

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

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Title	:	Time-resolved serial femtosecond crystallography reveals early structural
		changes in channelrhodopsin: Dark state structure
Authors	:	Oda, K.; Nomura, T.; Nakane, T.; Yamashita, K.; Inoue, K.; Ito, S.; Vierock,
		J.; Hirata, K.; Maturana, A.D.; Katayama, K.; Ikuta, T.; Ishigami, I.; Izume,
		T.; Umeda, R.; Eguma, R.; Oishi, S.; Kasuya, G.; Kato, T.; Kusakizako,
		T.; Shihoya, W.; Shimada, H.; Takatsuji, T.; Takemoto, M.; Taniguchi, R.;
		Tomita, A.; Nakamura, R.; Fukuda, M.; Miyauchi, H.; Lee, Y.; Nango, E.;
		Tanaka, R.; Tanaka, T.; Sugahara, M.; Kimura, T.; Shimamura, T.; Fujiwara,
		T.; Yamanaka, Y.; Owada, S.; Joti, Y.; Tono, K.; Ishitani, R.; Hayashi, S.;
		Kandori, H.; Hegemann, P.; Iwata, S.; Kubo, M.; Nishizawa, T.; Nureki, O.
Deposited on	:	2020-05-28
Resolution	:	2.30 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 (1)) were used in the production of this report:

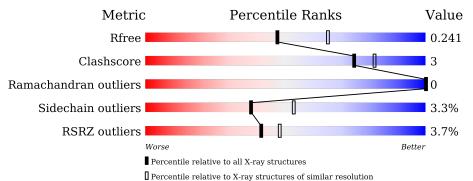
 $\begin{array}{rcl} {\rm MolProbity} &:& 4.02{\rm b}{\text{-}}467\\ {\rm Mogul} &:& 1.8.5\ (274361),\ {\rm CSD}\ {\rm as541be}\ (2020)\\ {\rm Xtriage}\ ({\rm Phenix}) &:& 1.13\\ {\rm EDS} &:& 2.35 \end{array}$ 

# 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 2.30 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	$\begin{array}{l} \textbf{Whole archive} \\ (\#\textbf{Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries,\ resolution\ range}({ m \AA}))$
R <sub>free</sub>	130704	5042 (2.30-2.30)
Clashscore	141614	5643 (2.30-2.30)
Ramachandran outliers	138981	5575 (2.30-2.30)
Sidechain outliers	138945	5575 (2.30-2.30)
RSRZ outliers	127900	4938 (2.30-2.30)

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	356	3% 76%	6% • 17%			
				Continued on next page			

Refmac	: : : :	20191225.v01 (using entries in the PDB archive December 25th 2019) 5.8.0158 7.0.044 (Gargrove) Engh & Huber (2001) Parkinson et al. (1996)
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Mol	Chain	Length	Quality	of chain
2	Е	2	50%	50%



# 2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 2529 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 Sensory opsin A,Channelrhodopsin (ChR) chimera between ChR1 & amp; ChR2.

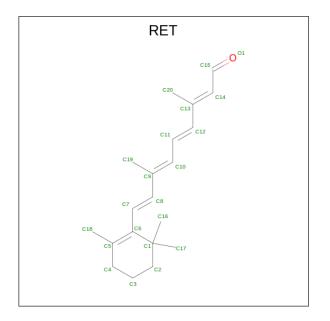
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
1	А	296	Total 2317	C 1521	N 369	O 412	S 15	0	0	0

• Molecule 2 is an oligosaccharide called 2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-a cetamido-2-deoxy-beta-D-glucopyranose.



Mol	Chain	Residues	Atoms		ZeroOcc	AltConf	Trace		
2	Е	2	Total 28	C 16	N 2	O 10	0	0	0

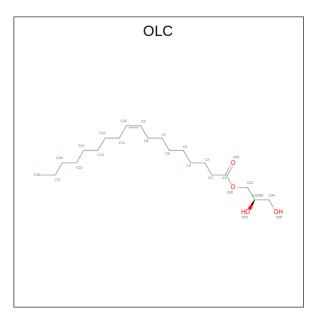
• Molecule 3 is RETINAL (three-letter code: RET) (formula:  $C_{20}H_{28}O$ ).





