wwPDB X-ray Structure Validation Summary Report (i)

Aug 5, 2026 – 09:53 PM EDT

PDB ID	:	3N6B / pdb_00003n6b
Title	:	Structure of endothelial nitric oxide synthase H373S single mutant 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	:	3.10 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 (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:

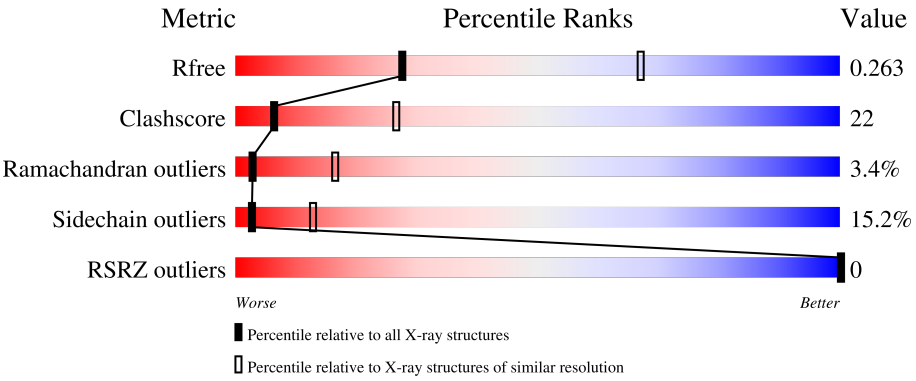
MolProbity	:	4-5-2 with Phenix2.0
Mogul	:	2022.3.0, CSD as543be (2022)
Xtriage (Phenix)	:	2.0
EDS	:	3.0
Buster-report	:	wwPDB partial adaption of 1.1.7 (2018)
Percentile statistics	:	20250101.v01 (using entries in the PDB archive January 1st 2025)
CCP4	:	9.0.015 (Gargrove)
Density-Fitness	:	1.0.12
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.50

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 3.10 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	1456 (3.10-3.10)
Clashscore	190562	1539 (3.10-3.10)
Ramachandran outliers	187476	1467 (3.10-3.10)
Sidechain outliers	187428	1467 (3.10-3.10)
RSRZ outliers	180081	1456 (3.10-3.10)

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	444	48% 35% 8% 9%
1	B	444	44% 36% 11% 9%

2 Entry composition (i)

There are 7 unique types of molecules in this entry. The entry contains 6600 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.

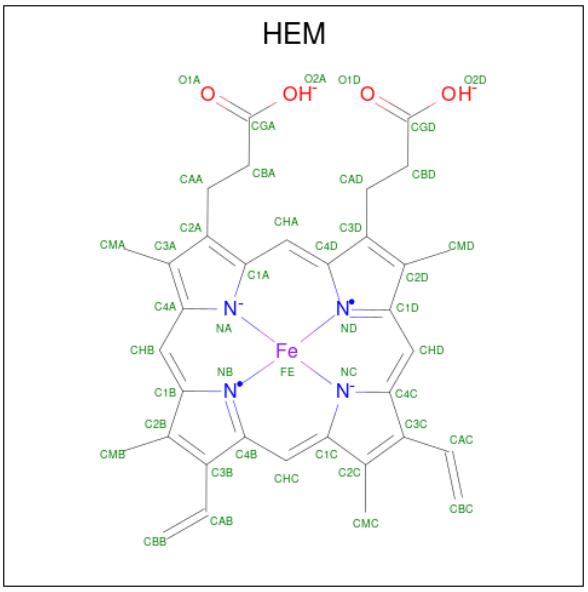
- Molecule 1 is a protein called Nitric oxide synthase.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	404	Total	C	N	O	S	0	0	0
			3205	2038	562	589	16			
1	B	402	Total	C	N	O	S	0	1	0
			3206	2040	561	589	16			

There are 4 discrepancies between the modelled and reference sequences:

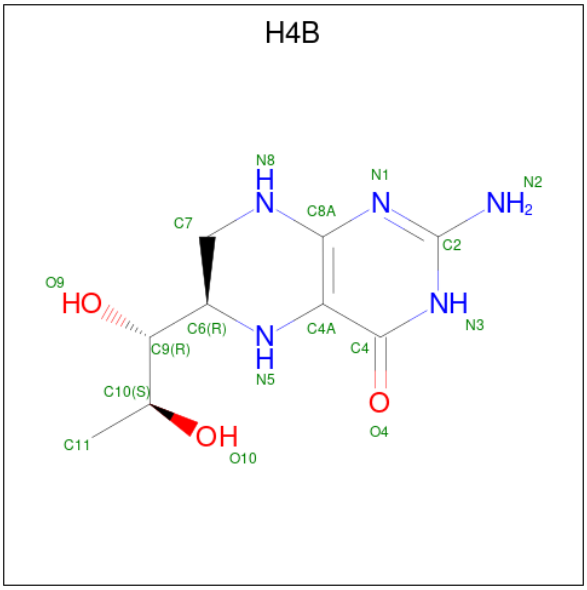
Chain	Residue	Modelled	Actual	Comment	Reference
A	100	ARG	CYS	SEE REMARK 999	UNP P29476
A	373	SER	HIS	engineered mutation	UNP P29476
B	100	ARG	CYS	SEE REMARK 999	UNP P29476
B	373	SER	HIS	engineered mutation	UNP P29476

