Full wwPDB X-ray Structure Validation Report ⓘ

Dec 9, 2023 – 08:44 am GMT

PDB ID	:	8AM0
Title	:	Crystal structure of human T1061E PI3Kalpha in complex with its regulatory subunit and the inhibitor GDC-0077 (Inavolisib)
Authors	:	Goncalves, M.; Johnson, J.L.; Roewer, K.M.
Deposited on	:	2022-08-02
Resolution	:	2.82 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:

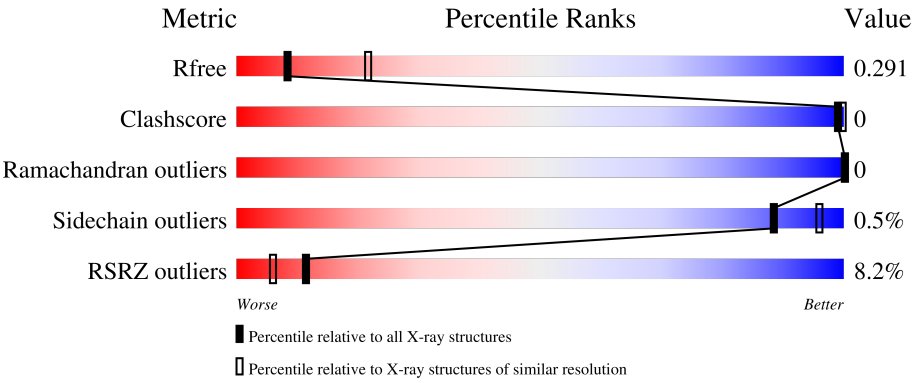
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
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 2.82 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	3617 (2.84-2.80)
Clashscore	141614	4060 (2.84-2.80)
Ramachandran outliers	138981	3978 (2.84-2.80)
Sidechain outliers	138945	3980 (2.84-2.80)
RSRZ outliers	127900	3552 (2.84-2.80)

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	1068	6% 95%
2	B	293	14% 86% 13%

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
4	EDO	B	601	-	-	-	X

2 Entry composition (i)

There are 7 unique types of molecules in this entry. The entry contains 10845 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 Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	1031	8443	5401	1447	1526	69	0	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1061	GLU	THR	engineered mutation	UNP P42336

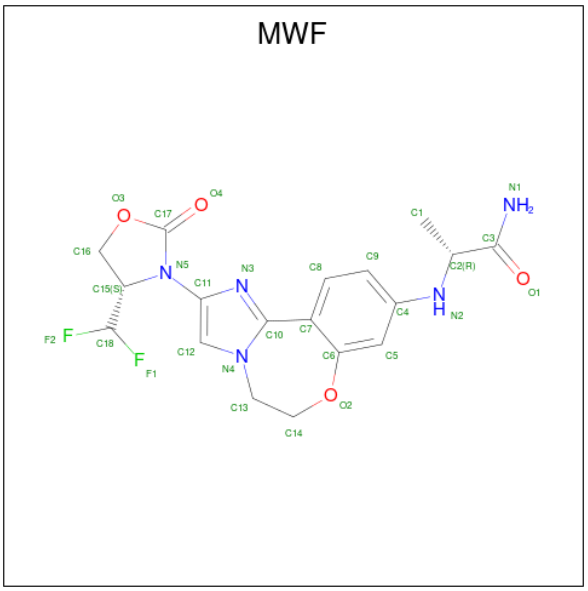
- Molecule 2 is a protein called Isoform 3 of Phosphatidylinositol 3-kinase regulatory subunit alpha.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
2	B	255	2161	1353	385	418	5	0	0	0

There are 7 discrepancies between the modelled and reference sequences:

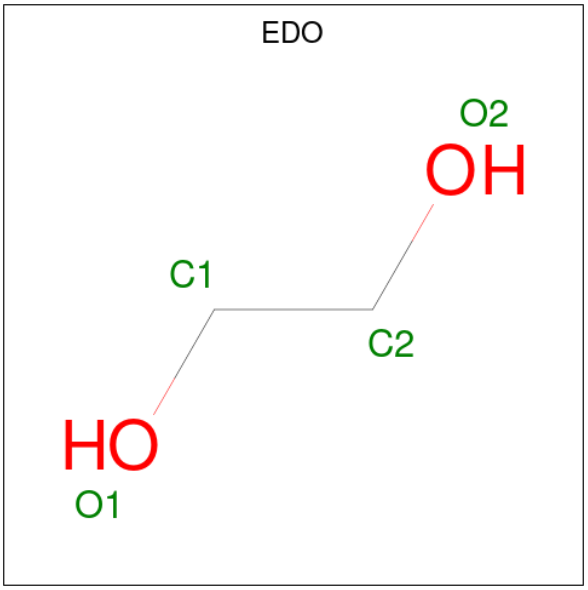
Chain	Residue	Modelled	Actual	Comment	Reference
B	301	MET	-	initiating methionine	UNP P27986-3
B	302	HIS	-	expression tag	UNP P27986-3
B	303	HIS	-	expression tag	UNP P27986-3
B	304	HIS	-	expression tag	UNP P27986-3
B	305	HIS	-	expression tag	UNP P27986-3
B	306	HIS	-	expression tag	UNP P27986-3
B	307	HIS	-	expression tag	UNP P27986-3

- Molecule 3 is (2R)-2-[[2-[(4S)-4-[bis(fluoranyl)methyl]-2-oxidanylidene-1,3-oxazolidin-3-yl]-5,6-dihydroimidazo[1,2-d][1,4]benzoxazepin-9-yl]amino]propanamide (three-letter code: MWF) (formula: $C_{18}H_{19}F_2N_5O_4$) (labeled as "Ligand of Interest" by depositor).



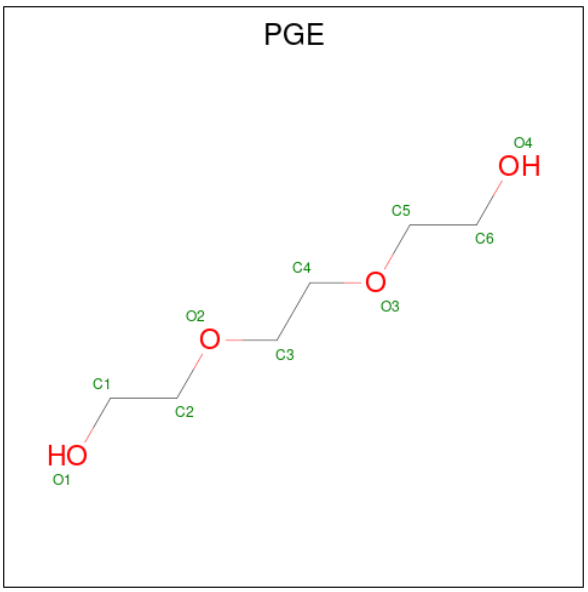
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	A	1	Total C F N O 29 18 2 5 4	0	0

- Molecule 4 is 1,2-ETHANEDIOL (three-letter code: EDO) (formula: $C_2H_6O_2$).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	A	1	Total C O 4 2 2	0	0
4	A	1	Total C O 4 2 2	0	0
4	B	1	Total C O 4 2 2	0	0

- Molecule 5 is TRIETHYLENE GLYCOL (three-letter code: PGE) (formula: $C_6H_{14}O_4$).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	A	1	Total C O 10 6 4	0	0

- Molecule 6 is CHLORIDE ION (three-letter code: CL) (formula: Cl).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
6	A	1	Total Cl 1 1	0	0

- Molecule 7 is water.

