

Full wwPDB X-ray Structure Validation Report (i)

Dec 2, 2024 – 08:08 PM JST

PDB ID : 9K8W

Title : Crystal structure of the calcium indicator GCaMP6s-BrUS in calcium-bound

state

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Deposited on : 2024-10-24

Resolution : 2.65 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:

 $Mol Probity \quad : \quad 4.02b\text{--}467$

Mogul: 1.8.5 (274361), CSD as541be (2020)

Xtriage (Phenix) : 1.21 EDS : 3.0

Percentile statistics : 20231227.v01 (using entries in the PDB archive December 27th 2023)

CCP4 : 9.0.004 (Gargrove)

Density-Fitness : 1.0.11

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

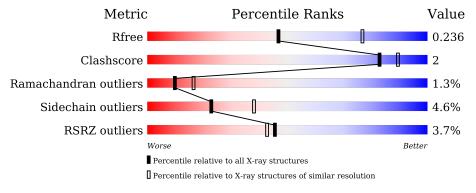
Validation Pipeline (wwPDB-VP) : 2.40

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 2.65 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	Whole archive $(\# \mathrm{Entries})$	$\begin{array}{c} {\rm Similar\ resolution} \\ (\#{\rm Entries},\ {\rm resolution\ range}({\rm \AA})) \end{array}$
R_{free}	164625	1003 (2.66-2.66)
Clashscore	180529	1063 (2.66-2.66)
Ramachandran outliers	177936	1052 (2.66-2.66)
Sidechain outliers	177891	1052 (2.66-2.66)
RSRZ outliers	164620	1003 (2.66-2.66)

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			
			3%			
1	A	461	70%	15%	•	13%



2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 3230 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 Calcium indicator GCaMP6s-BrUS, Calmodulin-1.

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
1	Λ	403	Total	С	N	О	S	0	0	0
1	A	403	3136	1975	524	621	16	U	0	U

There are 6 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	316	PRO	ALA	engineered mutation	UNP P0DP23
A	375	ASP	ASN	engineered mutation	UNP P0DP23
A	393	TYR	ASP	engineered mutation	UNP P0DP23
A	394	ARG	THR	engineered mutation	UNP P0DP23
A	396	THR	SER	engineered mutation	UNP P0DP23
A	405	GLY	ARG	engineered mutation	UNP P0DP23

• Molecule 2 is CALCIUM ION (three-letter code: CA) (formula: Ca) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	A	4	Total Ca 4 4	0	0

• Molecule 3 is water.

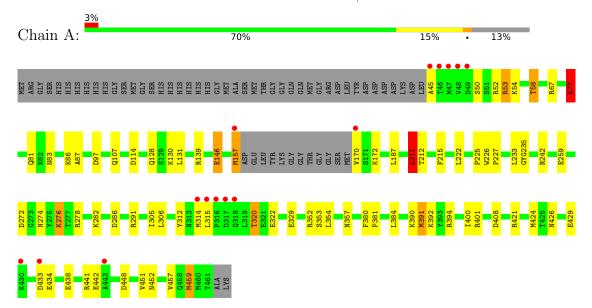
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	A	90	Total O 90 90	0	0



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: Calcium indicator GCaMP6s-BrUS, Calmodulin-1





4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 41 21 2	Depositor
Cell constants	121.54Å 121.54Å 97.90Å	Donogitor
a, b, c, α , β , γ	90.00° 90.00° 90.00°	Depositor
Resolution (Å)	47.52 - 2.65	Depositor
Resolution (A)	47.52 - 2.65	EDS
% Data completeness	98.9 (47.52-2.65)	Depositor
(in resolution range)	98.9 (47.52-2.65)	EDS
R_{merge}	0.18	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) > 1$	1.50 (at 2.65Å)	Xtriage
Refinement program	REFMAC 5.8.0430	Depositor
D D	0.181 , 0.236	Depositor
R, R_{free}	0.181 , 0.236	DCC
R_{free} test set	1080 reflections (5.01%)	wwPDB-VP
Wilson B-factor (Å ²)	49.9	Xtriage
Anisotropy	0.047	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$, $B_{sol}(Å^2)$	0.32, 51.2	EDS
L-test for twinning ²	$ < L > = 0.50, < L^2> = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.95	EDS
Total number of atoms	3230	wwPDB-VP
Average B, all atoms $(Å^2)$	54.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 5.58% 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: ${\rm CA},$ ${\rm CR2}$

