

wwPDB X-ray Structure Validation Summary Report (i)

Aug 27, 2023 – 07:42 PM EDT

PDB ID : 3IQ5

Title: Crystal structure of an engineered metal-free tetrameric cytochrome cb562

complex templated by Zn-coordination

Authors: Brodin, J.N.; Tezcan, F.A.

Deposited on : 2009-08-19

Resolution : 2.05 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 (1)) 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 : 2.35

buster-report : 1.1.7 (2018)

Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)

 $Refmac \quad : \quad 5.8.0158$

CCP4 : 7.0.044 (Gargrove)

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

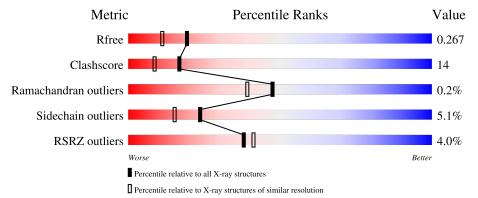
Validation Pipeline (wwPDB-VP) : 2.35

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure: X-RAY DIFFRACTION

The reported resolution of this entry is 2.05 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	$\begin{array}{c} \text{Whole archive} \\ (\#\text{Entries}) \end{array}$	Similar resolution $(\# \text{Entries, resolution range}(\text{\AA}))$
R_{free}	130704	1692 (2.04-2.04)
Clashscore	141614	1773 (2.04-2.04)
Ramachandran outliers	138981	1752 (2.04-2.04)
Sidechain outliers	138945	1752 (2.04-2.04)
RSRZ outliers	127900	1672 (2.04-2.04)

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	106	83%	14%				
1	В	106	72%	25%				
1	С	106	75%	21%	-			
1	D	106	71%	27%				



2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 3680 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 Soluble cytochrome b562.

Mol	Chain	Residues		At	oms			ZeroOcc	AltConf	Trace
1	Λ	106	Total	С	N	О	S	0	0	0
1	A	100	818	505	145	162	6	0	0	
1	В	106	Total	С	N	О	S	0	0	0
1	Б	100	818	505	145	162	6	0	0	
1	С	106	Total	С	N	О	S	0	0	0
1		100	818	505	145	162	6	0	0	
1	D	106	Total	С	N	О	S	0	0	0
1	ע	100	818	505	145	162	6			

There are 48 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	34	ALA	ARG	engineered mutation	UNP P0ABE7
A	38	ALA	LEU	engineered mutation	UNP P0ABE7
A	41	TRP	GLN	engineered mutation	UNP P0ABE7
A	42	SER	LYS	engineered mutation	UNP P0ABE7
A	59	HIS	LYS	engineered mutation	UNP P0ABE7
A	66	TRP	ASP	engineered mutation	UNP P0ABE7
A	69	ILE	VAL	engineered mutation	UNP P0ABE7
A	73	HIS	ASP	engineered mutation	UNP P0ABE7
A	77	HIS	LYS	engineered mutation	UNP P0ABE7
A	96	CYS	THR	engineered mutation	UNP P0ABE7
A	98	CYS	ARG	engineered mutation	UNP P0ABE7
A	101	CYS	TYR	engineered mutation	UNP P0ABE7
В	34	ALA	ARG	engineered mutation	UNP P0ABE7
В	38	ALA	LEU	engineered mutation	UNP P0ABE7
В	41	TRP	GLN	engineered mutation	UNP P0ABE7
В	42	SER	LYS	engineered mutation	UNP P0ABE7
В	59	HIS	LYS	engineered mutation	UNP P0ABE7
В	66	TRP	ASP	engineered mutation	UNP P0ABE7
В	69	ILE	VAL	engineered mutation	UNP P0ABE7
В	73	HIS	ASP	engineered mutation	UNP P0ABE7
В	77	HIS	LYS	engineered mutation	UNP P0ABE7