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



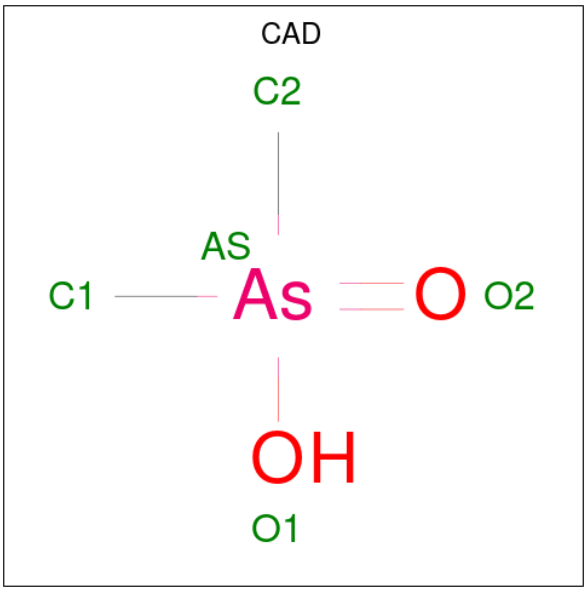
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total	C	Fe	N	O	0	0
			43	34	1	4	4		
2	B	1	Total	C	Fe	N	O	0	0
			43	34	1	4	4		

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



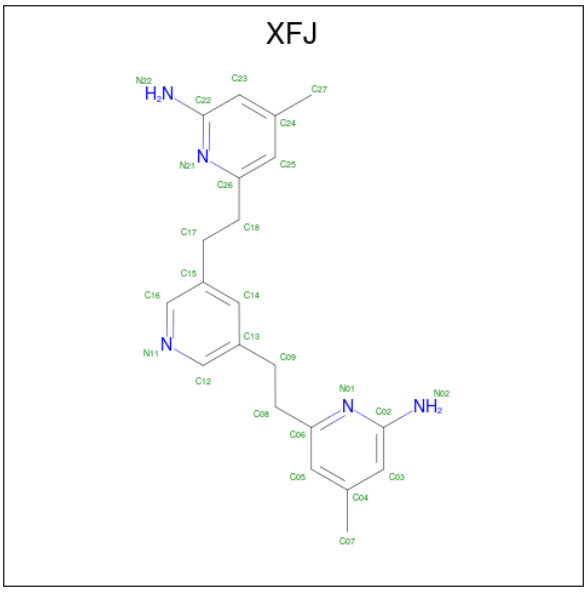
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
3	A	1	Total	C	N	O	0	0
			17	9	5	3		
3	B	1	Total	C	N	O	0	0
			17	9	5	3		

- Molecule 4 is CACODYLIC ACID (CCD ID: CAD) (formula: $C_2H_7AsO_2$).



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

- Molecule 5 is 6,6'-(pyridine-3,5-diyldiethane-2,1-diyl)bis(4-methylpyridin-2-amine) (CCD ID: XFJ) (formula: $C_{21}H_{25}N_5$).



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 26 21 5	0	0

- Molecule 6 is ZINC ION (CCD ID: ZN) (formula: Zn).

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

- Molecule 7 is water.

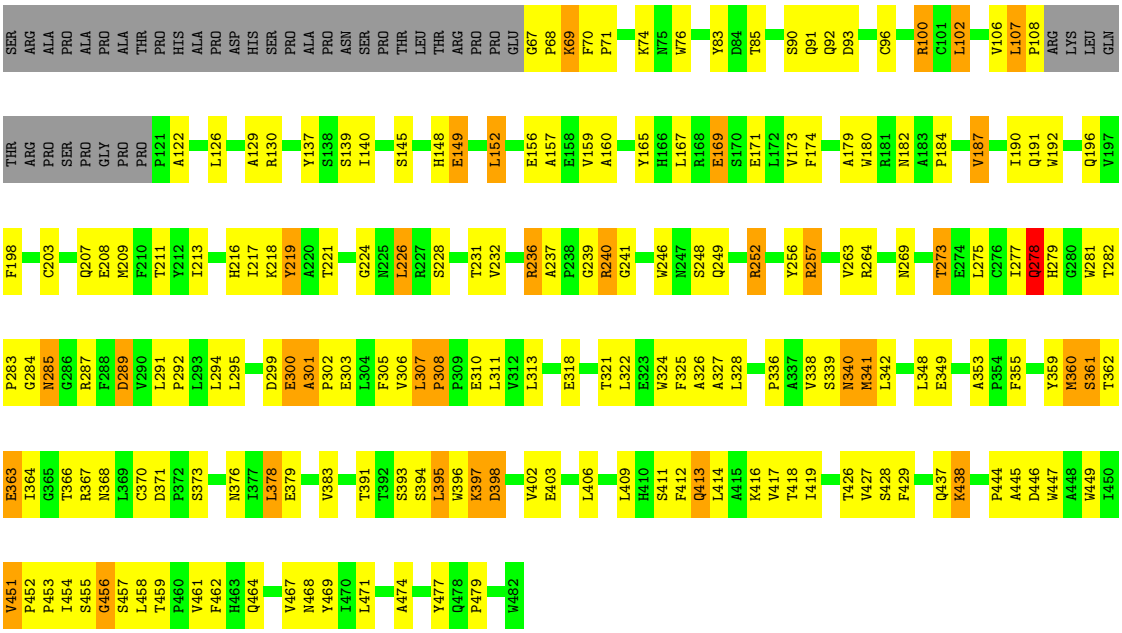
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
7	A	5	Total O 5 5	0	0
7	B	5	Total O 5 5	0	0

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.

