



# wwPDB X-ray Structure Validation Summary Report ⓘ

May 18, 2020 – 05:02 pm BST

PDB ID : 3N5T  
Title : Structure of endothelial nitric oxide synthase heme domain complexed with 6,6'-(2,2'-(pyridine-3,5-diyl)bis(ethane-2,1-diyl))bis(4-methylpyridin-2-amine)  
Authors : Delker, S.L.; Li, H.; Poulos, T.L.  
Deposited on : 2010-05-25  
Resolution : 2.52 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](mailto: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.

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The following versions of software and data (see [references ⓘ](#)) were used in the production of this report:

MolProbity : 4.02b-467  
Mogul : 1.8.5 (274361), CSD as541be (2020)  
Xtriage (Phenix) : 1.13  
EDS : 2.11  
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) : 2.11

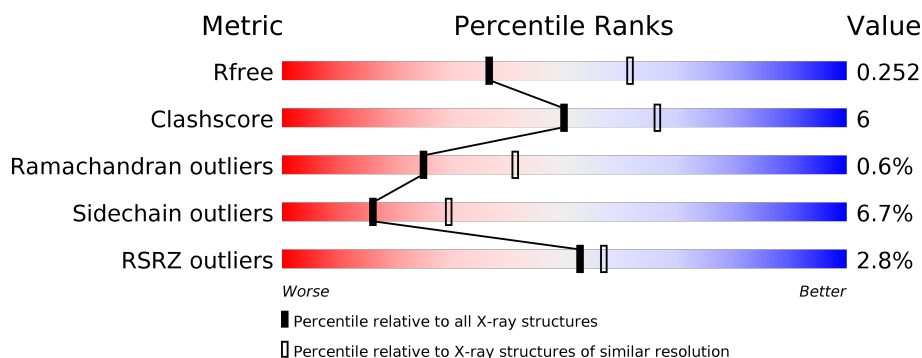
# 1 Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:

## *X-RAY DIFFRACTION*

The reported resolution of this entry is 2.52 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))
$R_{free}$	130704	5743 (2.54-2.50)
Clashscore	141614	6463 (2.54-2.50)
Ramachandran outliers	138981	6335 (2.54-2.50)
Sidechain outliers	138945	6337 (2.54-2.50)
RSRZ outliers	127900	5630 (2.54-2.50)

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 on the lower bar indicate the fraction of residues that contain outliers for  $\geq 3$ , 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	444	<div> <div>3%</div> <div> <div></div> <div>80%</div> <div>11%</div> <div>•</div> <div>9%</div> </div> </div>
1	B	444	<div> <div>2%</div> <div> <div></div> <div>72%</div> <div>18%</div> <div>9%</div> </div> </div>

## 2 Entry composition [i](#)

There are 9 unique types of molecules in this entry. The entry contains 6766 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 Nitric oxide synthase.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	405	Total	C	N	O	S	0	0	0
			3220	2047	568	589	16			
1	B	403	Total	C	N	O	S	0	0	0
			3209	2040	566	587	16			

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	100	ARG	CYS	SEE REMARK 999	UNP P29473
B	100	ARG	CYS	SEE REMARK 999	UNP P29473

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



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

- Molecule 3 is ACETATE ION (three-letter code: ACT) (formula:  $C_2H_3O_2$ ).



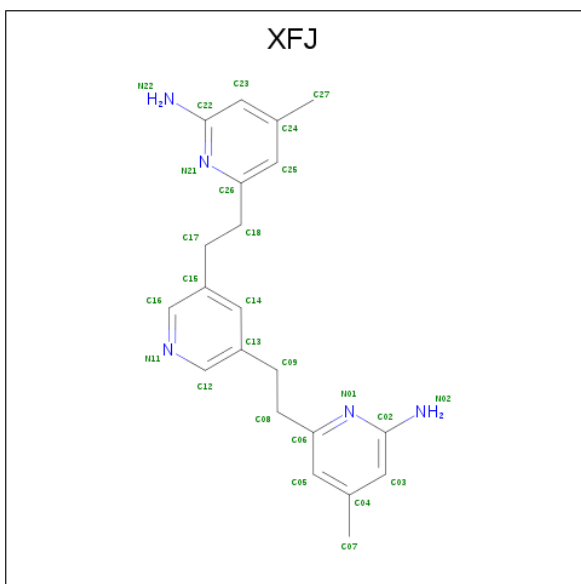
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
3	A	1	Total	C	O	0	0
			4	2	2		
3	B	1	Total	C	O	0	0
			4	2	2		

- Molecule 4 is GLYCEROL (three-letter code: GOL) (formula:  $C_3H_8O_3$ ).



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

- Molecule 5 is 6,6'-(pyridine-3,5-diyl)diethane-2,1-diylbis(4-methylpyridin-2-amine) (three-letter code: XFJ) (formula: C<sub>21</sub>H<sub>25</sub>N<sub>5</sub>).