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	Во	ond angles
IVIOI	Chain	RMSZ	lengths $\# Z > 5$	RMSZ	# Z > 5
1	A	0.62	0/3174	1.52	53/4286 (1.2%)

There are no bond length outliers.

All (53) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
1	A	242	ARG	CG-CD-NE	-10.48	89.78	111.80
1	A	81	GLN	N-CA-CB	9.88	128.38	110.60
1	A	81	GLN	CB-CA-C	-9.09	92.23	110.40
1	A	53	ARG	CA-CB-CG	8.94	133.06	113.40
1	A	394	ARG	NE-CZ-NH2	-8.41	116.10	120.30
1	A	131	LEU	CB-CG-CD2	-7.93	97.51	111.00
1	A	312	TYR	CB-CA-C	7.93	126.25	110.40
1	A	401	ARG	NE-CZ-NH1	7.47	124.03	120.30
1	A	276	LYS	CA-CB-CG	7.41	129.69	113.40
1	A	441	ARG	CD-NE-CZ	7.35	133.90	123.60
1	A	441	ARG	CA-CB-CG	7.18	129.19	113.40
1	A	276	LYS	CB-CG-CD	7.06	129.95	111.60
1	A	77	LYS	CA-CB-CG	6.88	128.53	113.40
1	A	146	GLU	N-CA-CB	-6.86	98.25	110.60
1	A	272	ASP	CB-CA-C	6.62	123.64	110.40
1	A	97	ASP	CB-CA-C	6.58	123.56	110.40
1	A	54	LYS	CB-CG-CD	6.54	128.60	111.60
1	A	170	VAL	CA-CB-CG1	6.53	120.70	110.90
1	A	322	GLU	CB-CA-C	6.27	122.94	110.40
1	A	211	LEU	CB-CG-CD2	6.26	121.64	111.00
1	A	130	LYS	CB-CA-C	6.17	122.75	110.40
1	A	128	GLN	N-CA-CB	6.02	121.44	110.60
1	A	392	LYS	N-CA-CB	6.02	121.44	110.60
1	A	114	ASP	CB-CA-C	5.97	122.34	110.40

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Mol	Chain	Res	Type		\mathbf{Z}	$Observed(^o)$	$\operatorname{Ideal}({}^{o})$
1	A	401	ARG	CD-NE-CZ	5.93	131.90	123.60
1	A	278	ARG	NE-CZ-NH1	5.91	123.26	120.30
1	A	442	GLU	N-CA-CB	5.90	121.22	110.60
1	A	67	ARG	NE-CZ-NH2	-5.87	117.36	120.30
1	A	286	ASP	CB-CA-C	5.87	122.14	110.40
1	A	421	ARG	N-CA-CB	5.80	121.04	110.60
1	A	354	LEU	CB-CG-CD1	-5.79	101.17	111.00
1	A	352	ARG	NE-CZ-NH2	-5.71	117.45	120.30
1	A	172	LYS	CB-CA-C	5.61	121.61	110.40
1	A	390	LYS	CB-CG-CD	5.60	126.15	111.60
1	A	187	LEU	CB-CG-CD1	-5.56	101.55	111.00
1	A	401	ARG	NE-CZ-NH2	-5.51	117.55	120.30
1	A	211	LEU	N-CA-CB	-5.47	99.45	110.40
1	A	45	ALA	CB-CA-C	5.34	118.11	110.10
1	A	391	MET	CG-SD-CE	-5.34	91.66	100.20
1	A	291	ARG	CG-CD-NE	-5.33	100.60	111.80
1	A	282	LYS	CA-CB-CG	5.29	125.05	113.40
1	A	83	ASN	CB-CA-C	5.22	120.83	110.40
1	A	139	ARG	NE-CZ-NH1	5.21	122.91	120.30
1	A	259	GLU	N-CA-CB	5.18	119.93	110.60
1	A	291	ARG	CD-NE-CZ	5.18	130.86	123.60
1	A	408	ASP	CB-CA-C	5.17	120.73	110.40
1	A	320	THR	CA-CB-OG1	5.15	119.81	109.00
1	A	392	LYS	CA-CB-CG	5.15	124.72	113.40
1	A	329	GLU	CB-CA-C	5.14	120.68	110.40
1	A	52	ARG	CB-CA-C	5.08	120.56	110.40
1	A	384	LEU	CB-CG-CD2	5.04	119.56	111.00
1	A	392	LYS	CB-CA-C	-5.03	100.34	110.40
1	A	157	MET	CB-CG-SD	5.03	127.49	112.40