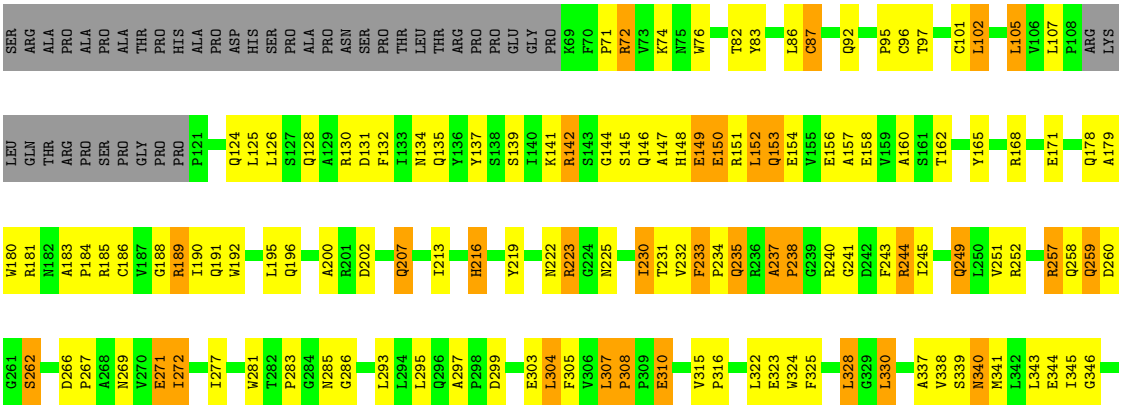
- Molecule 1: Nitric oxide synthase

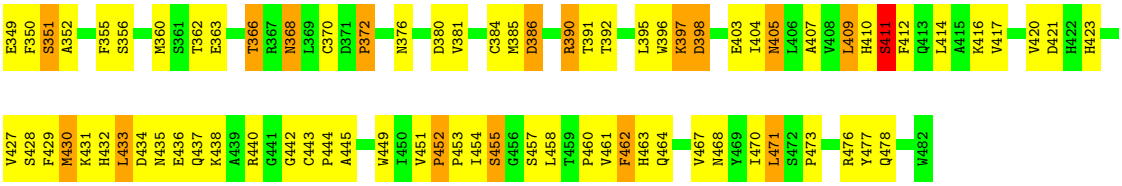
Chain A:



- Molecule 1: Nitric oxide synthase

Chain B:




E349
F350
S351
A352
F355
S356
M360
S361
T362
E363
T366
R367
N368
L369
C370
D371
P372
N376
D380
V381
C384
M385
D386
R390
T391
T392
L395
W396
K397
D398
E403
I404
N405
L406
A407
V408
L409
H410
S411
F412
Q413
L414
A415
K416
V417
V420
D421
H422
H423
V427
S428
F429
M430
K431
H432
L433
D434
N435
E436
Q437
K438
A439
R440
G441
G442
C443
P444
A445
W449
I450
V451
P452
P453
I454
S455
G456
S457
L458
T459
P460
V461
F462
H463
Q464
V467
N468
Y469
I470
L471
S472
P473
R476
Y477
Q478
W482

4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α, β, γ	57.76Å 106.55Å 157.82Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	39.46 – 3.10 39.46 – 3.10	Depositor EDS
% Data completeness (in resolution range)	99.1 (39.46-3.10) 99.3 (39.46-3.10)	Depositor EDS
R_{merge}	0.10	Depositor
R_{sym}	0.10	Depositor
$< I/\sigma(I) >$ [1]	2.13 (at 3.12Å)	Xtriage
Refinement program	REFMAC 5.5.0109, CNS	Depositor
R, R_{free}	0.187 , 0.274 0.185 , 0.263	Depositor DCC
R_{free} test set	900 reflections (4.88%)	wwPDB-VP
Wilson B-factor (Å^2)	75.2	Xtriage
Anisotropy	0.683	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.32 , 77.7	EDS
L-test for twinning[2]	$< \|L\| > = 0.46$, $< L^2 > = 0.29$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.95	EDS
Total number of atoms	6600	wwPDB-VP
Average B, all atoms (Å^2)	79.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.62% of the height of the origin peak. No significant pseudotranslation is detected.*

[1] Intensities estimated from amplitudes.

[2] Theoretical 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.

5 Model quality

5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: HEM, ZN, CAD, XFJ, H4B

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	0.93	1/3294 (0.0%)	1.21	22/4487 (0.5%)
1	B	0.91	1/3295 (0.0%)	1.24	24/4488 (0.5%)
All	All	0.92	2/6589 (0.0%)	1.22	46/8975 (0.5%)

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	A	0	1

All (2) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	470	ILE	CA-CB	5.67	1.60	1.54
1	A	179	ALA	CA-CB	-5.14	1.45	1.53

The worst 5 of 46 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	301	ALA	CA-C-N	9.92	130.54	119.83
1	A	301	ALA	C-N-CA	9.92	130.54	119.83
1	B	145	SER	N-CA-C	9.84	118.20	108.75
1	A	451	VAL	CA-C-N	9.80	130.48	120.38
1	A	451	VAL	C-N-CA	9.80	130.48	120.38

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	301	ALA	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 the asymmetric unit, whereas Symm-Clashes lists symmetry-related clashes.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	3205	0	3113	130	0
1	B	3206	0	3112	155	0
2	A	43	0	30	6	0
2	B	43	0	30	8	0
3	A	17	0	15	3	0
3	B	17	0	15	1	0
4	A	3	0	0	0	0
4	B	3	0	0	2	0
5	A	26	0	25	0	0
5	B	26	0	25	3	0
6	A	1	0	0	0	0
7	A	5	0	0	0	0
7	B	5	0	0	0	0
All	All	6600	0	6365	284	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 22.

