

# Full wwPDB X-ray Structure Validation Report (i)

#### May 20, 2024 - 03:29 pm BST

PDB ID	:	8QZ4
Title	:	Crystal structure of human two pore domain potassium ion channel TREK-2
		(K2P10.1) in complex with an activatory nanobody $(Nb76)$
Authors	:	Rodstrom, K.E.J.; Pike, A.C.W.; Baronina, A.; Ang, J.; Bushell, S.R.; Chalk,
		R.; Mukhopadhyay, S.M.M.; Pardon, E.; Arrowsmith, C.H.; Edwards, A.M.;
		Bountra, C.; Burgess-Brown, N.A.; Tucker, S.J.; Steyaert, J.; Carpenter, E.P.;
		Structural Genomics Consortium (SGC)
Deposited on	:	2023-10-26
Resolution	:	3.20 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:

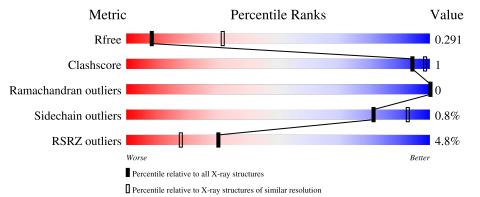
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as $541$ be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36.2
buster-report	:	1.1.7(2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber $(2001)$

# 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 3.20 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	$\begin{array}{c} \textbf{Whole archive} \\ (\#\textbf{Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries,\ resolution\ range}({ m \AA}))$
$R_{free}$	130704	1133 (3.20-3.20)
Clashscore	141614	1253 (3.20-3.20)
Ramachandran outliers	138981	1234 (3.20-3.20)
Sidechain outliers	138945	1233 (3.20-3.20)
RSRZ outliers	127900	1095 (3.20-3.20)

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	А	282	5% 86%	• 11%
1	В	282	2% <b>8</b> 9%	• 8%
2	С	123	87%	• 9%
			Continued on	next page

Ideal geometry (DNA, RNA) : Parkinson et al. (1996) Validation Pipeline (wwPDB-VP) : 2.36.2



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Mol	Chain	Length	Quality of chain		
			11%		
2	D	123	89%	•	9%

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	Y01	А	403	-	-	-	Х
4	Y01	А	404	-	-	-	Х



# 2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 5473 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.

Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace	
1	Δ	250	Total	С	Ν	Ο	S	0	0	0
	A	230	1794	1194	278	318	4	0	0	0
1	р	259	Total	С	Ν	0	S	0	0	0
	D	239	1882	1254	288	336	4	0	U	U

• Molecule 1 is a protein called Potassium channel subfamily K member 10.

Chain	Residue	Modelled	Actual	Actual Comment	
А	66	MET	- initiating methionine		UNP P57789
А	341	ALA	-	expression tag	UNP P57789
А	342	GLU	-	expression tag	UNP P57789
А	343	ASN	-	expression tag	UNP P57789
А	344	LEU	-	expression tag	UNP P57789
А	345	TYR	-	expression tag	UNP P57789
А	346	PHE	-	expression tag	UNP P57789
А	347	GLN	-	expression tag	UNP P57789
В	66	MET	-	initiating methionine	UNP P57789
В	341	ALA	-	expression tag	UNP P57789
В	342	GLU	-	expression tag	UNP P57789
В	343	ASN	-	expression tag	UNP P57789
В	344	LEU	-	expression tag	UNP P57789
В	345	TYR	-	expression tag	UNP P57789
В	346	PHE	-	expression tag	UNP P57789
В	347	GLN	-	expression tag	UNP P57789

There are 16 discrepancies between the modelled and reference sequences:

• Molecule 2 is a protein called Nanobody 76.

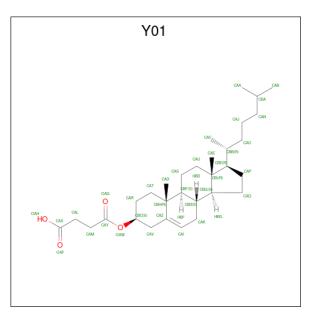
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
9	С	112	Total	С	Ν	0	S	0	0	0
	U	112	839	527	150	158	4	0	0	0
0	р	112	Total	С	Ν	0	S	0	0	0
	D	112	806	508	141	153	4	0	0	0



• Molecule 3 is BARIUM ION (three-letter code: BA) (formula: Ba).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	А	2	Total Ba 2 2	0	0
3	В	2	Total Ba 2 2	0	0

• Molecule 4 is CHOLESTEROL HEMISUCCINATE (three-letter code: Y01) (formula:  $C_{31}H_{50}O_4$ ).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	1	Total C O 35 31 4	0	0
4	А	1	Total C O	0	0
			$\begin{array}{cccc} 35 & 31 & 4 \\ \hline \text{Total} & \text{C} & \text{O} \end{array}$		
4	А	1	35 31 4	0	0
4	В	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 35 & 31 & 4 \end{array}$	0	0

• Molecule 5 is water.

