

Full wwPDB X-ray Structure Validation Report (i)

Aug 1, 2024 – 04:33 pm BST

PDB ID : 8RFU

Title: Low pH (5.5) nitrite-bound MSOX movie series dataset 40 of the copper nitrite

reductase (NirK) from Bradyrhizobium japonicum USDA110 [24.4 MGy] -

water ligand + decarboxylated AspCAT (final)

Authors: Rose, S.L.; Ferroni, F.M.; Antonyuk, S.V.; Eady, R.R.; Hasnain, S.S.

Deposited on : 2023-12-13

Resolution : 2.18 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 (1)) were used in the production of this report:

Mol Probity : 4.02b-467

Mogul : 1.8.4, CSD as541be (2020)

Xtriage (Phenix) : 1.13 EDS : 2.37.1

Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)

 $Refmac \quad : \quad 5.8.0158$

CCP4 : 7.0.044 (Gargrove)

Ideal geometry (proteins) : Engh & Huber (2001) Ideal geometry (DNA, RNA) : Parkinson et al. (1996)

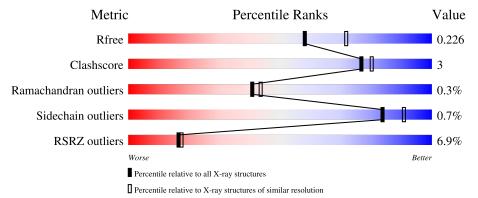
Validation Pipeline (wwPDB-VP) : 2.37.1

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 2.18 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	$\begin{array}{c} \text{Whole archive} \\ (\#\text{Entries}) \end{array}$	Similar resolution $(\# \text{Entries, resolution range}(\text{\AA}))$
R_{free}	130704	6864 (2.20-2.16)
Clashscore	141614	7689 (2.20-2.16)
Ramachandran outliers	138981	7564 (2.20-2.16)
Sidechain outliers	138945	7564 (2.20-2.16)
RSRZ outliers	127900	6738 (2.20-2.16)

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	340	7% 87%	11%	-	
2	С	2	100%			



2 Entry composition (i)

There are 6 unique types of molecules in this entry. The entry contains 2760 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 Copper-containing nitrite reductase.

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
1	Λ	334	Total	С	N	О	S	0	15	0
1	A	334	2635	1691	446	479	19	U	10	U

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	MET	-	initiating methionine	UNP Q89EJ6
A	310	CYS	GLY	conflict	UNP Q89EJ6

• Molecule 2 is an oligosaccharide called beta-D-fructofuranose-(2-1)-alpha-D-glucopyranose.



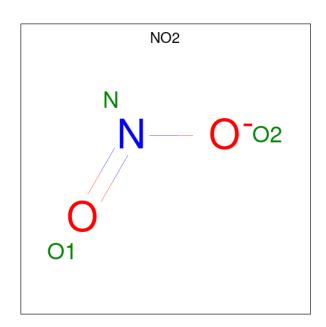
Mol	Chain	Residues	At	oms		ZeroOcc	AltConf	Trace
2	С	2	Total 23	C 12	O 11	0	0	0

• Molecule 3 is COPPER (II) ION (three-letter code: CU) (formula: Cu) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	A	2	Total Cu 2 2	0	0

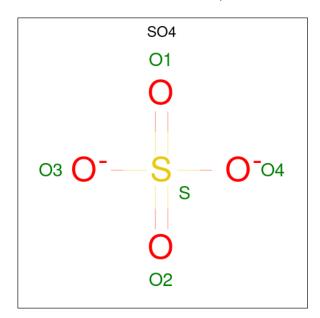
• Molecule 4 is NITRITE ION (three-letter code: NO2) (formula: NO₂).





