

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

Jan 9, 2023 – 12:31 pm GMT

PDB ID	:	7QOU
Title	:	A mutant of the nitrile hydratase from Geobacillus pallidus having enhanced
		thermostability
Authors	:	Van Wyk, J.C.; Cowan, D.A.; Danson, M.J.; Tsekoa, T.L.; Sayed, M.F.; Sewell,
		B.T.
Deposited on	:	2021-12-29
Resolution	:	1.30  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:

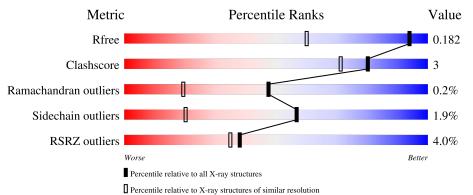
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as $541$ be (2020)
Xtriage (Phenix)	:	1.13
$\mathrm{EDS}$	:	2.31.3
buster-report	:	1.1.7(2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.31.3

# 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 1.30 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	$\begin{array}{c} \textbf{Whole archive} \\ (\#\textbf{Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries,\ resolution\ range}({ m \AA}))$
$R_{free}$	130704	1058 (1.30-1.30)
Clashscore	141614	1101 (1.30-1.30)
Ramachandran outliers	138981	1058 (1.30-1.30)
Sidechain outliers	138945	1058 (1.30-1.30)
RSRZ outliers	127900	1029 (1.30-1.30)

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	216	3% 85%	8% • 6%				
2	В	229	86%	12% ••				



#### $7 \mathrm{QOU}$

## 2 Entry composition (i)

There are 6 unique types of molecules in this entry. The entry contains 3951 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 Nitrile hydratase.

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
1	А	202	Total 1668	C 1062	N 289	O 309	S 8	0	5	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
А	188	VAL	MET	engineered mutation	UNP Q84FS5

• Molecule 2 is a protein called Nitrile hydratase subunit beta.

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
2	В	227	Total 1888	C 1215	N 316	O 348	S 9	0	4	0

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
В	96	GLU	ASP	engineered mutation	UNP Q84FS6
В	167	VAL	ASP	engineered mutation	UNP Q84FS6

• Molecule 3 is COBALT (II) ION (three-letter code: CO) (formula: Co) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	А	1	Total Co 1 1	0	0

• Molecule 4 is MAGNESIUM ION (three-letter code: MG) (formula: Mg) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	2	Total Mg 2 2	0	0

• Molecule 5 is CHLORIDE ION (three-letter code: CL) (formula: Cl) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	А	1	Total Cl 1 1	0	0

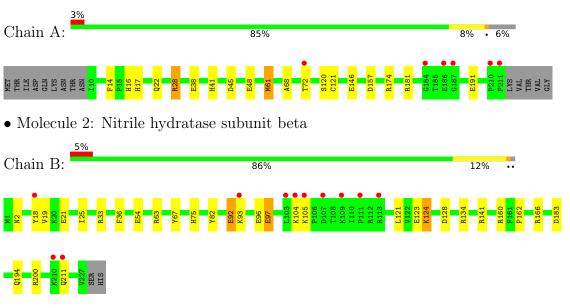
• Molecule 6 is water.

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
6	А	175	Total O 175 175	0	0
6	В	216	Total         O           216         216	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: Nitrile hydratase



## 4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 41 21 2	Depositor
Cell constants	106.08Å $106.08$ Å $82.97$ Å	Depositor
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $90.00^{\circ}$ $90.00^{\circ}$	Depositor
Resolution (Å)	74.95 - 1.30	Depositor
Resolution (A)	36.30 - 1.30	EDS
% Data completeness	98.6 (74.95-1.30)	Depositor
(in resolution range)	98.6 (36.30-1.30)	EDS
R <sub>merge</sub>	0.05	Depositor
R <sub>sym</sub>	(Not available)	Depositor
$< I/\sigma(I) > 1$	$2.15 (at 1.30 \text{\AA})$	Xtriage
Refinement program	PHENIX 1.10.1	Depositor
D D	0.153 , $0.182$	Depositor
$R, R_{free}$	0.157 , $0.182$	DCC
$R_{free}$ test set	5740 reflections $(5.02\%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	14.3	Xtriage
Anisotropy	0.076	Xtriage
Bulk solvent $k_{sol}(e/Å^3), B_{sol}(Å^2)$	0.38, 46.2	EDS
L-test for twinning <sup>2</sup>	$ < L >=0.51, < L^2>=0.35$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.97	EDS
Total number of atoms	3951	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 2.88% 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: CSD, CO, MG, CL