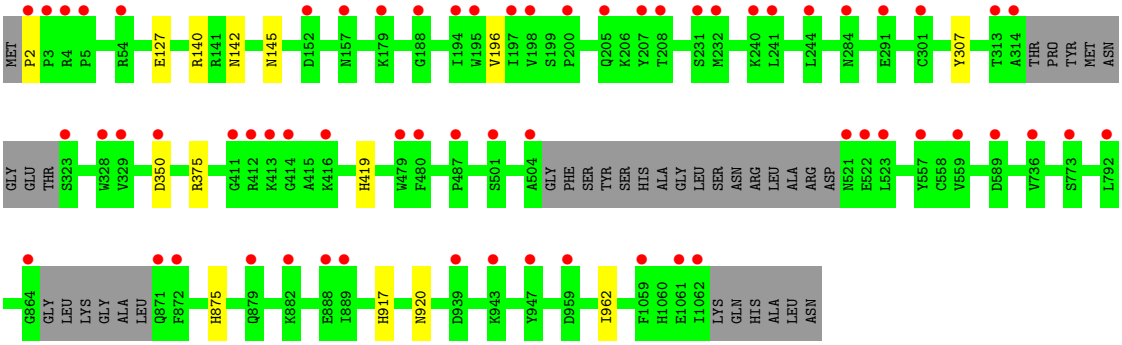
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
7	A	169	Total O 169 169	0	0
7	B	20	Total O 20 20	0	0

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.

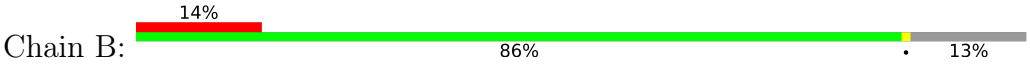
• Molecule 1: Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform

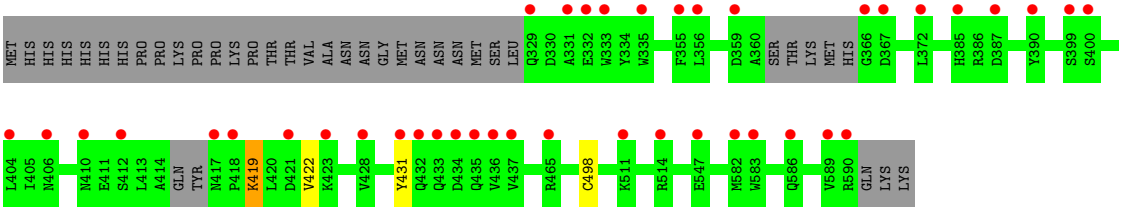
Chain A:



• Molecule 2: Isoform 3 of Phosphatidylinositol 3-kinase regulatory subunit alpha

Chain B:





4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α, β, γ	99.81Å 107.68Å 136.30Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	47.39 – 2.82 47.39 – 2.82	Depositor EDS
% Data completeness (in resolution range)	100.0 (47.39-2.82) 100.0 (47.39-2.82)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.33 (at 2.81Å)	Xtriage
Refinement program	BUSTER 2.11.8 (8-JUN-2022)	Depositor
R, R_{free}	0.285 , 0.300 0.272 , 0.291	Depositor DCC
R_{free} test set	1768 reflections (4.89%)	wwPDB-VP
Wilson B-factor (Å^2)	59.8	Xtriage
Anisotropy	0.308	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.30 , 40.0	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	0.90	EDS
Total number of atoms	10845	wwPDB-VP
Average B, all atoms (Å^2)	68.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 2.96% 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

Bond lengths and bond angles in the following residue types are not validated in this section: MWF, CL, PGE, EDO

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	0.36	0/8635	0.46	0/11667
2	B	0.35	0/2195	0.44	0/2939
All	All	0.36	0/10830	0.46	0/14606

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

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	8443	0	8462	6	0
2	B	2161	0	2129	1	0
3	A	29	0	0	0	0
4	A	8	0	12	0	0
4	B	4	0	6	0	0
5	A	10	0	14	0	0
6	A	1	0	0	0	0
7	A	169	0	0	0	0
7	B	20	0	0	0	0
All	All	10845	0	10623	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 0.