The worst 5 of 284 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:244:ARG:HG3	1:B:244:ARG:HH11	1.20	1.02
1:B:260:ASP:OD1	1:B:262:SER:HB2	1.63	0.99
1:B:409:LEU:HD11	1:B:421:ASP:HB3	1.47	0.95
1:A:236:ARG:HH11	1:A:236:ARG:HB2	1.29	0.94
1:A:257:ARG:HG3	1:A:257:ARG:HH11	1.36	0.90

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	400/444 (90%)	339 (85%)	51 (13%)	10 (2%)	4 21
1	B	399/444 (90%)	319 (80%)	63 (16%)	17 (4%)	2 12
All	All	799/888 (90%)	658 (82%)	114 (14%)	27 (3%)	3 16

5 of 27 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	257	ARG
1	A	90	SER
1	A	122	ALA
1	A	239	GLY
1	A	361	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 sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
1	A	343/377 (91%)	292 (85%)	51 (15%)	3 13
1	B	343/377 (91%)	290 (84%)	53 (16%)	2 12
All	All	686/754 (91%)	582 (85%)	104 (15%)	3 13

5 of 104 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	107	LEU

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Mol	Chain	Res	Type
1	B	235	GLN
1	B	411	SER
1	B	139	SER
1	B	153	GLN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 28 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	166	HIS
1	B	478	GLN
1	B	196	GLN
1	B	405	ASN
1	B	191	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)

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 9 ligands modelled in this entry, 1 is monoatomic - leaving 8 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	B	500	-	50,50,50	1.88	14 (28%)	67,82,82	1.28	7 (10%)
3	H4B	B	600	-	17,18,18	0.88	0	14,26,26	2.22	7 (50%)
4	CAD	A	950	1	0,2,4	-	-	0,1,6	-	-
5	XFJ	A	800	-	28,28,28	0.48	0	36,38,38	1.97	12 (33%)
3	H4B	A	600	-	17,18,18	0.95	0	14,26,26	1.73	6 (42%)
5	XFJ	B	800	-	28,28,28	0.77	0	36,38,38	1.63	6 (16%)
2	HEM	A	500	1	50,50,50	1.95	10 (20%)	67,82,82	1.68	17 (25%)
4	CAD	B	950	-	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
2	HEM	B	500	-	-	2/14/54/54	-
3	H4B	B	600	-	-	0/8/17/17	0/2/2/2
5	XFJ	A	800	-	-	0/10/10/10	0/3/3/3
3	H4B	A	600	-	-	0/8/17/17	0/2/2/2
5	XFJ	B	800	-	-	3/10/10/10	0/3/3/3
2	HEM	A	500	1	-	7/14/54/54	-

The worst 5 of 24 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	500	HEM	C3D-C2D	6.74	1.51	1.36
2	A	500	HEM	FE-ND	6.50	2.14	1.94
2	A	500	HEM	C3D-C2D	6.13	1.50	1.36
2	A	500	HEM	FE-NB	5.19	2.10	1.94
2	B	500	HEM	FE-NB	3.34	2.05	1.94

The worst 5 of 55 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
5	A	800	XFJ	C02-N01-C06	6.11	122.64	118.07
5	B	800	XFJ	C02-N01-C06	5.12	121.90	118.07
2	A	500	HEM	C4D-ND-C1D	4.42	110.44	105.21
2	A	500	HEM	CAD-C3D-C4D	3.89	131.48	124.70
5	A	800	XFJ	C14-C15-C16	3.89	120.50	116.90

There are no chirality outliers.

5 of 12 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
5	B	800	XFJ	C15-C17-C18-C26
2	A	500	HEM	C3D-CAD-CBD-CGD
2	A	500	HEM	C2B-C3B-CAB-CBB
2	A	500	HEM	C4D-C3D-CAD-CBD
2	B	500	HEM	C4B-C3B-CAB-CBB

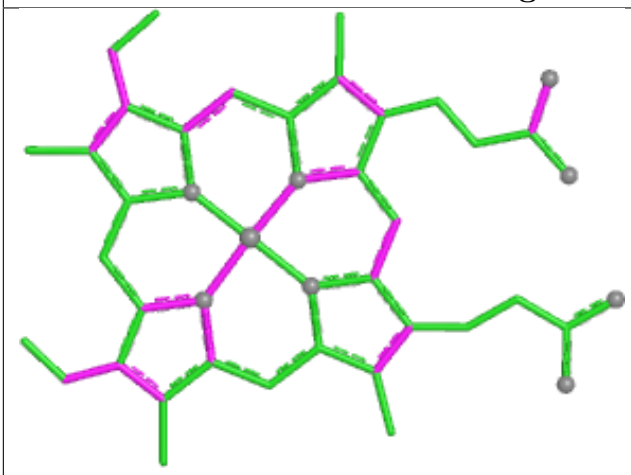
There are no ring outliers.

