



# wwPDB X-ray Structure Validation Summary Report ⓘ

May 16, 2020 – 05:26 pm BST

PDB ID : 3JWW  
Title : Structure of endothelial nitric oxide synthase heme domain complexed with N  
1-[(3'S,4'S)-4'-((6"-amino-4"-methylpyridin-2"-yl)methyl)pyrrolidin-3'-yl]-N2  
- (3'-fluorophenethyl)ethane-1,2-diamine tetrahydrochloride  
Authors : Delker, S.L.; Li, H.; Poulos, T.L.  
Deposited on : 2009-09-18  
Resolution : 2.20 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

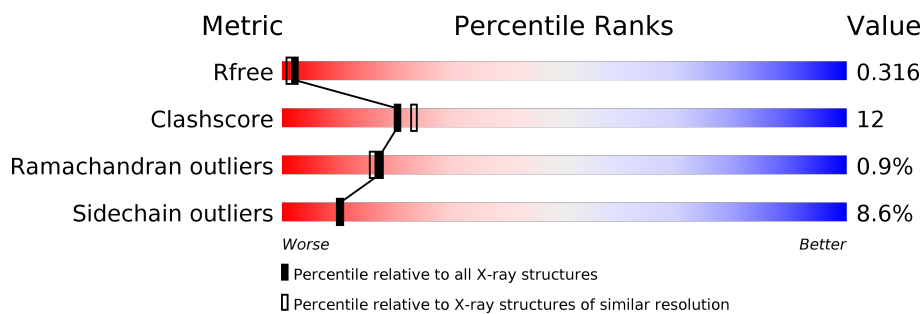
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*X-RAY DIFFRACTION*

The reported resolution of this entry is 2.20 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	4898 (2.20-2.20)
Clashscore	141614	5594 (2.20-2.20)
Ramachandran outliers	138981	5503 (2.20-2.20)
Sidechain outliers	138945	5504 (2.20-2.20)

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  $\leq 5\%$ .

Mol	Chain	Length	Quality of chain
1	A	444	
1	B	444	

## 2 Entry composition [i](#)

There are 9 unique types of molecules in this entry. The entry contains 6863 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, endothelial.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	404	Total	C	N	O	S	0	0	0
			3213	2042	567	588	16			
1	B	403	Total	C	N	O	S	0	3	0
			3242	2058	577	591	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:  $C_{34}H_{32}FeN_4O_4$ ).



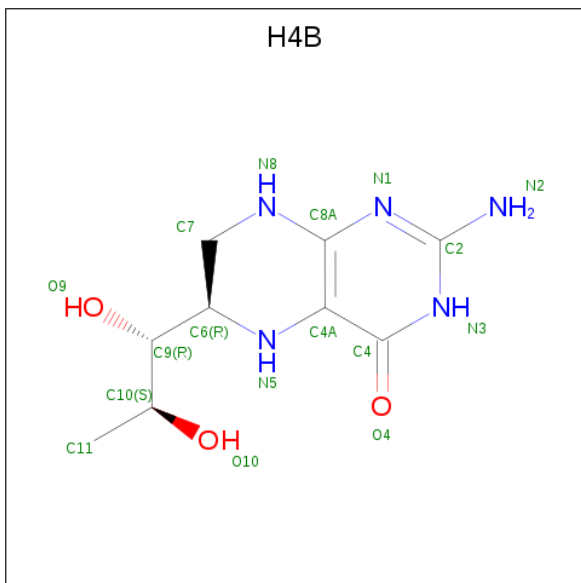
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf
2	A	1	Total	C	Fe	N	O	0	0
			43	34	1	4	4		

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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 5,6,7,8-TETRAHYDROBIOPTERIN (three-letter code: 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 ACETATE ION (three-letter code: ACT) (formula:  $C_2H_3O_2$ ).



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

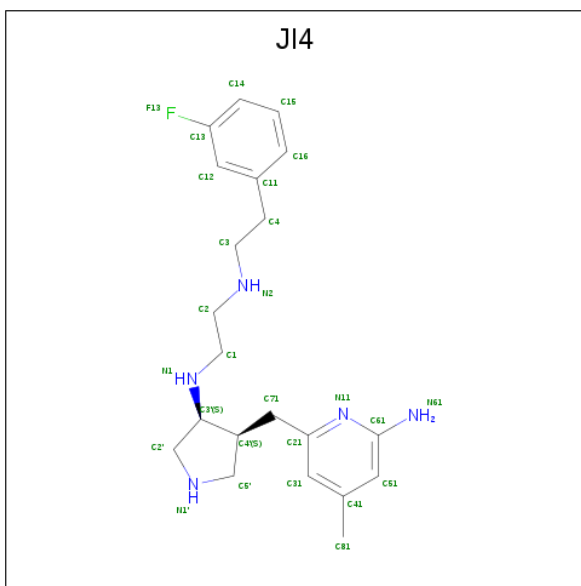
- Molecule 5 is GLYCEROL (three-letter code: GOL) (formula:  $C_3H_8O_3$ ).