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	I Chain Bo		nd lengths	Bond angles	
IVIOI	Chain	RMSZ	# Z  > 5	RMSZ	# Z  > 5
1	А	1.22	6/1697~(0.4%)	0.99	5/2308~(0.2%)
2	В	1.37	13/1941~(0.7%)	1.10	8/2623~(0.3%)
All	All	1.30	19/3638~(0.5%)	1.05	13/4931~(0.3%)

All (19) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	В	21	GLU	CD-OE2	9.59	1.36	1.25
2	В	25	ILE	CA-CB	7.79	1.72	1.54
2	В	97	GLU	CG-CD	-7.41	1.40	1.51
1	А	191	GLU	CG-CD	7.28	1.62	1.51
2	В	194	GLN	CB-CG	-7.22	1.33	1.52
2	В	82	TYR	CE1-CZ	-7.08	1.29	1.38
2	В	194	GLN	CD-OE1	6.82	1.39	1.24
1	А	48	GLU	CD-OE1	6.57	1.32	1.25
2	В	123	GLU	CB-CG	-6.33	1.40	1.52
1	А	191	GLU	CD-OE1	6.14	1.32	1.25
2	В	63	ARG	CZ-NH2	-5.63	1.25	1.33
1	А	38	GLU	CD-OE1	5.46	1.31	1.25
2	В	96	GLU	CG-CD	5.42	1.60	1.51
1	А	48	GLU	CD-OE2	-5.40	1.19	1.25
2	В	96	GLU	CD-OE1	5.27	1.31	1.25
1	А	146	GLU	CD-OE1	5.13	1.31	1.25
2	В	18	TYR	CG-CD2	5.06	1.45	1.39
2	В	54	GLU	CD-OE1	-5.03	1.20	1.25
2	В	19	VAL	CB-CG1	-5.02	1.42	1.52

All (13) bond angle outliers are listed below:



Mol	Chain	Res	Type	Atoms	Ζ	$Observed(^{o})$	$Ideal(^{o})$
2	В	128	ASP	CB-CG-OD2	-6.93	112.06	118.30
1	А	28	ARG	NE-CZ-NH1	-6.85	116.88	120.30
2	В	200	ARG	NE-CZ-NH2	-6.60	117.00	120.30
1	А	157	ASP	CB-CG-OD2	6.17	123.85	118.30
2	В	33	ARG	NE-CZ-NH1	-5.71	117.44	120.30
1	А	181	ARG	NE-CZ-NH2	-5.64	117.48	120.30
2	В	134	ARG	NE-CZ-NH2	-5.55	117.53	120.30
2	В	141	ARG	NE-CZ-NH2	-5.32	117.64	120.30
1	А	22	GLN	CB-CA-C	5.32	121.04	110.40
2	В	54	GLU	OE1-CD-OE2	-5.16	117.11	123.30
1	А	45	ASP	CB-CG-OD2	-5.12	113.69	118.30
2	В	92	GLU	OE1-CD-OE2	5.07	129.39	123.30
2	В	21	GLU	OE1-CD-OE2	5.02	129.33	123.30

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	1668	0	1647	8	0
2	В	1888	0	1844	11	0
3	А	1	0	0	0	0
4	А	2	0	0	0	0
5	А	1	0	0	0	0
6	А	175	0	0	1	0
6	В	216	0	0	3	0
All	All	3951	0	3491	19	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.