Mol	Chain	Residues	Atoms		ZeroOcc	AltConf	
4	A	1	Total 3	N 1	O 2	0	0

 \bullet Molecule 5 is SULFATE ION (three-letter code: SO4) (formula: $\mathrm{O_4S}).$



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	A	1	Total O S 5 4 1	0	0
5	A	1	Total O S 5 4 1	0	0
5	A	1	Total O S 5 4 1	0	0

Continued on next page...



Continued from previous page...

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	A	1	Total O S 5 4 1	0	0
5	A	1	Total O S 5 4 1	0	0

• Molecule 6 is water.

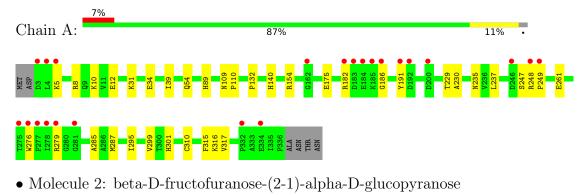
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
6	A	72	Total O 72 72	0	1



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: Copper-containing nitrite reductase



Chain C: 100%

GLC1 FRU2



4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 63	Depositor
Cell constants	104.52Å 104.52Å 64.69Å	Depositor
a, b, c, α , β , γ	90.00° 90.00° 120.00°	Depositor
Resolution (Å)	30.00 - 2.18	Depositor
Resolution (A)	45.26 - 2.18	EDS
% Data completeness	99.9 (30.00-2.18)	Depositor
(in resolution range)	99.9 (45.26-2.18)	EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) > 1$	1.59 (at 2.18Å)	Xtriage
Refinement program	REFMAC 5.8.0419	Depositor
D D.	0.186 , 0.222	Depositor
R, R_{free}	0.191 , 0.226	DCC
R_{free} test set	1050 reflections (4.97%)	wwPDB-VP
Wilson B-factor (Å ²)	40.1	Xtriage
Anisotropy	0.356	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$, $B_{sol}(Å^2)$	0.34, 42.9	EDS
L-test for twinning ²	$< L > = 0.49, < L^2> = 0.32$	Xtriage
Estimated twinning fraction	0.056 for h,-h-k,-l	Xtriage
F_o, F_c correlation	0.96	EDS
Total number of atoms	2760	wwPDB-VP
Average B, all atoms (Å ²)	46.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 3.65% of the height of the origin peak. No significant pseudotranslation is detected.

²Theoretical 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.



¹Intensities 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: FRU, SO4, NO2, CU, GLC

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		
IVIOI	Chain	RMSZ	# Z > 5	RMSZ	# Z > 5	
1	A	0.49	0/2743	0.89	2/3720 (0.1%)	

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a maintain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
1	A	0	2

There are no bond length outliers.

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$Observed(^o)$	$\operatorname{Ideal}({}^{o})$
1	A	229	THR	CA-CB-OG1	-6.02	96.37	109.00
1	A	315	PHE	CB-CA-C	-5.55	99.31	110.40

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	154	ARG	Sidechain
1	A	301	HIS	Peptide

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



Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	2635	0	2636	16	0
2	С	23	0	21	0	0
3	A	2	0	0	0	0
4	A	3	0	0	0	0
5	A	25	0	0	0	0
6	A	72	0	0	1	0
All	All	2760	0	2657	16	0

the asymmetric unit, whereas Symm-Clashes lists symmetry-related clashes.