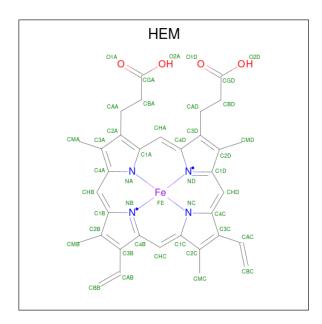


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Chain	Residue	Modelled	Actual	Comment	Reference
В	96	CYS	THR	engineered mutation	UNP P0ABE7
В	98	CYS	ARG	engineered mutation	UNP P0ABE7
В	101	CYS	TYR	engineered mutation	UNP P0ABE7
С	34	ALA	ARG	engineered mutation	UNP P0ABE7
С	38	ALA	LEU	engineered mutation	UNP P0ABE7
С	41	TRP	GLN	engineered mutation	UNP P0ABE7
С	42	SER	LYS	engineered mutation	UNP P0ABE7
С	59	HIS	LYS	engineered mutation	UNP P0ABE7
С	66	TRP	ASP	engineered mutation	UNP P0ABE7
С	69	ILE	VAL	engineered mutation	UNP P0ABE7
С	73	HIS	ASP	engineered mutation	UNP P0ABE7
С	77	HIS	LYS	engineered mutation	UNP P0ABE7
С	96	CYS	THR	engineered mutation	UNP P0ABE7
С	98	CYS	ARG	engineered mutation	UNP P0ABE7
С	101	CYS	TYR	engineered mutation	UNP P0ABE7
D	34	ALA	ARG	engineered mutation	UNP P0ABE7
D	38	ALA	LEU	engineered mutation	UNP P0ABE7
D	41	TRP	GLN	engineered mutation	UNP P0ABE7
D	42	SER	LYS	engineered mutation	UNP P0ABE7
D	59	HIS	LYS	engineered mutation	UNP P0ABE7
D	66	TRP	ASP	engineered mutation	UNP P0ABE7
D	69	ILE	VAL	engineered mutation	UNP P0ABE7
D	73	HIS	ASP	engineered mutation	UNP P0ABE7
D	77	HIS	LYS	engineered mutation	UNP P0ABE7
D	96	CYS	THR	engineered mutation	UNP P0ABE7
D	98	CYS	ARG	engineered mutation	UNP P0ABE7
D	101	CYS	TYR	engineered mutation	UNP P0ABE7

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





Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
2	Λ	1	Total	С	Fe	N	О	0	0
2	A	1	43	34	1	4	4	0	U
2	В	1	Total	С	Fe	N	О	0	0
2	Б	1	43	34	1	4	4	0	U
2	C	1	Total	С	Fe	N	О	0	0
2		1	43	34	1	4	4	0	U
2	D	1	Total	С	Fe	N	О	0	0
	ש	1	43	34	1	4	4		U

• Molecule 3 is water.

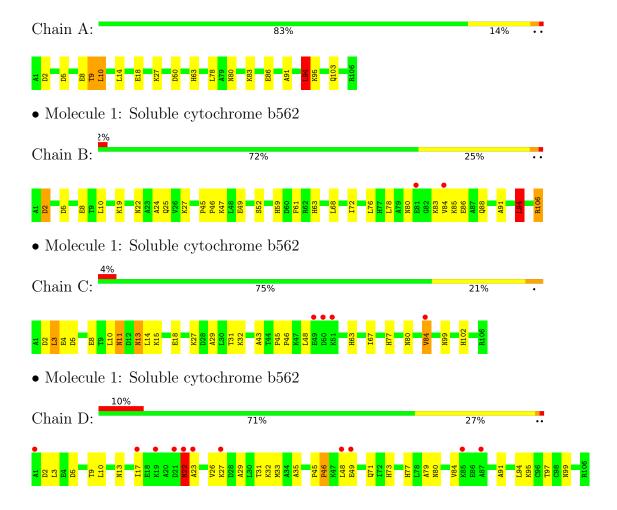
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	A	87	Total O 87 87	0	0
3	В	66	Total O 66 66	0	0
3	С	49	Total O 49 49	0	0
3	D	34	Total O 34 34	0	0