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

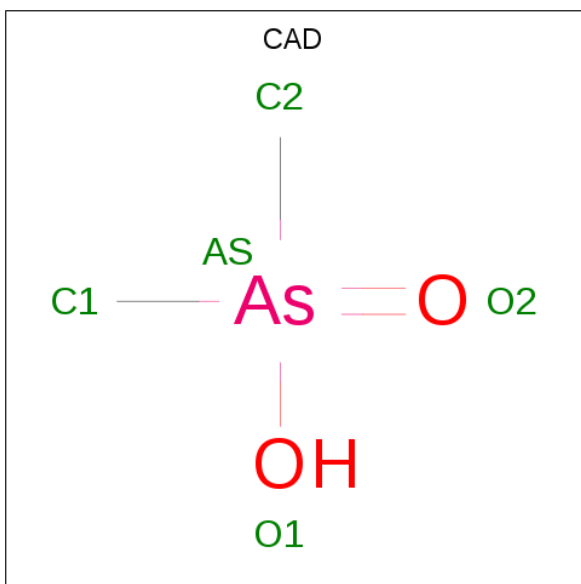
- Molecule 6 is N-{(3S,4S)-4-[(6-amino-4-methylpyridin-2-yl)methyl]pyrrolidin-3-yl}-N'-[2-(3-

fluorophenyl)ethyl]ethane-1,2-diamine (three-letter code: JI4) (formula:  $C_{21}H_{30}FN_5$ ).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
6	A	1	Total	C	F	N	0	0
			27	21	1	5		
6	B	1	Total	C	F	N	0	0
			27	21	1	5		

- 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		

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Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
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		

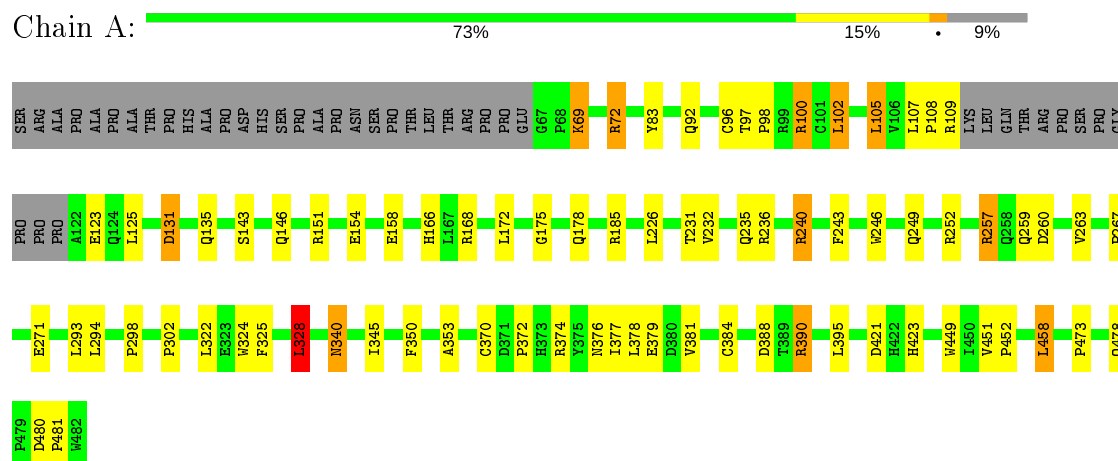
- Molecule 9 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
9	A	104	Total	O	0	0
			104	104		
9	B	107	Total	O	0	0
			107	107		

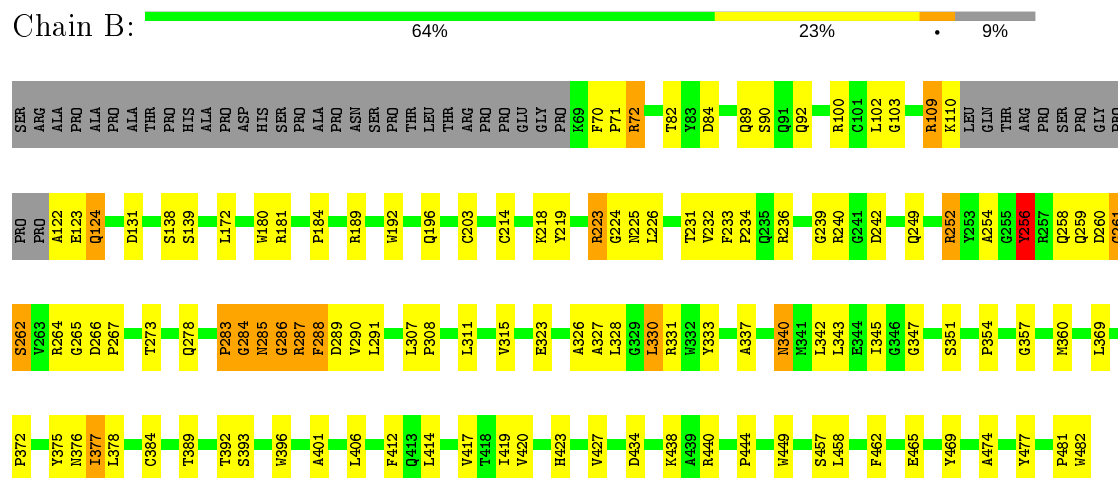
### 3 Residue-property plots

These plots are drawn for all protein, RNA and DNA 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. 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. 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, endothelial



