

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

#### Nov 13, 2023 - 03:51 PM EST

PDB ID	:	8CVA
Title	:	Structure of Hyoscyamine 6-beta Hydroxylase in complex with iron, succinate,
		and 6-OH-hyoscyamine
Authors	:	Wenger, E.W.; Boal, A.K.; Bollinger, J.M.; Krebs, C.
Deposited on		
Resolution	:	1.58  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:

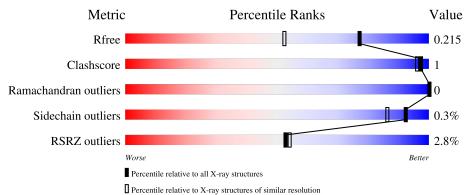
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix)		
EDS	:	2.36
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (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:  $X\text{-}RAY \, DIFFRACTION$ 

The reported resolution of this entry is 1.58 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	$egin{array}{c} { m Whole \ archive} \ (\#{ m Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries,\ resolution\ range}({ m \AA}))$
$R_{free}$	130704	5534 (1.60-1.56)
Clashscore	141614	5861 (1.60-1.56)
Ramachandran outliers	138981	5708 (1.60-1.56)
Sidechain outliers	138945	5703 (1.60-1.56)
RSRZ outliers	127900	5431 (1.60-1.56)

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		
			2%		
1	А	385	83%	•	16%



# 2 Entry composition (i)

There are 8 unique types of molecules in this entry. The entry contains 2913 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 Hyoscyamine 6-beta-hydroxylase.

Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace	
1	А	325	Total	C	N 412	O 404	S	0	0	0
			2578	1660	413	494	11			

Chain	Residue	Modelled	Actual	Comment	Reference
А	344	GLY	_	expression tag	UNP Q9XJ43
А	345	GLY	_	expression tag	UNP Q9XJ43
А	346	LYS	_	expression tag	UNP Q9XJ43
А	347	GLY	-	expression tag	UNP Q9XJ43
А	348	PRO	-	expression tag	UNP Q9XJ43
А	349	GLY	-	expression tag	UNP Q9XJ43
А	350	ASP	-	expression tag	UNP Q9XJ43
А	351	GLY	-	expression tag	UNP Q9XJ43
А	352	PRO	-	expression tag	UNP Q9XJ43
А	353	GLY	-	expression tag	UNP Q9XJ43
А	354	GLY	-	expression tag	UNP Q9XJ43
А	355	SER	-	expression tag	UNP Q9XJ43
А	356	GLY	-	expression tag	UNP Q9XJ43
А	357	GLY	-	expression tag	UNP Q9XJ43
А	358	PRO	-	expression tag	UNP Q9XJ43
А	359	ARG	-	expression tag	UNP Q9XJ43
А	360	GLY	-	expression tag	UNP Q9XJ43
А	361	GLY	-	expression tag	UNP Q9XJ43
А	362	GLU	-	expression tag	UNP Q9XJ43
А	363	ASN	-	expression tag	UNP Q9XJ43
А	364	LEU	-	expression tag	UNP Q9XJ43
А	365	TYR	-	expression tag	UNP Q9XJ43
А	366	PHE	-	expression tag	UNP Q9XJ43
А	367	GLN	-	expression tag	UNP Q9XJ43
А	368	SER	-	expression tag	UNP Q9XJ43
А	369	GLY	-	expression tag	UNP Q9XJ43
А	370	HIS	-	expression tag	UNP Q9XJ43

There are 42 discrepancies between the modelled and reference sequences:

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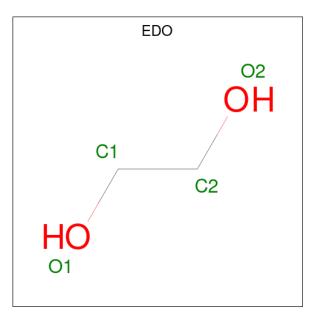
Chain	Residue	Modelled	Actual	Comment	Reference
А	371	HIS	-	expression tag	UNP Q9XJ43
А	372	HIS	-	expression tag	UNP Q9XJ43
А	373	HIS	-	expression tag	UNP Q9XJ43
А	374	HIS	-	expression tag	UNP Q9XJ43
А	375	HIS	-	expression tag	UNP Q9XJ43
А	376	HIS	-	expression tag	UNP Q9XJ43
Α	377	HIS	-	expression tag	UNP Q9XJ43
А	378	GLY	-	expression tag	UNP Q9XJ43
A	379	GLY	-	expression tag	UNP Q9XJ43
А	380	ARG	-	expression tag	UNP Q9XJ43
A	381	GLY	-	expression tag	UNP Q9XJ43
А	382	GLY	-	expression tag	UNP Q9XJ43
А	383	ALA	-	expression tag	UNP Q9XJ43
А	384	ALA	-	expression tag	UNP Q9XJ43
А	385	ALA	-	expression tag	UNP Q9XJ43

