



wwPDB X-ray Structure Validation Summary Report ⓘ

May 22, 2020 – 02:04 pm BST

PDB ID : 3PI9
Title : Site-specific Glycosylation of Hemoglobin Utilizing Oxime Ligation Chemistry as a Viable Alternative to PEGylation
Authors : Bhatt, V.S.; Styslinger, T.J.; Zhang, N.; Wang, P.G.; Palmer, A.F.
Deposited on : 2010-11-05
Resolution : 2.90 Å(reported)

This is a wwPDB X-ray Structure Validation Summary Report for a publicly released PDB entry.

We welcome your comments at validation@mail.wwpdb.org

A user guide is available at

<https://www.wwpdb.org/validation/2017/XrayValidationReportHelp>

with specific help available everywhere you see the ⓘ symbol.

The 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

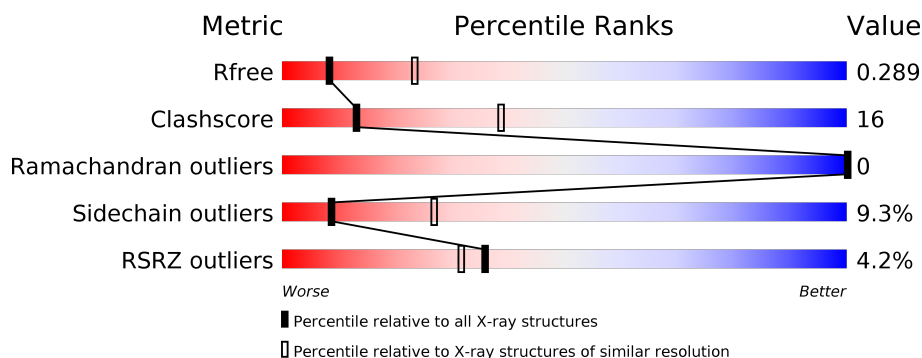
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.90 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	1957 (2.90-2.90)
Clashscore	141614	2172 (2.90-2.90)
Ramachandran outliers	138981	2115 (2.90-2.90)
Sidechain outliers	138945	2117 (2.90-2.90)
RSRZ outliers	127900	1906 (2.90-2.90)

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 ≥ 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	141	<div> <div style="width: 100%; height: 10px; background: linear-gradient(to right, red 1%, green 71%, yellow 25%, orange 4%, red 2%);"></div> <div style="display: flex; justify-content: space-between; width: 100%;"> % 71% 25% • • </div> </div>
1	C	141	<div> <div style="width: 100%; height: 10px; background: linear-gradient(to right, red 2%, green 70%, yellow 25%, orange 3%, red 2%);"></div> <div style="display: flex; justify-content: space-between; width: 100%;"> 2% 70% 25% • • </div> </div>
2	B	145	<div> <div style="width: 100%; height: 10px; background: linear-gradient(to right, red 2%, green 62%, yellow 30%, orange 8%, red 2%);"></div> <div style="display: flex; justify-content: space-between; width: 100%;"> 2% 62% 30% 8% </div> </div>
2	D	145	<div> <div style="width: 100%; height: 10px; background: linear-gradient(to right, red 12%, green 59%, yellow 30%, orange 10%, red 2%);"></div> <div style="display: flex; justify-content: space-between; width: 100%;"> 12% 59% 30% 10% • </div> </div>

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

ria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
4	CMO	B	148	-	-	X	-

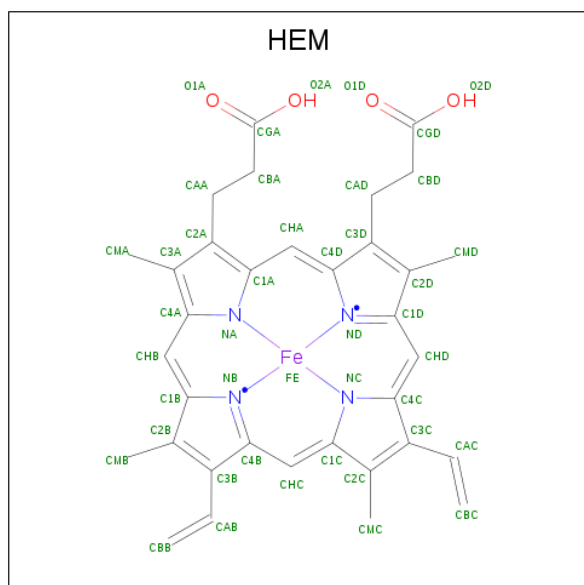
In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 2 is a protein called Hemoglobin subunit beta.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	141	Total 1066	C 683	N 186	O 196	S 1	10	0	0
1	C	141	Total 1066	C 683	N 186	O 196	S 1	1	0	0

