wwPDB X-ray Structure Validation Summary Report (i)

Feb 3, 2025 – 06:06 pm GMT

PDB ID	:	9GC4
Title	:	Highly optimized CNS penetrant inhibitors of EGFR Exon20 Insertion Mutations
Authors	:	Hargreaves, D.
Deposited on	:	2024-08-01
Resolution	:	2.42 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 (i)) were used in the production of this report:

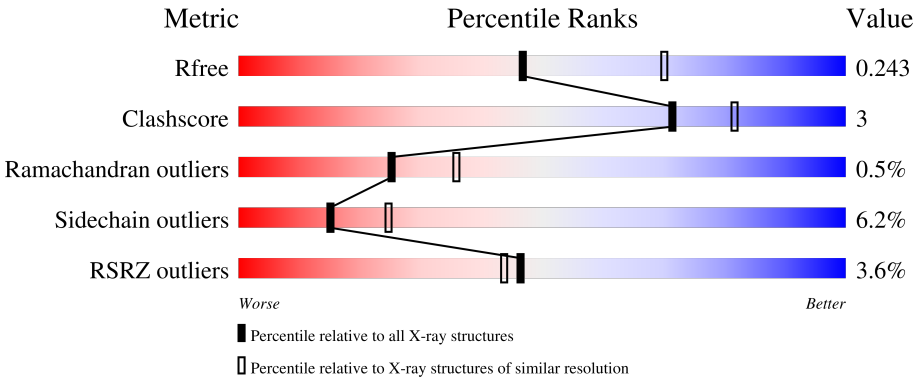
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	3.0
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
CCP4	:	9.0.003 (Gargrove)
Density-Fitness	:	1.0.11
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.40

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 2.42 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}	164625	5670 (2.44-2.40)
Clashscore	180529	6299 (2.44-2.40)
Ramachandran outliers	177936	6232 (2.44-2.40)
Sidechain outliers	177891	6233 (2.44-2.40)
RSRZ outliers	164620	5670 (2.44-2.40)

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	332	2% 75% 11% 14%
1	B	332	4% 76% 9% 14%
1	D	332	% 95%

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 4963 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.

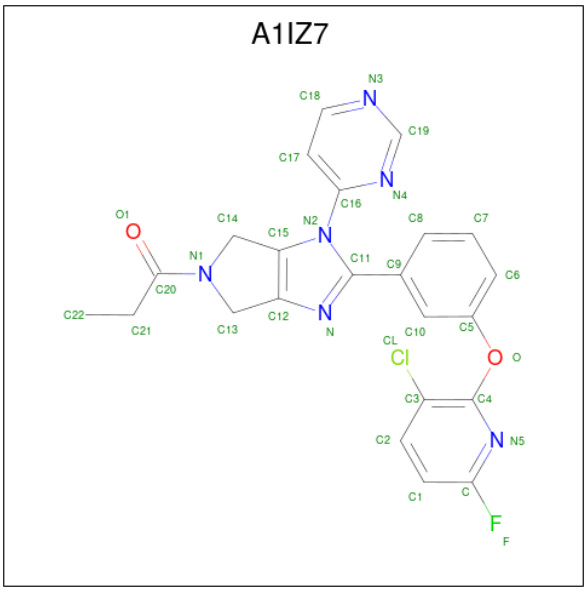
- Molecule 1 is a protein called Epidermal growth factor receptor.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	285	Total	C	N	O	S	0	0	0
			2282	1470	389	408	15			
1	B	285	Total	C	N	O	S	0	0	0
			2282	1470	389	408	15			
1	D	17	Total	C	N	O	S	0	0	0
			138	83	17	36	2			

There are 15 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	694	GLY	-	expression tag	UNP P00533
A	771	ASN	-	insertion	UNP P00533
A	772	PRO	-	insertion	UNP P00533
A	773	GLY	-	insertion	UNP P00533
A	951	ARG	VAL	engineered mutation	UNP P00533
B	694	GLY	-	expression tag	UNP P00533
B	771	ASN	-	insertion	UNP P00533
B	772	PRO	-	insertion	UNP P00533
B	773	GLY	-	insertion	UNP P00533
B	951	ARG	VAL	engineered mutation	UNP P00533
D	694	GLY	-	expression tag	UNP P00533
D	771	ASN	-	insertion	UNP P00533
D	772	PRO	-	insertion	UNP P00533
D	773	GLY	-	insertion	UNP P00533
D	951	ARG	VAL	engineered mutation	UNP P00533