M	ol	Chain	Residues Atoms		ZeroOcc	AltConf
3		А	1	Total         C           20         20	0	0

• Molecule 4 is (2R)-2,3-dihydroxypropyl (9Z)-octadec-9-enoate (three-letter code: OLC) (formula:  $C_{21}H_{40}O_4$ ).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	1	Total         C         O           25         21         4	0	0
4	А	1	Total         C         O           16         14         2	0	0
4	А	1	Total         C         O           14         10         4	0	0
4	А	1	Total         C         O           16         12         4	0	0
4	А	1	Total C O 18 14 4	0	0
4	А	1	Total         C         O           10         8         2	0	0
4	А	1	Total         C         O           10         8         2	0	0
4	А	1	Total C 9 9	0	0
4	А	1	Total C 8 8	0	0

• Molecule 5 is water.



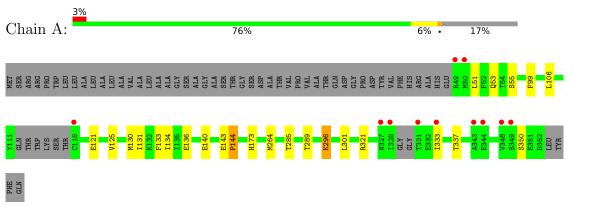
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	А	38	Total         O           38         38	0	0



## 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: Sensory opsin A, Channelrhodopsin (ChR) chimera between ChR1 & amp; ChR2



• Molecule 2: 2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-2-acetamido-2-deoxy-beta-D-glucopyranose

Chain E.	E 0.0/	E 09/
Unam L.	30%	50%

NAG1 NAG2



## 4 Data and refinement statistics (i)

Property	Value	Source
Space group	C 2 2 21	Depositor
Cell constants	61.80Å 142.20Å 94.70Å	Depositor
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $90.00^{\circ}$ $90.00^{\circ}$	Depositor
Resolution (Å)	15.00 - 2.30	Depositor
Resolution (A)	48.63 - 2.30	EDS
% Data completeness	99.4 (15.00-2.30)	Depositor
(in resolution range)	93.9 (48.63-2.30)	EDS
R <sub>merge</sub>	(Not available)	Depositor
R <sub>sym</sub>	(Not available)	Depositor
$< I/\sigma(I) > 1$	$0.97 (at 2.29 \text{\AA})$	Xtriage
Refinement program	REFMAC 5.8.0266	Depositor
D D.	0.182 , $0.232$	Depositor
$R, R_{free}$	0.191 , $0.241$	DCC
$R_{free}$ test set	905 reflections $(4.78\%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	53.4	Xtriage
Anisotropy	0.461	Xtriage
Bulk solvent $k_{sol}(e/Å^3), B_{sol}(Å^2)$	0.32 , $83.4$	EDS
L-test for twinning <sup>2</sup>	$ \langle L  \rangle = 0.49, \langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.96	EDS
Total number of atoms	2529	wwPDB-VP
Average B, all atoms $(Å^2)$	73.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 5.77% 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: RET, NAG, OLC

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).

Mal	Chain	Bond	lengths	Bond	angles
	Chain	RMSZ	# Z  > 5	RMSZ	# Z  > 5
1	А	0.67	0/2377	0.74	0/3237

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	2317	0	2261	13	0
2	Е	28	0	25	0	0
3	А	20	0	27	3	0
4	А	126	0	172	0	0
5	А	38	0	0	0	0
All	All	2529	0	2485	16	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 3.

The worst 5 of 16 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)
3:A:401:RET:H8	3:A:401:RET:H161	1.62	0.82
1:A:125:VAL:HG11	1:A:296:LYS:HG3	1.87	0.57
1:A:285:THR:O	1:A:289:THR:HG23	2.05	0.56
1:A:121:GLU:HG3	1:A:173:HIS:HB2	1.88	0.55
1:A:53:GLN:HE21	1:A:55:SER:H	1.54	0.54

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	Percer	ntiles
1	А	290/356~(82%)	284 (98%)	6~(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	nalysed Rotameric		Percentiles	
1	А	242/295~(82%)	234~(97%)	8(3%)	38 53	

5 of 8 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	350	SER
1	А	333	ILE

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Mol	Chain	Res	Type
1	А	296	LYS
1	А	144	PRO
1	А	301	LEU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	А	173	HIS