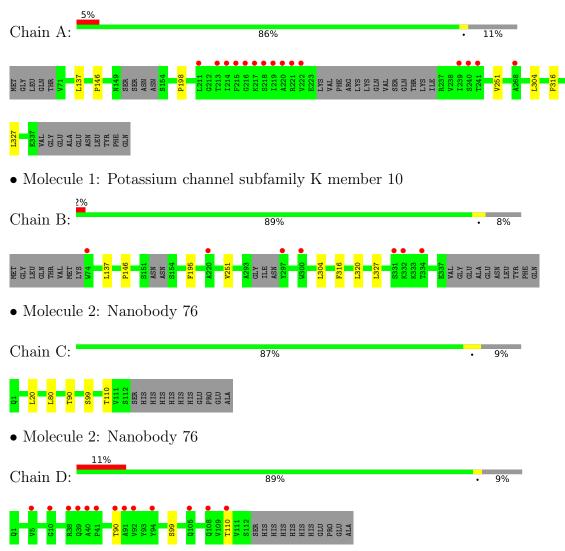
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	А	3	Total O 3 3	0	0
5	В	4	Total O 4 4	0	0
5	С	1	Total O 1 1	0	0



# 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: Potassium channel subfamily K member 10





## 4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants	68.28Å 75.58Å 259.15Å	Depositor
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $90.00^{\circ}$ $90.00^{\circ}$	Depositor
Resolution (Å)	31.11 - 3.20	Depositor
Resolution (A)	50.66 - 3.20	EDS
% Data completeness	77.8 (31.11-3.20)	Depositor
(in resolution range)	77.8(50.66-3.20)	EDS
R <sub>merge</sub>	(Not available)	Depositor
R <sub>sym</sub>	(Not available)	Depositor
$< I/\sigma(I) > 1$	$1.53 (at 3.19 \text{\AA})$	Xtriage
Refinement program	BUSTER 2.10.4	Depositor
B B.	0.270 , $0.298$	Depositor
$R, R_{free}$	0.267 , $0.291$	DCC
$R_{free}$ test set	896 reflections $(5.02\%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	70.8	Xtriage
Anisotropy	0.105	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$ , $B_{sol}(Å^2)$	0.29 , $62.2$	EDS
L-test for twinning <sup>2</sup>	$ < L >=0.48, < L^2>=0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.82	EDS
Total number of atoms	5473	wwPDB-VP
Average B, all atoms $(Å^2)$	77.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 4.18% of the height of the origin peak. No significant pseudotranslation is detected.

<sup>&</sup>lt;sup>2</sup>Theoretical 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.



<sup>&</sup>lt;sup>1</sup>Intensities estimated from amplitudes.

# 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: Y01, BA  $\,$ 

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
	Unam	RMSZ	# Z  > 5	RMSZ	# Z  > 5
1	А	0.22	0/1839	0.39	0/2523
1	В	0.23	0/1929	0.40	0/2644
2	С	0.22	0/856	0.43	0/1165
2	D	0.21	0/823	0.43	0/1127
All	All	0.22	0/5447	0.40	0/7459

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	А	1794	0	1672	7	0
1	В	1882	0	1776	8	0
2	С	839	0	801	2	0
2	D	806	0	728	1	0
3	А	2	0	0	0	0
3	В	2	0	0	0	0
4	А	105	0	147	5	0
4	В	35	0	49	2	0
5	А	3	0	0	0	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
5	В	4	0	0	0	0
5	С	1	0	0	0	0
All	All	5473	0	5173	14	0

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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 1.