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	A	3136	0	2977	14	0
2	A	4	0	0	0	0
3	A	90	0	0	0	0
All	All	3230	0	2977	14	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 2.

All (14) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic	Clash
Atom-1	Atom-2	${f distance}({ m \AA})$	overlap (Å)
1:A:77:LYS:HE3	1:A:86:LYS:HD2	1.75	0.69
1:A:58:THR:HG21	1:A:424:MET:HG2	1.84	0.58
1:A:353:SER:HB2	1:A:426:ASN:HB3	1.87	0.56
1:A:87:ALA:HB3	1:A:107:GLN:HB3	1.90	0.52
1:A:380:PHE:HB3	1:A:381:PRO:HD3	1.97	0.47
1:A:400:ILE:HG21	1:A:457:VAL:HG22	1.97	0.47
1:A:451:VAL:HG11	1:A:459:MET:HE2	1.97	0.46
1:A:146:GLU:HB3	1:A:211:LEU:HB3	1.97	0.46
1:A:222:LEU:HD22	1:A:226:TRP:CE2	2.52	0.45
1:A:215:PHE:CZ	1:A:233:LEU:HB3	2.53	0.43
1:A:50:SER:HA	1:A:53:ARG:HB3	2.02	0.41
1:A:225:PRO:HD3	1:A:305:ILE:O	2.20	0.41
1:A:306:LEU:HD23	1:A:306:LEU:HA	1.94	0.41
1:A:451:VAL:HG11	1:A:459:MET:CE	2.51	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	A	396/461 (86%)	375 (95%)	16 (4%)	5 (1%)	10 16	



All (5) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	429	GLU
1	A	434	GLU
1	A	314	MET
1	A	433	ASP
1	A	315	LEU

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	A	328/391 (84%)	313 (95%)	15 (5%)	23 38		

All (15) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	58	THR
1	A	77	LYS
1	A	157	MET
1	A	211	LEU
1	A	212	THR
1	A	227	PRO
1	A	274	ASN
1	A	276	LYS
1	A	320	THR
1	A	357	ASN
1	A	391	MET
1	A	438	GLU
1	A	448	ASP
1	A	452	ASN
1	A	459	MET

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (2) such sidechains are listed below:



Mol	Chain	Res	Type
1	A	56	ASN
1	A	356	GLN

5.3.3 RNA (i)

There are no RNA molecules in this entry.