- Molecule 1: Nitric oxide synthase, endothelial



## 4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	58.61Å 107.13Å 157.71Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	39.43 – 2.20 39.43 – 2.20	Depositor EDS
% Data completeness (in resolution range)	96.6 (39.43-2.20) 96.6 (39.43-2.20)	Depositor EDS
$R_{merge}$	0.07	Depositor
$R_{sym}$	0.07	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.24 (at 2.20Å)	Xtriage
Refinement program	REFMAC 5.5.0089, CNS	Depositor
R, $R_{free}$	0.210 , 0.284 0.258 , 0.316	Depositor DCC
$R_{free}$ test set	2450 reflections (4.97%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	42.5	Xtriage
Anisotropy	0.718	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.32 , 38.4	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.49$ , $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.93	EDS
Total number of atoms	6863	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	54.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.90% 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, H4B, JI4, 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	0.84	1/3302 (0.0%)	0.82	1/4497 (0.0%)
1	B	1.17	11/3330 (0.3%)	1.02	6/4531 (0.1%)
All	All	1.02	12/6632 (0.2%)	0.92	7/9028 (0.1%)

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

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

The worst 5 of 12 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	284	GLY	N-CA	23.14	1.80	1.46
1	B	286	GLY	N-CA	10.56	1.61	1.46
1	B	256	TYR	CB-CG	8.56	1.64	1.51
1	B	203	CYS	CB-SG	-7.96	1.68	1.82
1	B	289	ASP	CB-CG	6.69	1.65	1.51

The worst 5 of 7 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	283	PRO	C-N-CA	-7.19	107.20	122.30
1	B	440	ARG	NE-CZ-NH1	6.86	123.73	120.30
1	B	285	ASN	OD1-CG-ND2	-6.41	107.16	121.90
1	B	285	ASN	CB-CG-OD1	6.30	134.20	121.60
1	B	285	ASN	N-CA-CB	-5.20	101.25	110.60

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	B	239	GLY	Peptide
1	B	256	TYR	Sidechain

## 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	3213	0	3120	57	0
1	B	3242	0	3154	104	0
2	A	43	0	30	6	0
2	B	43	0	30	5	0
3	A	17	0	15	1	0
3	B	17	0	15	1	0
4	A	4	0	3	0	0
5	A	6	0	8	0	0
5	B	6	0	8	0	0
6	A	27	0	30	1	0
6	B	27	0	30	4	0
7	A	3	0	0	3	0
7	B	3	0	0	2	0
8	A	1	0	0	0	0
9	A	104	0	0	2	0
9	B	107	0	0	9	0
All	All	6863	0	6443	162	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 12.

The worst 5 of 162 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:284:GLY:N	1:B:284:GLY:CA	1.80	1.41
1:B:444:PRO:HB3	1:B:469:TYR:CZ	1.71	1.25
1:A:384:CYS:SG	7:A:950:CAD:AS	2.60	1.18

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:B:109[A]:ARG:HG3	1:B:109[A]:ARG:HH11	1.08	1.15
1:A:72:ARG:H	1:B:109[B]:ARG:NH2	1.45	1.14

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%)	381 (95%)	18 (4%)	1 (0%)	41	46
1	B	402/444 (90%)	372 (92%)	24 (6%)	6 (2%)	10	8
All	All	802/888 (90%)	753 (94%)	42 (5%)	7 (1%)	17	16

5 of 7 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	327	ALA
1	B	103	GLY
1	B	261	GLY
1	B	286	GLY
1	A	108	PRO

### 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%)	315 (92%)	28 (8%)	11	11
1	B	346/377 (92%)	312 (90%)	34 (10%)	8	7
All	All	689/754 (91%)	627 (91%)	62 (9%)	10	9

5 of 62 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	72	ARG
1	B	109[A]	ARG
1	B	377	ILE
1	B	92	GLN
1	B	110	LYS

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 12 such sidechains are listed below:

Mol	Chain	Res	Type
1	A	468	ASN
1	B	146	GLN
1	B	340	ASN
1	A	376	ASN
1	B	258	GLN

### 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 12 ligands modelled in this entry, 1 is monoatomic - leaving 11 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
5	GOL	B	880	-	5,5,5	0.42	0	5,5,5	0.92	0
3	H4B	A	600	-	16,18,18	1.13	1 (6%)	11,26,26	2.93	6 (54%)
2	HEM	A	500	1	27,50,50	2.22	7 (25%)	17,82,82	2.41	6 (35%)
2	HEM	B	500	1	27,50,50	2.28	8 (29%)	17,82,82	2.73	5 (29%)
4	ACT	A	860	-	1,3,3	2.37	1 (100%)	0,3,3	0.00	-
7	CAD	B	950	-	0,2,4	0.00	-	0,1,6	0.00	-
3	H4B	B	600	-	16,18,18	1.04	0	11,26,26	2.80	6 (54%)
5	GOL	A	880	-	5,5,5	0.38	0	5,5,5	1.13	0
6	JI4	A	800	-	27,29,29	1.03	1 (3%)	30,38,38	1.57	6 (20%)
7	CAD	A	950	-	0,2,4	0.00	-	0,1,6	0.00	-
6	JI4	B	800	-	27,29,29	0.70	0	30,38,38	2.01	6 (20%)

