



wwPDB X-ray Structure Validation Summary Report ⓘ

Mar 9, 2026 – 05:49 AM UTC

PDB ID : 4IMX / pdb_00004imx
Title : Structure of bovine endothelial nitric oxide synthase heme domain in complex with 3,5-bis(2-(6-amino-4-methylpyridin-2-yl)ethyl)benzotrile
Authors : Li, H.; Poulos, T.L.
Deposited on : 2013-01-03
Resolution : 2.25 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 ⓘ 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 ⓘ](#)) were used in the production of this report:

MolProbity : 4-5-2 with Phenix2.0
Mogul : 2022.3.0, CSD as543be (2022)
Xtrriage (Phenix) : 2.0
EDS : 3.0
Buster-report : wwPDB partial adaption of 1.1.7 (2018)
Percentile statistics : 20250101.v01 (using entries in the PDB archive January 1st 2025)
CCP4 : 9.0.010 (Gargrove)
Density-Fitness : 1.0.12
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.49

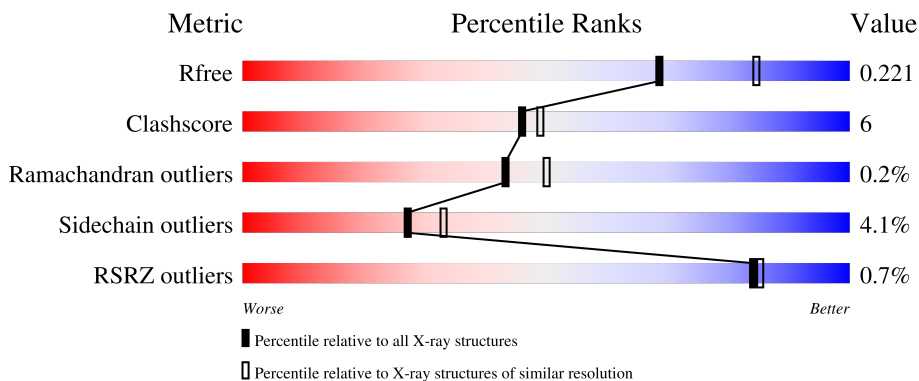
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.25 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 (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	180053	1898 (2.26-2.26)
Clashscore	190562	2005 (2.26-2.26)
Ramachandran outliers	187476	1965 (2.26-2.26)
Sidechain outliers	187428	1966 (2.26-2.26)
RSRZ outliers	180081	1898 (2.26-2.26)

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 $\leq 5\%$. 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	442	 81% 9% • 8%
1	B	442	 79% 10% • 8%

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
4	1EV	B	503	-	-	X	-

2 Entry composition [i](#)

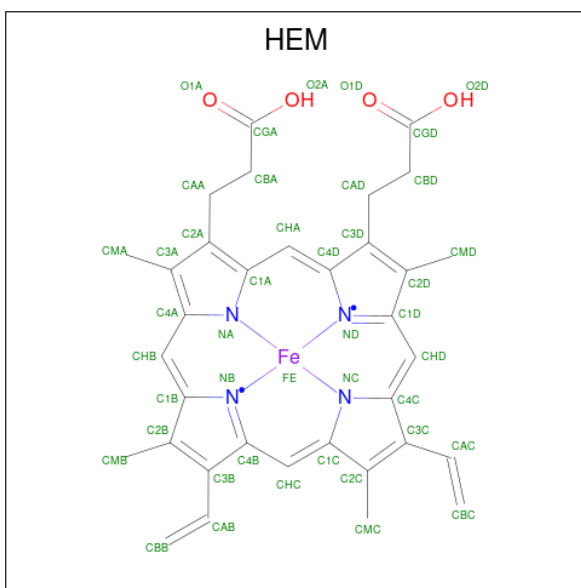
There are 9 unique types of molecules in this entry. The entry contains 6906 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 subunit A.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	405	Total 3220	C 2047	N 568	O 589	S 16	0	0	0
1	B	405	Total 3226	C 2052	N 569	O 589	S 16	0	0	0

- Molecule 2 is PROTOPORPHYRIN IX CONTAINING FE (CCD ID: HEM) (formula: $C_{34}H_{32}FeN_4O_4$).



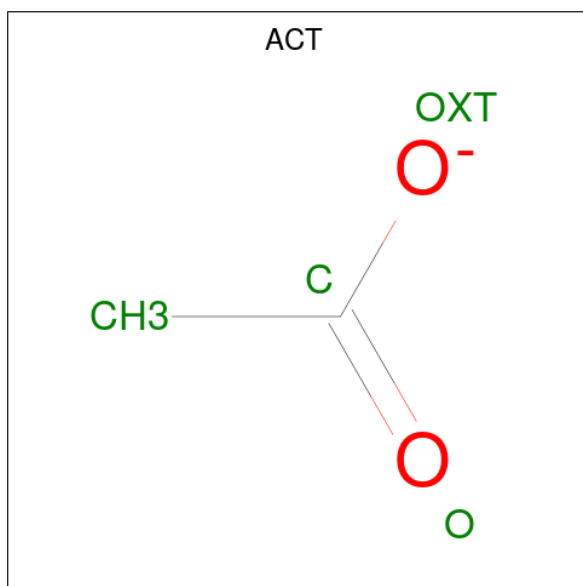
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
			Total	C	Fe	N	O		
2	A	1	Total 43	C 34	Fe 1	N 4	O 4	0	0
2	B	1	Total 43	C 34	Fe 1	N 4	O 4	0	0

- Molecule 3 is 5,6,7,8-TETRAHYDROBIOPTERIN (CCD ID: H4B) (formula: $C_9H_{15}N_5O_3$).

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Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
4	B	1	Total	C	N	0	0
			28	23	5		

- Molecule 5 is ACETATE ION (CCD ID: ACT) (formula: $C_2H_3O_2$).