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

1 non-standard protein/DNA/RNA residue is 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	Type	Chain	Res	$\mathbf{s} \mid \mathbf{Link} \mid$	Bo	Bond lengths			Bond angles		
Mol	туре				Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2	
1	CR2	A	235	1	20,20,21	1.08	1 (5%)	25,27,29	2.47	9 (36%)	

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	CR2	A	235	1	-	1/6/25/26	0/2/2/2

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(A)	$\operatorname{Ideal}(\text{\AA})$
1	A	235	CR2	C1-N3	3.59	1.42	1.37

All (9) bond angle outliers are listed below:

Mol	Chain	Res	Type	ype Atoms		$\mathbf{Observed}(^o)$	$\operatorname{Ideal}({}^{o})$
1	A	235	CR2	C2-N3-C1	-6.92	104.61	107.99
1	A	235	CR2	CA2-C2-N3	5.51	105.97	103.37
1	A	235	CR2	CA1-C1-N2	-4.67	118.02	124.28

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Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$\mathbf{Observed}(^o)$	$\operatorname{Ideal}({}^o)$
1	A	235	CR2	N3-C1-N2	3.10	115.48	111.76
1	A	235	CR2	C1-CA1-N1	-2.97	106.27	112.85
1	A	235	CR2	CA1-C1-N3	2.86	126.35	122.52
1	A	235	CR2	O3-C3-CA3	-2.76	118.06	126.39
1	A	235	CR2	O2-C2-CA2	-2.35	129.64	130.96
1	A	235	CR2	CG2-CB2-CA2	2.01	132.41	129.94

There are no chirality outliers.

All (1) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	A	235	CR2	C3-CA3-N3-C2

There are no ring outliers.

No monomer is involved in short contacts.

5.5 Carbohydrates (i)

There are no oligosaccharides 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	$\langle { m RSRZ} \rangle$	$\# \mathrm{RSRZ}{>}2$	$OWAB(Å^2)$	Q<0.9
1	A	402/461 (87%)	-0.55	15 (3%) 45 43	28, 45, 107, 144	0

All (15) RSRZ outliers are listed below:

Mol	Chain	$\operatorname{Res} \mid \operatorname{Type} \mid$		RSRZ
1	A	45	ALA	5.0
1	A	316	PRO	4.6
1	A	49	ASP	4.5
1	A	48	VAL	4.3
1	A	317	ASP	4.0
1	A	157	MET	3.4
1	A	433	ASP	3.3
1	A	170	VAL	3.3
1	A	315	LEU	3.2
1	A	46	THR	3.0
1	A	47	MET	3.0
1	A	314	MET	2.7
1	A	430	LYS	2.5
1	A	318	GLN	2.3
1	A	443	ALA	2.2

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	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
1	CR2	A	235	19/20	0.97	0.07	29,36,58,59	0



6.3 Carbohydrates (i)

There are no monosaccharides in this entry.

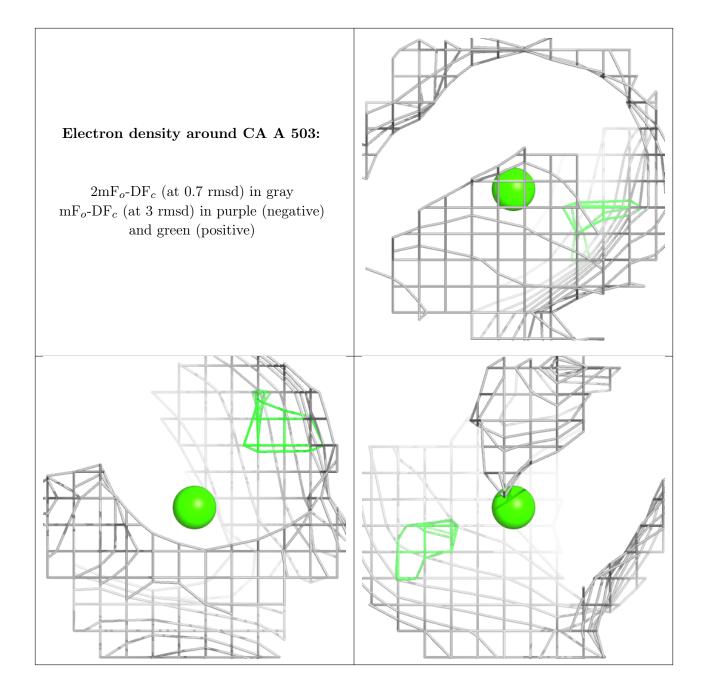
6.4 Ligands (i)

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	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
2	CA	A	503	1/1	0.98	0.07	68,68,68,68	1
2	CA	A	502	1/1	0.99	0.02	37,37,37,37	0
2	CA	A	504	1/1	0.99	0.03	61,61,61,61	1
2	CA	A	501	1/1	1.00	0.01	38,38,38,38	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.