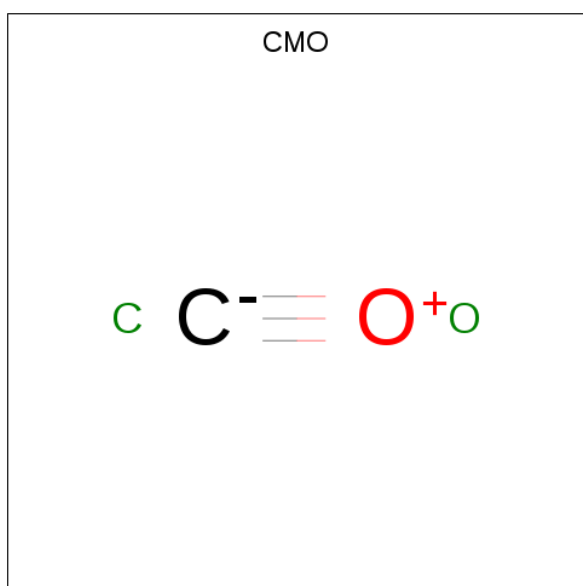
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	B	145	Total 1127	C 726	N 194	O 203	S 4	3	0	0
2	D	145	Total 1127	C 726	N 194	O 203	S 4	6	0	0

- Molecule 3 is PROTOPORPHYRIN IX CONTAINING FE (three-letter code: HEM) (formula: $C_{34}H_{32}FeN_4O_4$).



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

- Molecule 4 is CARBON MONOXIDE (three-letter code: CMO) (formula: CO).



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

- Molecule 5 is water.

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

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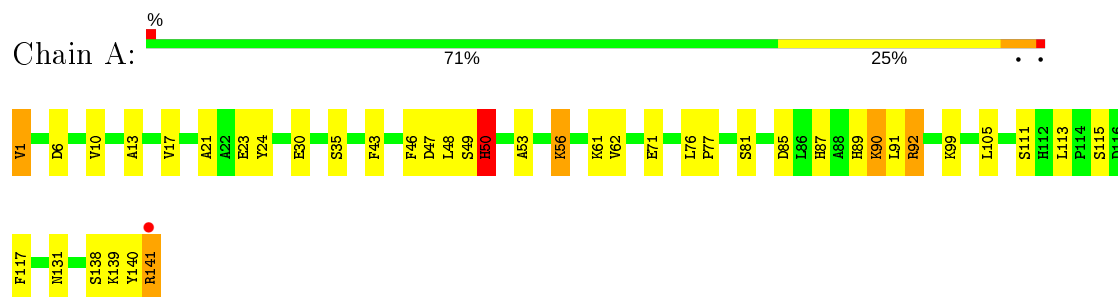
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Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	C	2	Total	O	0	0
			2	2		
5	D	4	Total	O	0	0
			4	4		

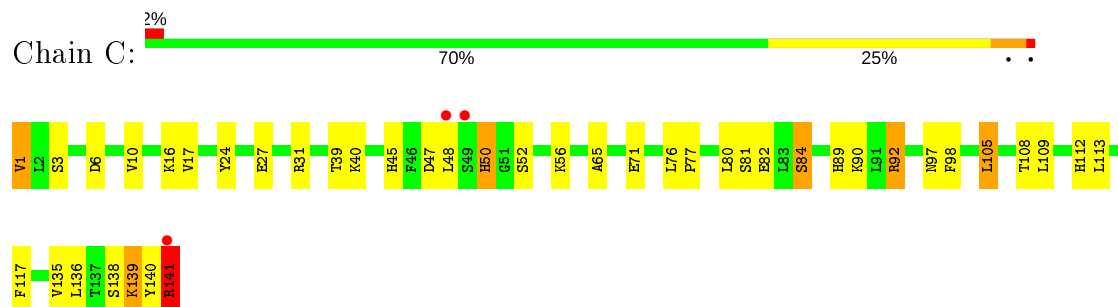
3 Residue-property plots [i](#)

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 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.