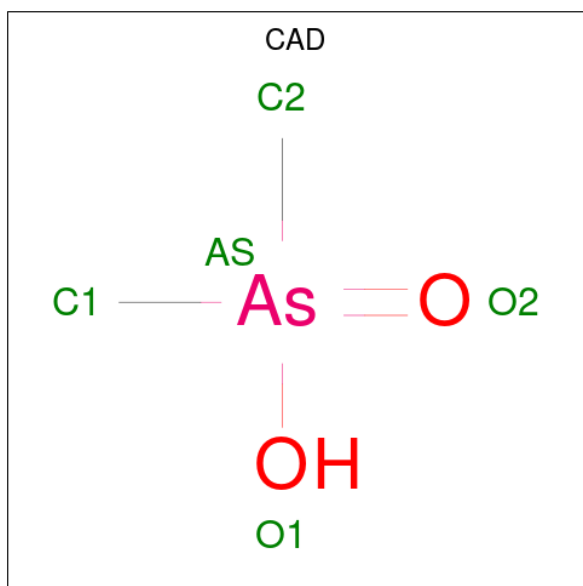
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	O	0	0
			4	2	2		
5	A	1	Total	C	O	0	0
			4	2	2		
5	B	1	Total	C	O	0	0
			4	2	2		
5	B	1	Total	C	O	0	0
			4	2	2		

- Molecule 6 is GLYCEROL (CCD ID: GOL) (formula: $C_3H_8O_3$).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
6	A	1	Total C O 6 3 3	0	0
6	B	1	Total C O 6 3 3	0	0

- Molecule 7 is CACODYLIC ACID (CCD ID: CAD) (formula: $C_2H_7AsO_2$).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
7	A	1	Total As C 3 1 2	0	0
7	B	1	Total As C 3 1 2	0	0

- Molecule 8 is ZINC ION (CCD ID: ZN) (formula: Zn).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
8	A	1	Total 1	Zn 1	0	0

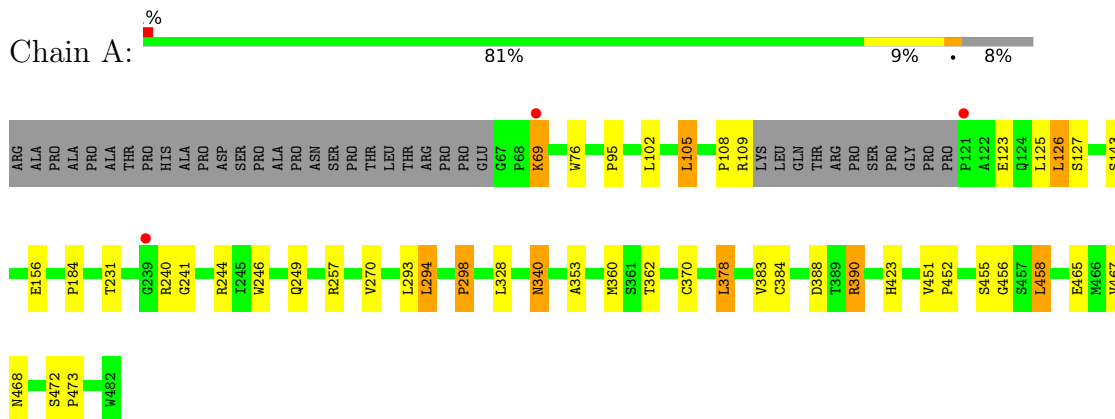
- Molecule 9 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
9	A	134	Total 134	O 134	0	0
9	B	115	Total 115	O 115	0	0

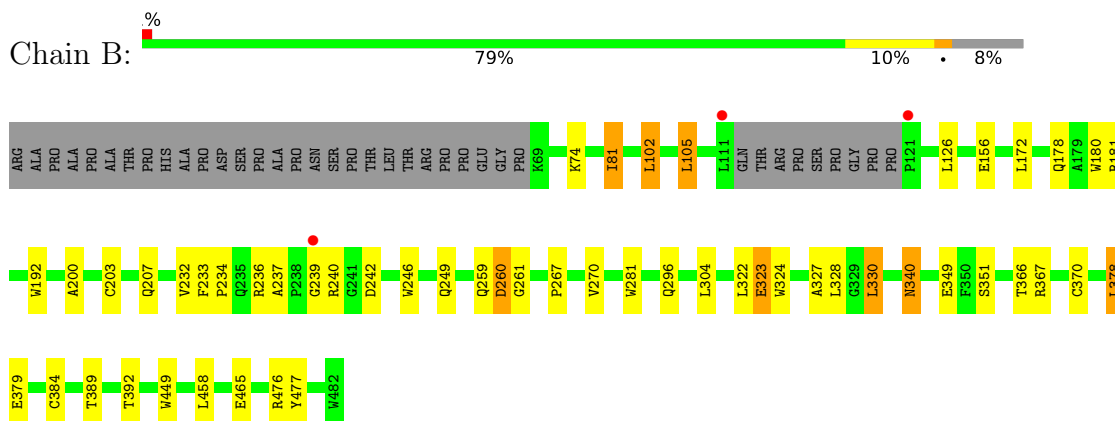
3 Residue-property plots [i](#)

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: subunit A



- Molecule 1: subunit A



4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α , β , γ	58.06Å 106.60Å 156.46Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	39.26 – 2.25 39.26 – 2.25	Depositor EDS
% Data completeness (in resolution range)	99.3 (39.26-2.25) 99.2 (39.26-2.25)	Depositor EDS
R_{merge}	0.06	Depositor
R_{sym}	0.06	Depositor
$\langle I/\sigma(I) \rangle$ ¹	2.05 (at 2.24Å)	Xtrriage
Refinement program	REFMAC	Depositor
R, R_{free}	0.176 , 0.222 0.179 , 0.221	Depositor DCC
R_{free} test set	2320 reflections (4.95%)	wwPDB-VP
Wilson B-factor (Å ²)	40.4	Xtrriage
Anisotropy	0.453	Xtrriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.35 , 34.0	EDS
L-test for twinning ²	$\langle L \rangle = 0.49$, $\langle L^2 \rangle = 0.32$	Xtrriage
Estimated twinning fraction	No twinning to report.	Xtrriage
F_o, F_c correlation	0.96	EDS
Total number of atoms	6906	wwPDB-VP
Average B, all atoms (Å ²)	44.0	wwPDB-VP

Xtrriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.29% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical 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.