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: Soluble cytochrome b562





4 Data and refinement statistics (i)

Property	Value	Source
Space group	I 41	Depositor
Cell constants	69.15Å 69.15Å 186.94Å	Donositon
a, b, c, α , β , γ	90.00° 90.00° 90.00°	Depositor
Resolution (Å)	50.00 - 2.05	Depositor
rtesolution (A)	46.29 - 2.05	EDS
% Data completeness	99.3 (50.00-2.05)	Depositor
(in resolution range)	99.3 (46.29-2.05)	EDS
R_{merge}	0.08	Depositor
R_{sym}	0.08	Depositor
$< I/\sigma(I) > 1$	3.16 (at 2.05Å)	Xtriage
Refinement program	CNS	Depositor
R, R_{free}	0.229 , 0.277	Depositor
It, It free	0.219 , 0.267	DCC
R_{free} test set	1885 reflections (6.92%)	wwPDB-VP
Wilson B-factor (Å ²)	28.2	Xtriage
Anisotropy	0.459	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$, $B_{sol}(Å^2)$	0.37, 62.9	EDS
L-test for twinning ²	$< L > = 0.47, < L^2> = 0.30$	Xtriage
Estimated twinning fraction	0.054 for -h,k,-l	Xtriage
F_o, F_c correlation	0.95	EDS
Total number of atoms	3680	wwPDB-VP
Average B, all atoms (Å ²)	37.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The analyses of the Patterson function reveals a significant off-origin peak that is 38.11 % of the origin peak, indicating pseudo-translational symmetry. The chance of finding a peak of this or larger height randomly in a structure without pseudo-translational symmetry is equal to 3.8733e-04. The detected translational NCS is most likely also responsible for the elevated intensity ratio.

²Theoretical values of <|L|>, $<L^2>$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.



¹Intensities estimated from amplitudes.

5 Model quality (i)

5.1 Standard geometry (i)

Bond lengths and bond angles in the following residue types are not validated in this section: HEM

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		
WIOI		RMSZ	# Z > 5	RMSZ	# Z > 5	
1	A	0.52	0/833	0.69	2/1125~(0.2%)	
1	В	0.52	0/833	0.71	2/1125~(0.2%)	
1	С	0.47	0/833	0.59	0/1125	
1	D	0.42	0/833	0.60	0/1125	
All	All	0.48	0/3332	0.65	4/4500 (0.1%)	

There are no bond length outliers.

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$Observed(^o)$	$\operatorname{Ideal}({}^{o})$
1	A	94	LEU	CA-CB-CG	7.96	133.62	115.30
1	В	94	LEU	CA-CB-CG	7.67	132.93	115.30
1	В	106	ARG	NE-CZ-NH1	-5.03	117.78	120.30
1	A	10	LEU	CA-CB-CG	5.03	126.86	115.30

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	818	0	789	15	0
1	В	818	0	789	25	0
1	С	818	0	789	24	0



Continued	trom	mmoninonic	maaa
COHABABACA		DIEUIUU	DUIUE
0 0 1000100000			

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	D	818	0	789	30	0
2	A	43	0	30	0	0
2	В	43	0	30	1	0
2	С	43	0	30	2	0
2	D	43	0	30	4	0
3	A	87	0	0	4	0
3	В	66	0	0	3	0
3	С	49	0	0	4	0
3	D	34	0	0	4	0
All	All	3680	0	3276	95	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 14.

The worst 5 of 95 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	$\begin{array}{c} {\rm Interatomic} \\ {\rm distance} \ ({\rm \AA}) \end{array}$	$\begin{array}{c} \text{Clash} \\ \text{overlap } (\text{\AA}) \end{array}$
1:D:22:ASN:H	1:D:22:ASN:ND2	1.63	0.96
1:D:22:ASN:HD22	1:D:22:ASN:N	1.63	0.94
1:B:91:ALA:HA	1:B:94:LEU:HD13	1.59	0.82
1:D:13:ASN:HD21	1:D:32:LYS:HD2	1.44	0.82
1:D:22:ASN:H	1:D:22:ASN:HD22	0.83	0.75

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	nalysed Favoured		Outliers	Perce	entiles
1	A	104/106 (98%)	103 (99%)	1 (1%)	0	100	100
1	В	104/106 (98%)	102 (98%)	2 (2%)	0	100	100