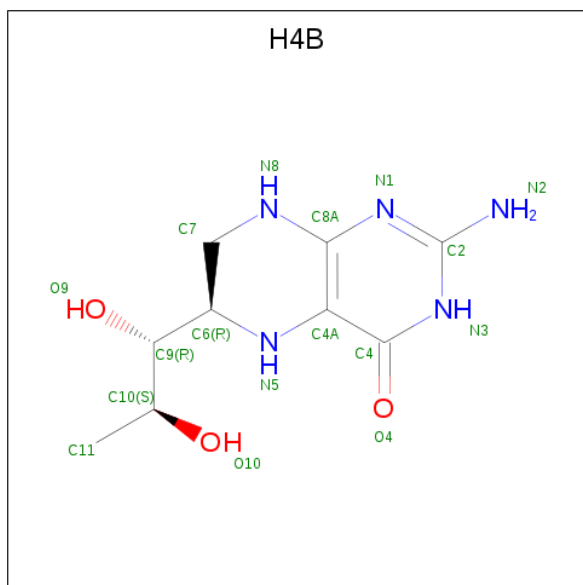
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	A	1	Total	C	N	0	0
			26	21	5		

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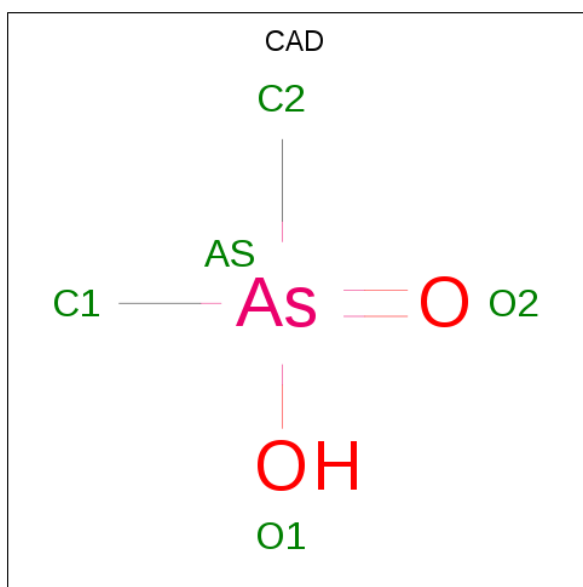
Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
5	B	1	Total	C	N	0	0
			26	21	5		

- Molecule 6 is 5,6,7,8-TETRAHYDROBIOPTERIN (three-letter code: H4B) (formula:  $C_9H_{15}N_5O_3$ ).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
6	A	1	Total	C	N	O	0	0
			17	9	5	3		
6	B	1	Total	C	N	O	0	0
			17	9	5	3		

- Molecule 7 is CACODYLIC ACID (three-letter code: CAD) (formula:  $C_2H_7AsO_2$ ).



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

- Molecule 8 is ZINC ION (three-letter code: ZN) (formula: Zn).

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

- Molecule 9 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
9	A	64	Total	O	0	0
			64	64		
9	B	74	Total	O	0	0
			74	74		





## 4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	58.12Å 106.74Å 157.38Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	42.82 – 2.52 42.82 – 2.52	Depositor EDS
% Data completeness (in resolution range)	99.8 (42.82-2.52) 99.8 (42.82-2.52)	Depositor EDS
$R_{merge}$	0.08	Depositor
$R_{sym}$	0.08	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.14 (at 2.51Å)	Xtriage
Refinement program	REFMAC 5.5.0089, CNS	Depositor
R, $R_{free}$	0.186 , 0.248 0.198 , 0.252	Depositor DCC
$R_{free}$ test set	1666 reflections (4.93%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	43.8	Xtriage
Anisotropy	0.298	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.33 , 42.8	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.49$ , $\langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.95	EDS
Total number of atoms	6766	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	46.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 4.02% of the height of the origin peak. No significant pseudotranslation is detected.*

<sup>1</sup>Intensities estimated from amplitudes.

<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.

## 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: GOL, ZN, XFJ, H4B, ACT, HEM, CAD

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	0.66	0/3310	0.70	0/4508
1	B	0.69	0/3298	0.73	1/4491 (0.0%)
All	All	0.67	0/6608	0.71	1/8999 (0.0%)

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	252	ARG	NE-CZ-NH2	-5.18	117.71	120.30

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	A	3220	0	3127	29	0
1	B	3209	0	3118	40	0
2	A	43	0	30	3	0
2	B	43	0	30	8	0
3	A	4	0	3	0	0
3	B	4	0	3	0	0
4	A	6	0	8	0	0
4	B	6	0	8	0	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
5	A	26	0	25	1	0
5	B	26	0	25	2	0
6	A	17	0	15	0	0
6	B	17	0	15	0	0
7	A	3	0	0	0	0
7	B	3	0	0	3	0
8	A	1	0	0	0	0
9	A	64	0	0	2	0
9	B	74	0	0	2	0
All	All	6766	0	6407	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:B:384:CYS:SG	7:B:950:CAD:AS	2.60	1.19
1:A:236:ARG:HG3	1:A:236:ARG:NH1	1.67	1.04
1:A:236:ARG:HG3	1:A:236:ARG:HH11	0.85	1.00
1:A:236:ARG:CG	1:A:236:ARG:HH11	1.74	1.00
2:B:500:HEM:HBB2	2:B:500:HEM:HHC	1.69	0.74