- Molecule 2 is 1-[2-[3-(3-chloranyl-6-fluoranyl-pyridin-2-yl)oxyphenyl]-3-pyrimidin-4-yl-4,6-dihydropyrrolo[3,4-d]imidazol-5-yl]propan-1-one (three-letter code: A1IZ7) (formula: $C_{23}H_{18}ClFN_6O_2$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms						ZeroOcc	AltConf
2	A	1	Total	C	Cl	F	N	O	0	0
			33	23	1	1	6	2		
2	B	1	Total	C	Cl	F	N	O	0	0
			33	23	1	1	6	2		

- Molecule 3 is water.

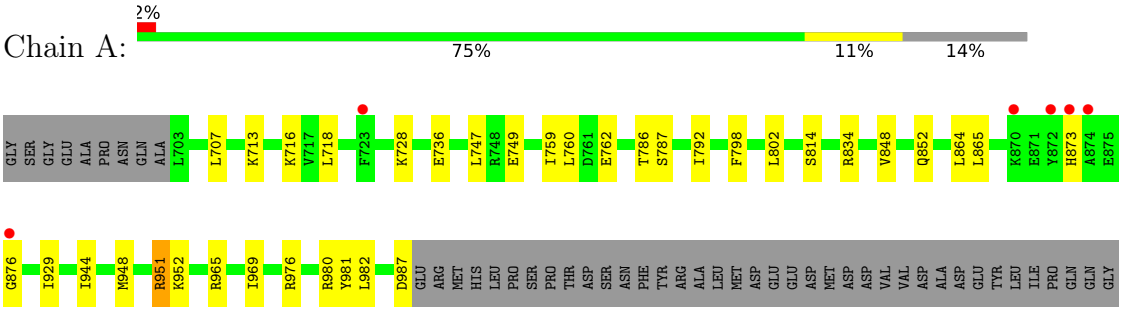
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	A	105	Total	O	0	0
			105	105		
3	B	85	Total	O	0	0
			85	85		
3	D	5	Total	O	0	0
			5	5		

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.

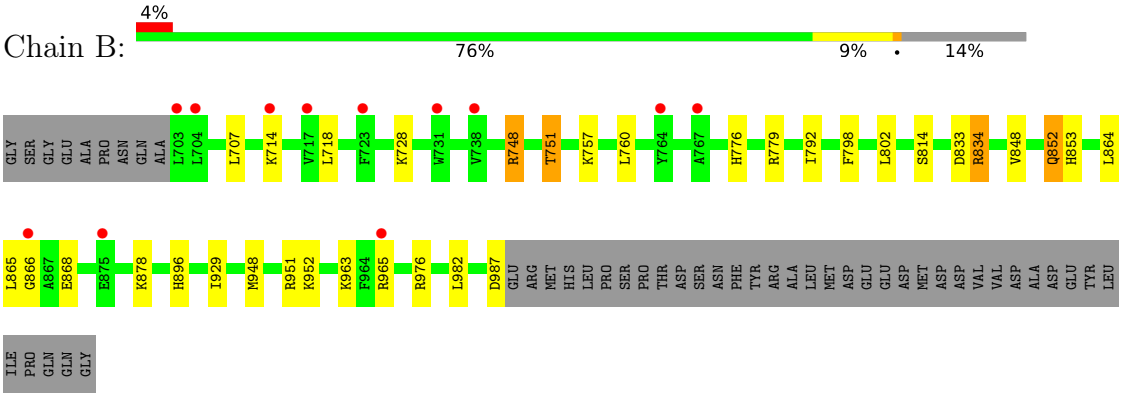
• Molecule 1: Epidermal growth factor receptor

Chain A:



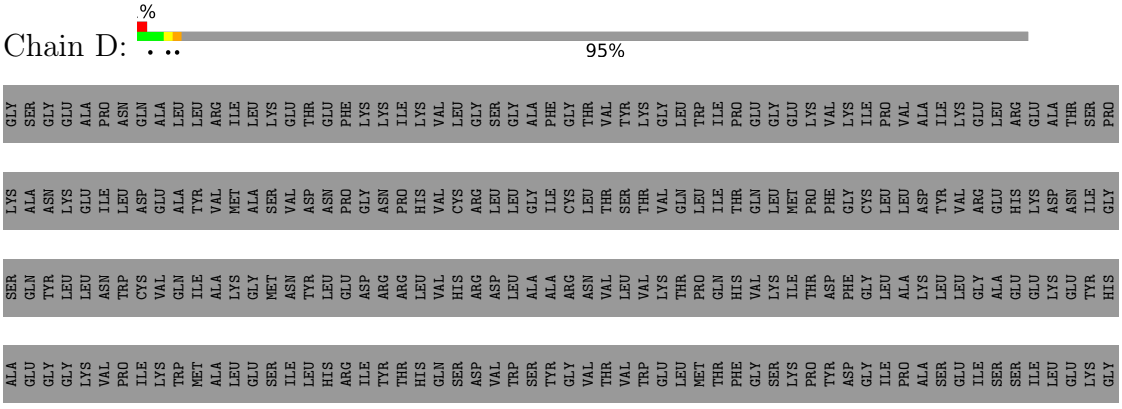
• Molecule 1: Epidermal growth factor receptor

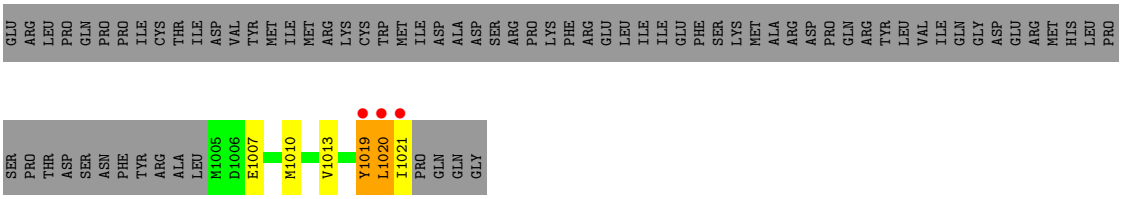
Chain B:



• Molecule 1: Epidermal growth factor receptor

Chain D:




GLU ARG LEU PRO GLN PRO PRO ILE CYS THR ILE ASP VAL TYR MET ILE MET ARG LYS CYS TRP MET ILE ASP ALA ASP SER ARG PRO LYS PHE ARG GLU LEU ILE ILE GLU PHE SER LYS MET ALA ARG ASP PRO GLN ARG TYR LEU VAL ILE GLN GLY ASP GLU ARG MET HIS LEU PRO
SER PRO THR ASP SER ASN PHE TYR ARG ALA LEU M1005 D1006 E1007 M1010 V1013 Y1019 L1020 T1021 PRO GLN GLN GLY

4 Data and refinement statistics (i)

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants a, b, c, α, β, γ	134.52Å 84.25Å 96.47Å 90.00° 121.29° 90.00°	Depositor
Resolution (Å)	26.91 – 2.42 26.91 – 2.42	Depositor EDS
% Data completeness (in resolution range)	66.2 (26.91-2.42) 66.1 (26.91-2.42)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.60 (at 2.42Å)	Xtriage
Refinement program	BUSTER 2.11.8 (26-JUL-2023)	Depositor
R, R_{free}	0.216 , 0.248 0.206 , 0.243	Depositor DCC
R_{free} test set	13128 reflections (4.86%)	wwPDB-VP
Wilson B-factor (Å^2)	50.3	Xtriage
Anisotropy	0.064	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.32 , 46.7	EDS
L-test for twinning[2]	$< \|L\| > = 0.48$, $< L^2 > = 0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.93	EDS
Total number of atoms	4963	wwPDB-VP
Average B, all atoms (Å^2)	53.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.03% 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: A1IZ7

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	0/2333	0.56	0/3155
1	B	0.36	0/2333	0.54	0/3155
1	D	0.47	0/138	0.73	0/186
All	All	0.36	0/4804	0.56	0/6496

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	2282	0	2340	17	1
1	B	2282	0	2340	14	0
1	D	138	0	113	8	0
2	A	33	0	0	0	0
2	B	33	0	0	0	0
3	A	105	0	0	0	0
3	B	85	0	0	2	0
3	D	5	0	0	0	0
All	All	4963	0	4793	31	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 3.