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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percer	ntiles
1	С	104/106~(98%)	102 (98%)	2 (2%)	0	100	100
1	D	104/106~(98%)	96 (92%)	7 (7%)	1 (1%)	15	6
All	All	$416/424 \ (98\%)$	403 (97%)	12 (3%)	1 (0%)	47	39

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	D	22	ASN

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	84/84 (100%)	81 (96%)	3 (4%)	35	28	
1	В	84/84 (100%)	79 (94%)	5 (6%)	19	11	
1	C	84/84 (100%)	79 (94%)	5 (6%)	19	11	
1	D	84/84 (100%)	80 (95%)	4 (5%)	25	18	
All	All	336/336 (100%)	319 (95%)	17 (5%)	24	15	

5 of 17 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	D	22	ASN
1	D	73	HIS
1	В	94	LEU
1	С	3	LEU
1	С	10	LEU

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	С	11	ASN



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Mol	Chain	Res	Type
1	D	103	GLN
1	С	77	HIS
1	D	73	HIS
1	С	73	HIS

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 monosaccharides in this entry.

5.6 Ligand geometry (i)

4 ligands are modelled in this entry.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with |Z| > 2 is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Chain Res Lin		Bond lengths				Bond angles		
IVIOI	туре	Chain	nes	Link	Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z >2	
2	HEM	D	150	1	41,50,50	1.69	9 (21%)	45,82,82	2.07	11 (24%)	
2	HEM	С	150	1	41,50,50	1.43	5 (12%)	45,82,82	1.82	11 (24%)	
2	HEM	В	150	1	41,50,50	1.43	10 (24%)	45,82,82	1.74	10 (22%)	
2	HEM	A	150	1	41,50,50	1.65	10 (24%)	45,82,82	2.11	11 (24%)	

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	D	150	1	-	6/12/54/54	-
2	HEM	С	150	1	-	8/12/54/54	-
2	HEM	В	150	1	-	3/12/54/54	-
2	HEM	A	150	1	-	3/12/54/54	-

The worst 5 of 34 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$\operatorname{Observed}(\text{\AA})$	$Ideal(\AA)$
2	D	150	HEM	C3C-CAC	-4.54	1.38	1.47
2	A	150	HEM	C2C-C1C	4.12	1.51	1.42
2	С	150	HEM	C2C-C1C	3.95	1.51	1.42
2	D	150	HEM	C1B-C2B	3.74	1.51	1.44
2	A	150	HEM	C1B-NB	-3.64	1.34	1.40

The worst 5 of 43 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	\mathbf{Z}	$Observed(^o)$	$\operatorname{Ideal}({}^{o})$
2	D	150	HEM	CBA-CAA-C2A	7.21	124.93	112.62
2	A	150	HEM	C4B-CHC-C1C	6.23	130.78	122.56
2	A	150	HEM	C2C-C3C-C4C	-5.75	102.88	106.90
2	D	150	HEM	C4B-CHC-C1C	5.02	129.18	122.56
2	A	150	HEM	C4B-C3B-C2B	-4.93	103.20	107.11

There are no chirality outliers.

5 of 20 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	С	150	HEM	C3A-C2A-CAA-CBA
2	D	150	HEM	C1A-C2A-CAA-CBA
2	D	150	HEM	C3A-C2A-CAA-CBA
2	D	150	HEM	C2A-CAA-CBA-CGA
2	A	150	HEM	C2B-C3B-CAB-CBB