6 monomers are involved in 18 short contacts:

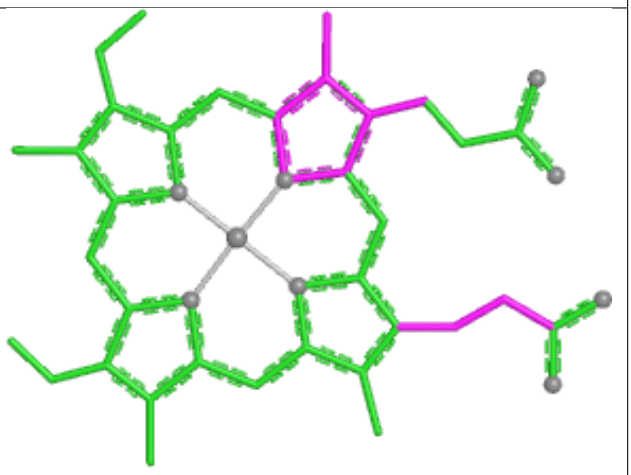
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	B	500	HEM	8	0
3	B	600	H4B	1	0
3	A	600	H4B	3	0
5	B	800	XFJ	3	0
2	A	500	HEM	6	0
4	B	950	CAD	2	0

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 then 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.

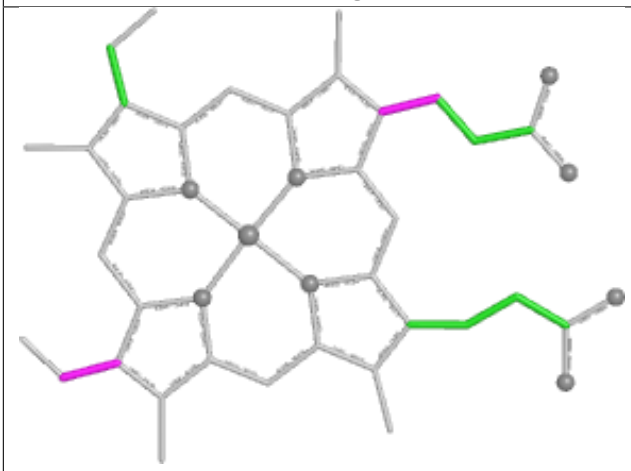
Ligand HEM B 500



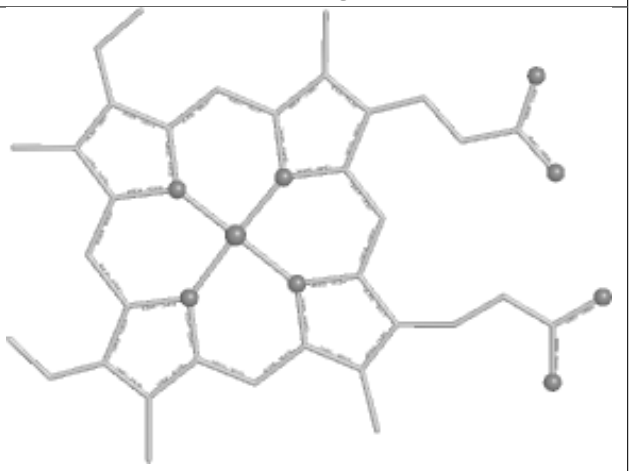
Bond lengths



Bond angles

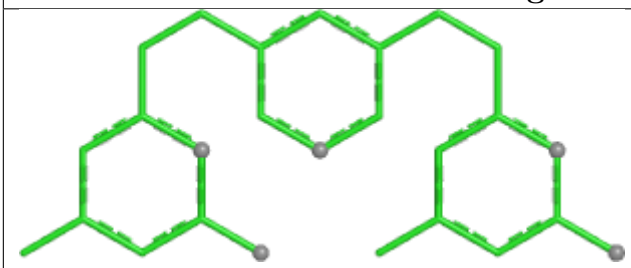


Torsions

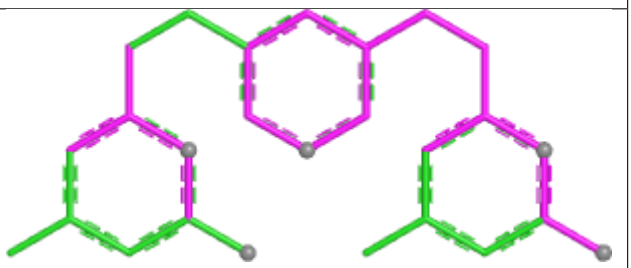


Rings

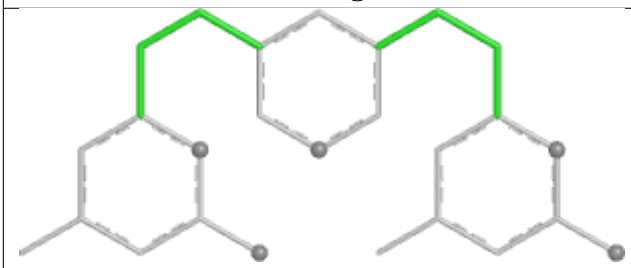
Ligand XFJ A 800



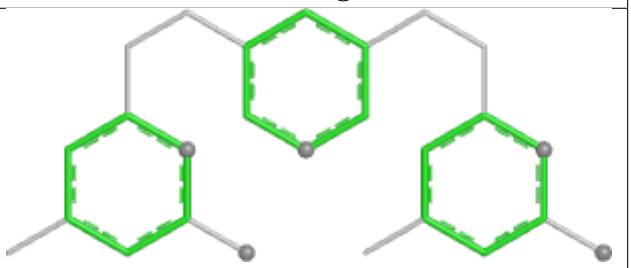
Bond lengths



Bond angles

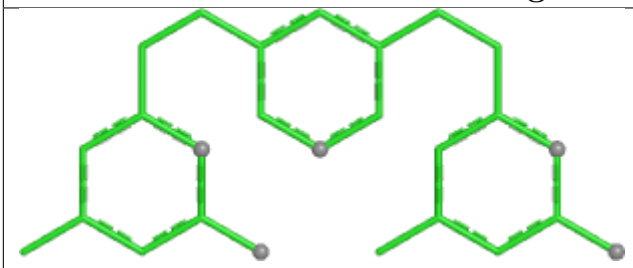


Torsions

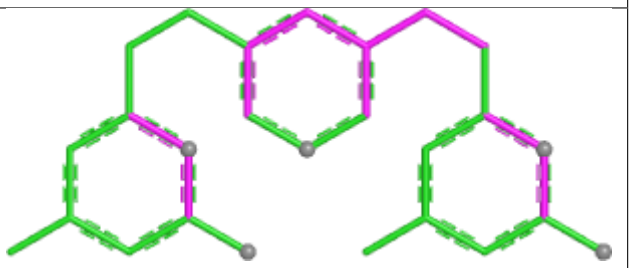


Rings

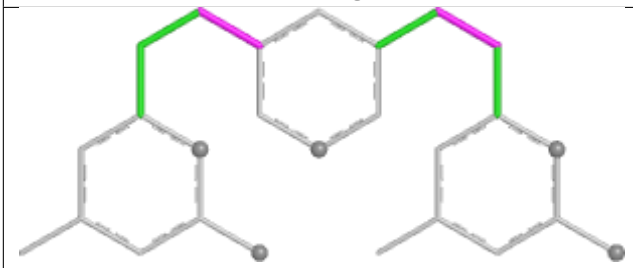
Ligand XFJ B 800



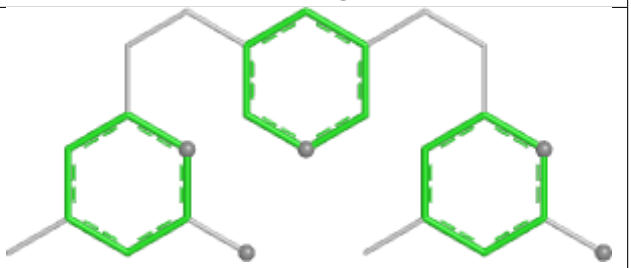
Bond lengths