All (14) 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:304:LEU:HG	4:B:403:Y01:HAN1	1.73	0.70
1:A:137:LEU:HD23	1:B:137:LEU:HD23	1.88	0.56
2:C:90:THR:HG23	2:C:110:THR:HA	1.91	0.53
2:D:90:THR:HG23	2:D:110:THR:HA	1.91	0.52
1:A:304:LEU:HG	4:A:403:Y01:HAN1	1.93	0.49
1:A:137:LEU:HD11	1:B:146:PRO:HG2	1.98	0.46
2:C:20:LEU:HD12	2:C:80:LEU:HD23	1.98	0.44
1:A:251:VAL:HB	4:A:403:Y01:HAQ1	2.00	0.44
4:A:405:Y01:HAS2	1:B:327:LEU:HD22	2.01	0.43
4:A:404:Y01:HAJ1	1:B:195:PHE:HD2	1.85	0.41
1:B:251:VAL:HB	4:B:403:Y01:HAQ1	2.01	0.41
1:A:146:PRO:HG2	1:B:137:LEU:HD11	2.01	0.41
1:A:198:PRO:HD3	1:B:320:LEU:HD22	2.01	0.41
1:A:327:LEU:HD22	4:A:404:Y01:HAS2	2.02	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	А	244/282~(86%)	240~(98%)	4(2%)	0	100 100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percent	iles
1	В	253/282~(90%)	248~(98%)	5(2%)	0	100 1	00
2	С	110/123~(89%)	108 (98%)	2(2%)	0	100 1	00
2	D	110/123~(89%)	108 (98%)	2(2%)	0	100 1	00
All	All	717/810 (88%)	704 (98%)	13 (2%)	0	100 1	00

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There are no Ramachandran outliers to report.

#### 5.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent side chain 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	А	164/233~(70%)	163~(99%)	1 (1%)	86 94
1	В	178/233~(76%)	177~(99%)	1 (1%)	86 94
2	С	84/102~(82%)	83~(99%)	1 (1%)	71 88
2	D	74/102~(72%)	73~(99%)	1 (1%)	67 86
All	All	500/670~(75%)	496 (99%)	4 (1%)	81 93

All (4) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	316	PHE
1	В	316	PHE
2	С	99	SER
2	D	99	SER

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)

Of 8 ligands modelled in this entry, 4 are monoatomic - leaving 4 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	Turne	Chain	Res	Link	Bo	ond leng	ths	В	ond ang	les
	Type	Chain	nes		Counts	RMSZ	# Z >2	Counts	RMSZ	# Z  > 2
4	Y01	А	405	-	38,38,38	0.23	0	57,57,57	0.30	0
4	Y01	А	403	-	38,38,38	0.27	0	57,57,57	0.35	0
4	Y01	А	404	-	38,38,38	0.24	0	57,57,57	0.29	0
4	Y01	В	403	-	38,38,38	0.25	0	57,57,57	0.34	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	Y01	А	405	-	-	5/19/77/77	0/4/4/4
4	Y01	А	403	-	-	6/19/77/77	0/4/4/4
4	Y01	А	404	-	-	5/19/77/77	0/4/4/4
4	Y01	В	403	-	-	6/19/77/77	0/4/4/4

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (22) torsion outliers are listed below:



Mol	Chain	Res	Type	Atoms
4	А	404	Y01	CAJ-CAO-CBB-CAC
4	А	405	Y01	CAJ-CAO-CBB-CAC
4	А	404	Y01	CAJ-CAO-CBB-CBE
4	А	405	Y01	CAJ-CAO-CBB-CBE
4	В	403	Y01	CAO-CAJ-CAN-CBA
4	А	403	Y01	CAO-CAJ-CAN-CBA
4	А	403	Y01	CAN-CAJ-CAO-CBB
4	В	403	Y01	CAN-CAJ-CAO-CBB
4	А	405	Y01	CAM-CAL-CAX-OAH
4	А	404	Y01	CAM-CAL-CAX-OAH
4	А	404	Y01	CAM-CAL-CAX-OAF
4	В	403	Y01	CAM-CAL-CAX-OAH
4	А	405	Y01	CAM-CAL-CAX-OAF
4	В	403	Y01	CAM-CAL-CAX-OAF
4	А	403	Y01	CAM-CAL-CAX-OAH
4	А	403	Y01	CAM-CAL-CAX-OAF
4	А	403	Y01	CAJ-CAO-CBB-CBE
4	А	405	Y01	CAL-CAM-CAY-OAW
4	В	403	Y01	CAJ-CAO-CBB-CBE
4	А	404	Y01	CAL-CAM-CAY-OAW
4	А	403	Y01	CAL-CAM-CAY-OAW
4	В	403	Y01	CAL-CAM-CAY-OAW