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	401/444 (90%)	383 (96%)	15 (4%)	3 (1%)	22	37
1	B	399/444 (90%)	381 (96%)	16 (4%)	2 (0%)	29	47
All	All	800/888 (90%)	764 (96%)	31 (4%)	5 (1%)	25	41

All (5) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	122	ALA
1	B	260	ASP
1	B	328	LEU
1	A	161	SER
1	A	456	GLY

### 5.3.2 Protein sidechains ⓘ

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	344/377 (91%)	323 (94%)	21 (6%)	18	34
1	B	343/377 (91%)	318 (93%)	25 (7%)	14	26
All	All	687/754 (91%)	641 (93%)	46 (7%)	16	29

5 of 46 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	438	LYS
1	B	124	GLN
1	B	393	SER
1	B	92	GLN
1	B	102	LEU

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 16 such sidechains are listed below:

Mol	Chain	Res	Type
1	A	468	ASN
1	B	178	GLN
1	B	278	GLN
1	A	376	ASN
1	B	340	ASN

### 5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

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

## 5.5 Carbohydrates ⓘ

There are no carbohydrates in this entry.

## 5.6 Ligand geometry ⓘ

Of 13 ligands modelled in this entry, 1 is monoatomic - leaving 12 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	$\# Z  > 2$
2	HEM	A	500	1	27,50,50	2.08	6 (22%)	17,82,82	2.00	3 (17%)
4	GOL	A	880	-	5,5,5	0.48	0	5,5,5	0.45	0
7	CAD	B	950	-	0,2,4	0.00	-	0,1,6	0.00	-
3	ACT	B	860	-	1,3,3	1.39	0	0,3,3	0.00	-
2	HEM	B	500	1	27,50,50	2.31	6 (22%)	17,82,82	1.94	4 (23%)
6	H4B	B	600	-	16,18,18	0.97	1 (6%)	11,26,26	2.98	7 (63%)
3	ACT	A	860	-	1,3,3	2.03	1 (100%)	0,3,3	0.00	-
6	H4B	A	600	-	16,18,18	1.03	1 (6%)	11,26,26	2.81	6 (54%)
4	GOL	B	880	-	5,5,5	0.37	0	5,5,5	0.87	0
7	CAD	A	950	-	0,2,4	0.00	-	0,1,6	0.00	-
5	XFJ	A	800	-	28,28,28	0.65	0	38,38,38	2.19	11 (28%)
5	XFJ	B	800	-	28,28,28	0.47	0	38,38,38	2.15	13 (34%)

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
2	HEM	A	500	1	-	0/6/54/54	-
4	GOL	A	880	-	-	2/4/4/4	-
2	HEM	B	500	1	-	0/6/54/54	-
6	H4B	B	600	-	-	0/8/17/17	0/2/2/2
6	H4B	A	600	-	-	3/8/17/17	0/2/2/2
4	GOL	B	880	-	-	4/4/4/4	-
5	XFJ	A	800	-	-	0/10/10/10	0/3/3/3
5	XFJ	B	800	-	-	2/10/10/10	0/3/3/3

The worst 5 of 15 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	500	HEM	C3B-C2B	-6.04	1.32	1.40
2	A	500	HEM	C3B-C2B	-5.50	1.32	1.40
2	B	500	HEM	C3D-C2D	5.22	1.53	1.37
2	B	500	HEM	C3C-C2C	-4.87	1.33	1.40
2	A	500	HEM	C3D-C2D	4.52	1.51	1.37

The worst 5 of 44 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
5	A	800	XFJ	C02-N01-C06	6.88	123.31	118.10
5	A	800	XFJ	C22-N21-C26	5.96	122.62	118.10
5	B	800	XFJ	C02-N01-C06	5.66	122.39	118.10
2	A	500	HEM	CBD-CAD-C3D	-5.21	102.88	112.48
2	B	500	HEM	CBA-CAA-C2A	-5.06	103.15	112.49

There are no chirality outliers.