#### 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)

2 monosaccharides 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	Turne	Chain	Dec	Link	Bo	ond leng	$\mathbf{ths}$	В	ond ang	les
	Type	Unam	Res	LIIIK	Counts	RMSZ	# Z >2	Counts	RMSZ	# Z  > 2
2	NAG	Е	1	2,1	14,14,15	0.41	0	17,19,21	0.83	0
2	NAG	Е	2	2	14,14,15	0.29	0	17,19,21	1.17	1 (5%)

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	NAG	Е	1	2,1	-	2/6/23/26	0/1/1/1
2	NAG	Е	2	2	-	3/6/23/26	0/1/1/1

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
2	Е	2	NAG	O5-C1-C2	-3.23	106.19	111.29

There are no chirality outliers.

All (5) torsion outliers are listed below:

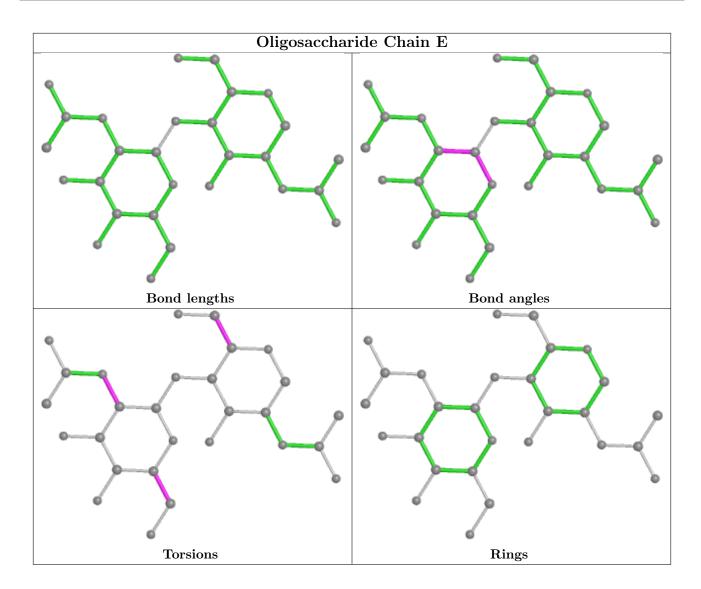
Mol	Chain	Res	Type	Atoms
2	Е	1	NAG	O5-C5-C6-O6
2	Е	2	NAG	C4-C5-C6-O6
2	Е	2	NAG	O5-C5-C6-O6
2	Е	1	NAG	C4-C5-C6-O6
2	Е	2	NAG	C3-C2-N2-C7

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 oligosaccharide.





### 5.6 Ligand geometry (i)

10 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	Ros	Bos	Link	ink Bond lengths			Bond angles			
	туре			LIIIK	Counts	RMSZ	# Z >2	Counts	RMSZ	# Z >2			
3	RET	А	401	1	20,20,21	2.80	6 (30%)	27,27,28	1.35	4 (14%)			
4	OLC	А	407	-	9,9,24	0.35	0	9,9,25	0.39	0			
4	OLC	А	405	-	15,15,24	0.36	0	16,16,25	0.27	0			



Mol	Turne	Chain	Res	Link	Bo	ond leng	ths	Bond angles		
INIOI	Type	Unam	nes	LIIIK	Counts	RMSZ	# Z >2	Counts	RMSZ	# Z >2
4	OLC	А	410	-	7,7,24	0.15	0	$6,\!6,\!25$	0.16	0
4	OLC	А	409	-	8,8,24	0.26	0	7,7,25	0.16	0
4	OLC	А	402	-	24,24,24	0.25	0	$25,\!25,\!25$	0.28	0
4	OLC	А	408	-	9,9,24	0.38	0	9,9,25	0.35	0
4	OLC	А	404	-	13,13,24	0.28	0	14,14,25	0.31	0
4	OLC	А	403	-	$15,\!15,\!24$	0.32	0	$15,\!15,\!25$	0.28	0
4	OLC	А	406	-	17,17,24	0.34	0	$18,\!18,\!25$	0.40	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
3	RET	А	401	1	-	0/13/30/31	0/1/1/1
4	OLC	А	407	-	-	3/7/7/24	-
4	OLC	А	405	-	-	11/15/15/24	-
4	OLC	А	410	-	-	1/5/5/24	-
4	OLC	А	409	-	-	2/6/6/24	-
4	OLC	А	402	-	-	9/24/24/24	-
4	OLC	А	408	-	-	3/7/7/24	-
4	OLC	А	404	-	-	2/13/13/24	-
4	OLC	А	403	-	-	6/13/13/24	-
4	OLC	А	406	-	-	12/17/17/24	-