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: CAD, GOL, ZN, HEM, H4B, ACT, 1EV

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	
		RMSZ	# $ Z > 5$	RMSZ	# $ Z > 5$
1	A	0.83	1/3310 (0.0%)	0.89	0/4508
1	B	0.83	0/3315	0.95	3/4513 (0.1%)
All	All	0.83	1/6625 (0.0%)	0.92	3/9021 (0.0%)

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 mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
1	B	0	1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	383	VAL	CA-CB	5.01	1.60	1.54

All (3) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	237	ALA	CA-C-N	8.12	129.99	119.84
1	B	237	ALA	C-N-CA	8.12	129.99	119.84
1	B	240	ARG	N-CA-C	5.15	117.24	109.41

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	B	239	GLY	Peptide

5.2 Too-close contacts

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	3220	0	3128	32	0
1	B	3226	0	3142	38	0
2	A	43	0	30	1	0
2	B	43	0	30	8	0
3	A	17	0	15	1	0
3	B	17	0	15	2	0
4	A	28	0	25	2	0
4	B	28	0	25	11	0
5	A	8	0	6	0	0
5	B	8	0	6	0	0
6	A	6	0	8	1	0
6	B	6	0	8	0	0
7	A	3	0	0	1	0
7	B	3	0	0	3	0
8	A	1	0	0	0	0
9	A	134	0	0	1	0
9	B	115	0	0	2	0
All	All	6906	0	6438	76	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 6.

The worst 5 of 76 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)
1:A:384:CYS:SG	7:A:507:CAD:AS	2.59	1.21
1:B:384:CYS:SG	7:B:507:CAD:AS	2.59	1.20
1:B:249:GLN:HE21	4:B:503:1EV:H2	1.11	1.05
1:A:249:GLN:HE21	4:A:503:1EV:H2	1.32	0.95
1:B:249:GLN:NE2	4:B:503:1EV:H2	1.82	0.94

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	401/442 (91%)	392 (98%)	9 (2%)	0	100	100
1	B	401/442 (91%)	386 (96%)	13 (3%)	2 (0%)	24	24
All	All	802/884 (91%)	778 (97%)	22 (3%)	2 (0%)	43	50

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	260	ASP
1	B	328	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	344/375 (92%)	327 (95%)	17 (5%)	22	24
1	B	345/375 (92%)	334 (97%)	11 (3%)	34	43
All	All	689/750 (92%)	661 (96%)	28 (4%)	27	33

5 of 28 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	378	LEU
1	B	458	LEU
1	B	81	ILE
1	B	378	LEU

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Mol	Chain	Res	Type
1	A	458	LEU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 21 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	249	GLN
1	B	376	ASN
1	B	478	GLN
1	B	405	ASN
1	B	340	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](#)

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

5.5 Carbohydrates [i](#)

There are no oligosaccharides in this entry.

5.6 Ligand geometry [i](#)

Of 15 ligands modelled in this entry, 1 is monoatomic - leaving 14 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			Bond angles		
					Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
3	H4B	B	502	-	17,18,18	0.88	1 (5%)	14,26,26	2.08	7 (50%)
5	ACT	B	505	-	3,3,3	0.97	0	3,3,3	0.90	0

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
7	CAD	A	507	-	0,2,4	-	-	0,1,6	-	-
4	1EV	B	503	-	30,30,30	1.57	1 (3%)	39,41,41	1.90	10 (25%)
2	HEM	A	501	1	50,50,50	1.66	7 (14%)	67,82,82	1.25	9 (13%)
5	ACT	B	504	-	3,3,3	0.82	0	3,3,3	0.67	0
2	HEM	B	501	1	50,50,50	1.79	10 (20%)	67,82,82	1.34	7 (10%)
6	GOL	A	506	-	5,5,5	0.48	0	5,5,5	0.87	0
6	GOL	B	506	-	5,5,5	0.38	0	5,5,5	0.58	0
4	1EV	A	503	-	30,30,30	1.70	1 (3%)	39,41,41	2.17	15 (38%)
5	ACT	A	504	-	3,3,3	0.68	0	3,3,3	0.94	0
3	H4B	A	502	-	17,18,18	0.84	0	14,26,26	1.88	5 (35%)
5	ACT	A	505	-	3,3,3	0.70	0	3,3,3	1.00	0
7	CAD	B	507	-	0,2,4	-	-	0,1,6	-	-

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	H4B	B	502	-	-	0/8/17/17	0/2/2/2
4	1EV	B	503	-	-	5/12/12/12	0/3/3/3
2	HEM	A	501	1	-	0/14/54/54	-
2	HEM	B	501	1	-	0/14/54/54	-
6	GOL	A	506	-	-	2/4/4/4	-
6	GOL	B	506	-	-	2/4/4/4	-
4	1EV	A	503	-	-	3/12/12/12	0/3/3/3
3	H4B	A	502	-	-	0/8/17/17	0/2/2/2

The worst 5 of 20 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
4	A	503	1EV	C11-C10	-7.51	1.28	1.44
4	B	503	1EV	C11-C10	-7.38	1.29	1.44
2	B	501	HEM	C3D-C2D	6.87	1.51	1.36
2	A	501	HEM	C3D-C2D	6.62	1.51	1.36
2	A	501	HEM	FE-ND	4.17	2.07	1.94

The worst 5 of 53 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
4	A	503	1EV	C08-C06-N01	6.07	125.25	116.06
4	B	503	1EV	C02-N01-C06	4.72	121.60	118.07
4	B	503	1EV	C09-C13-C14	4.52	128.14	120.54
3	A	502	H4B	C2-N1-C8A	4.51	121.32	113.36
4	A	503	1EV	C08-C06-C05	-4.23	115.52	121.65

There are no chirality outliers.