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 (16) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	$\begin{array}{c} \text{Interatomic} \\ \text{distance (Å)} \end{array}$	Clash overlap (Å)
1:A:235[A]:ASN:ND2	1:A:287:MET:SD	2.48	0.85
1:A:295:ILE:O	1:A:295:ILE:HD12	2.07	0.54
1:A:249:PRO:HA	1:A:299:VAL:O	2.13	0.48
1:A:10:LYS:HE2	1:A:12:GLU:OE2	2.14	0.48
1:A:182:ARG:NH1	1:A:186:GLY:O	2.47	0.48
1:A:8:ARG:HD3	1:A:34:GLU:OE2	2.15	0.46
1:A:230:ALA:O	1:A:317:VAL:HA	2.16	0.46
1:A:54:GLN:NE2	1:A:191:TYR:O	2.48	0.44
1:A:237:LEU:HD11	1:A:285:ALA:HB1	2.00	0.43
1:A:132:PRO:HG2	1:A:140:HIS:CE1	2.54	0.43
1:A:109:ASN:HB3	1:A:110:PRO:HD2	2.00	0.42
1:A:175:GLU:OE2	1:A:247:SER:HB2	2.19	0.42
1:A:31:LYS:HG3	6:A:504:HOH:O	2.18	0.42
1:A:39:ILE:HD13	1:A:89:HIS:HB2	2.02	0.41
1:A:248:ARG:HA	1:A:276:TRP:O	2.20	0.41
1:A:261:GLU:HG3	1:A:279[A]:ARG:HH12	1.84	0.41

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	Percentil	$\mathbf{e}\mathbf{s}$
1	A	347/340 (102%)	338 (97%)	8 (2%)	1 (0%)	41 43	

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	310	CYS

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	Analysed Rotameric		Percentiles	
1	A	282/275 (102%)	280 (99%)	2 (1%)	84 91	

All (2) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	5	LYS
1	A	316	LYS

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)

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).

Mal	Mol Type Chain		Res	Dog	Link	Bo	Bond lengths			ond ang	cles
IVIOI	Type	Chain	nes	LIIIK	Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2	
2	GLC	С	1	2	11,11,12	0.79	0	15,15,17	1.96	6 (40%)	
2	FRU	С	2	2	11,12,12	1.22	1 (9%)	10,18,18	1.46	1 (10%)	

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.

N	Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
	2	GLC	С	1	2	-	2/2/19/22	0/1/1/1
	2	FRU	С	2	2	-	3/5/24/24	0/1/1/1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	$Ideal(\AA)$
2	С	2	FRU	O2-C2	2.41	1.44	1.40

All (7) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$Observed(^o)$	$Ideal(^{o})$
2	С	2	FRU	O2-C2-O5	3.44	116.14	109.50
2	С	1	GLC	C3-C4-C5	-3.28	104.38	110.24
2	С	1	GLC	O5-C5-C6	3.27	112.33	107.20
2	С	1	GLC	O3-C3-C2	2.94	115.62	109.99
2	С	1	GLC	O5-C5-C4	-2.66	104.36	110.83
2	С	1	GLC	C2-C3-C4	-2.45	106.65	110.89
2	С	1	GLC	O4-C4-C5	2.17	114.67	109.30

There are no chirality outliers.

All (5) torsion outliers are listed below:

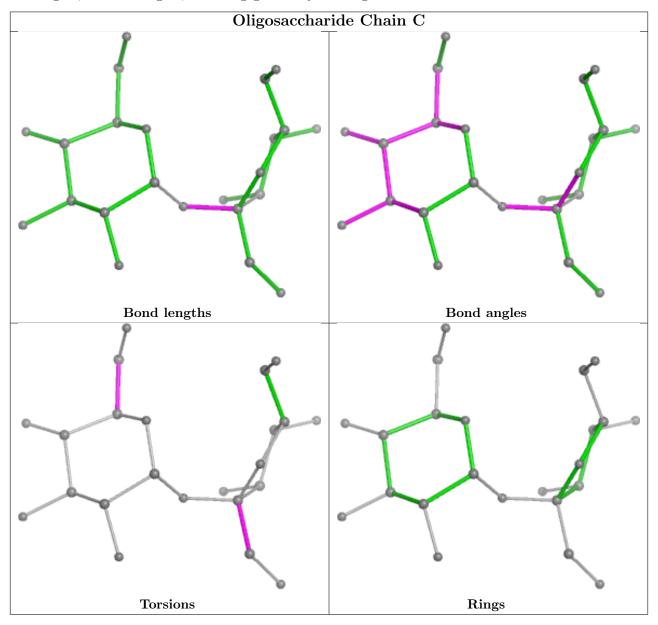


Mol	Chain	Res	Type	Atoms
2	С	2	FRU	O1-C1-C2-C3
2	С	2	FRU	O1-C1-C2-O2
2	С	2	FRU	O1-C1-C2-O5
2	С	1	GLC	O5-C5-C6-O6
2	С	1	GLC	C4-C5-C6-O6