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• Molecule 2 is FE (II) ION (three-letter code: FE2) (formula: Fe) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	А	1	Total Fe 1 1	0	0

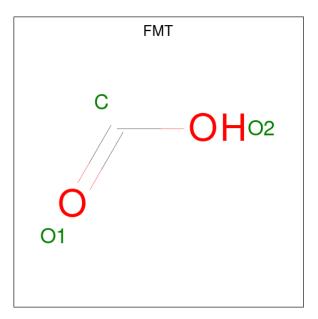
• Molecule 3 is 1,2-ETHANEDIOL (three-letter code: EDO) (formula:  $C_2H_6O_2$ ).





Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 4 & 2 & 2 \end{array}$	0	0
3	А	1	$\begin{array}{ccc} \text{Total}  \text{C}  \text{O} \\ 4  2  2 \end{array}$	0	0
3	А	1	$\begin{array}{ccc} \text{Total}  \text{C}  \text{O} \\ 4  2  2 \end{array}$	0	0
3	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 4 & 2 & 2 \end{array}$	0	0

• Molecule 4 is FORMIC ACID (three-letter code: FMT) (formula:  $CH_2O_2$ ).

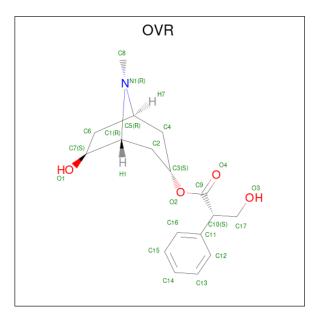


Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 3 & 1 & 2 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 3 & 1 & 2 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total}  \text{C}  \text{O} \\ 3  1  2 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total}  \text{C}  \text{O} \\ 3  1  2 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total}  \text{C}  \text{O} \\ 3  1  2 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total}  \text{C}  \text{O} \\ 3  1  2 \end{array}$	0	0
4	А	1	$\begin{array}{ccc} \text{Total} & \text{C} & \text{O} \\ 3 & 1 & 2 \end{array}$	0	0

• Molecule 5 is (1R,3S,5R,6S)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (2S)-3-hydrox

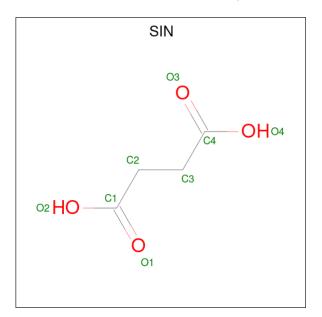


y-2-phenyl propanoate (three-letter code: OVR) (formula:  $\rm C_{17}H_{23}NO_4)$  (labeled as "Ligand of Interest" by depositor).



Μ	bl	Chain	Residues	A	ton	ıs		ZeroOcc	AltConf
5		А	1	Total 22	C 17	N 1	0 4	0	0

• Molecule 6 is SUCCINIC ACID (three-letter code: SIN) (formula:  $C_4H_6O_4$ ) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
6	А	1	Total 8	С 4	0 4	0	0



• Molecule 7 is STRONTIUM ION (three-letter code: SR) (formula: Sr).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
7	А	2	$\begin{array}{cc} \text{Total} & \text{Sr} \\ 2 & 2 \end{array}$	0	0

• Molecule 8 is water.