The worst 5 of 31 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:B:852:GLN:HB2	1:D:1010:MET:HG2	1.68	0.75
1:A:786:THR:HG22	1:A:787:SER:H	1.50	0.73
1:A:976:ARG:HH21	1:D:1019:TYR:HB2	1.62	0.65
1:A:736:GLU:OE1	1:B:976:ARG:NH1	2.30	0.63
1:B:748:ARG:O	1:B:751:THR:HB	2.07	0.55

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:749:GLU:OE2	1:A:951:ARG:NH2[4_546]	2.14	0.06

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	283/332 (85%)	274 (97%)	8 (3%)	1 (0%)	30	42
1	B	283/332 (85%)	271 (96%)	11 (4%)	1 (0%)	30	42
1	D	15/332 (4%)	12 (80%)	2 (13%)	1 (7%)	1	0
All	All	581/996 (58%)	557 (96%)	21 (4%)	3 (0%)	25	36

All (3) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	866	GLY
1	D	1020	LEU
1	A	876	GLY

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	250/290 (86%)	238 (95%)	12 (5%)	21 35
1	B	250/290 (86%)	232 (93%)	18 (7%)	12 19
1	D	16/290 (6%)	14 (88%)	2 (12%)	3 4
All	All	516/870 (59%)	484 (94%)	32 (6%)	15 25

5 of 32 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	965	ARG
1	B	987	ASP
1	B	714	LYS
1	A	987	ASP
1	D	1007	GLU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 15 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	756	ASN
1	B	896	HIS
1	B	774	ASN
1	B	938	GLN
1	B	845	ASN

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 oligosaccharides in this entry.

5.6 Ligand geometry (i)

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	A1IZ7	B	1101	1	32,37,37	0.53	0	33,53,53	1.49	2 (6%)
2	A1IZ7	A	1101	1	32,37,37	0.48	0	33,53,53	1.27	1 (3%)

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	A1IZ7	B	1101	1	-	2/15/26/26	0/5/5/5
2	A1IZ7	A	1101	1	-	1/15/26/26	0/5/5/5

There are no bond length outliers.

All (3) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	B	1101	A1IZ7	C16-N2-C11	6.48	133.28	124.57
2	A	1101	A1IZ7	C16-N2-C11	5.79	132.35	124.57
2	B	1101	A1IZ7	C12-C13-N1	3.69	105.75	102.35

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	1101	A1IZ7	N1-C20-C21-C22

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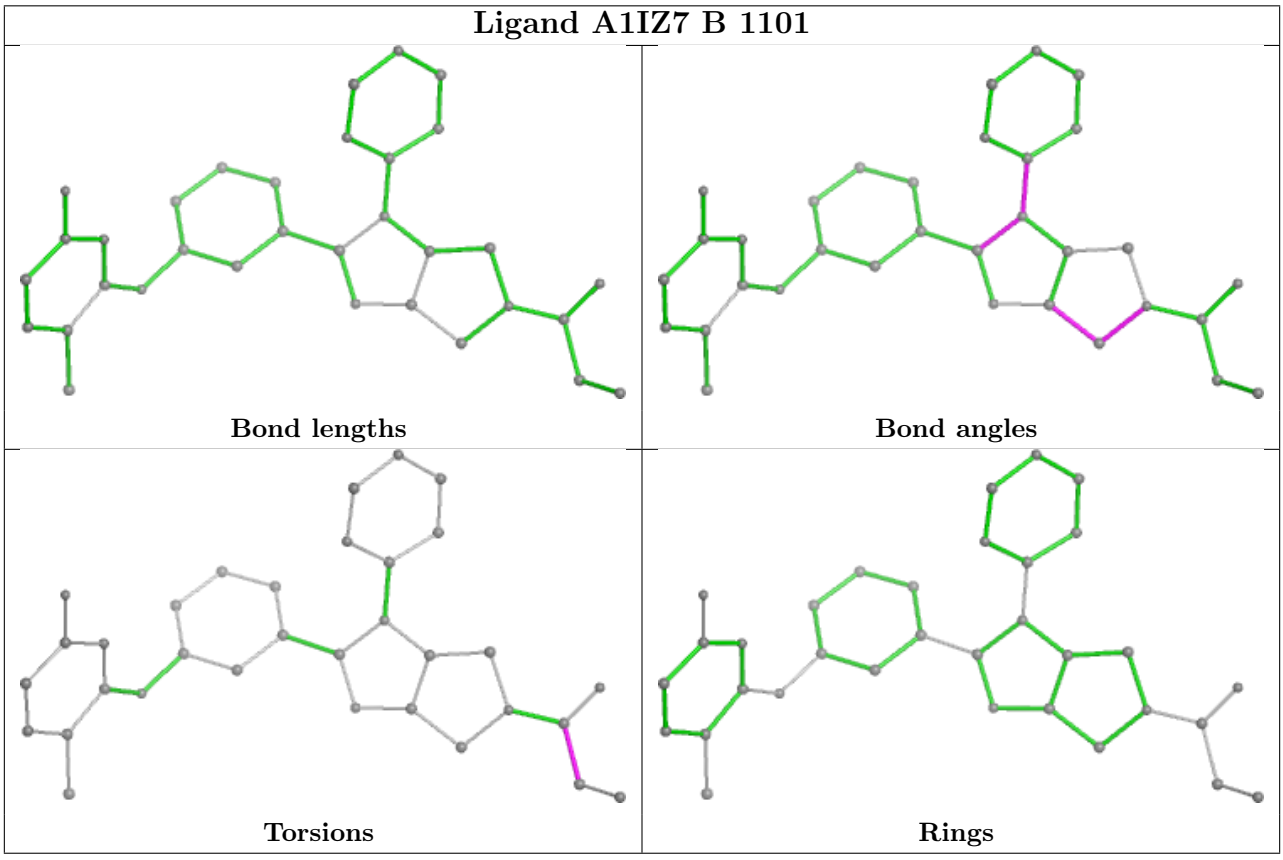
Mol	Chain	Res	Type	Atoms
2	B	1101	A1IZ7	N1-C20-C21-C22
2	B	1101	A1IZ7	O1-C20-C21-C22