Bond angles

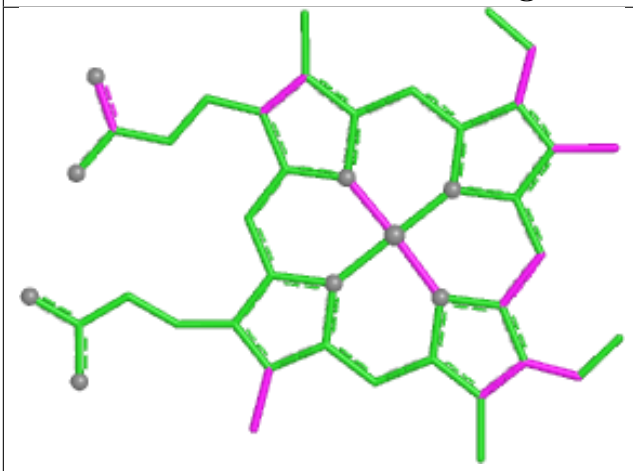


Torsions

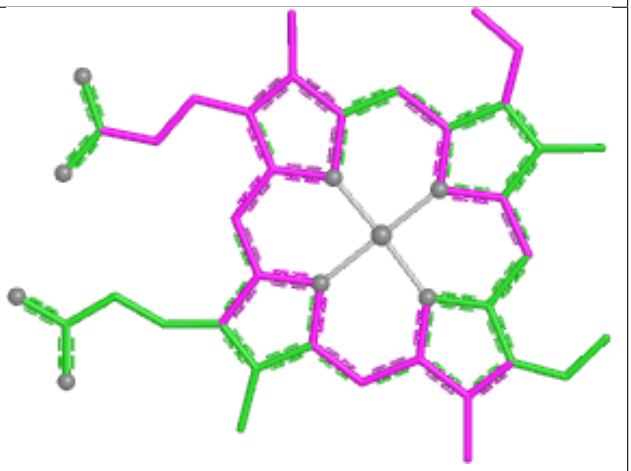


Rings

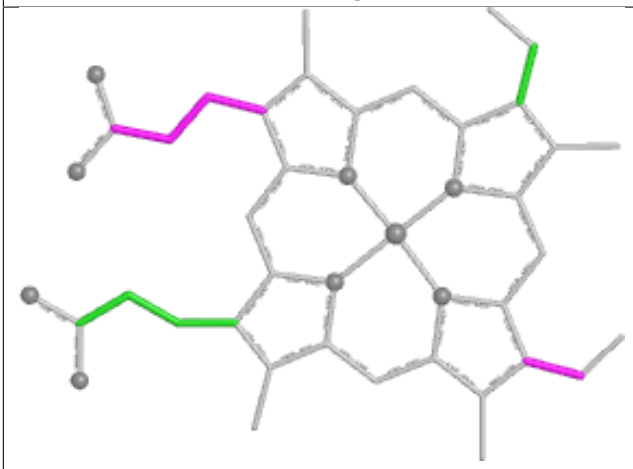
Ligand HEM A 500



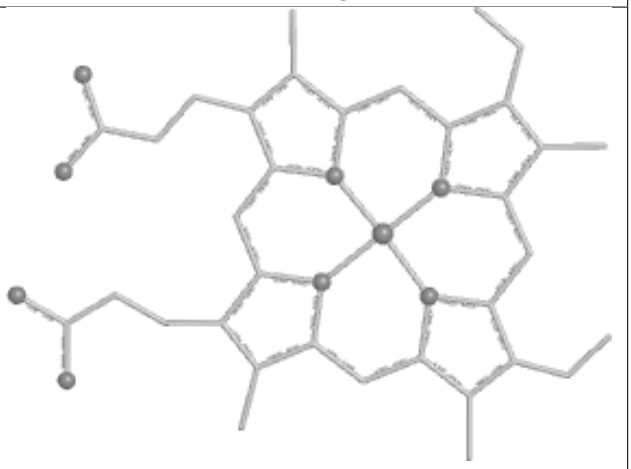
Bond lengths



Bond angles



Torsions



Rings

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

6.1 Protein, DNA and RNA chains

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>2			OWAB(Å^2)	Q<0.9
1	A	404/444 (90%)	-0.62	0	100	100	52, 77, 108, 120	0
1	B	402/444 (90%)	-0.58	0	100	100	35, 77, 114, 145	1 (0%)
All	All	806/888 (90%)	-0.60	0	100	100	35, 77, 110, 145	1 (0%)

There are no RSRZ outliers to report.

6.2 Non-standard residues in protein, DNA, RNA chains

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

6.3 Carbohydrates

There are no oligosaccharides in this entry.

6.4 Ligands

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	B-factors(Å^2)	Q<0.9
3	H4B	B	600	17/17	0.90	0.10	77,82,84,84	0