All (19) 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)
2:B:2:ASN:HD21	2:B:166:ARG:HH12	1.24	0.83

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		Interatomic	Clash
Atom-1	Atom-2	distance $(\text{\AA})$	overlap (Å)
1:A:61[B]:MET:HE3	1:A:61[B]:MET:H	1.56	0.68
2:B:124:LYS:NZ	2:B:124:LYS:HB3	2.09	0.66
1:A:41:HIS:HE1	6:B:428:HOH:O	1.82	0.62
1:A:68:ALA:O	1:A:72[B]:THR:HG22	2.01	0.61
2:B:75:HIS:HD2	6:B:481:HOH:O	1.85	0.59
2:B:67:TYR:O	2:B:75:HIS:HE1	1.87	0.57
1:A:16:HIS:H	1:A:16:HIS:CD2	2.21	0.56
2:B:93:LYS:O	2:B:97:GLU:HG3	2.07	0.55
1:A:17:HIS:HD2	6:A:560:HOH:O	1.89	0.54
2:B:124:LYS:HB3	2:B:124:LYS:HZ2	1.73	0.54
2:B:124:LYS:NZ	6:B:302:HOH:O	2.41	0.54
1:A:121:CSD:HB2	1:A:174:ARG:CZ	2.38	0.53
1:A:61[B]:MET:H	1:A:61[B]:MET:CE	2.23	0.52
1:A:14:PHE:HB3	1:A:17:HIS:CD2	2.49	0.47
2:B:104:LYS:O	2:B:105:LYS:HD2	2.16	0.45
2:B:121:LEU:HD23	2:B:121:LEU:C	2.36	0.45
2:B:160:ARG:O	2:B:162:PRO:HD3	2.18	0.43
2:B:2:ASN:ND2	2:B:166:ARG:HH12	2.05	0.41

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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	Perce	ntiles
1	А	203/216~(94%)	196~(97%)	6 (3%)	1 (0%)	29	6
2	В	228/229~(100%)	222~(97%)	6 (3%)	0	100	100
All	All	431/445~(97%)	418 (97%)	12 (3%)	1 (0%)	47	19

All (1) Ramachandran outliers are listed below:



Mol	Chain	Res	Type
1	А	120	SER

#### 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 side chain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles		
1	А	182/190~(96%)	179~(98%)	3~(2%)	62	28	
2	В	198/196~(101%)	193~(98%)	5 (2%)	47	10	
All	All	380/386~(98%)	372~(98%)	8 (2%)	57	16	

All (8) residues with a non-rotameric sidechain are listed below:

Mol	Chain	$\operatorname{Res}$	Type
1	А	28	ARG
1	А	61[A]	MET
1	А	61[B]	MET
2	В	36	PHE
2	В	92	GLU
2	В	124	LYS
2	В	183	ASP
2	В	211	GLN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (8) such sidechains are listed below:

Mol	Chain	Res	Type
1	А	16	HIS
1	А	17	HIS
1	А	41	HIS
2	В	2	ASN
2	В	29	HIS
2	В	75	HIS
2	В	152	ASN
2	В	192	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)

2 non-standard protein/DNA/RNA residues 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		ı Res	Res Link	Bond lengths			Bond angles		
IVIOI	Moi Type Chain F	nes		Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z >2	
1	CSD	А	119	1	3,7,8	1.01	0	1,8,10	0.00	0
1	CSD	А	121	3,1	3,7,8	0.98	0	1,8,10	1.03	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
1	CSD	А	119	1	-	0/2/6/8	-
1	CSD	А	121	3,1	-	0/2/6/8	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

1 monomer is involved in 1 short contact:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	А	121	CSD	1	0



### 5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

## 5.6 Ligand geometry (i)

Of 4 ligands modelled in this entry, 4 are monoatomic - leaving 0 for Mogul analysis.