5 of 12 torsion outliers are listed below:

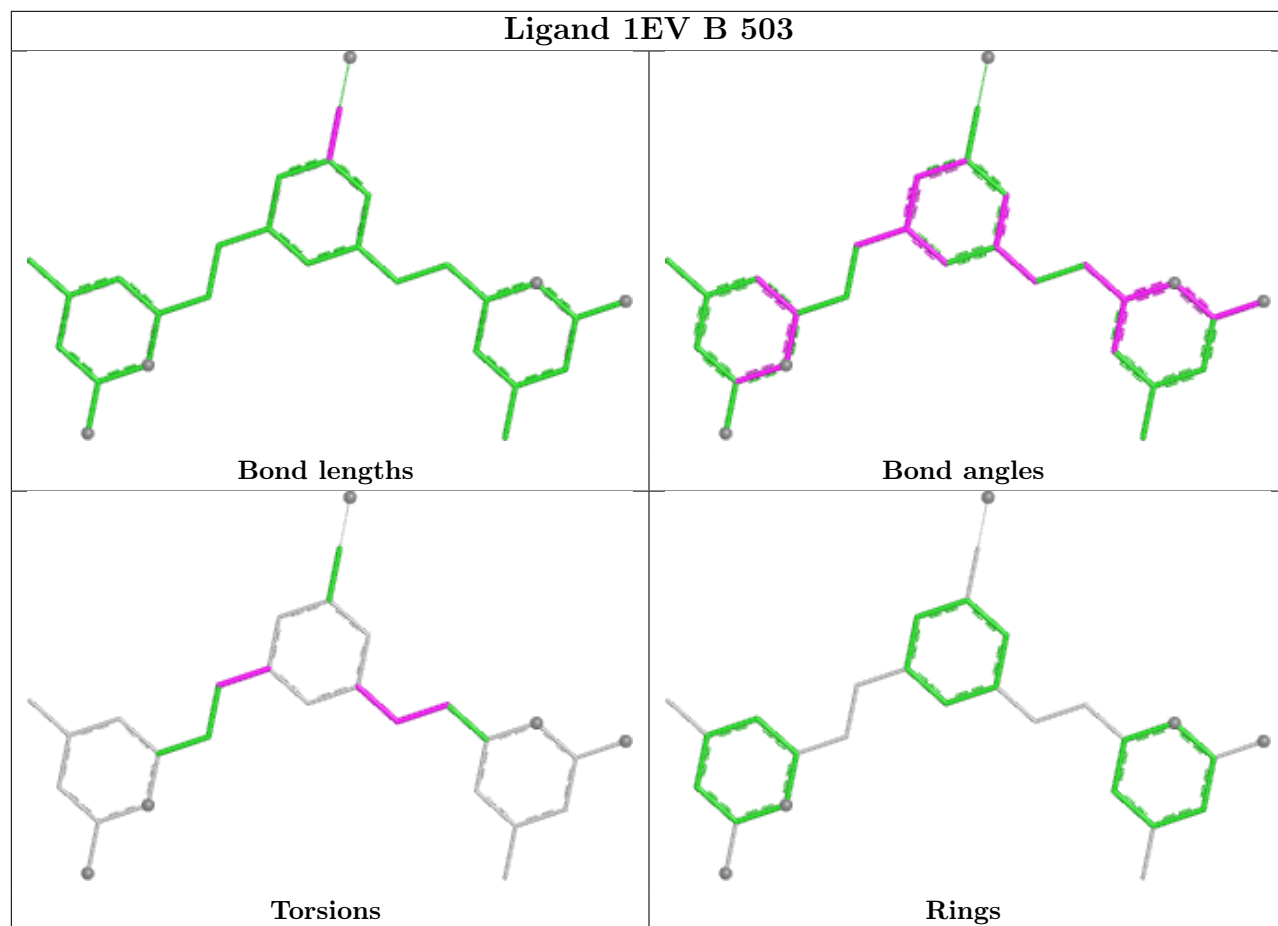
Mol	Chain	Res	Type	Atoms
4	A	503	1EV	C26-C28-C29-C15
4	B	503	1EV	C26-C28-C29-C15
6	A	506	GOL	C1-C2-C3-O3
6	A	506	GOL	O2-C2-C3-O3
6	B	506	GOL	C1-C2-C3-O3