5	XFJ	B	800	26/26	0.91	0.13	57,89,105,106	0
4	CAD	B	950	3/5	0.94	0.17	122,122,122,124	0
3	H4B	A	600	17/17	0.94	0.08	71,73,77,79	0
5	XFJ	A	800	26/26	0.95	0.13	81,104,110,111	0
4	CAD	A	950	3/5	0.95	0.13	103,103,106,106	0
2	HEM	A	500	43/43	0.98	0.07	51,57,63,69	0

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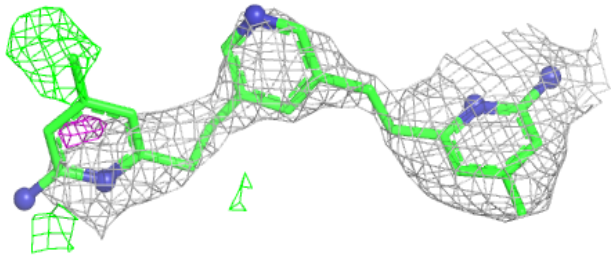
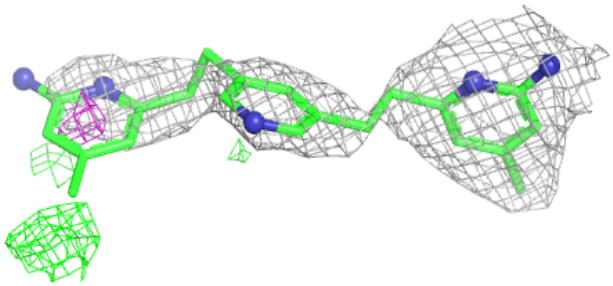
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
2	HEM	B	500	43/43	0.99	0.06	46,51,65,74	0
6	ZN	A	900	1/1	1.00	0.02	73,73,73,73	0

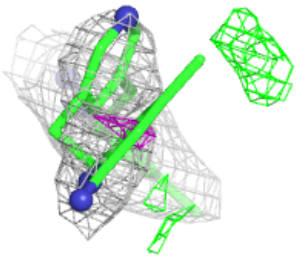
The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation to approximate a three-dimensional view.

Electron density around XFJ B 800:

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

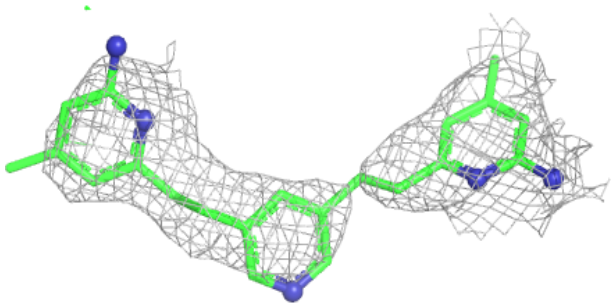


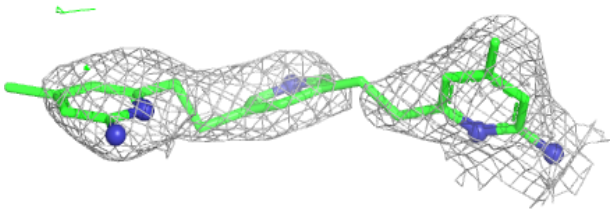


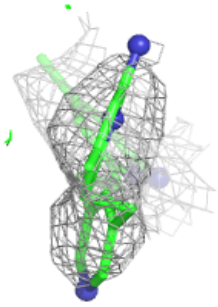


Electron density around XFJ A 800:

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

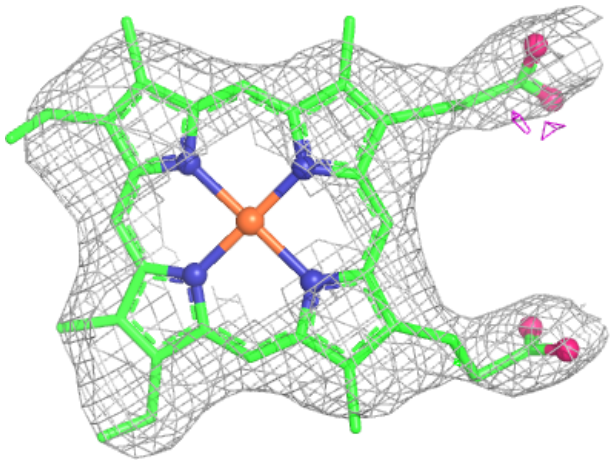


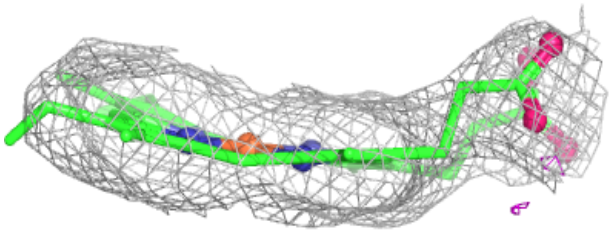


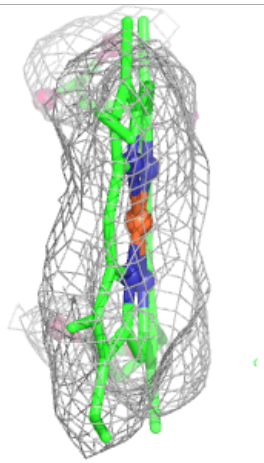


Electron density around HEM A 500:

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

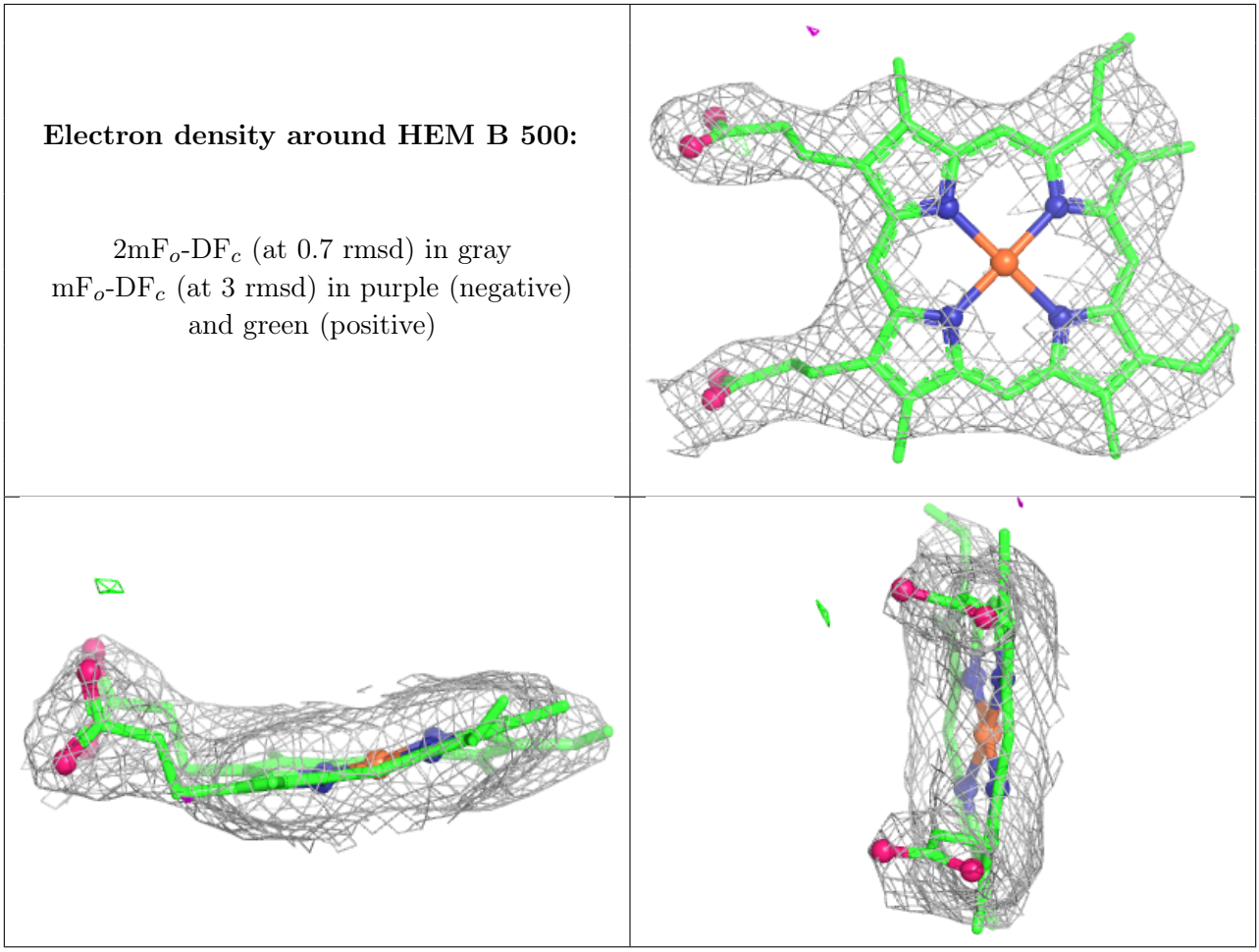






Electron density around HEM B 500:

$2mF_o$-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.