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
5	GOL	B	880	-	-	4/4/4/4	-
3	H4B	A	600	-	-	0/8/17/17	0/2/2/2
2	HEM	A	500	1	-	0/6/54/54	-
2	HEM	B	500	1	-	0/6/54/54	-
3	H4B	B	600	-	-	0/8/17/17	0/2/2/2
5	GOL	A	880	-	-	2/4/4/4	-
6	JI4	A	800	-	-	8/13/23/23	0/3/3/3
6	JI4	B	800	-	-	4/13/23/23	0/3/3/3

The worst 5 of 18 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	500	HEM	C3B-C2B	-5.54	1.32	1.40
2	B	500	HEM	C3B-C2B	-5.25	1.33	1.40

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Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	500	HEM	C3C-C2C	-4.68	1.33	1.40
2	B	500	HEM	C3D-C2D	4.62	1.51	1.37
2	A	500	HEM	C3C-CAC	4.30	1.56	1.47

The worst 5 of 35 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	B	500	HEM	CBA-CAA-C2A	-7.90	97.91	112.49
2	A	500	HEM	CBA-CAA-C2A	-6.05	101.32	112.49
2	B	500	HEM	C1D-C2D-C3D	-6.03	102.80	107.00
3	A	600	H4B	C4-C4A-N5	5.29	123.56	119.12
6	B	800	JI4	C61-N11-C21	5.01	121.89	118.10

There are no chirality outliers.

5 of 18 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
5	B	880	GOL	C1-C2-C3-O3
5	A	880	GOL	C1-C2-C3-O3
6	A	800	JI4	C2-C1-N1-C3'
6	A	800	JI4	C2'-C3'-N1-C1
6	A	800	JI4	C4'-C3'-N1-C1

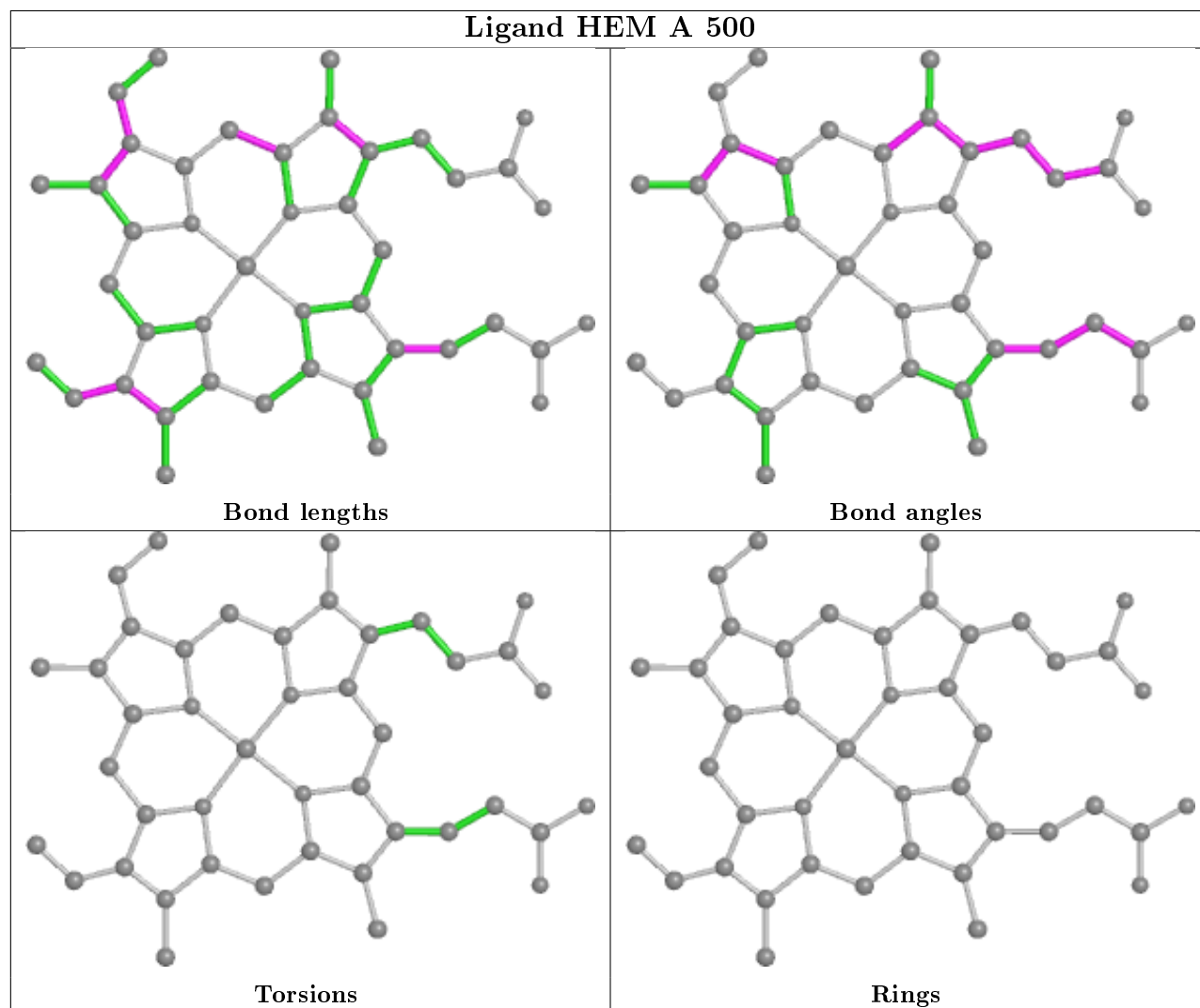
There are no ring outliers.