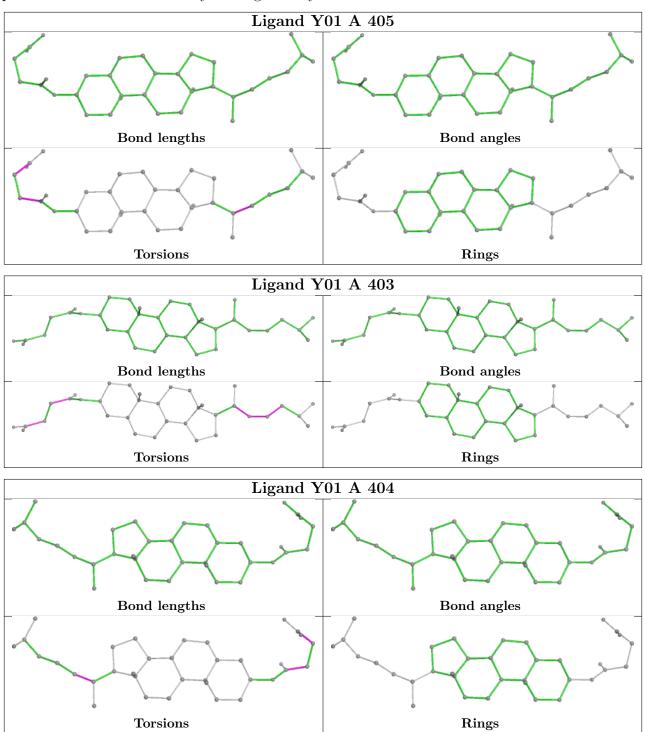
There are no ring outliers.

4 monomers are involved in 7 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	А	405	Y01	1	0
4	А	403	Y01	2	0
4	А	404	Y01	2	0
4	В	403	Y01	2	0

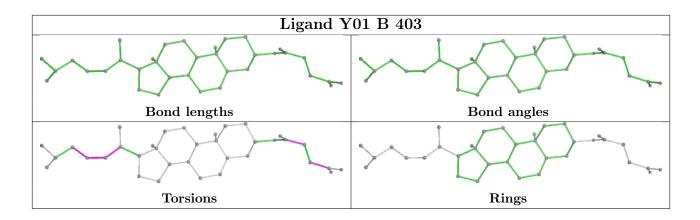
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. 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.





## 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,  $95^{th}$  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	$\mathbf{OWAB}(\mathbf{\mathring{A}}^2)$	$\mathbf{Q}{<}0.9$
1	А	250/282~(88%)	0.15	15 (6%) 21 12	45, 75, 142, 181	0
1	В	259/282~(91%)	0.18	7 (2%) 54 39	39, 74, 105, 135	0
2	С	112/123~(91%)	-0.00	0 100 100	41, 59, 72, 78	0
2	D	112/123~(91%)	0.60	13 (11%) 4 3	65, 87, 113, 119	0
All	All	733/810~(90%)	0.21	35 (4%) 30 18	39, 73, 123, 181	0

All (35) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	А	240	SER	5.9
1	В	220	ALA	5.7
1	А	220	ALA	5.5
2	D	91	ALA	5.4
1	А	221	ARG	5.3
1	А	218	SER	5.0
2	D	90	THR	4.7
1	В	334	THR	4.0
1	А	214	ILE	3.9
1	А	215	PHE	3.6
2	D	10	GLY	3.5
2	D	40	ALA	3.3
2	D	39	GLN	3.2
1	А	213	THR	3.1
1	А	219	ILE	3.0
2	D	108	GLN	3.0
1	А	241	THR	2.9
1	А	222	VAL	2.8
1	В	332	LYS	2.7
2	D	92	VAL	2.7
1	В	74	TRP	2.7

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Mol	Chain	Res	Type	RSRZ	
1	А	239	ILE	2.6	
2	D	38	ARG	2.5	
1	А	216	GLY	2.5	
2	D	41	PRO	2.4	
2	D	105	GLN	2.4	
2	D	5	VAL	2.4	
1	В	297	TYR	2.3	
1	А	217	LYS	2.3	
2	D	94	TYR	2.3	
1	В	300	TRP	2.2	
1	А	268	ALA	2.2	
2	D	110	THR	2.1	
1	В	331	SER	2.0	
1	А	211	LEU	2.0	

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#### 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.

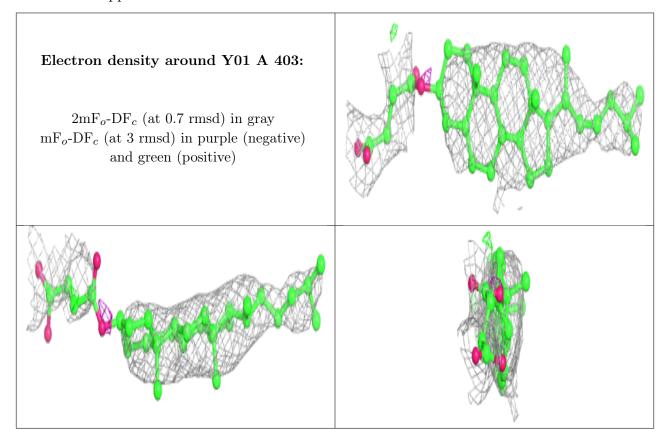
#### 6.4 Ligands (i)

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.