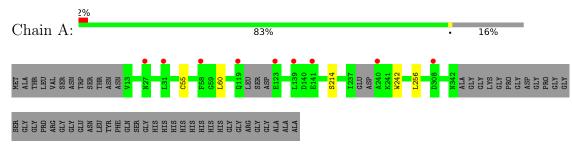
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
8	А	265	Total         O           265         265	0	0



# 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: Hyoscyamine 6-beta-hydroxylase





## 4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants	44.84Å 79.26Å 55.96Å	Depositor
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $111.22^{\circ}$ $90.00^{\circ}$	Depositor
Resolution (Å)	43.61 - 1.58	Depositor
Resolution (A)	43.57 - 1.58	EDS
% Data completeness	$97.6\ (43.61-1.58)$	Depositor
(in resolution range)	$97.6\ (43.57\text{-}1.58)$	EDS
R <sub>merge</sub>	0.09	Depositor
$R_{sym}$	(Not available)	Depositor
$< I/\sigma(I) > 1$	$2.35 (at 1.58 \text{\AA})$	Xtriage
Refinement program	REFMAC 5.8.0267	Depositor
$R, R_{free}$	0.185 , $0.205$	Depositor
n, n <sub>free</sub>	0.196 , $0.215$	DCC
$R_{free}$ test set	2534 reflections $(5.20%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	14.0	Xtriage
Anisotropy	0.029	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$ , $B_{sol}(Å^2)$	0.37, $40.4$	EDS
L-test for $twinning^2$	$< L >=0.50, < L^2>=0.33$	Xtriage
Estimated twinning fraction	0.025 for h,-k,-h-l	Xtriage
$F_o, F_c$ correlation	0.95	EDS
Total number of atoms	2913	wwPDB-VP
Average B, all atoms $(Å^2)$	16.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 6.78% 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: FE2, OVR, SR, FMT, EDO, SIN

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	0.59	0/2639	0.66	0/3588	

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	2578	0	2559	3	0
2	А	1	0	0	0	0
3	А	16	0	24	1	0
4	А	21	0	7	0	0
5	А	22	0	0	0	0
6	А	8	0	4	1	0
7	А	2	0	0	0	0
8	А	265	0	0	1	0
All	All	2913	0	2594	4	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 1.

All (4) close contacts within the same asymmetric unit are listed below, sorted by their clash



Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
6:A:411:SIN:O4	8:A:501:HOH:O	2.02	0.76
1:A:55:CYS:SG	1:A:256:LEU:HD11	2.52	0.50
1:A:60:LEU:C	1:A:60:LEU:HD23	2.40	0.42
1:A:242:TRP:CD1	3:A:405:EDO:H12	2.56	0.40

magnitude.

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	319/385~(83%)	311 (98%)	8 (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	А	287/326~(88%)	286 (100%)	1 (0%)	92 86	

All (1) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	214	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 16 ligands modelled in this entry, 3 are monoatomic - leaving 13 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	Trune	Chain	Res	Link	Bo	ond leng	ths	В	ond ang	les
IVIOI	Type	Chain	nes		Counts	RMSZ	# Z >2	Counts	RMSZ	# Z  > 2
4	FMT	А	413	-	2,2,2	0.27	0	$1,\!1,\!1$	0.17	0
4	FMT	А	404	-	2,2,2	0.27	0	1,1,1	0.16	0
4	FMT	А	408	-	2,2,2	0.32	0	$1,\!1,\!1$	0.15	0
5	OVR	А	409	-	24,24,24	0.28	0	26,34,34	0.48	0
3	EDO	А	402	-	$3,\!3,\!3$	0.06	0	2,2,2	0.01	0
4	FMT	А	406	-	2,2,2	0.25	0	1,1,1	0.18	0
4	FMT	А	414	-	2,2,2	0.32	0	1,1,1	0.11	0
6	SIN	А	411	2	7,7,7	0.98	0	8,8,8	1.42	1 (12%)
3	EDO	А	405	-	3,3,3	0.05	0	2,2,2	0.26	0
4	FMT	А	412	-	2,2,2	0.30	0	1,1,1	0.19	0
3	EDO	А	410	-	3,3,3	0.08	0	2,2,2	0.27	0
3	EDO	А	403	-	3,3,3	0.06	0	2,2,2	0.19	0
4	FMT	А	407	-	2,2,2	0.25	0	$1,\!1,\!1$	0.18	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
5	OVR	А	409	-	-	1/14/38/38	0/4/3/3
3	EDO	А	402	-	-	0/1/1/1	-
6	SIN	А	411	2	-	4/5/5/5	-
3	EDO	А	405	-	-	1/1/1/1	-
3	EDO	А	410	-	-	0/1/1/1	-
3	EDO	А	403	-	-	0/1/1/1	-

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	vpe Atoms		$Observed(^{o})$	$Ideal(^{o})$
6	А	411	SIN	O1-C1-C2	-2.12	116.29	123.08

There are no chirality outliers.