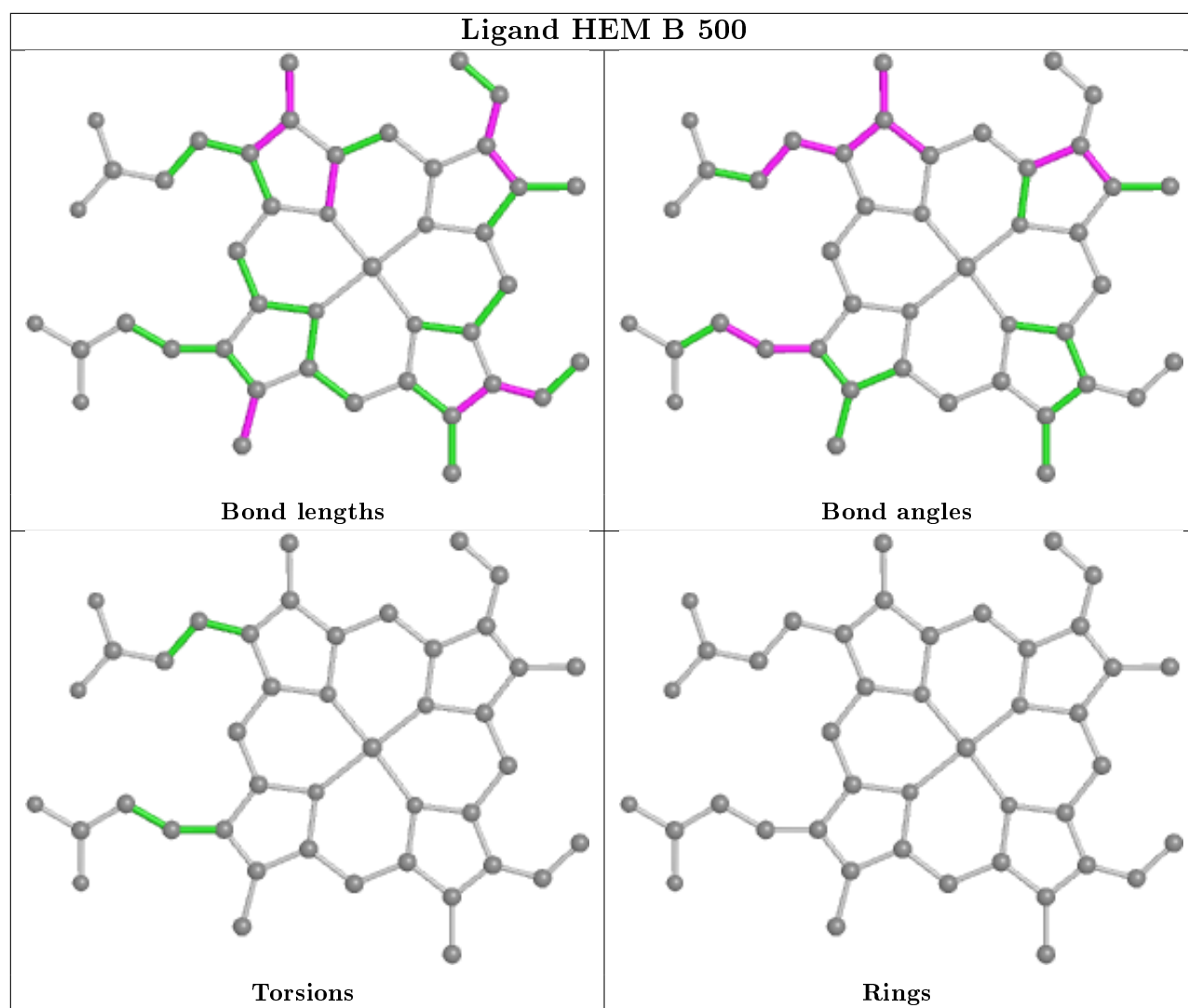
8 monomers are involved in 22 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	A	600	H4B	1	0
2	A	500	HEM	6	0
2	B	500	HEM	5	0
7	B	950	CAD	2	0
3	B	600	H4B	1	0
6	A	800	JI4	1	0
7	A	950	CAD	3	0
6	B	800	JI4	4	0