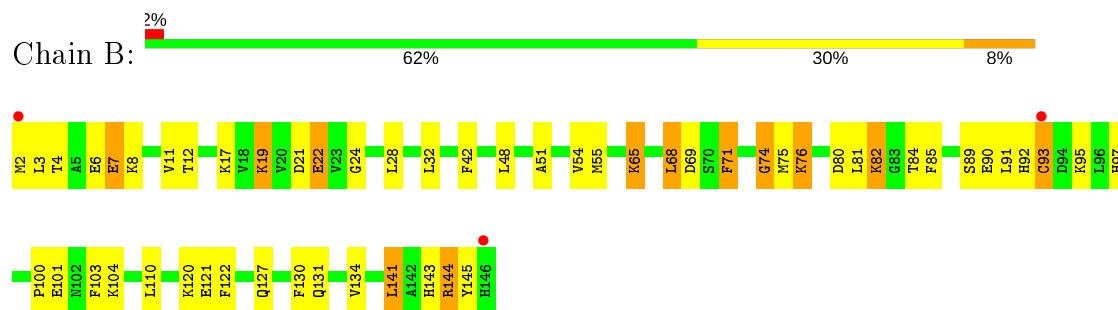
- Molecule 1: Hemoglobin subunit alpha



- Molecule 1: Hemoglobin subunit alpha

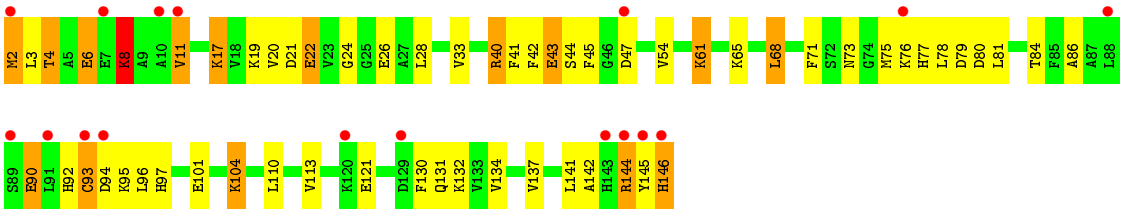


- Molecule 2: Hemoglobin subunit beta



- Molecule 2: Hemoglobin subunit beta





4 Data and refinement statistics

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α , β , γ	58.18Å 74.01Å 131.77Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	37.57 – 2.90 37.57 – 2.90	Depositor EDS
% Data completeness (in resolution range)	95.2 (37.57-2.90) 95.3 (37.57-2.90)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	12.09 (at 2.90Å)	Xtriage
Refinement program	REFMAC 5.5.0109	Depositor
R, R_{free}	0.261 , 0.293 0.255 , 0.289	Depositor DCC
R_{free} test set	614 reflections (4.87%)	wwPDB-VP
Wilson B-factor (Å ²)	41.5	Xtriage
Anisotropy	0.453	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.30 , 32.4	EDS
L-test for twinning ²	$\langle L \rangle = 0.50$, $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.89	EDS
Total number of atoms	4578	wwPDB-VP
Average B, all atoms (Å ²)	35.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.99% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

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

5 Model quality

5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: CMO, 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	
		RMSZ	# Z >5	RMSZ	# Z >5
1	A	1.46	15/1093 (1.4%)	1.01	5/1483 (0.3%)
1	C	1.49	14/1093 (1.3%)	1.01	7/1483 (0.5%)
2	B	1.47	11/1152 (1.0%)	1.10	4/1556 (0.3%)
2	D	1.86	20/1152 (1.7%)	1.25	12/1556 (0.8%)
All	All	1.58	60/4490 (1.3%)	1.10	28/6078 (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
1	C	0	1
All	All	0	2

The worst 5 of 60 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	D	6	GLU	CG-CD	28.30	1.94	1.51
1	C	50	HIS	CD2-NE2	23.37	1.91	1.42
2	B	22	GLU	CG-CD	22.20	1.85	1.51
1	A	92	ARG	CZ-NH1	17.81	1.56	1.33
2	D	146	HIS	C-OXT	15.56	1.52	1.23