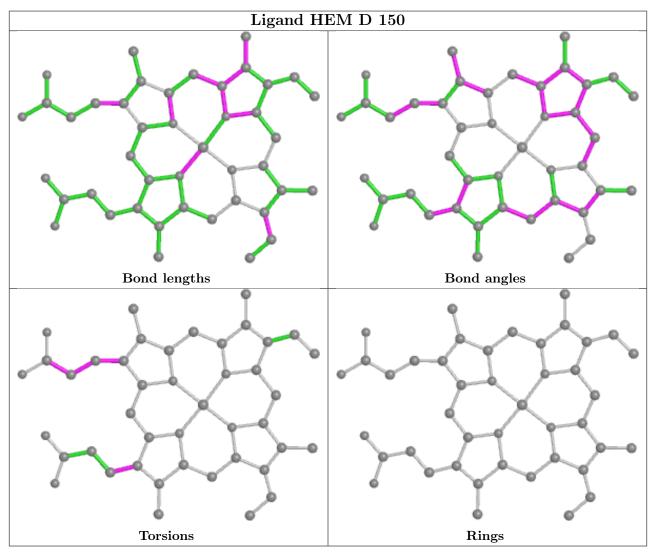
There are no ring outliers.

3 monomers are involved in 7 short contacts:

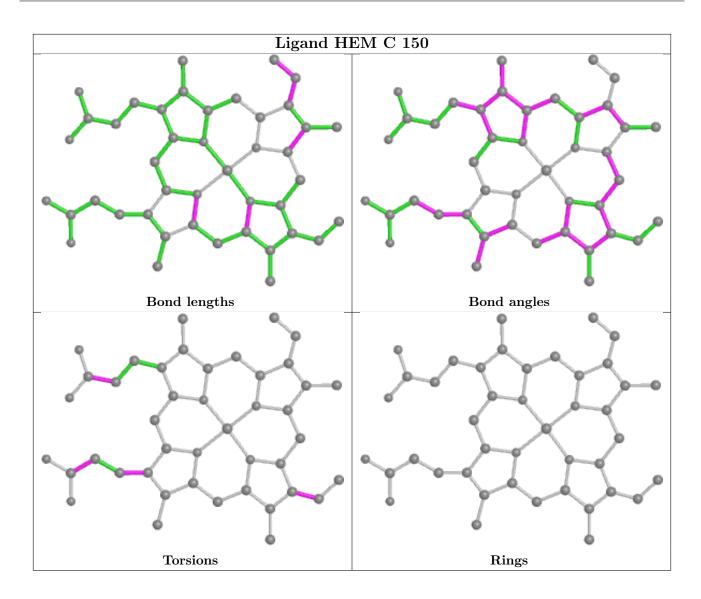
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	D	150	HEM	4	0
2	С	150	HEM	2	0
2	В	150	HEM	1	0



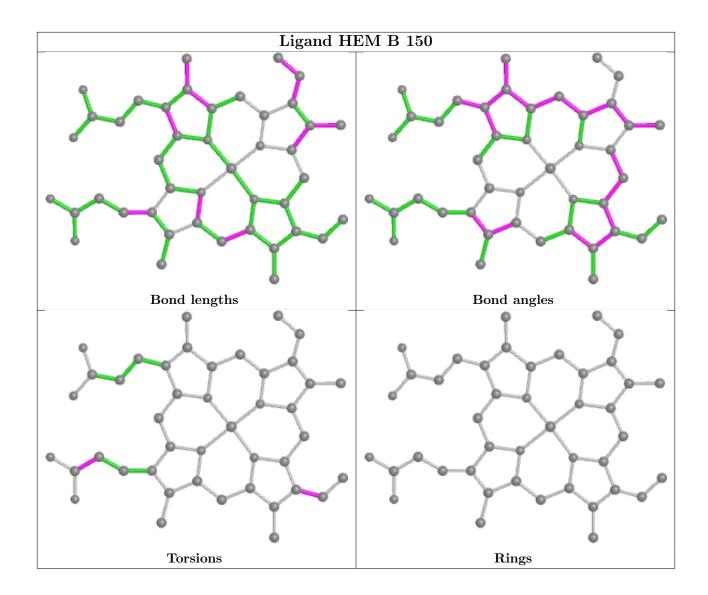
The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included.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