5 of 11 torsion outliers are listed below:

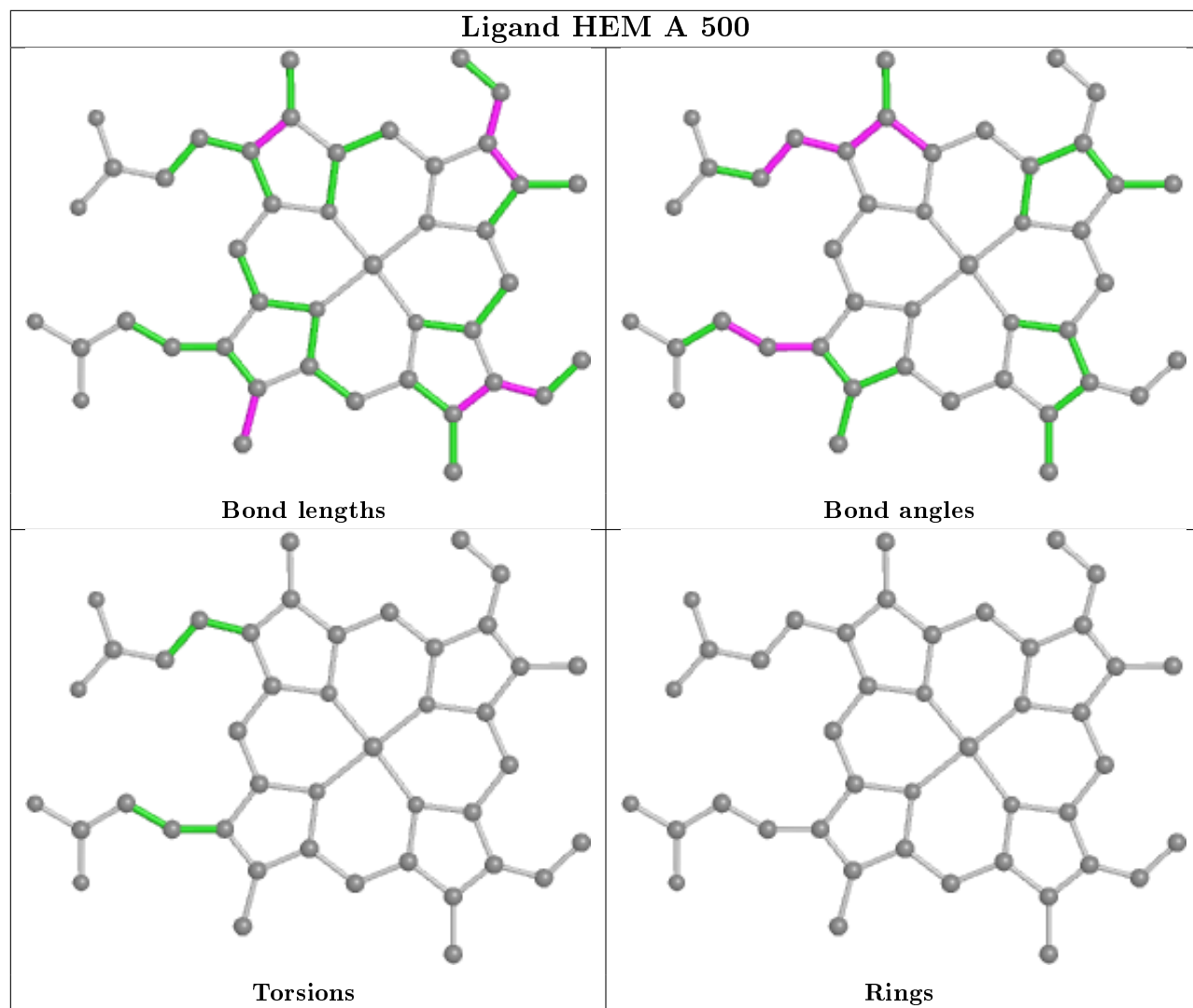
Mol	Chain	Res	Type	Atoms
4	A	880	GOL	C1-C2-C3-O3
4	B	880	GOL	O1-C1-C2-C3
4	B	880	GOL	C1-C2-C3-O3
4	B	880	GOL	O2-C2-C3-O3
4	B	880	GOL	O1-C1-C2-O2

There are no ring outliers.

