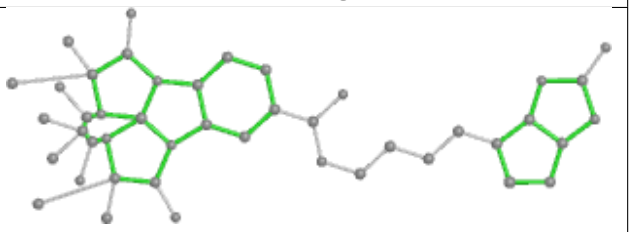
Bond lengths



Bond angles

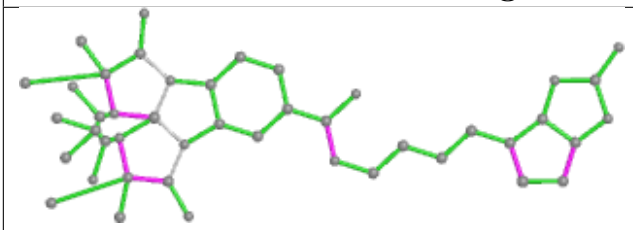


Torsions

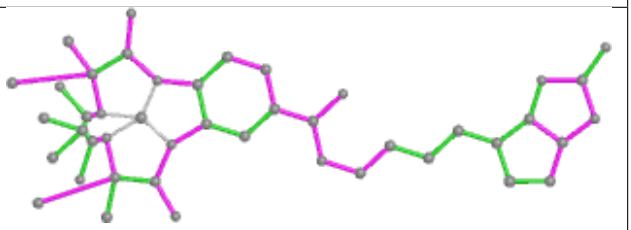


Rings

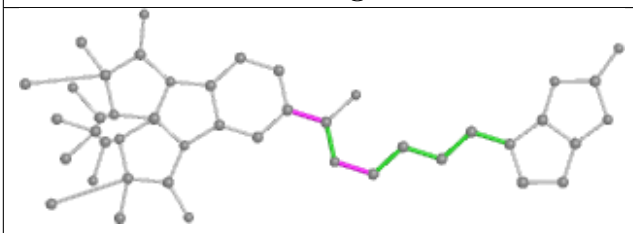
Ligand O6T AAA 201



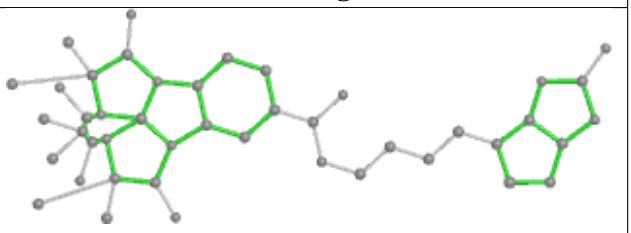
Bond lengths



Bond angles



Torsions



Rings

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	AAA	121/159 (76%)	0.04	8 (6%) 18 21	16, 23, 39, 58	0

All (8) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	AAA	66	THR	4.9
1	AAA	15	ALA	3.3
1	AAA	99	GLY	3.2
1	AAA	100	ALA	2.8
1	AAA	35	ALA	2.4
1	AAA	36	ASP	2.4
1	AAA	65	ALA	2.2
1	AAA	101	GLU	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 monosaccharides in this entry.

6.4 Ligands (i)

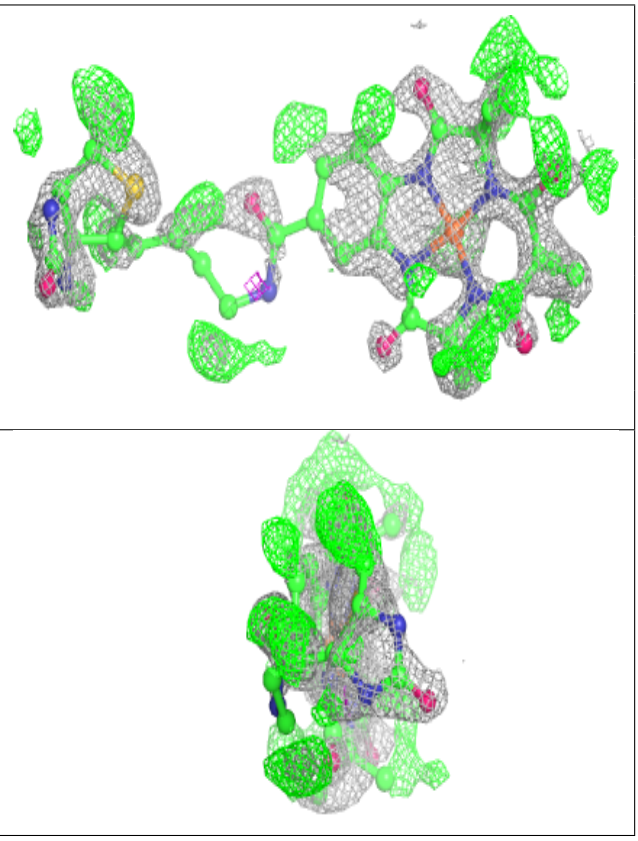
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95th percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists 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	O6T	AAA	203	44/44	0.80	0.22	26,32,47,49	44
3	GOL	AAA	204	6/6	0.83	0.16	50,62,77,77	2
3	GOL	AAA	202	6/6	0.94	0.24	45,48,58,58	14
2	O6T	AAA	201	44/44	0.95	0.18	16,37,43,47	27

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.

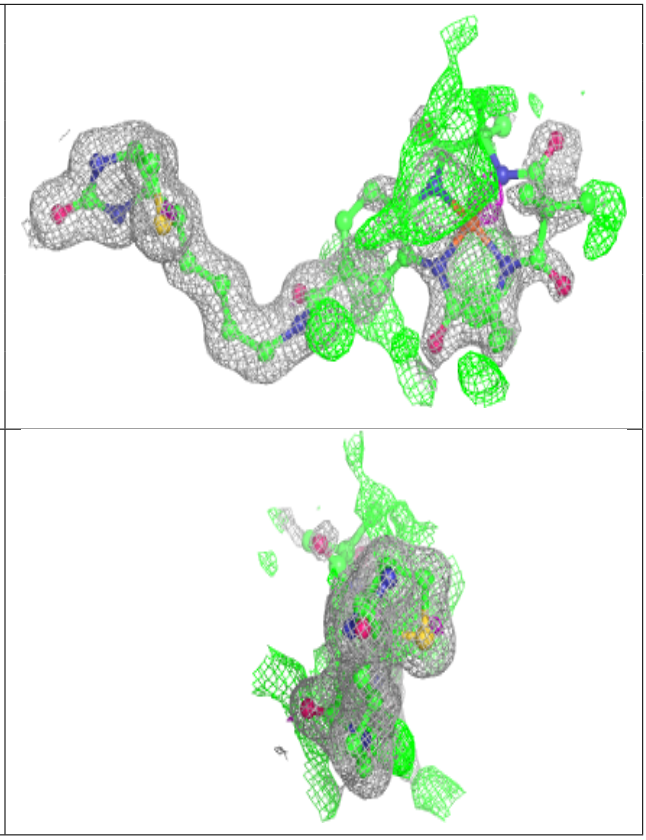
Electron density around O6T AAA 203:

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 O6T AAA 201:

$2mF_o$-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.