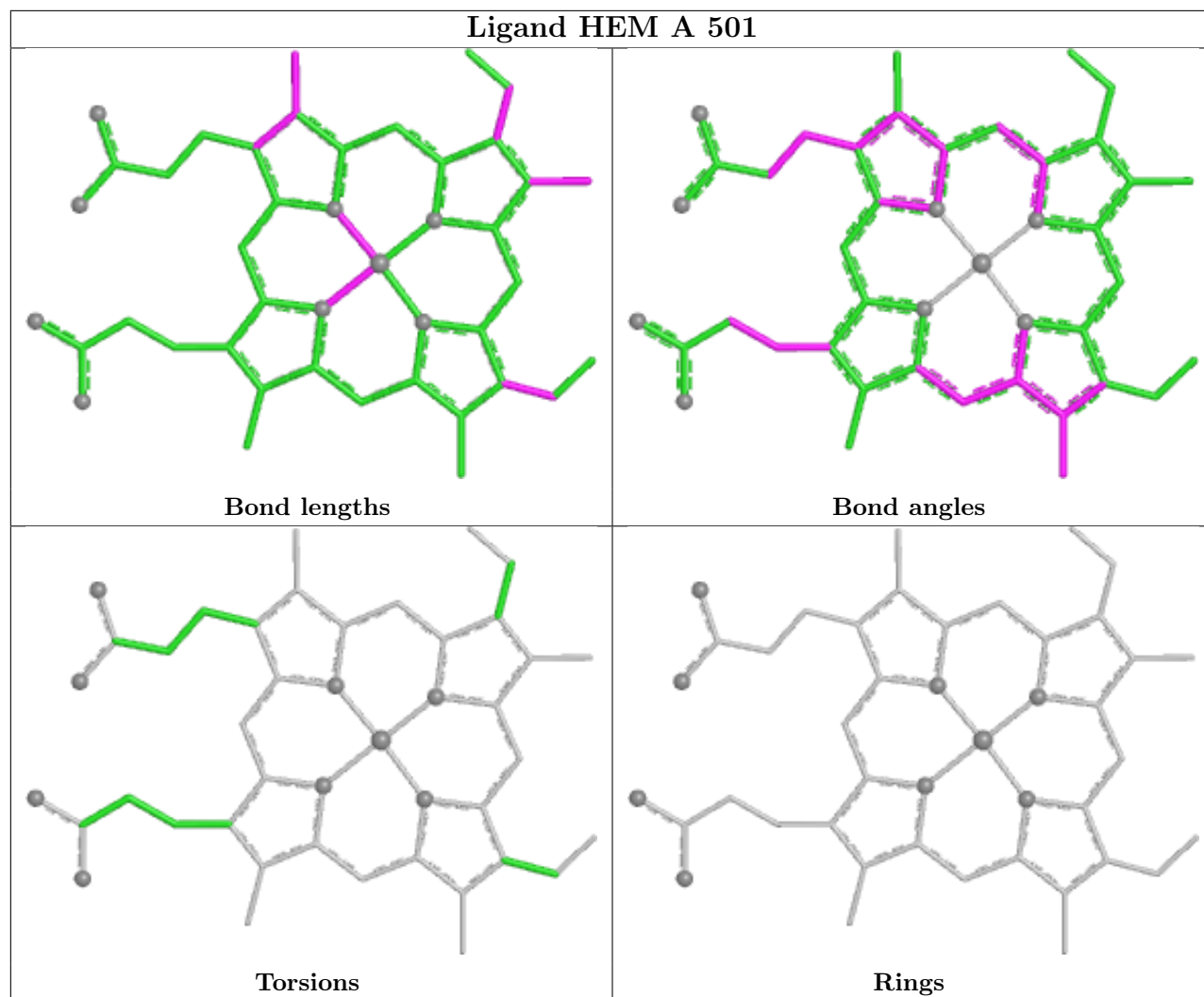
There are no ring outliers.

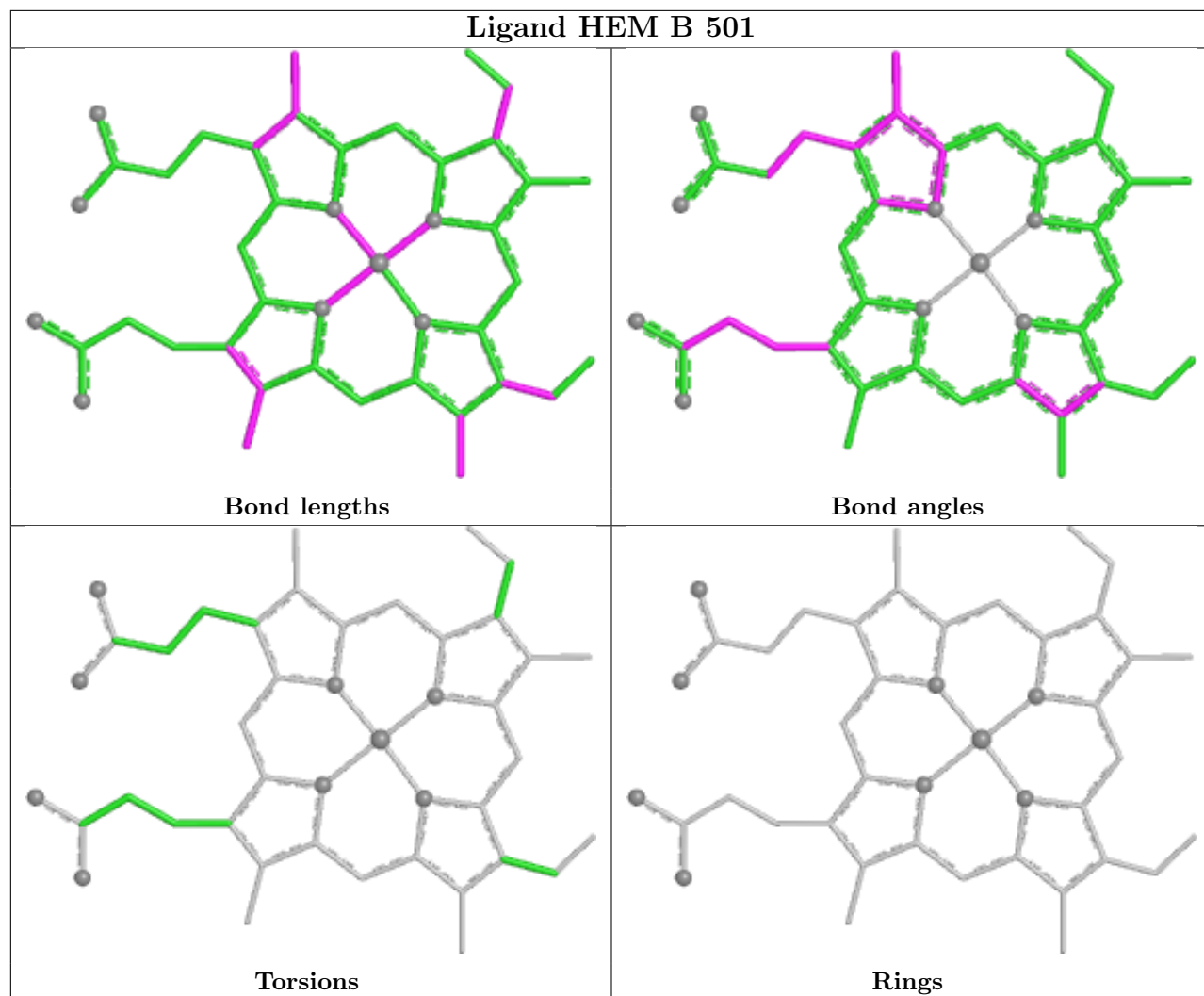
9 monomers are involved in 23 short contacts:

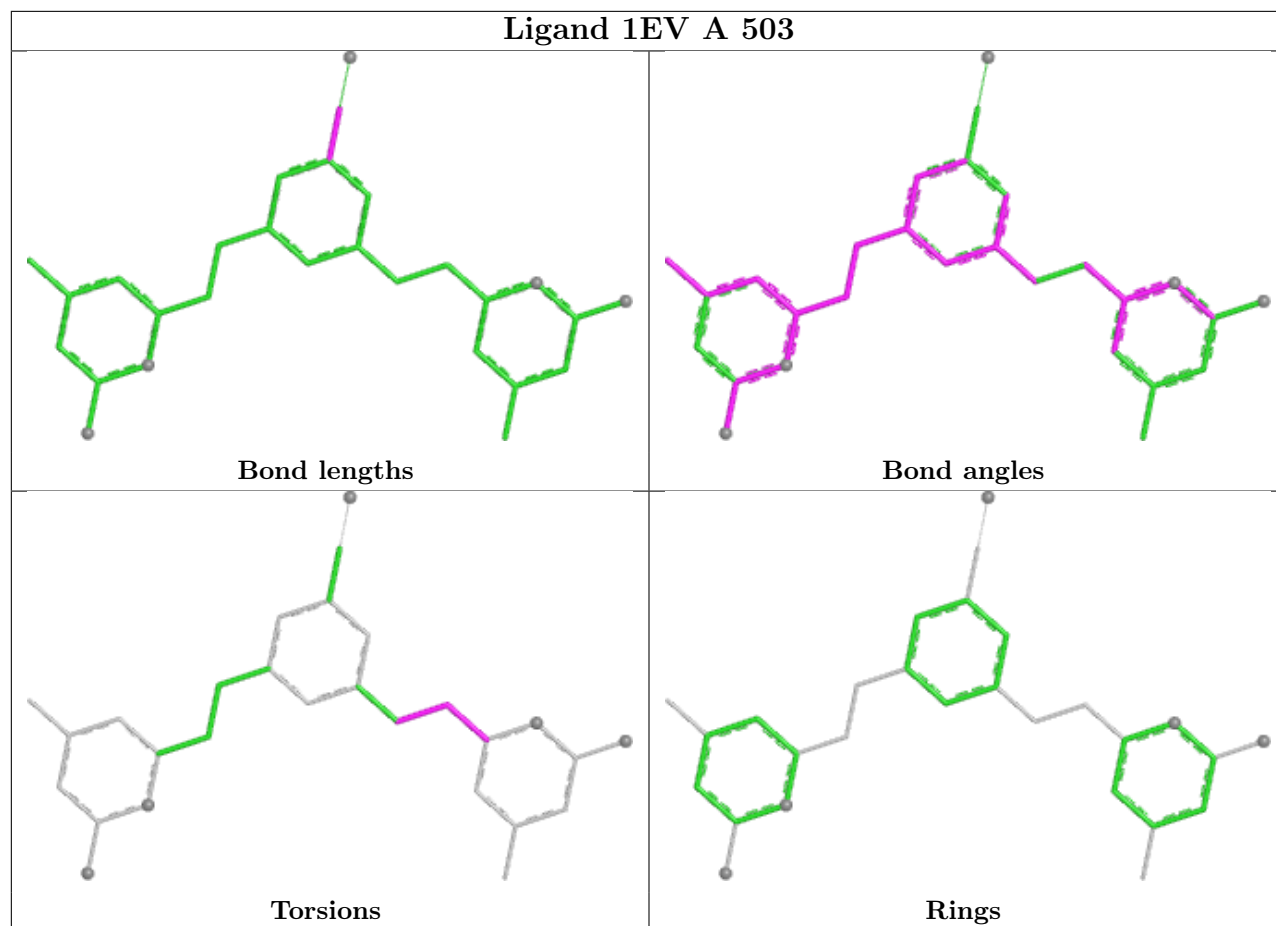
Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	B	502	H4B	2	0
7	A	507	CAD	1	0
4	B	503	1EV	11	0
2	A	501	HEM	1	0
2	B	501	HEM	8	0
6	A	506	GOL	1	0
4	A	503	1EV	2	0
3	A	502	H4B	1	0
7	B	507	CAD	3	0

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 than 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, 95th 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>2	OWAB(Å ²)	Q<0.9
1	A	405/442 (91%)	-0.32	3 (0%) 84 85	28, 41, 67, 81	0
1	B	405/442 (91%)	-0.26	3 (0%) 84 85	27, 44, 71, 95	0
All	All	810/884 (91%)	-0.29	6 (0%) 84 85	27, 42, 68, 95	0

The worst 5 of 6 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	239	GLY	3.8
1	B	111	LEU	3.8
1	A	121	PRO	3.4
1	B	121	PRO	2.5
1	B	239	GLY	2.2

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](#)