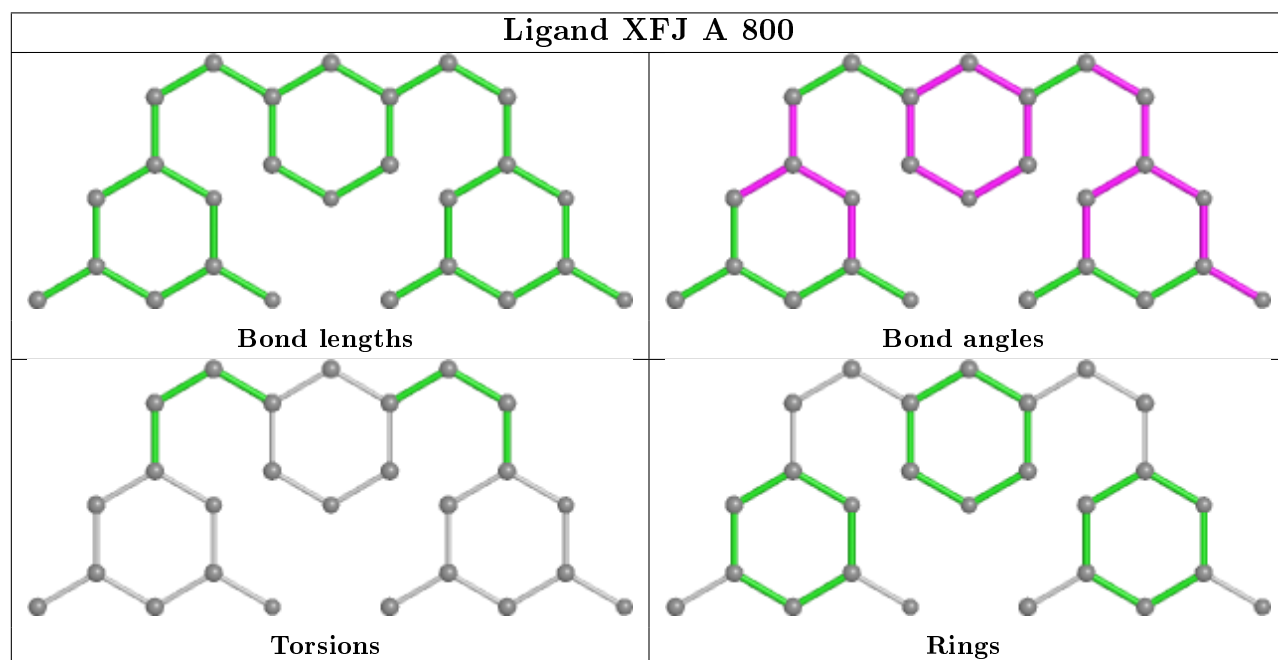
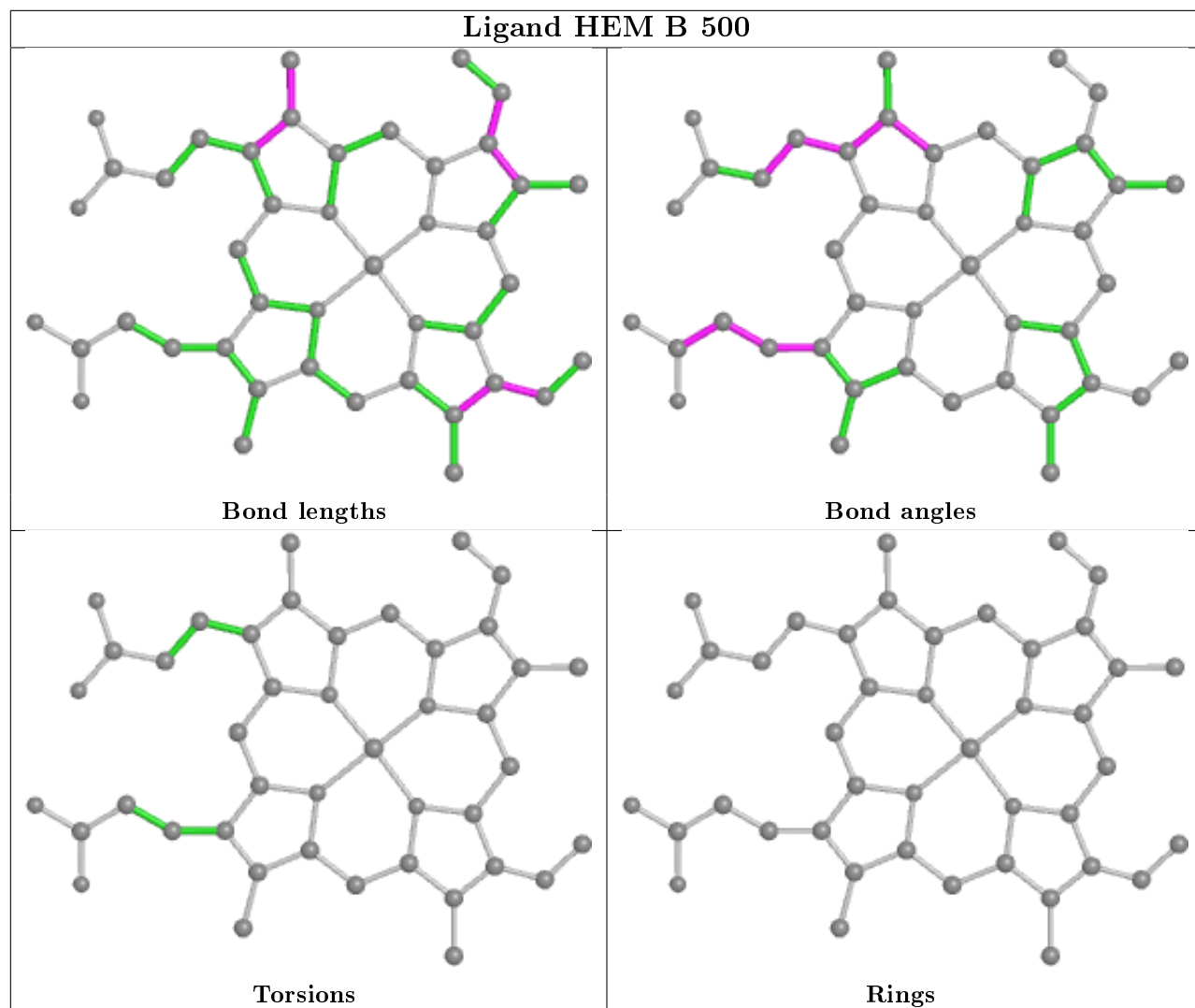
5 monomers are involved in 14 short contacts:

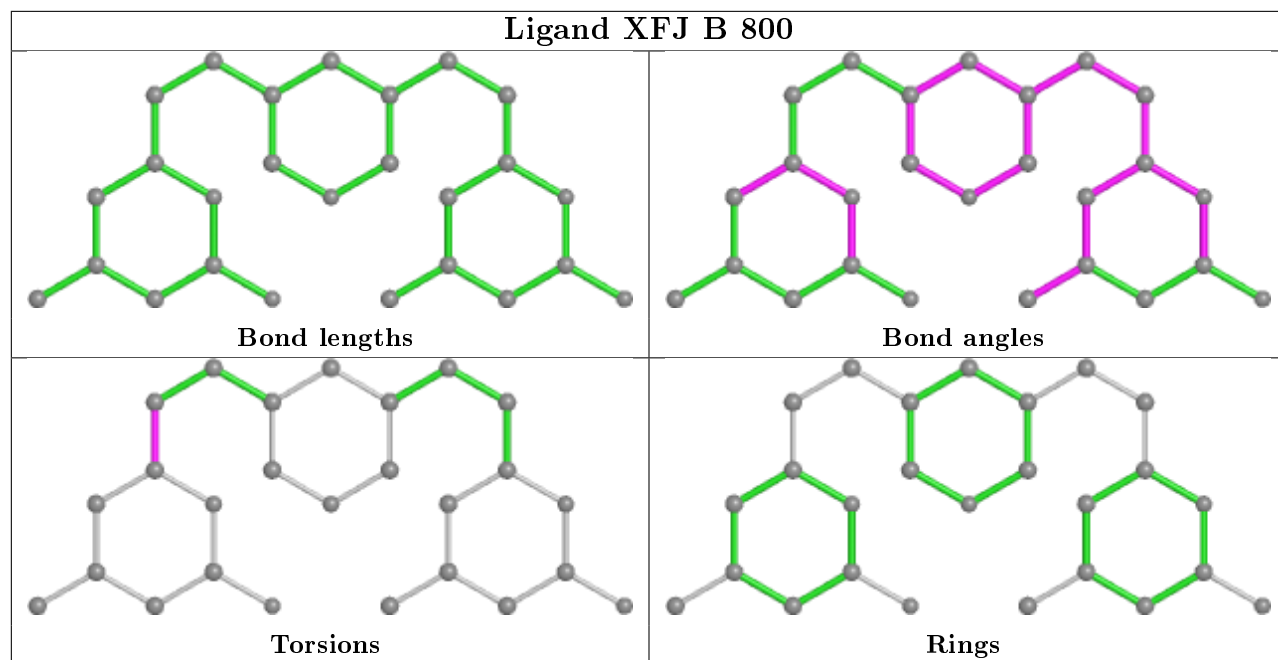
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	500	HEM	3	0
7	B	950	CAD	3	0
2	B	500	HEM	8	0
5	A	800	XFJ	1	0
5	B	800	XFJ	2	0

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, 95<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
1	A	405/444 (91%)	0.01	13 (3%) 47 52	27, 44, 72, 86	0
1	B	403/444 (90%)	-0.03	10 (2%) 57 61	25, 46, 74, 96	0
All	All	808/888 (90%)	-0.01	23 (2%) 53 57	25, 45, 73, 96	0

The worst 5 of 23 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	69	LYS	6.8
1	B	259	GLN	4.7
1	A	259	GLN	4.4
1	A	239	GLY	4.1
1	B	146	GLN	3.8