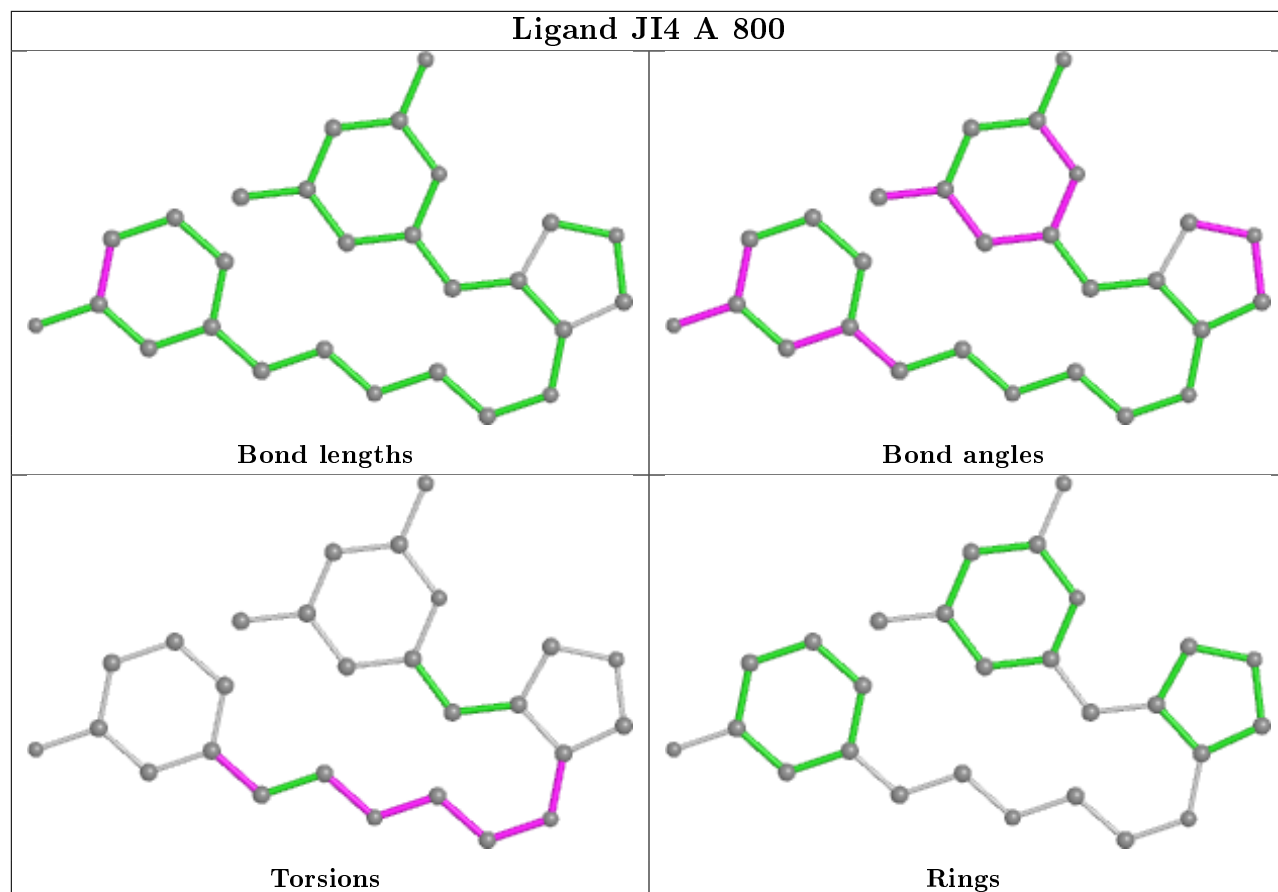
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. 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.