5 of 6 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	А	405	EDO	O1-C1-C2-O2
6	А	411	SIN	C2-C3-C4-O3
6	А	411	SIN	C2-C3-C4-O4
6	А	411	SIN	C1-C2-C3-C4
5	А	409	OVR	C9-C10-C17-O3

There are no ring outliers.

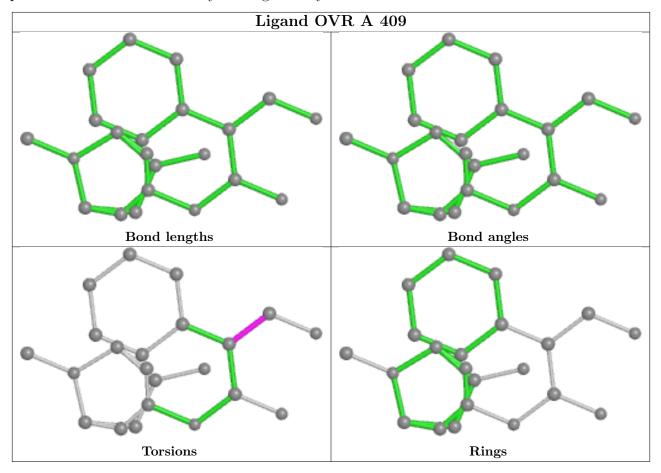
2 monomers are involved in 2 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
6	А	411	SIN	1	0
3	А	405	EDO	1	0

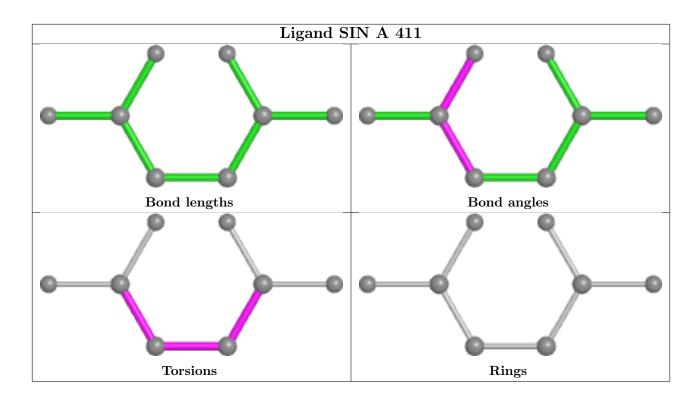
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. 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	$\langle RSRZ \rangle$	$\#RSRZ{>}2$		$OWAB(Å^2)$	Q < 0.9	
1	А	325/385~(84%)	0.15	9 (2%)	53	54	8, 14, 25, 37	0

The worst 5 of 9 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	А	240	ALA	4.8
1	А	119	GLN	4.0
1	А	308	ASP	3.6
1	А	27	ASN	3.5
1	А	123	GLU	3.1

#### 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,  $95^{th}$  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	$\mathbf{B} ext{-factors}(\mathrm{\AA}^2)$	Q<0.9
3	EDO	А	405	4/4	0.71	0.20	31,31,32,32	0
4	FMT	А	408	3/3	0.72	0.28	37,37,38,38	0