The worst 5 of 6 bond length outliers are listed below:

Mol	Chain	$\operatorname{Res}$	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	А	401	RET	C14-C13	9.17	1.40	1.33
3	А	401	RET	C10-C9	5.72	1.43	1.35
3	А	401	RET	C2-C3	-3.30	1.44	1.52
3	А	401	RET	C15-C14	-2.82	1.39	1.49
3	А	401	RET	C8-C9	-2.72	1.40	1.45

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
3	А	401	RET	C19-C9-C10	-3.17	118.49	122.92
3	А	401	RET	C8-C9-C10	2.99	123.53	118.94
3	А	401	RET	C2-C1-C6	2.92	114.97	110.48

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Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
3	А	401	RET	C11-C10-C9	2.12	130.33	127.31

There are no chirality outliers.

5 of 49 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
4	А	405	OLC	C21-C22-C24-O25
4	А	405	OLC	O20-C21-C22-C24
4	А	405	OLC	O20-C21-C22-O23
4	А	407	OLC	C1-C2-C3-C4
4	А	403	OLC	C1-C2-C3-C4

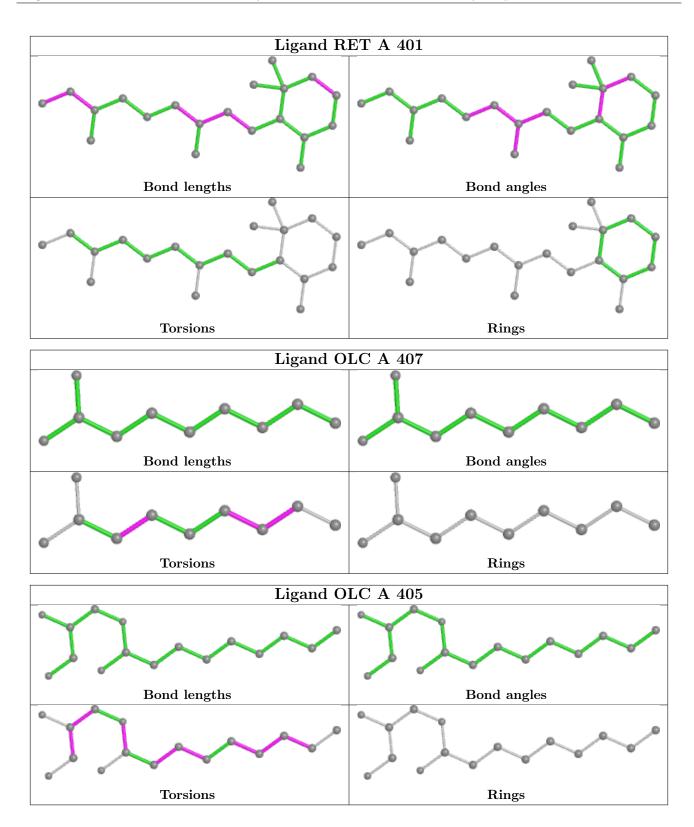
There are no ring outliers.