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)
1:A:2:PRO:N	1:A:127:GLU:OE1	2.34	0.60
1:A:917:HIS:NE2	1:A:920:ASN:ND2	2.62	0.48
2:B:419:LYS:HD2	2:B:419:LYS:O	2.17	0.44
1:A:142:ASN:O	1:A:145:ASN:OD1	2.38	0.42
1:A:875:HIS:ND1	1:A:962:ILE:HG21	2.36	0.41
1:A:140:ARG:NH2	1:A:307:TYR:CE2	2.89	0.40
1:A:375:ARG:NH1	1:A:419:HIS:HE1	2.20	0.40

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	A	1023/1068 (96%)	980 (96%)	43 (4%)	0	100	100
2	B	249/293 (85%)	246 (99%)	3 (1%)	0	100	100
All	All	1272/1361 (94%)	1226 (96%)	46 (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
1	A	946/974 (97%)	944 (100%)	2 (0%)	93 98
2	B	235/272 (86%)	231 (98%)	4 (2%)	60 86
All	All	1181/1246 (95%)	1175 (100%)	6 (0%)	88 96

All (6) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	196	VAL
1	A	350	ASP
2	B	419	LYS
2	B	422	VAL
2	B	431	TYR
2	B	498	CYS

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (2) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	495	HIS
1	A	665	HIS

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 monosaccharides in this entry.

5.6 Ligand geometry

Of 6 ligands modelled in this entry, 1 is 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 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	$\#\|Z\| > 2$
4	EDO	A	2002	-	3,3,3	0.27	0	2,2,2	0.25	0
5	PGE	A	2004	-	9,9,9	0.12	0	8,8,8	0.10	0
3	MWF	A	2001	-	28,32,32	1.03	2 (7%)	34,47,47	1.96	4 (11%)
4	EDO	B	601	-	3,3,3	0.26	0	2,2,2	0.26	0
4	EDO	A	2003	-	3,3,3	0.26	0	2,2,2	0.24	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
4	EDO	A	2002	-	-	1/1/1/1	-
5	PGE	A	2004	-	-	4/7/7/7	-
3	MWF	A	2001	-	-	4/12/39/39	0/3/4/4
4	EDO	B	601	-	-	1/1/1/1	-
4	EDO	A	2003	-	-	1/1/1/1	-

All (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	2001	MWF	C13-N4	-3.35	1.46	1.49
3	A	2001	MWF	C13-C14	-2.23	1.48	1.51

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	A	2001	MWF	O2-C14-C13	-9.00	106.30	112.62
3	A	2001	MWF	C4-N2-C2	3.71	129.22	122.49
3	A	2001	MWF	F2-C18-C15	2.90	112.52	109.33
3	A	2001	MWF	F1-C18-C15	2.78	112.40	109.33

There are no chirality outliers.

All (11) torsion outliers are listed below:

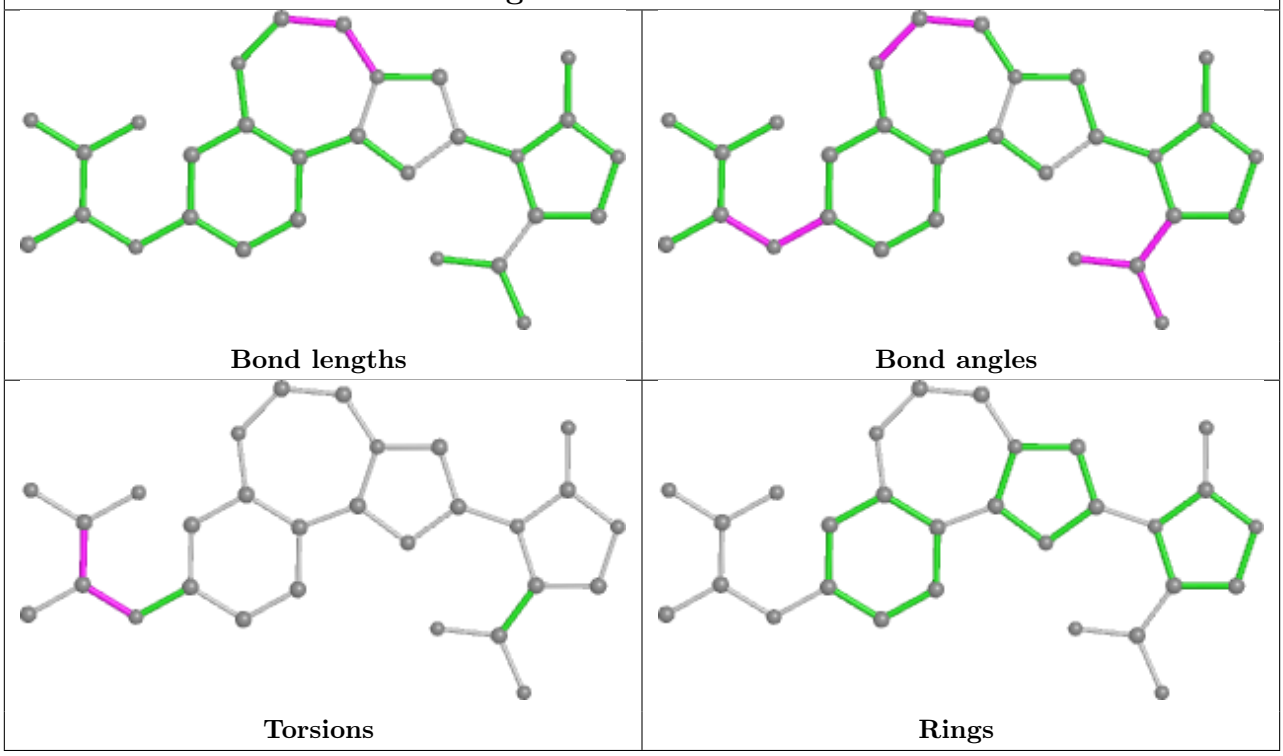
Mol	Chain	Res	Type	Atoms
3	A	2001	MWF	C1-C2-C3-N1
3	A	2001	MWF	C1-C2-C3-O1
5	A	2004	PGE	O2-C3-C4-O3
5	A	2004	PGE	O1-C1-C2-O2
4	B	601	EDO	O1-C1-C2-O2
3	A	2001	MWF	C3-C2-N2-C4
4	A	2002	EDO	O1-C1-C2-O2
5	A	2004	PGE	C4-C3-O2-C2
4	A	2003	EDO	O1-C1-C2-O2
3	A	2001	MWF	N2-C2-C3-O1
5	A	2004	PGE	O3-C5-C6-O4

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 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 MWF A 2001



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

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	1031/1068 (96%)	0.48	64 (6%) 20 13	37, 61, 116, 141	0
2	B	255/293 (87%)	0.91	41 (16%) 1 1	45, 83, 125, 138	0
All	All	1286/1361 (94%)	0.57	105 (8%) 11 6	37, 64, 120, 141	0

All (105) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	411	GLY	6.0
2	B	333	TRP	5.4
2	B	418	PRO	5.2
1	A	198	VAL	5.1
1	A	864	GLY	4.9
1	A	501	SER	4.9
1	A	3	PRO	4.8
1	A	2	PRO	4.7
1	A	522	GLU	4.6
2	B	431	TYR	4.6
2	B	332	GLU	4.2
1	A	231	SER	4.1
1	A	1062	ILE	4.0
1	A	291	GLU	3.9
1	A	1061	GLU	3.9
1	A	889	ILE	3.8
1	A	947	TYR	3.8
2	B	329	GLN	3.7
2	B	399	SER	3.7
2	B	589	VAL	3.7
1	A	871	GLN	3.6
2	B	406	ASN	3.5
2	B	331	ALA	3.5
1	A	521	ASN	3.5