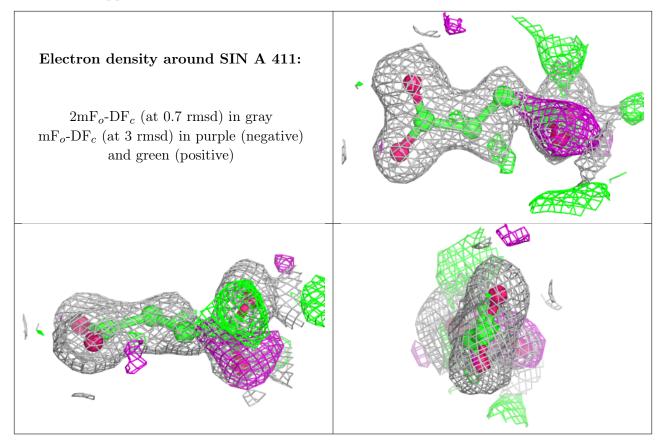
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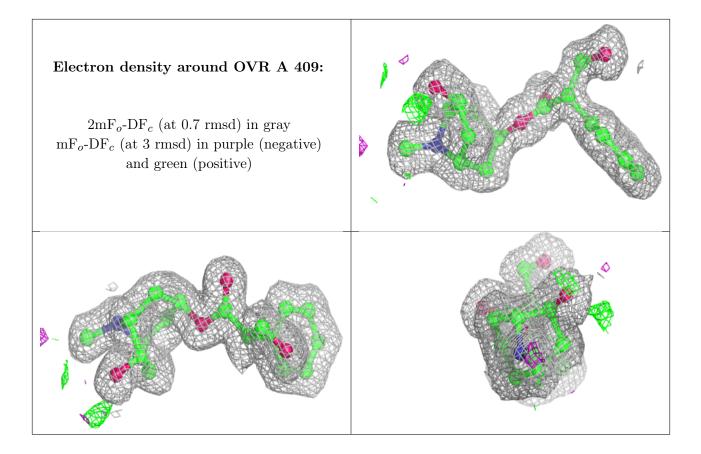
Mol	Type	Chain	Res	Atoms	RSCC	RSR	$B-factors(A^2)$	Q<0.9
4	FMT	А	413	3/3	0.73	0.22	37,37,38,38	0
3	EDO	А	410	4/4	0.74	0.20	33,33,33,34	0
4	FMT	А	404	3/3	0.76	0.19	34,34,35,37	0
4	FMT	А	412	3/3	0.83	0.22	32,32,34,34	0
6	SIN	А	411	8/8	0.84	0.21	$12,\!14,\!15,\!17$	8
4	FMT	А	414	3/3	0.86	0.28	29,29,30,30	0
4	FMT	А	406	3/3	0.89	0.09	39,39,39,40	0
3	EDO	А	402	4/4	0.90	0.11	$26,\!26,\!26,\!26$	0
4	FMT	А	407	3/3	0.92	0.12	44,44,44,44	0
5	OVR	А	409	22/22	0.93	0.08	11,12,12,14	0
3	EDO	А	403	4/4	0.95	0.07	22,23,23,23	0
7	SR	А	415	1/1	0.98	0.05	27,27,27,27	1
2	FE2	А	401	1/1	0.99	0.07	10,10,10,10	0
7	SR	А	416	1/1	1.00	0.08	10,10,10,10	0

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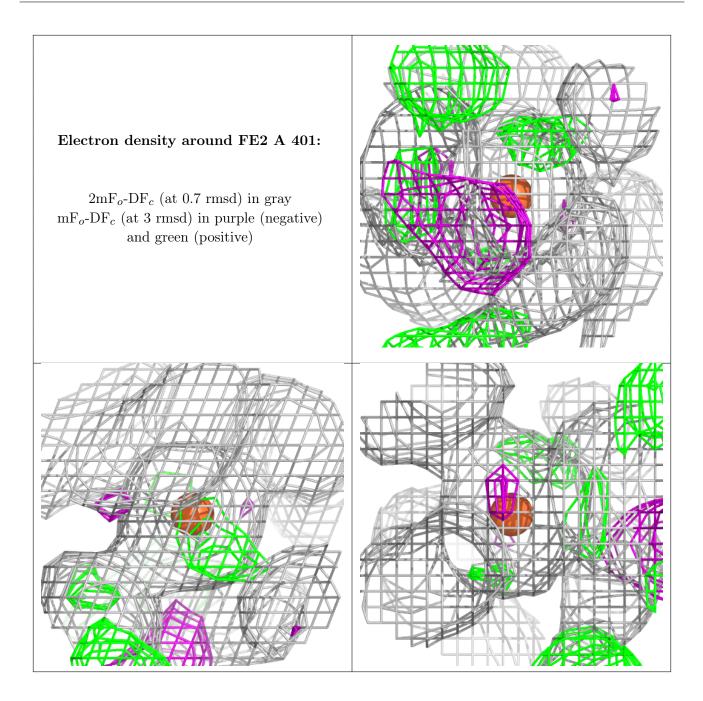
The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation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











### 6.5 Other polymers (i)

There are no such residues in this entry.