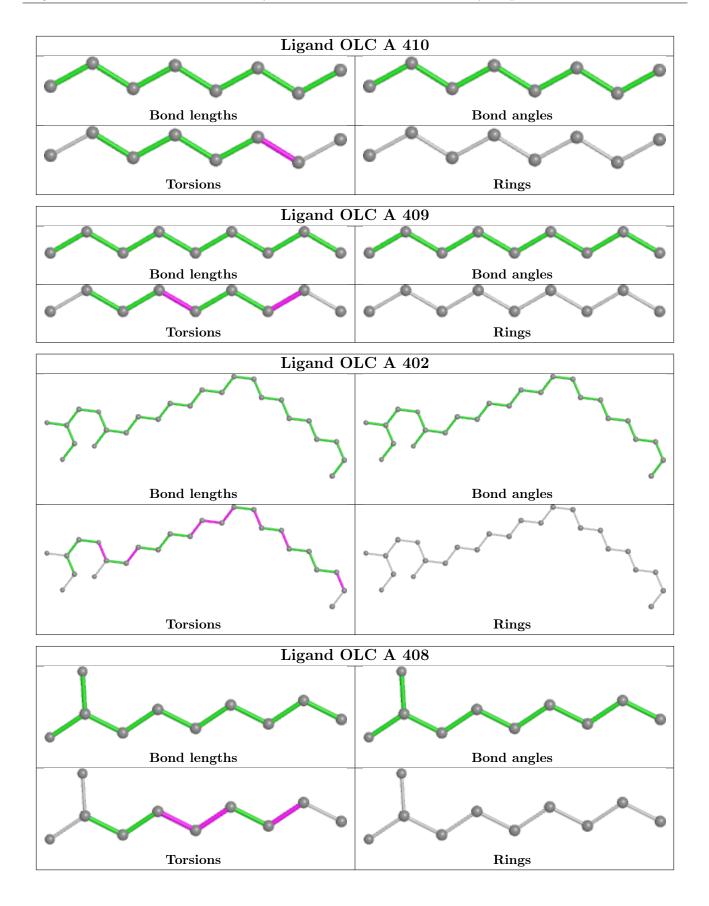
1 monomer is involved in 3 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	А	401	RET	3	0

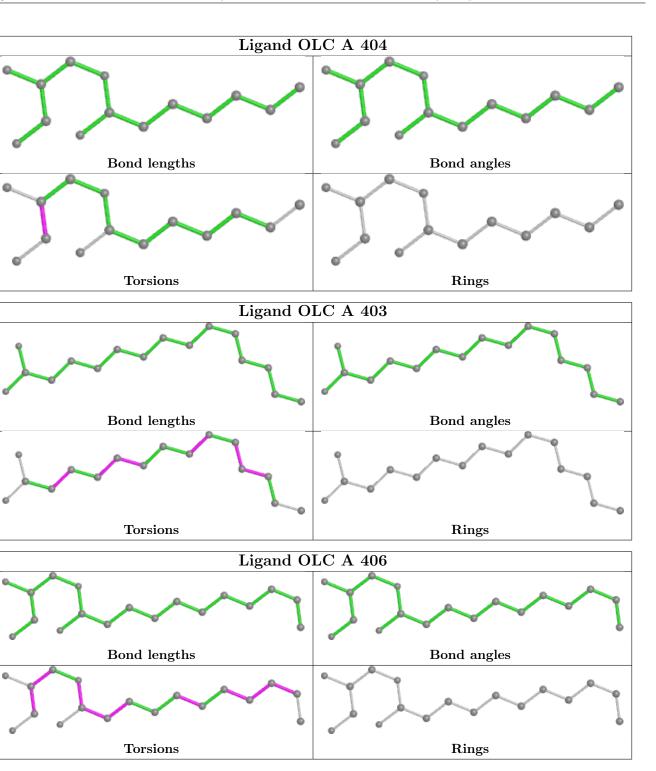
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included.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











### 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	< <b>RSRZ</b> >	#RSRZ>2	$\mathbf{OWAB}(\mathbf{\AA}^2)$	Q<0.9
1	А	296/356~(83%)	0.36	11 (3%) 41 48	39, 65, 123, 166	0

The worst 5 of 11 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	А	348	VAL	5.0
1	А	333	ILE	4.5
1	А	328	ILE	4.1
1	А	343	ALA	3.4
1	А	49	ARG	3.3

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

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

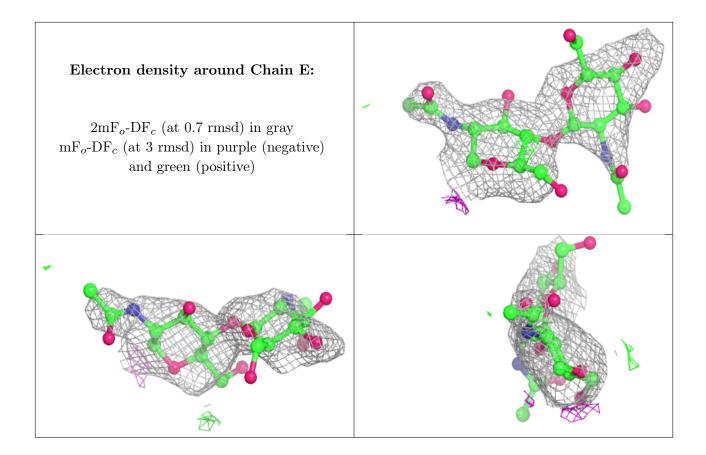
#### 6.3 Carbohydrates (i)