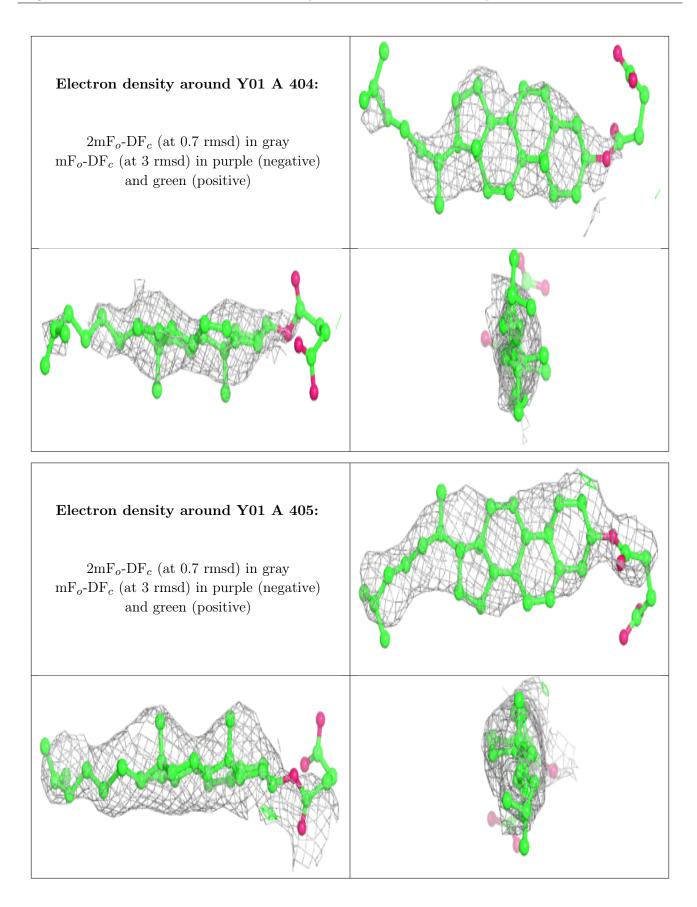
Mol	Type	Chain	Res	Atoms	RSCC	RSR	$B-factors(Å^2)$	Q < 0.9
4	Y01	А	403	35/35	0.75	0.47	$115,\!117,\!120,\!120$	0
4	Y01	А	404	35/35	0.78	0.60	122,123,125,125	0
4	Y01	А	405	35/35	0.80	0.43	118,120,122,122	0
4	Y01	В	403	35/35	0.87	0.29	93,95,98,98	0
3	BA	В	402	1/1	0.95	0.05	$151,\!151,\!151,\!151,\!151$	0
3	BA	А	401	1/1	0.97	0.05	109,109,109,109	0
3	BA	В	401	1/1	0.99	0.37	106,106,106,106	0
3	BA	А	402	1/1	1.00	0.16	50,50,50,50	0



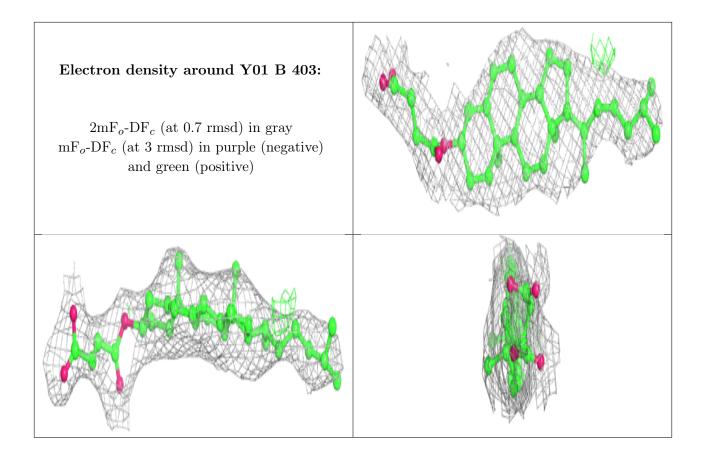
The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation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











## 6.5 Other polymers (i)

There are no such residues in this entry.