The worst 5 of 28 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	D	144	ARG	NE-CZ-NH1	24.42	132.51	120.30
2	B	80	ASP	CB-CG-OD2	-13.93	105.77	118.30

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	D	144	ARG	NH1-CZ-NH2	-13.09	105.00	119.40
1	A	50	HIS	CA-CB-CG	11.11	132.49	113.60
2	D	40	ARG	NE-CZ-NH2	-9.92	115.34	120.30

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	50	HIS	Sidechain
1	C	50	HIS	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	1066	0	1069	32	0
1	C	1066	0	1069	30	0
2	B	1127	0	1129	34	0
2	D	1127	0	1129	41	0
3	A	43	0	30	9	0
3	B	43	0	30	7	0
3	C	43	0	30	7	0
3	D	43	0	30	7	0
4	A	2	0	0	0	0
4	B	2	0	0	5	0
4	C	2	0	0	0	0
4	D	2	0	0	0	0
5	A	4	0	0	0	0
5	B	2	0	0	0	0
5	C	2	0	0	0	0
5	D	4	0	0	3	0
All	All	4578	0	4516	147	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 16.

The worst 5 of 147 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)
2:B:76:LYS:CD	2:B:76:LYS:CE	1.75	1.55
2:D:61:LYS:NZ	2:D:61:LYS:CE	1.78	1.45
1:A:90:LYS:NZ	1:A:90:LYS:CE	1.81	1.43
1:A:140:TYR:O	1:A:141:ARG:OXT	1.69	1.11
2:D:11:VAL:HG13	2:D:75:MET:HE1	1.49	0.95

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	139/141 (99%)	133 (96%)	6 (4%)	0	100	100
1	C	139/141 (99%)	136 (98%)	3 (2%)	0	100	100
2	B	143/145 (99%)	137 (96%)	6 (4%)	0	100	100
2	D	143/145 (99%)	134 (94%)	9 (6%)	0	100	100
All	All	564/572 (99%)	540 (96%)	24 (4%)	0	100	100

There are no Ramachandran outliers to report.

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	112/112 (100%)	103 (92%)	9 (8%)	12	33
1	C	112/112 (100%)	105 (94%)	7 (6%)	18	46

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
2	B	118/118 (100%)	104 (88%)	14 (12%)	5	15
2	D	118/118 (100%)	105 (89%)	13 (11%)	6	19
All	All	460/460 (100%)	417 (91%)	43 (9%)	9	27

5 of 43 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	120	LYS
1	C	81	SER
2	D	95	LYS
2	B	141	LEU
2	B	143	HIS

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (5) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	131	ASN
2	B	108	ASN
1	C	89	HIS
1	C	97	ASN
2	D	146	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 carbohydrates in this entry.

5.6 Ligand geometry [i](#)

8 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	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# $ Z > 2$	Counts	RMSZ	# $ Z > 2$
4	CMO	C	143	-	0,1,1	0.00	-	-		
3	HEM	C	142	1	27,50,50	2.52	9 (33%)	17,82,82	2.14	6 (35%)
3	HEM	A	142	1	27,50,50	2.43	9 (33%)	17,82,82	3.14	9 (52%)
4	CMO	B	148	3	0,1,1	0.00	-	-		
3	HEM	D	147	2	27,50,50	2.42	9 (33%)	17,82,82	3.01	7 (41%)
3	HEM	B	147	2,4	27,50,50	2.64	9 (33%)	17,82,82	2.63	7 (41%)
4	CMO	A	143	-	0,1,1	0.00	-	-		
4	CMO	D	148	-	0,1,1	0.00	-	-		

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
3	HEM	A	142	1	-	3/6/54/54	-
3	HEM	D	147	2	-	2/6/54/54	-
3	HEM	B	147	2,4	-	2/6/54/54	-
3	HEM	C	142	1	-	4/6/54/54	-

The worst 5 of 36 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	B	147	HEM	C3B-C2B	-7.67	1.29	1.40
3	B	147	HEM	C3C-C2C	-7.65	1.29	1.40
3	C	142	HEM	C3C-C2C	-7.57	1.29	1.40
3	A	142	HEM	C3C-C2C	-7.36	1.30	1.40
3	D	147	HEM	C3C-C2C	-6.94	1.30	1.40

The worst 5 of 29 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
3	D	147	HEM	CAA-CBA-CGA	-7.73	99.71	112.67

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
3	A	142	HEM	CAA-CBA-CGA	-6.79	101.28	112.67
3	B	147	HEM	CBD-CAD-C3D	-6.76	100.03	112.48
3	A	142	HEM	CBA-CAA-C2A	6.55	124.56	112.49
3	D	147	HEM	C1D-C2D-C3D	-6.47	102.50	107.00

There are no chirality outliers.