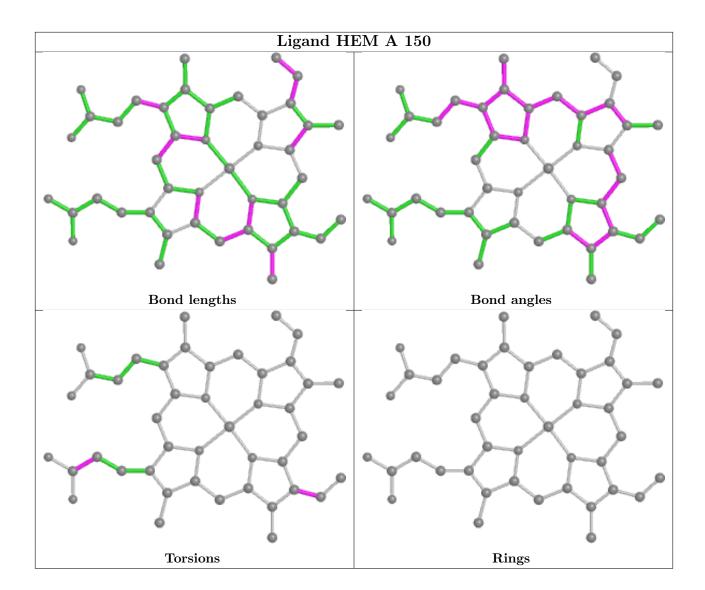












5.7 Other polymers (i)

There are no such residues in this entry.

5.8 Polymer linkage issues (i)

There are no chain breaks in this entry.



6 Fit of model and data (i)

6.1 Protein, DNA and RNA chains (i)

In the following table, the column labelled '#RSRZ>2' contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95^{th} percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled 'Q< 0.9' lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	$\langle { m RSRZ} \rangle$	$\#\mathrm{RSRZ}{>}2$	$OWAB(A^2)$	Q<0.9
1	A	106/106 (100%)	0.04	0 100 100	19, 29, 49, 57	0
1	В	106/106 (100%)	0.19	2 (1%) 66 71	18, 30, 55, 63	1 (0%)
1	С	106/106 (100%)	0.28	4 (3%) 40 44	22, 38, 56, 62	0
1	D	106/106 (100%)	0.63	11 (10%) 6 6	23, 45, 74, 85	0
All	All	424/424 (100%)	0.29	17 (4%) 38 41	18, 35, 59, 85	1 (0%)

The worst 5 of 17 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	D	27	LYS	5.5
1	D	21	ASP	3.6
1	D	22	ASN	3.2
1	D	48	LEU	2.7
1	D	19	LYS	2.5

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 monosaccharides in this entry.

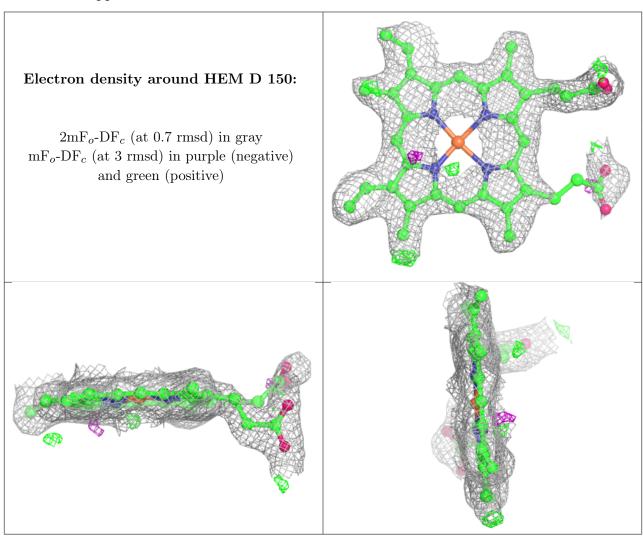
6.4 Ligands (i)