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

### 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	200/216~(92%)	0.04	6 (3%) 50 48	8, 14, 29, 39	0
2	В	227/229~(99%)	0.09	11 (4%) 30 28	9, 13, 29, 40	0
All	All	427/445~(95%)	0.07	17 (3%) 38 35	8, 14, 29, 40	0

All (17) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	В	210	LYS	4.4
2	В	18	TYR	4.4
1	А	211	PRO	3.8
2	В	104	LYS	3.7
2	В	105	LYS	3.3
2	В	107	ASP	3.1
2	В	109	LYS	2.8
2	В	111	PRO	2.8
1	А	184	GLY	2.6
1	А	210	PRO	2.6
2	В	93	LYS	2.5
2	В	211	GLN	2.4
1	А	72[A]	THR	2.2
2	В	103	LEU	2.2
1	А	186	GLU	2.1
1	А	187	GLY	2.1
2	В	113	ARG	2.1

## 6.2 Non-standard residues in protein, DNA, RNA chains (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
1	CSD	А	119	8/9	0.98	0.06	$9,\!11,\!12,\!12$	0
1	CSD	А	121	8/9	0.98	0.06	9,11,12,14	0

#### 6.3 Carbohydrates (i)

There are no monosaccharides in this entry.

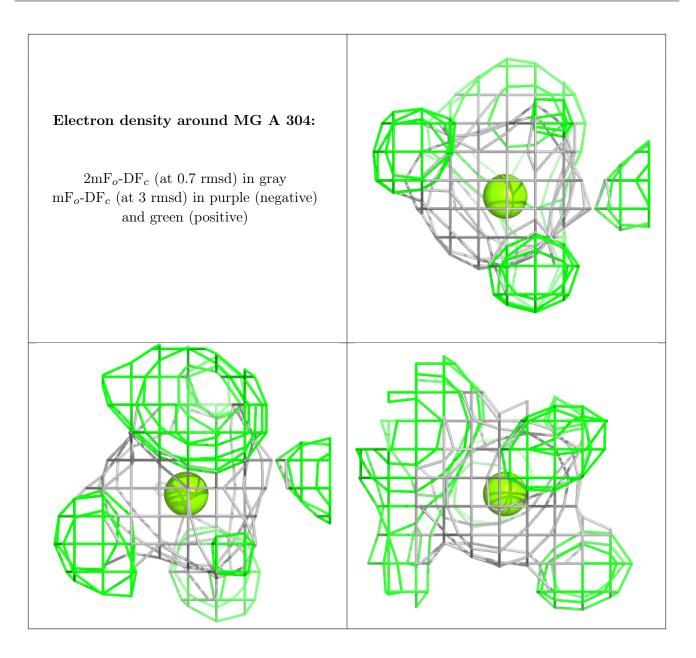
### 6.4 Ligands (i)

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.

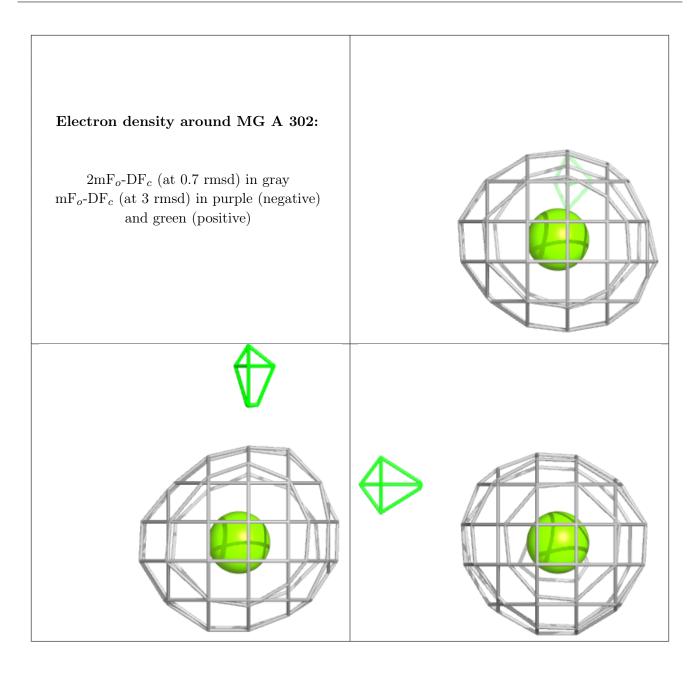
Mol	Type	Chain	Res	Atoms	RSCC	RSR	$\mathbf{B} ext{-factors}(\mathrm{\AA}^2)$	Q<0.9
4	MG	А	304	1/1	0.97	0.04	36, 36, 36, 36	0
4	MG	А	302	1/1	1.00	0.06	$17,\!17,\!17,\!17$	0
3	CO	А	301	1/1	1.00	0.07	8,8,8,8	1
5	CL	А	303	1/1	1.00	0.05	12,12,12,12	0

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.