### 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 carbohydrates in this entry.

### 6.4 Ligands [i](#)

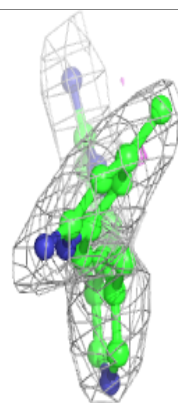
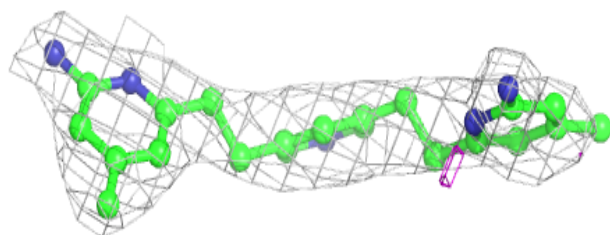
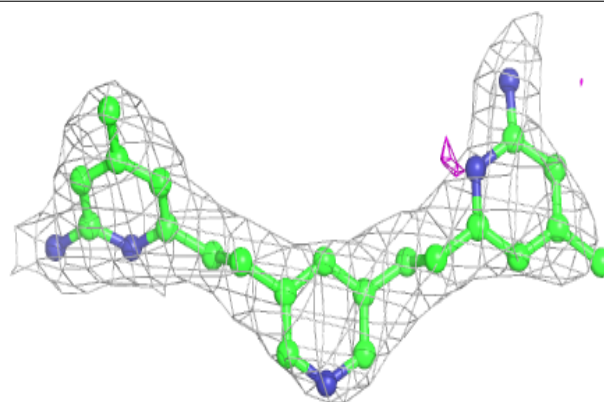
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95<sup>th</sup> percentile and maximum values of B factors of atoms in the group. The column labelled ‘Q< 0.9’ lists the number of atoms with occupancy less than 0.9.

Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors( $\text{\AA}^2$ )	Q<0.9
4	GOL	A	880	6/6	0.81	0.38	68,70,71,71	0
4	GOL	B	880	6/6	0.91	0.22	51,55,58,59	0
3	ACT	A	860	4/4	0.92	0.20	49,49,50,51	0
5	XFJ	B	800	26/26	0.92	0.23	40,54,62,62	0
5	XFJ	A	800	26/26	0.93	0.21	31,49,52,53	0
6	H4B	A	600	17/17	0.97	0.14	29,33,37,40	0
3	ACT	B	860	4/4	0.97	0.35	51,51,52,53	0
6	H4B	B	600	17/17	0.98	0.15	40,42,44,47	0
2	HEM	A	500	43/43	0.98	0.16	28,32,43,48	0
2	HEM	B	500	43/43	0.98	0.14	23,29,41,47	0
7	CAD	A	950	3/5	0.99	0.08	67,67,67,69	0
7	CAD	B	950	3/5	0.99	0.07	61,61,62,62	0
8	ZN	A	900	1/1	0.99	0.09	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.

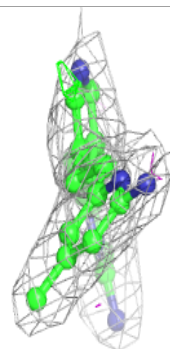
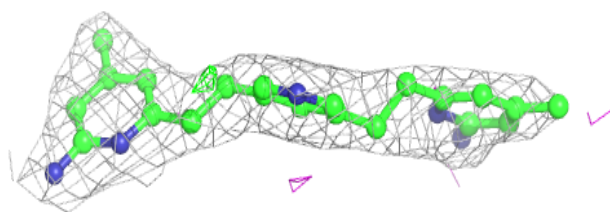
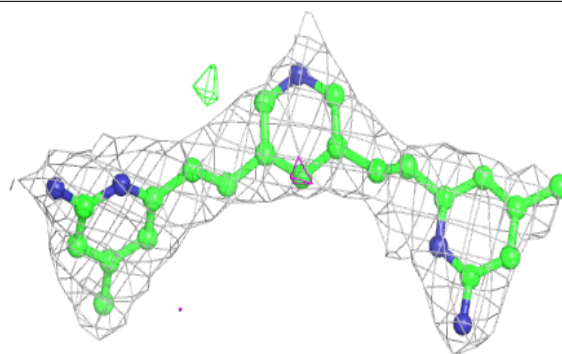
**Electron density around XFJ B 800:**

2mF<sub>o</sub>-DF<sub>c</sub> (at 0.7 rmsd) in gray  
mF<sub>o</sub>-DF<sub>c</sub> (at 3 rmsd) in purple (negative)  
and green (positive)



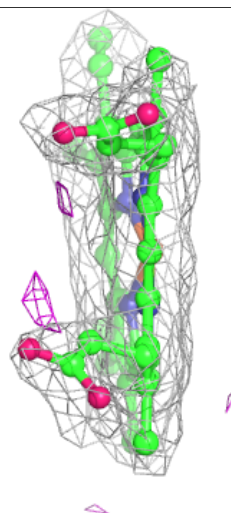
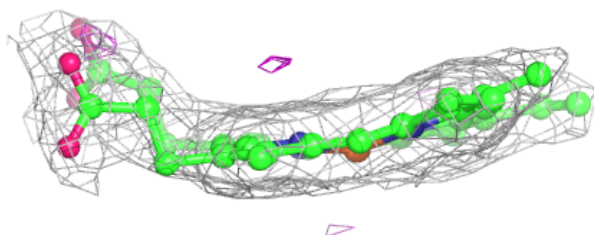
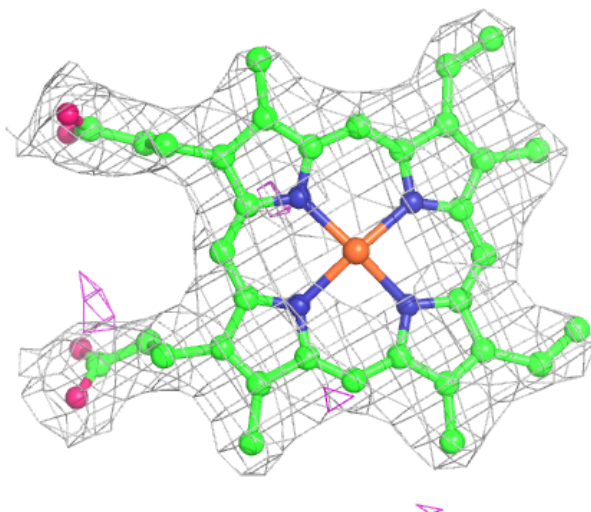
**Electron density around XFJ A 800:**