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

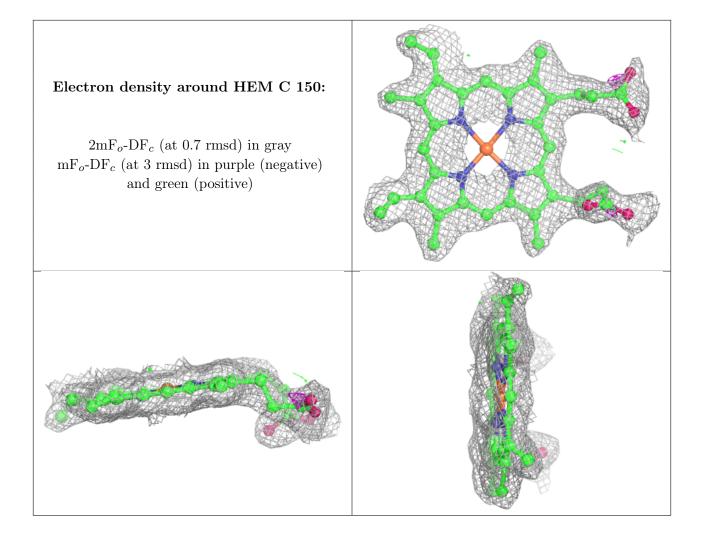


Mol	Type	Chain	Res	Atoms	RSCC	RSR	$\mathbf{B} ext{-}\mathbf{factors}(\mathbf{\mathring{A}}^2)$	Q<0.9
2	HEM	D	150	43/43	0.93	0.14	26,40,62,68	0
2	HEM	С	150	43/43	0.96	0.12	21,33,53,54	0
2	HEM	A	150	43/43	0.98	0.11	13,18,29,34	0
2	HEM	В	150	43/43	0.98	0.13	14,20,34,35	0

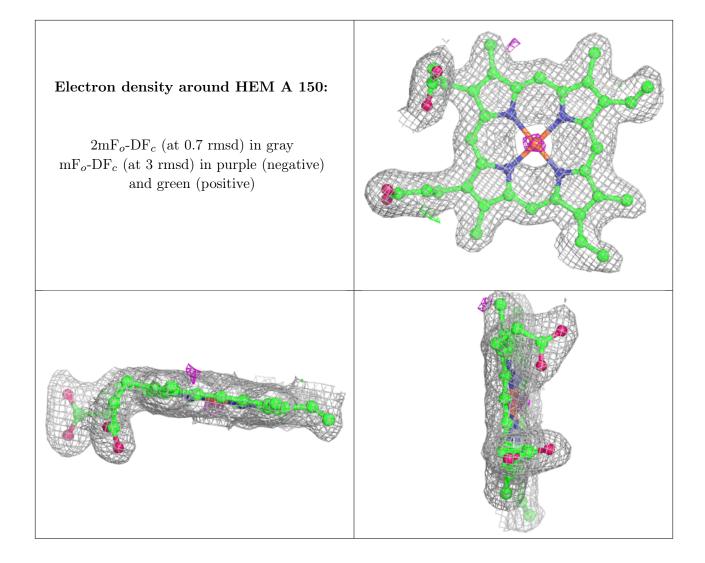
The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation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



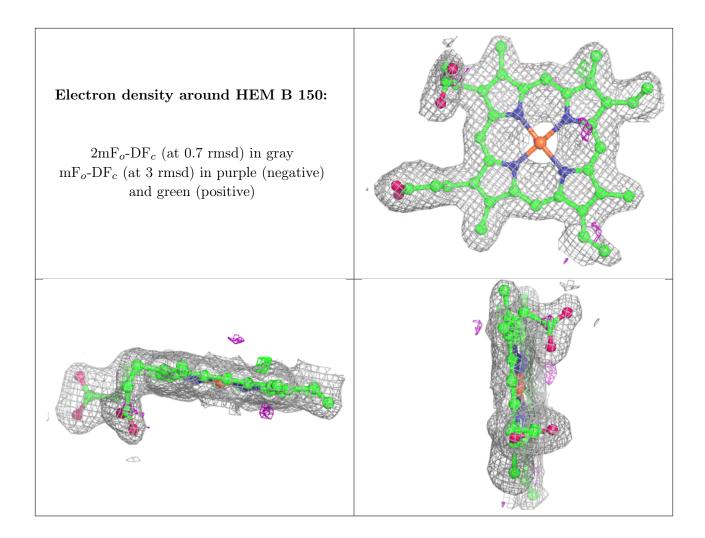












6.5 Other polymers (i)

There are no such residues in this entry.