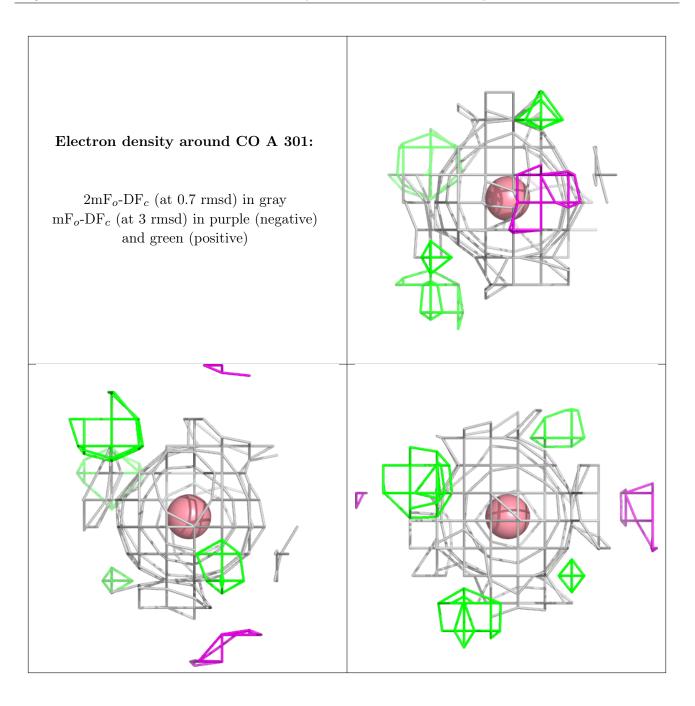




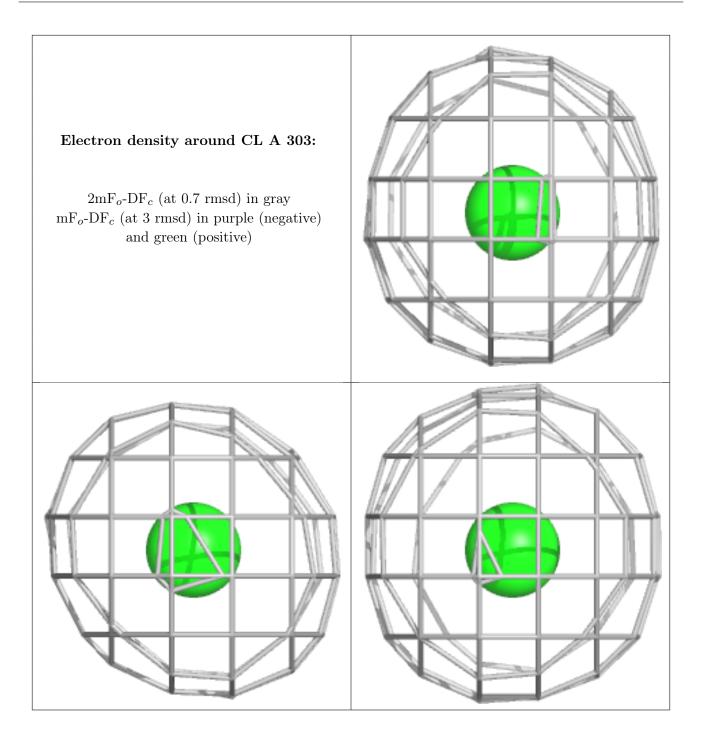












## 6.5 Other polymers (i)

There are no such residues in this entry.