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Mol	Chain	Res	Type	RSRZ
1	A	1059	PHE	3.4
2	B	433	GLN	3.4
2	B	435	GLN	3.3
1	A	412	ARG	3.3
2	B	417	ASN	3.3
1	A	197	ILE	3.3
1	A	888	GLU	3.2
2	B	428	VAL	3.2
2	B	387	ASP	3.2
1	A	313	THR	3.1
1	A	329	VAL	2.9
1	A	872	PHE	2.9
1	A	939	ASP	2.9
1	A	314	ALA	2.9
2	B	410	ASN	2.9
1	A	179	LYS	2.9
1	A	480	PHE	2.9
1	A	479	TRP	2.8
2	B	367	ASP	2.8
1	A	350	ASP	2.8
1	A	413	LYS	2.8
1	A	208	THR	2.8
1	A	232	MET	2.7
1	A	416	LYS	2.7
2	B	390	TYR	2.7
1	A	244	LEU	2.7
2	B	582	MET	2.6
1	A	504	ALA	2.6
2	B	372	LEU	2.6
1	A	195	TRP	2.6
2	B	400	SER	2.5
2	B	583	TRP	2.5
2	B	514	ARG	2.5
2	B	421	ASP	2.5
1	A	879	GLN	2.5
2	B	366	GLY	2.5
1	A	328	TRP	2.4
2	B	590	ARG	2.4
1	A	54	ARG	2.4
2	B	404	LEU	2.4
2	B	335	TRP	2.4
2	B	432	GLN	2.4

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Mol	Chain	Res	Type	RSRZ
2	B	355	PHE	2.3
2	B	586	GLN	2.3
1	A	157	ASN	2.3
1	A	559	VAL	2.3
2	B	436	VAL	2.3
1	A	188	GLY	2.3
2	B	356	LEU	2.3
1	A	284	ASN	2.2
1	A	323	SER	2.2
1	A	5	PRO	2.2
1	A	414	GLY	2.2
2	B	511	LYS	2.2
1	A	194	ILE	2.2
1	A	4	ARG	2.2
1	A	205	GLN	2.2
1	A	959	ASP	2.2
2	B	437	VAL	2.2
1	A	557	TYR	2.2
2	B	359	ASP	2.2
2	B	412	SER	2.2
1	A	523	LEU	2.2
2	B	465	ARG	2.2
1	A	207	TYR	2.2
1	A	152	ASP	2.1
1	A	241	LEU	2.1
1	A	589	ASP	2.1
1	A	943	LYS	2.1
1	A	736	VAL	2.1
2	B	547	GLU	2.1
1	A	240	LYS	2.1
1	A	792	LEU	2.0
1	A	200	PRO	2.0
1	A	487	PRO	2.0
2	B	385	HIS	2.0
2	B	423	LYS	2.0
1	A	301	CYS	2.0
1	A	773	SER	2.0
1	A	882	LYS	2.0
2	B	434	ASP	2.0

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 monosaccharides in this entry.