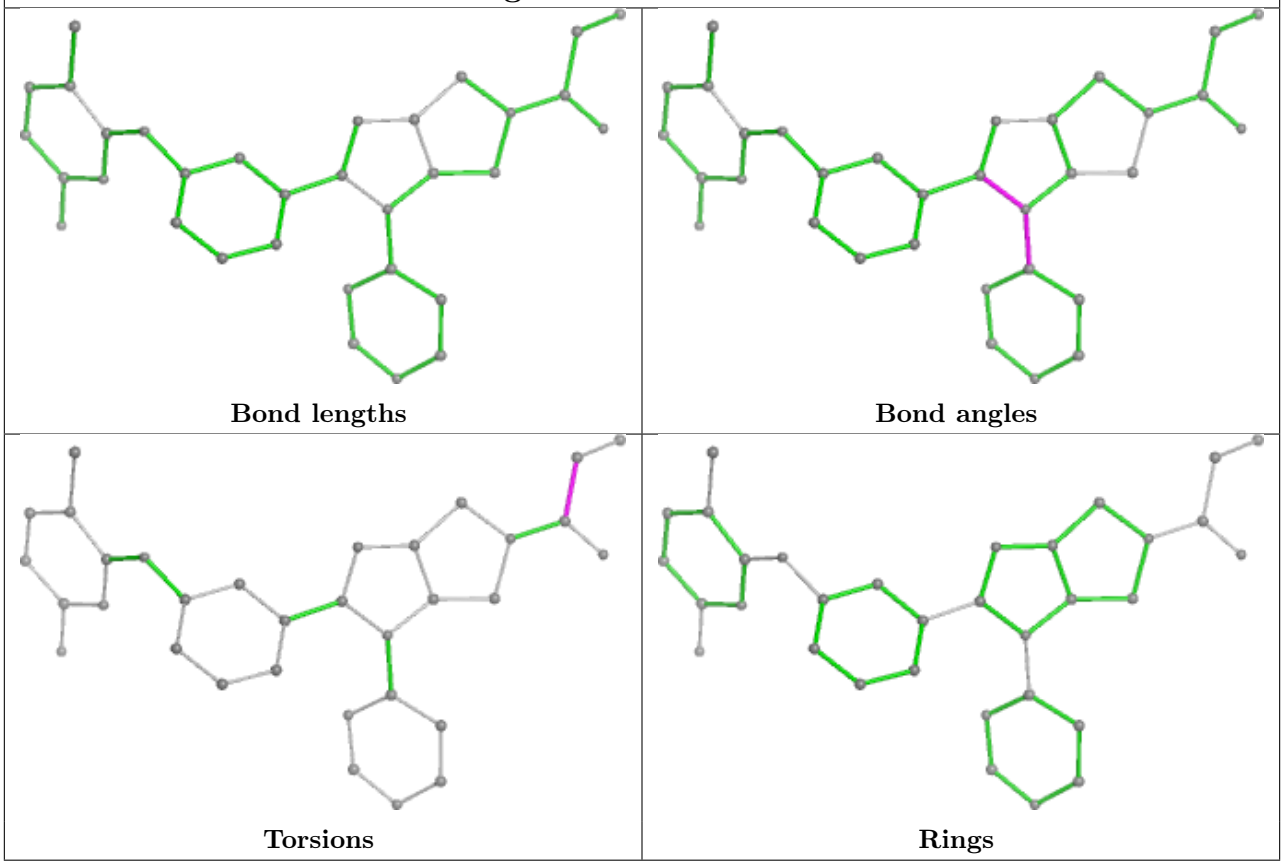
There are no ring outliers.