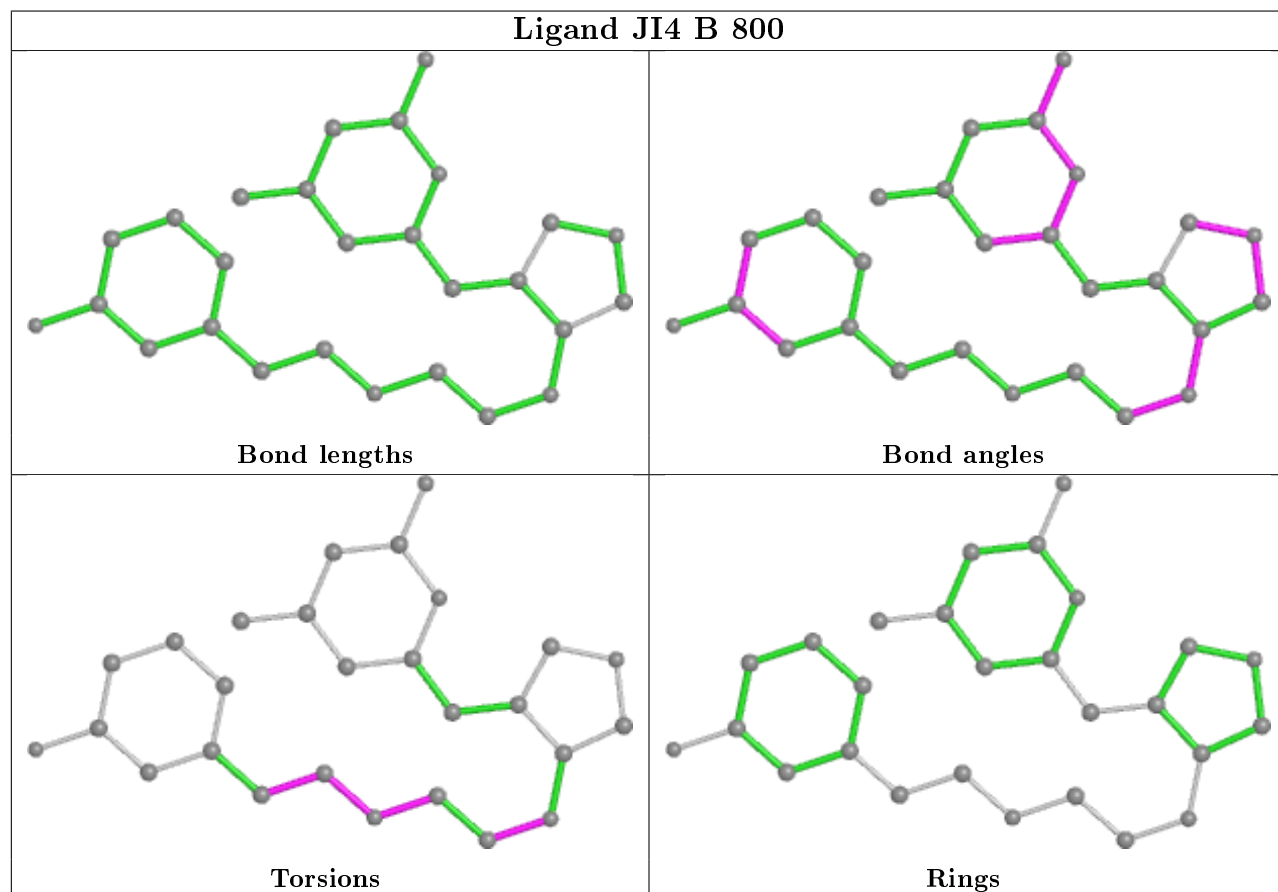




## Ligand JI4 A 800



## Ligand JI4 B 800



## 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

Unable to reproduce the depositors R factor - this section is therefore empty.

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

Unable to reproduce the depositors R factor - this section is therefore empty.

### 6.3 Carbohydrates

Unable to reproduce the depositors R factor - this section is therefore empty.

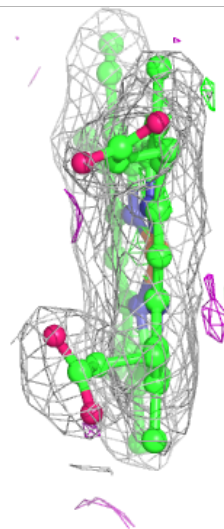
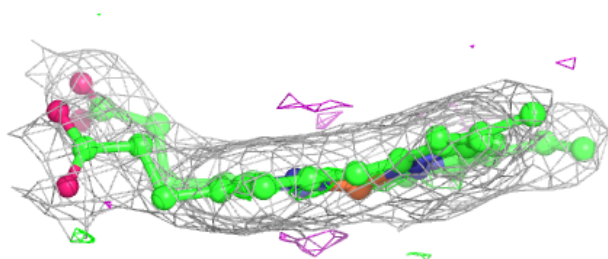
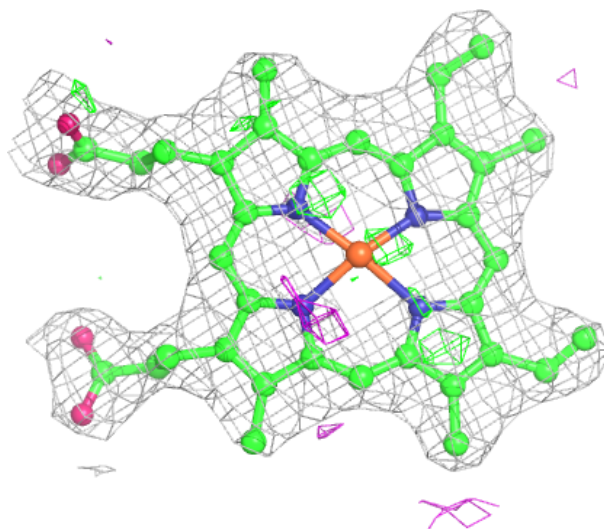
### 6.4 Ligands

Unable to reproduce the depositors R factor - this section is therefore empty.

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.