5 of 11 torsion outliers are listed below:

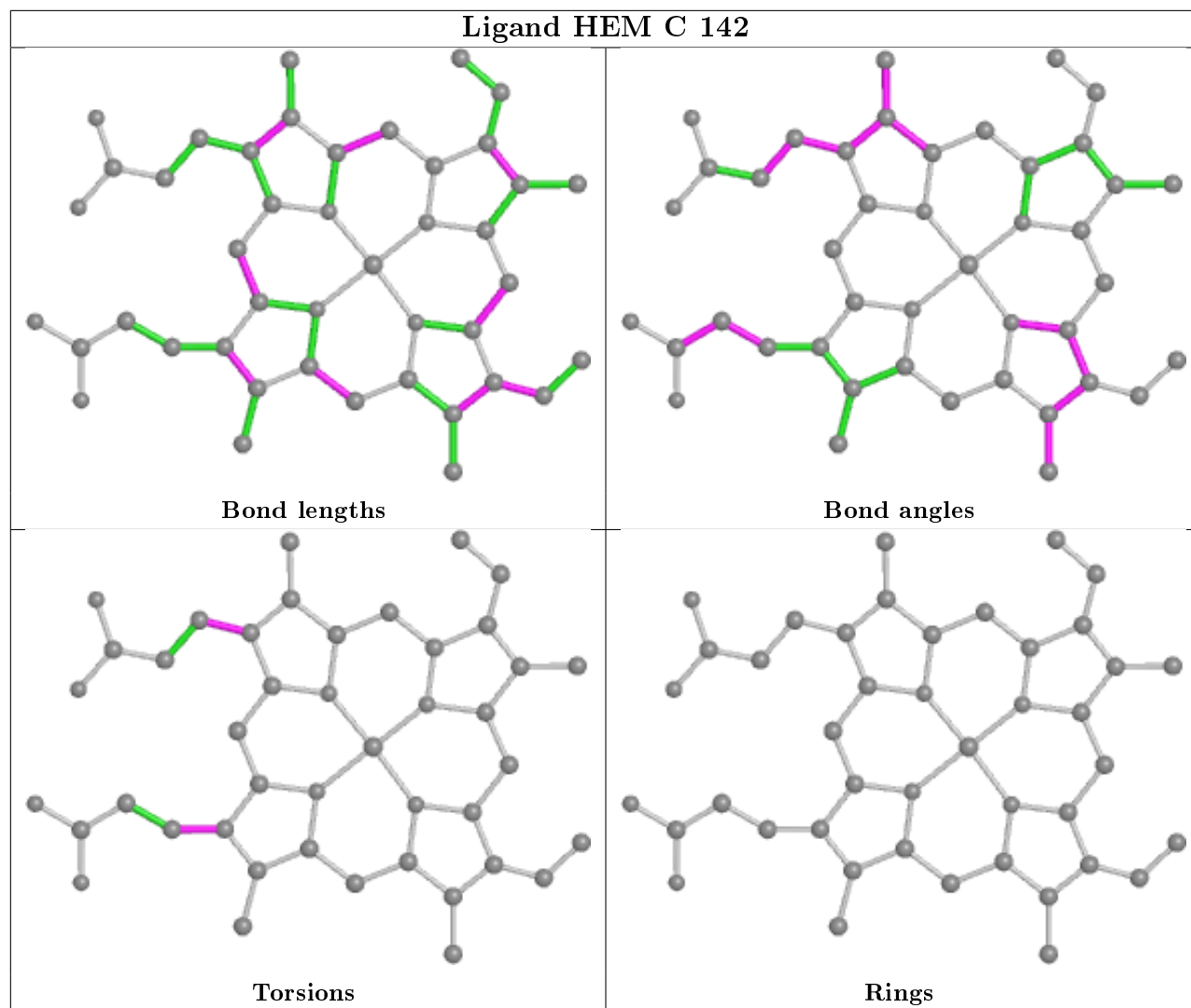
Mol	Chain	Res	Type	Atoms
3	C	142	HEM	C1A-C2A-CAA-CBA
3	C	142	HEM	C3A-C2A-CAA-CBA
3	C	142	HEM	C2D-C3D-CAD-CBD
3	C	142	HEM	C4D-C3D-CAD-CBD
3	A	142	HEM	C1A-C2A-CAA-CBA