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 A1IZ7 A 1101



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	285/332 (85%)	0.01	6 (2%)	63	61	33, 48, 74, 106	0
1	B	285/332 (85%)	0.26	12 (4%)	41	39	33, 52, 83, 100	0
1	D	17/332 (5%)	0.93	3 (17%)	4	4	46, 64, 97, 99	0
All	All	587/996 (58%)	0.16	21 (3%)	46	44	33, 50, 83, 106	0

The worst 5 of 21 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	D	1021	ILE	4.6
1	A	874	ALA	4.3
1	A	872	TYR	4.1
1	B	875	GLU	3.5
1	B	738	VAL	3.3

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

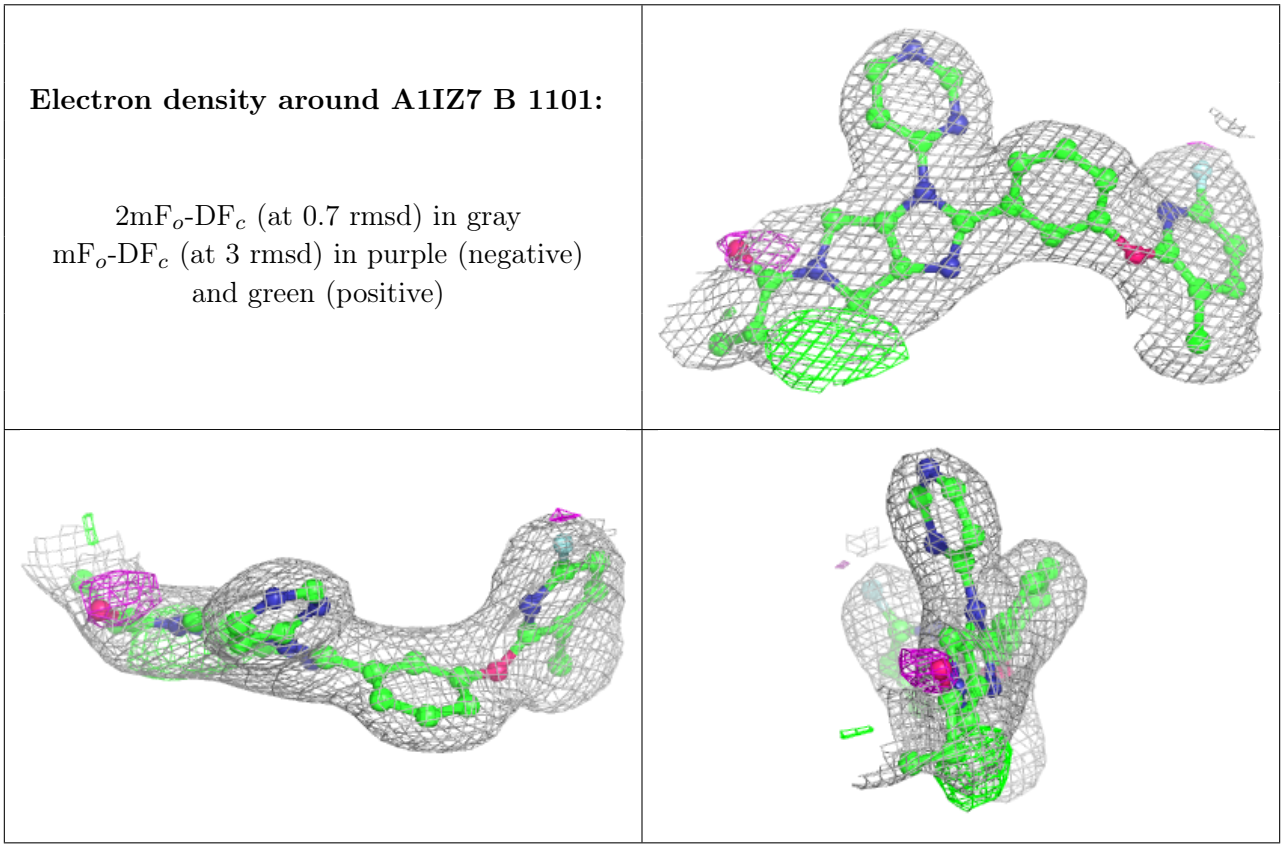
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95^{th} percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' 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
2	A1IZ7	B	1101	33/33	0.90	0.11	50,51,53,55	0
2	A1IZ7	A	1101	33/33	0.93	0.09	42,46,51,53	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.