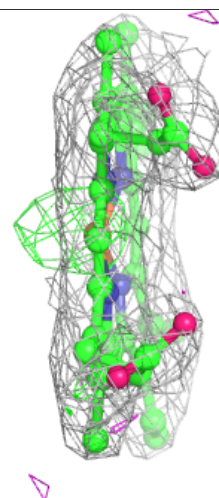
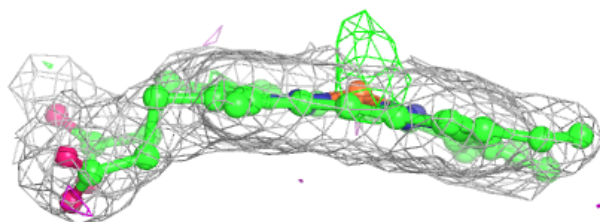
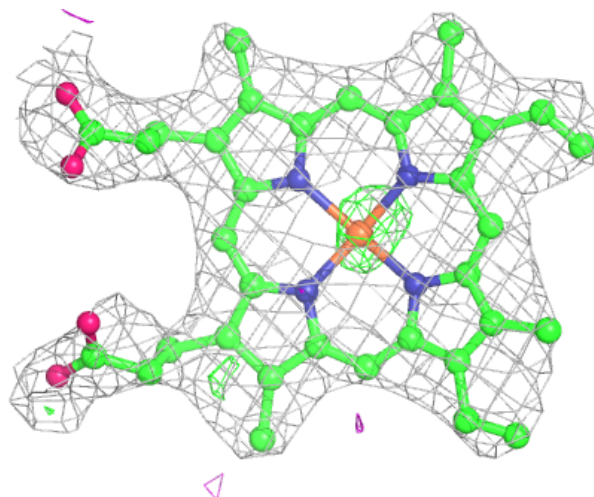
**Electron density around HEM A 500:**

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



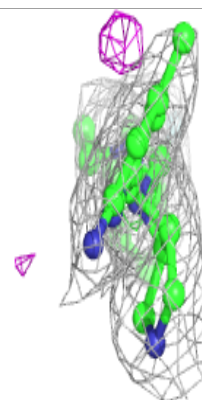
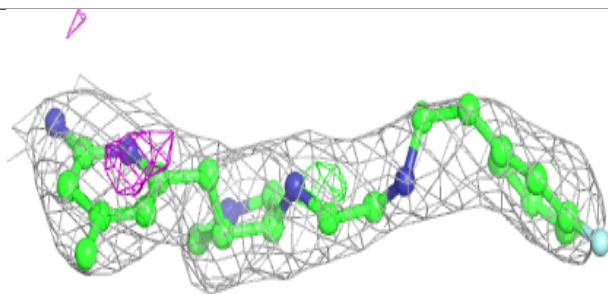
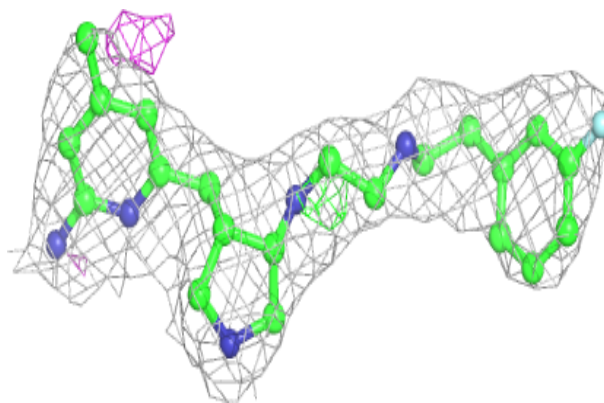
**Electron density around HEM B 500:**

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

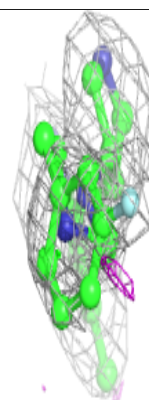
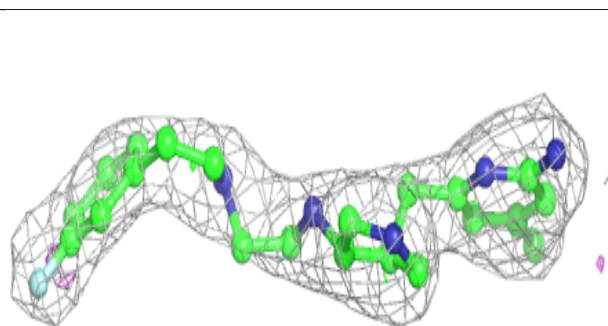
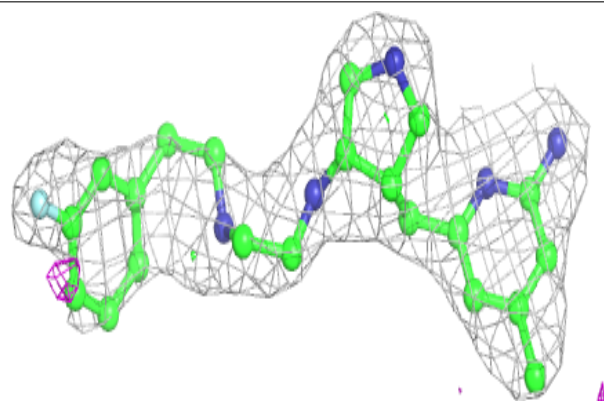


**Electron density around JI4 A 800:**

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

**Electron density around JI4 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)



## 6.5 Other polymers ⓘ

Unable to reproduce the depositors R factor - this section is therefore empty.