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)

Of 8 ligands modelled in this entry, 2 are monoatomic - leaving 6 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 Type		Chain Res Link Bond lengths				$_{ m gths}$	Bond angles			
MIOI	Type	Chain	nes	Lilik	Counts	RMSZ	# Z >2	Counts	RMSZ	# Z >2
4	NO2	A	403	-	1,2,2	0.95	0	0,1,1	-	-
5	SO4	A	408	-	4,4,4	0.38	0	6,6,6	0.13	0
5	SO4	A	407	_	4,4,4	0.37	0	6,6,6	0.15	0
5	SO4	A	404	_	4,4,4	0.23	0	6,6,6	0.21	0
5	SO4	A	405	_	4,4,4	0.37	0	6,6,6	0.17	0
5	SO4	A	406	-	4,4,4	0.28	0	6,6,6	0.09	0

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	<rsrz></rsrz>	#RS	RZ>2	$OWAB(Å^2)$	Q<0.9
1	A	334/340 (98%)	0.21	23 (6%)	16 17	33, 44, 62, 65	14 (4%)

All (23) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	4	LEU	5.2
1	A	186	GLY	4.5
1	A	183	ASP	4.2
1	A	278	ILE	4.1
1	A	185	LYS	4.1
1	A	5	LYS	3.9
1	A	182	ARG	3.7
1	A	277	PHE	3.4
1	A	3	ASP	3.2
1	A	184	GLU	3.1
1	A	276	TRP	2.9
1	A	275	THR	2.9
1	A	281	GLY	2.6
1	A	191	TYR	2.6
1	A	248	ARG	2.5
1	A	192	ASP	2.5
1	A	249	PRO	2.4
1	A	162[A]	GLY	2.4
1	A	334	GLU	2.3
1	A	279[A]	ARG	2.3
1	A	332	PRO	2.2
1	A	200	ASP	2.2
1	A	246	ASP	2.1



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

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

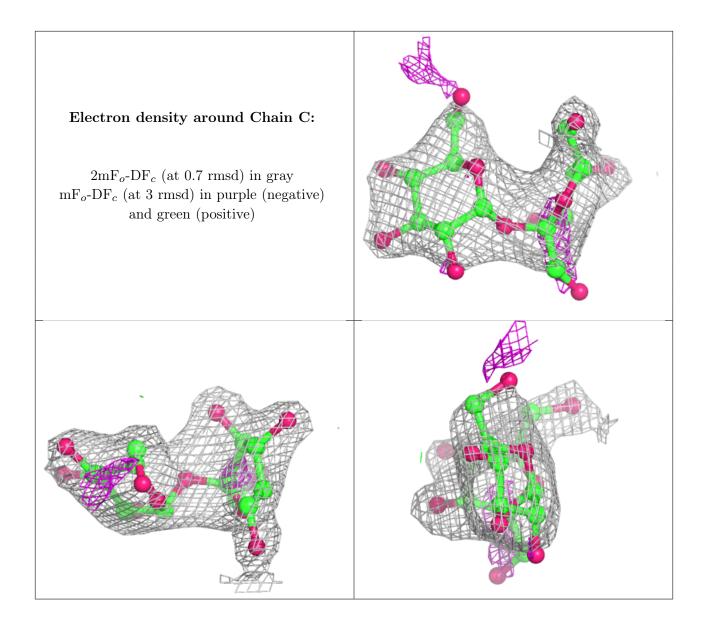
6.3 Carbohydrates (i)