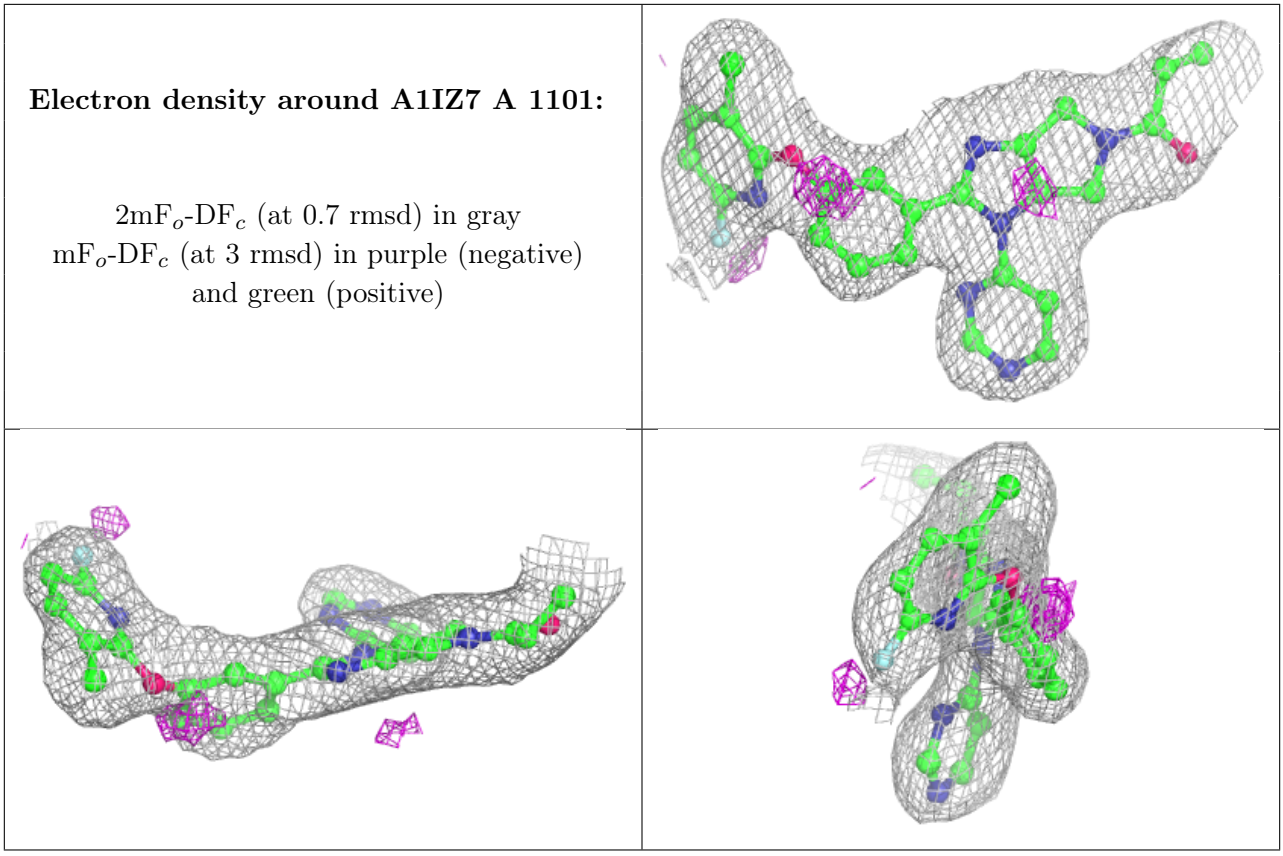
Electron density around A1IZ7 B 1101:

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



Electron density around A1IZ7 A 1101:

$2mF_o$-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.