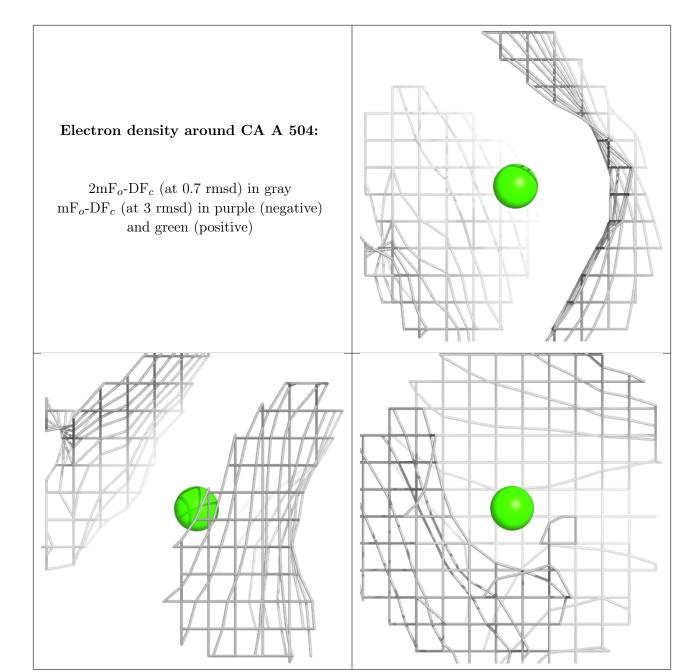




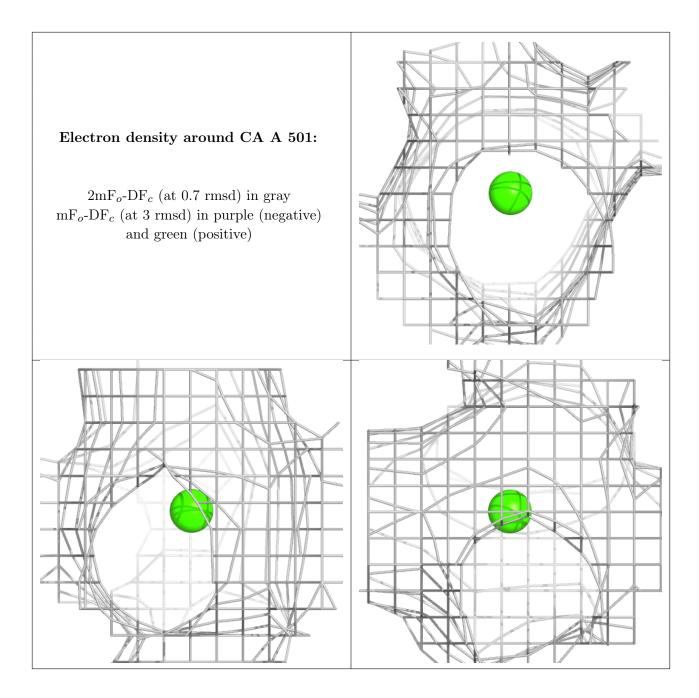


Electron density around CA A 502: $2 \mathrm{mF}_o\text{-}\mathrm{DF}_c$ (at 0.7 rmsd) in gray ${\rm mF}_o\text{-}{\rm DF}_c$ (at 3 rmsd) in purple (negative) and green (positive)









6.5 Other polymers (i)

There are no such residues in this entry.