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	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
2	GLC	С	1	11/12	0.92	0.34	65,65,65,65	0
2	FRU	С	2	12/12	0.92	0.28	60,65,65,65	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	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
4	NO2	A	403	3/3	0.86	0.31	65,65,65,65	0
5	SO4	A	408	5/5	0.88	0.31	49,57,63,64	5
5	SO4	A	404	5/5	0.90	0.27	57,63,65,65	5
5	SO4	A	407	5/5	0.95	0.13	55,56,57,62	5
5	SO4	A	406	5/5	0.95	0.39	65,65,65,65	5
5	SO4	A	405	5/5	0.97	0.08	61,62,65,65	5
3	CU	A	401	1/1	0.99	0.08	42,42,42,42	0

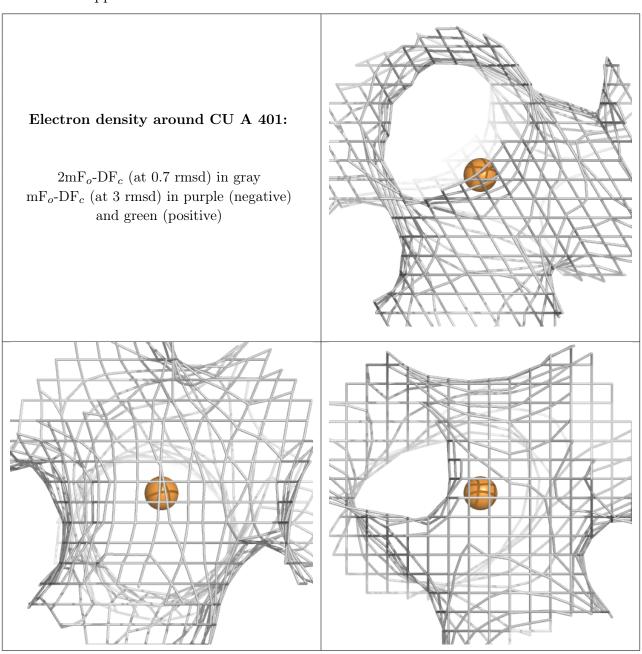
Continued on next page...



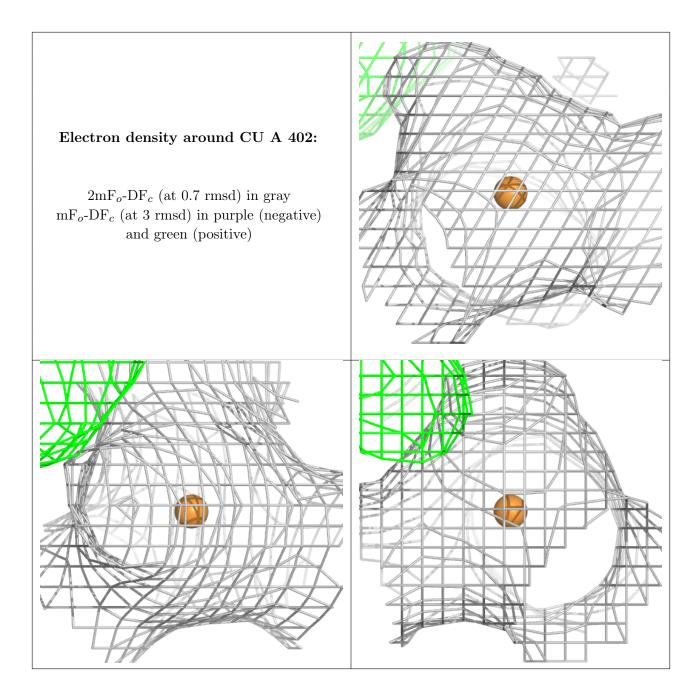
Continued from previous page...

Mol	Type	Chain	Res	Atoms	RSCC	RSR	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
3	CU	A	402	1/1	1.00	0.14	39,39,39,39	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.