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	NAG	Е	2	14/15	0.75	0.37	129,152,157,169	0
2	NAG	Е	1	14/15	0.87	0.26	126,140,157,171	0

The following is a graphical depiction of the model fit to experimental electron density for oligosaccharide. Each fit is shown from different orientation to approximate a three-dimensional view.





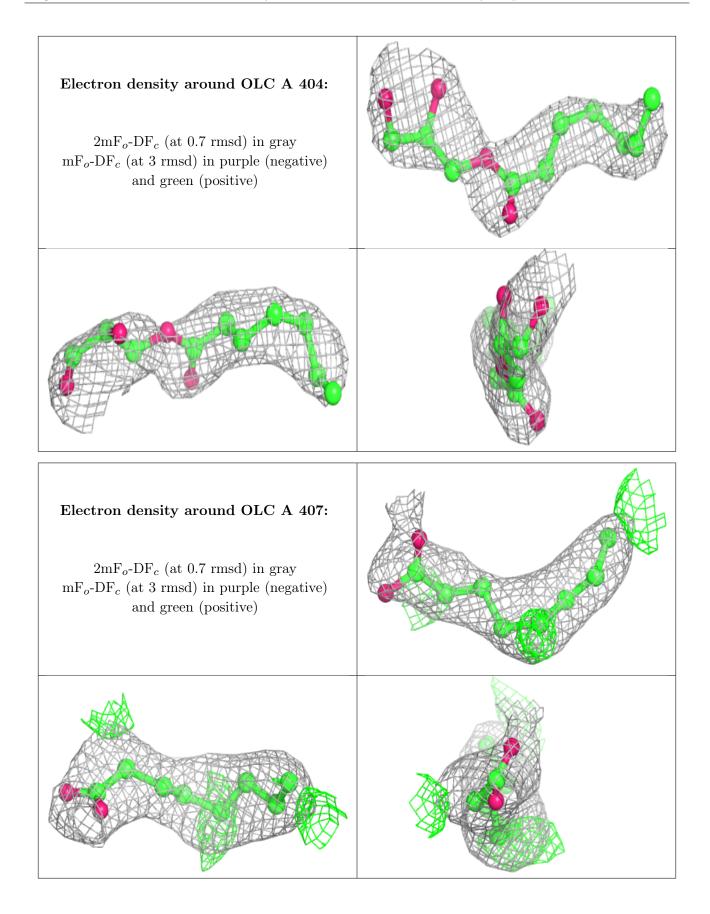
#### 6.4 Ligands (i)

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.