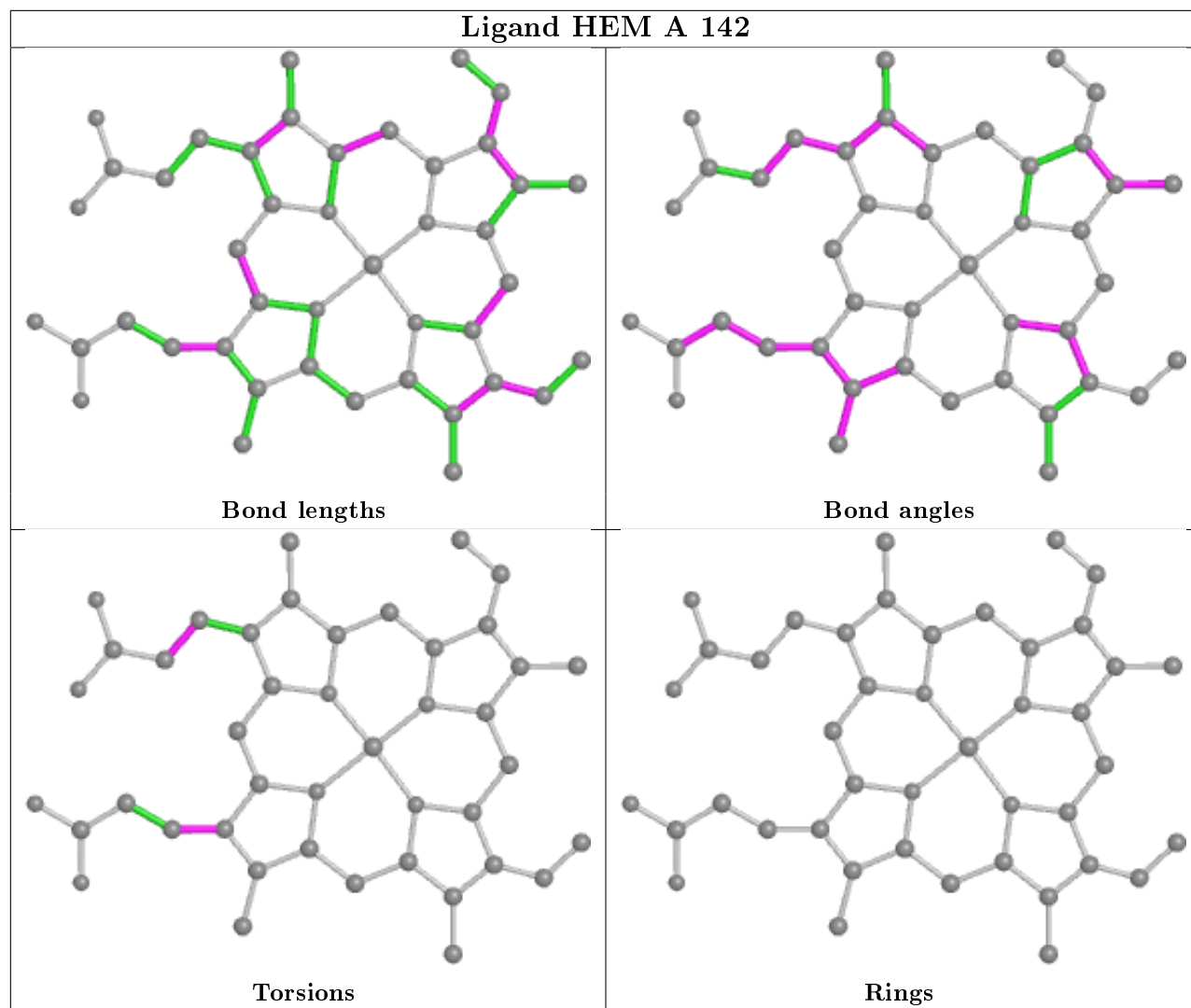
There are no ring outliers.