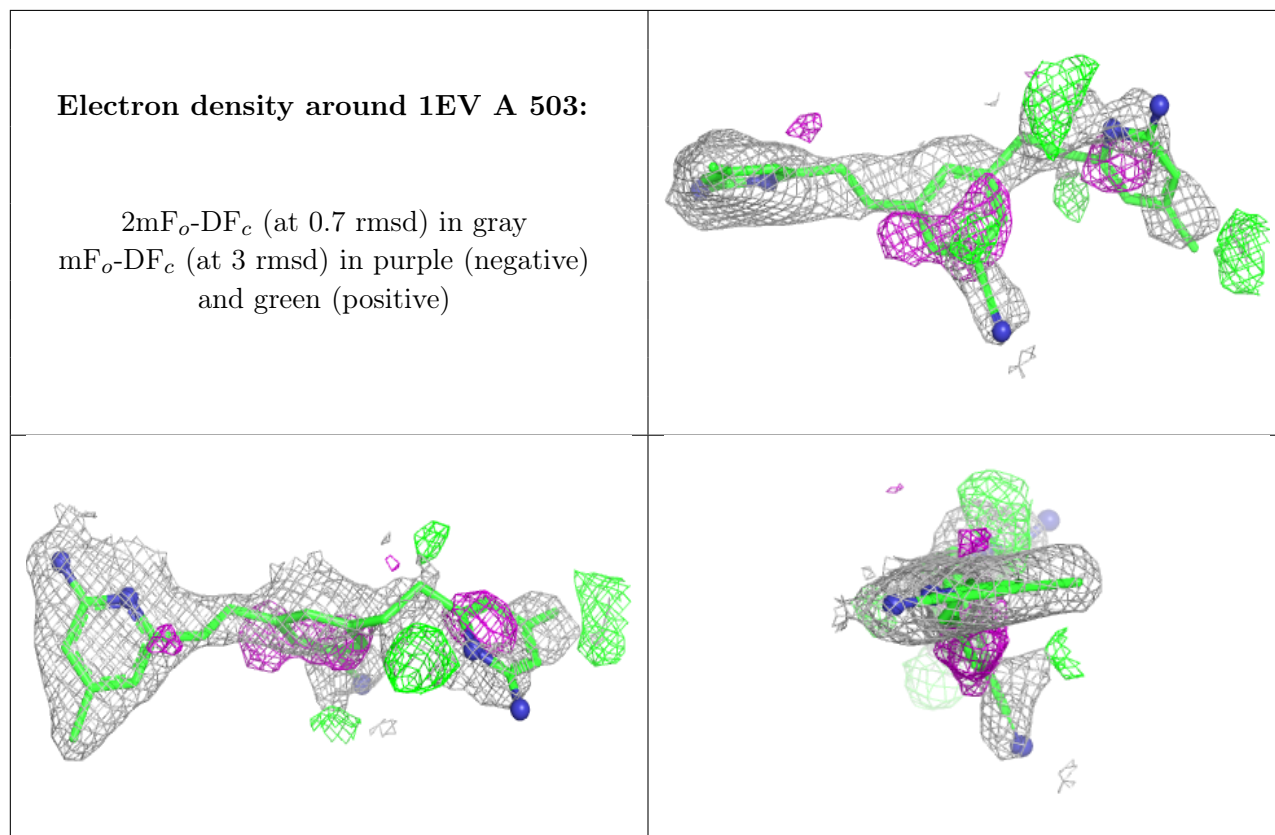
There are no oligosaccharides in this entry.

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, 95th 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.

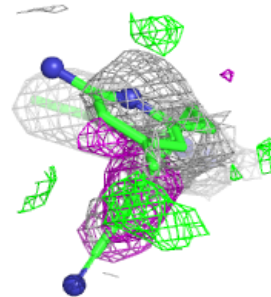
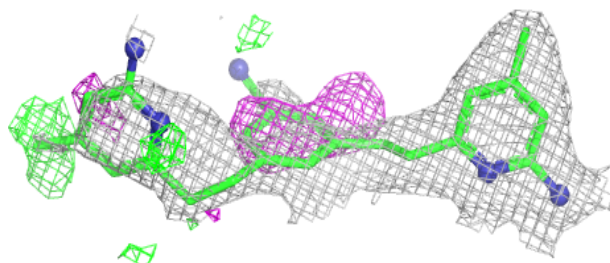
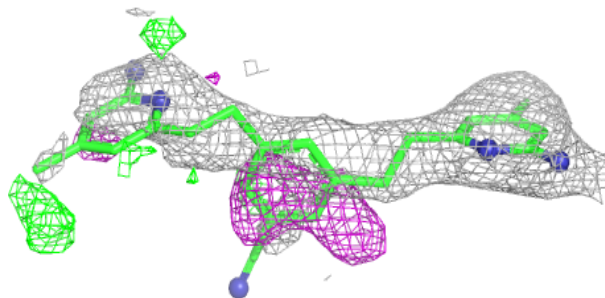
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(\AA^2)	Q<0.9
4	1EV	A	503	28/28	0.78	0.19	27,76,90,91	0
4	1EV	B	503	28/28	0.82	0.18	32,74,90,91	0
6	GOL	A	506	6/6	0.88	0.15	56,57,60,60	0
6	GOL	B	506	6/6	0.89	0.13	55,56,58,58	0
7	CAD	A	507	3/5	0.90	0.18	84,84,84,85	0
5	ACT	A	505	4/4	0.92	0.10	44,45,45,47	0
5	ACT	A	504	4/4	0.93	0.08	43,43,46,47	0
3	H4B	A	502	17/17	0.94	0.08	37,41,44,46	0
3	H4B	B	502	17/17	0.94	0.08	36,39,43,47	0
5	ACT	B	504	4/4	0.94	0.10	51,52,53,55	0
7	CAD	B	507	3/5	0.95	0.14	70,70,70,71	0
5	ACT	B	505	4/4	0.96	0.10	43,43,46,47	0
2	HEM	A	501	43/43	0.98	0.07	24,29,44,46	0
2	HEM	B	501	43/43	0.98	0.06	26,32,43,46	0
8	ZN	A	508	1/1	0.99	0.02	35,35,35,35	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.