6.4 Ligands (i)

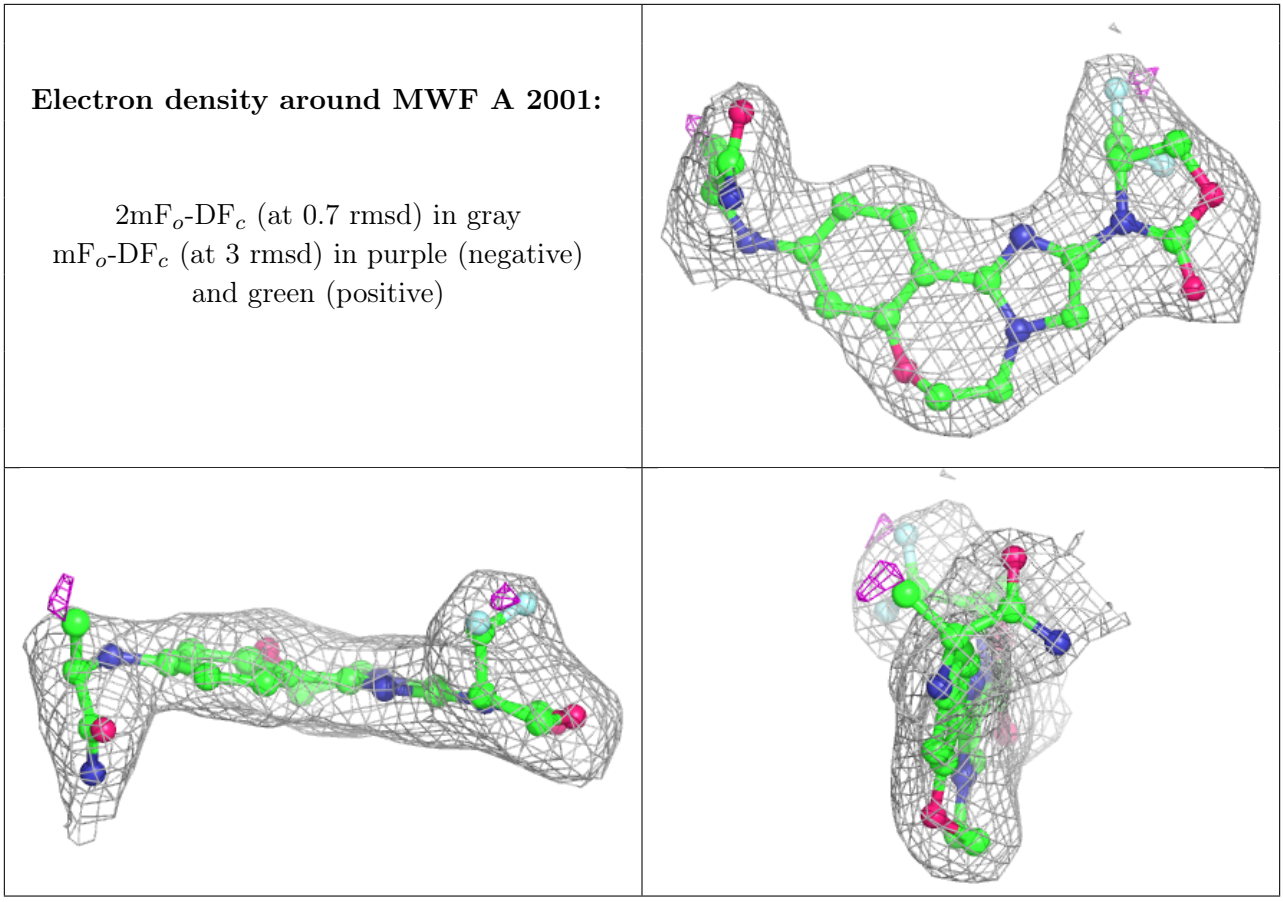
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95^{th} percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists the number of atoms with occupancy less than 0.9.

Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
4	EDO	B	601	4/4	0.49	0.46	73,73,73,73	0
4	EDO	A	2003	4/4	0.60	0.40	74,74,74,74	0
4	EDO	A	2002	4/4	0.64	0.34	51,51,51,51	0
5	PGE	A	2004	10/10	0.74	0.18	69,69,69,69	0
3	MWF	A	2001	29/29	0.93	0.20	45,45,45,45	0
6	CL	A	2005	1/1	0.95	0.13	64,64,64,64	0

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 MWF A 2001:

$2mF_o$-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.