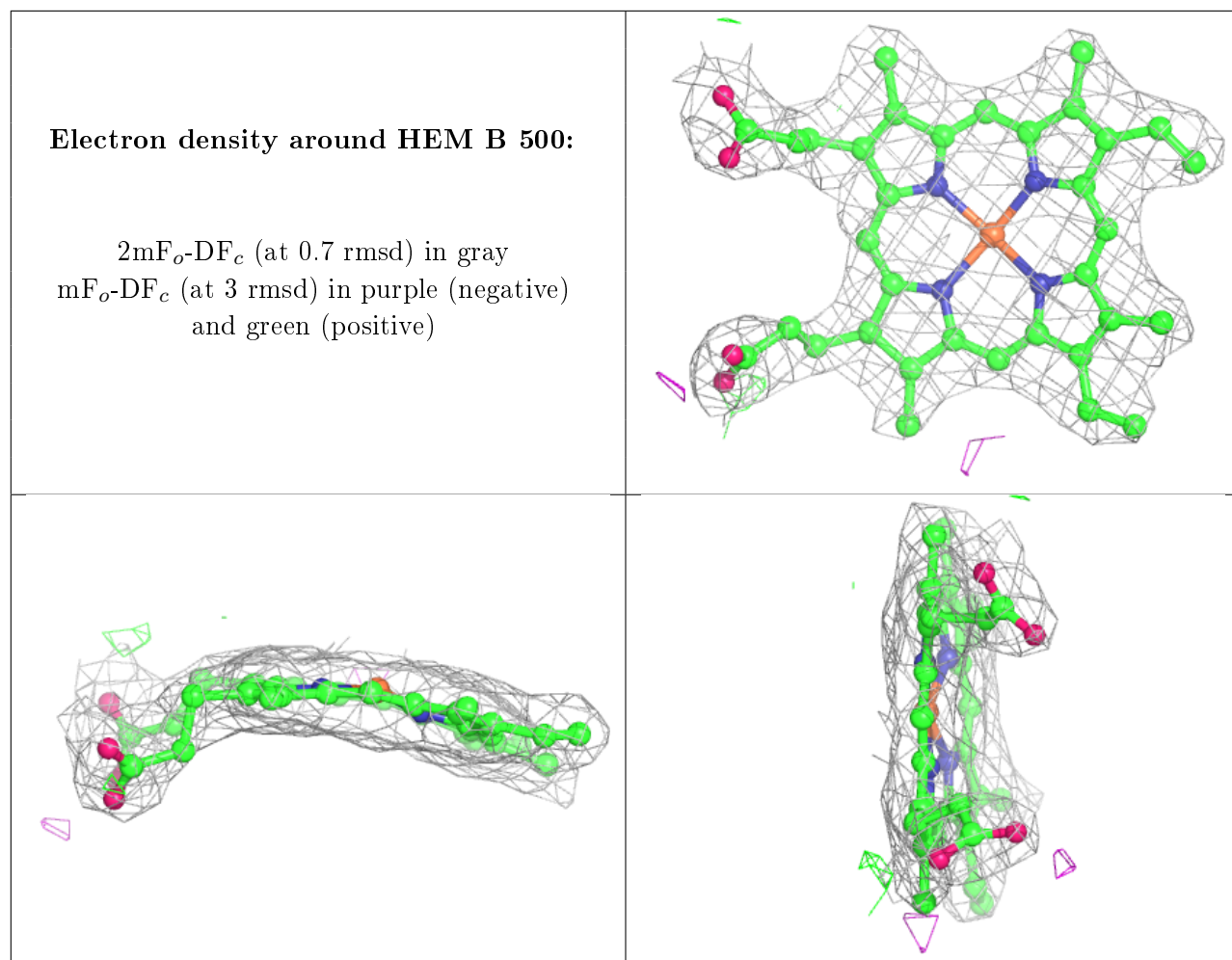
$2mF_o - DF_c$  (at 0.7 rmsd) in gray  
 $mF_o - DF_c$  (at 3 rmsd) in purple (negative)  
and green (positive)



**Electron density around HEM A 500:**

$2mF_o-DF_c$  (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.