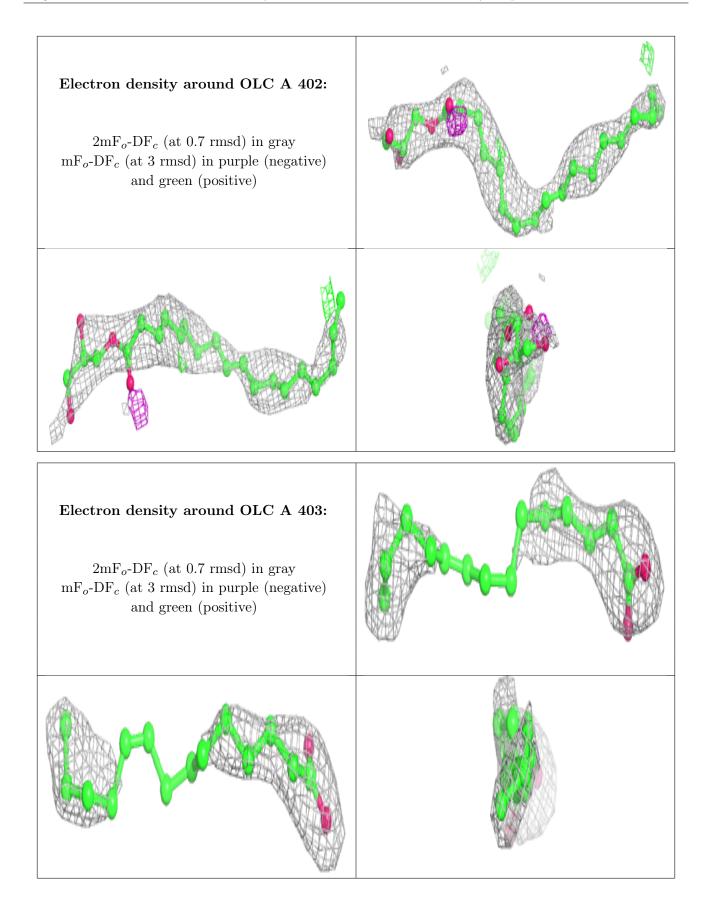
Mol	Type	Chain	Res	Atoms	RSCC	RSR	$B-factors(A^2)$	Q<0.9
4	OLC	А	404	14/25	0.66	0.22	109,125,138,148	0
4	OLC	А	407	10/25	0.68	0.24	91,98,126,139	0
4	OLC	А	402	25/25	0.69	0.26	92,119,132,137	0
4	OLC	А	403	16/25	0.70	0.31	104,122,141,143	0
4	OLC	А	406	18/25	0.72	0.28	75,93,127,128	0
4	OLC	А	410	8/25	0.76	0.22	115,124,130,131	0
4	OLC	А	409	9/25	0.85	0.27	91,103,108,111	0
4	OLC	А	408	10/25	0.88	0.17	108,115,123,125	0
3	RET	А	401	20/21	0.88	0.22	63,71,74,74	0
4	OLC	А	405	16/25	0.88	0.23	72,82,121,125	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.