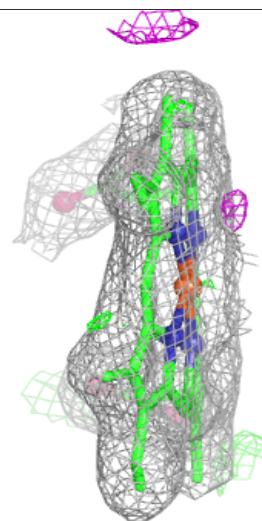
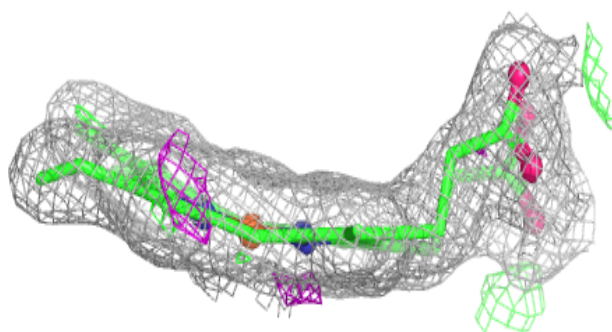
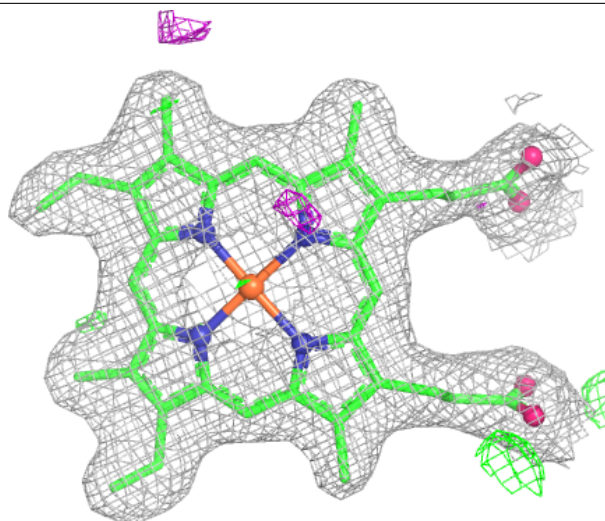
Electron density around 1EV B 503:

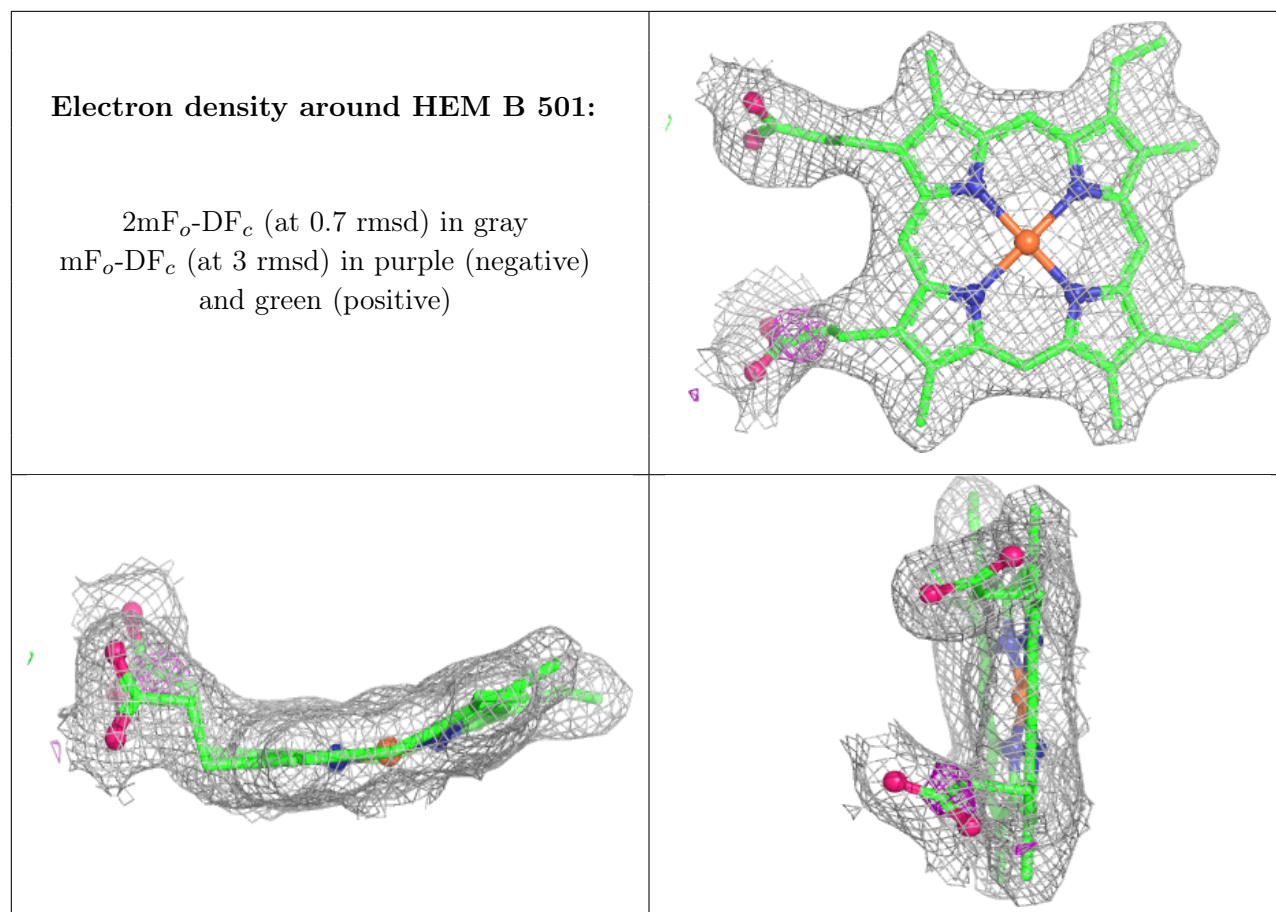
$2mF_o-DF_c$ (at 0.7 rmsd) in gray
 mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)



Electron density around HEM A 501:

$2mF_o-DF_c$ (at 0.7 rmsd) in gray
 mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)





6.5 Other polymers [i](#)

There are no such residues in this entry.