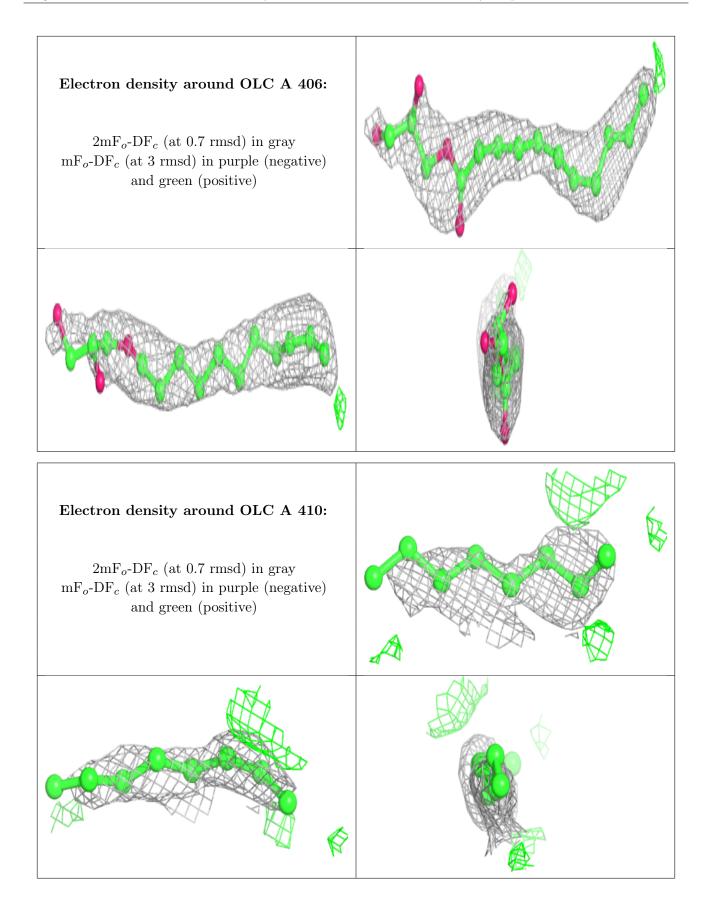




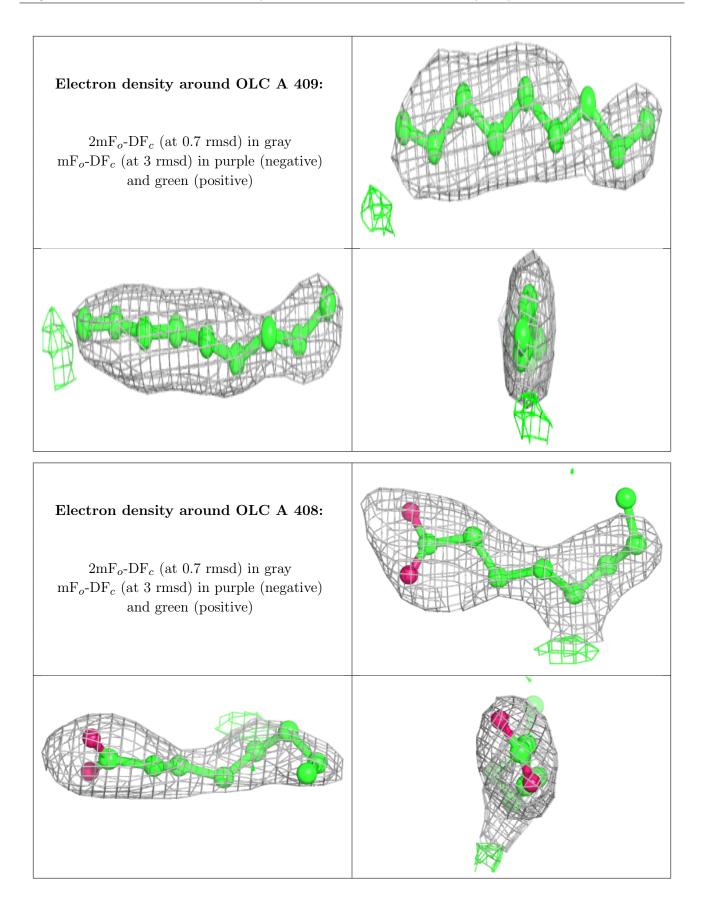






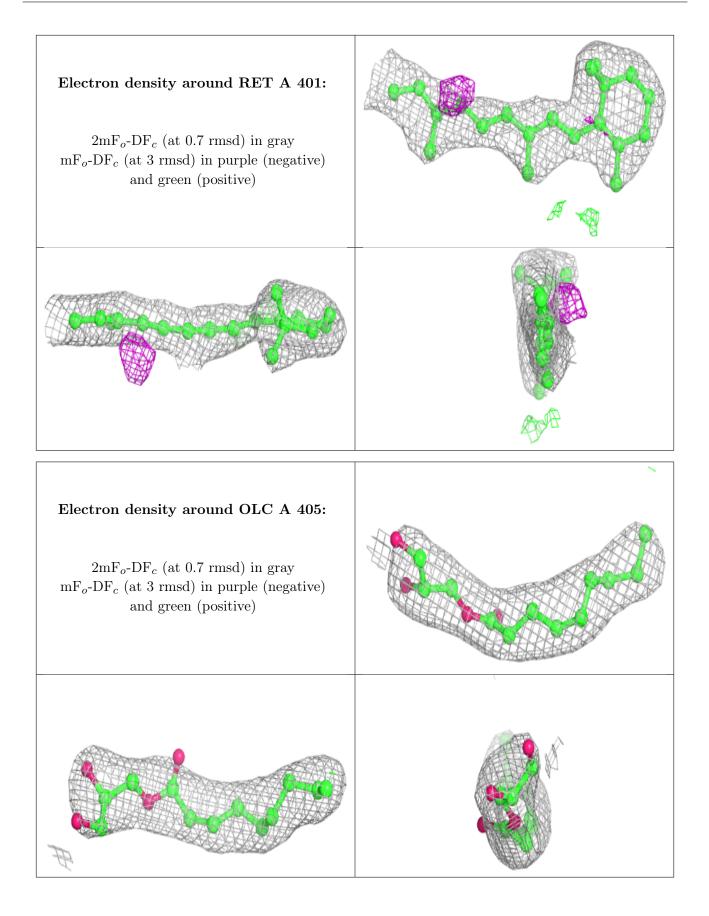














### 6.5 Other polymers (i)

There are no such residues in this entry.