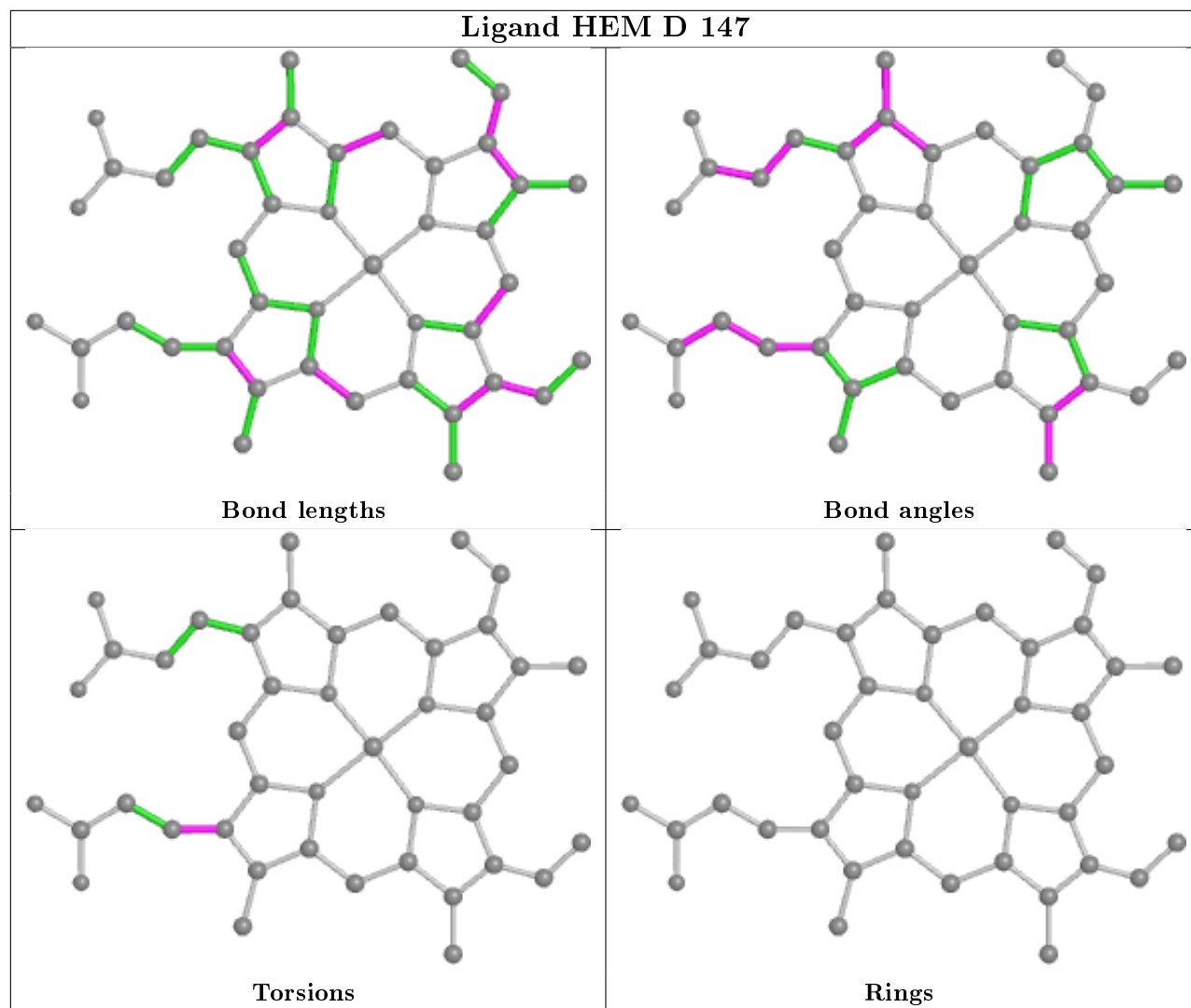
5 monomers are involved in 30 short contacts:

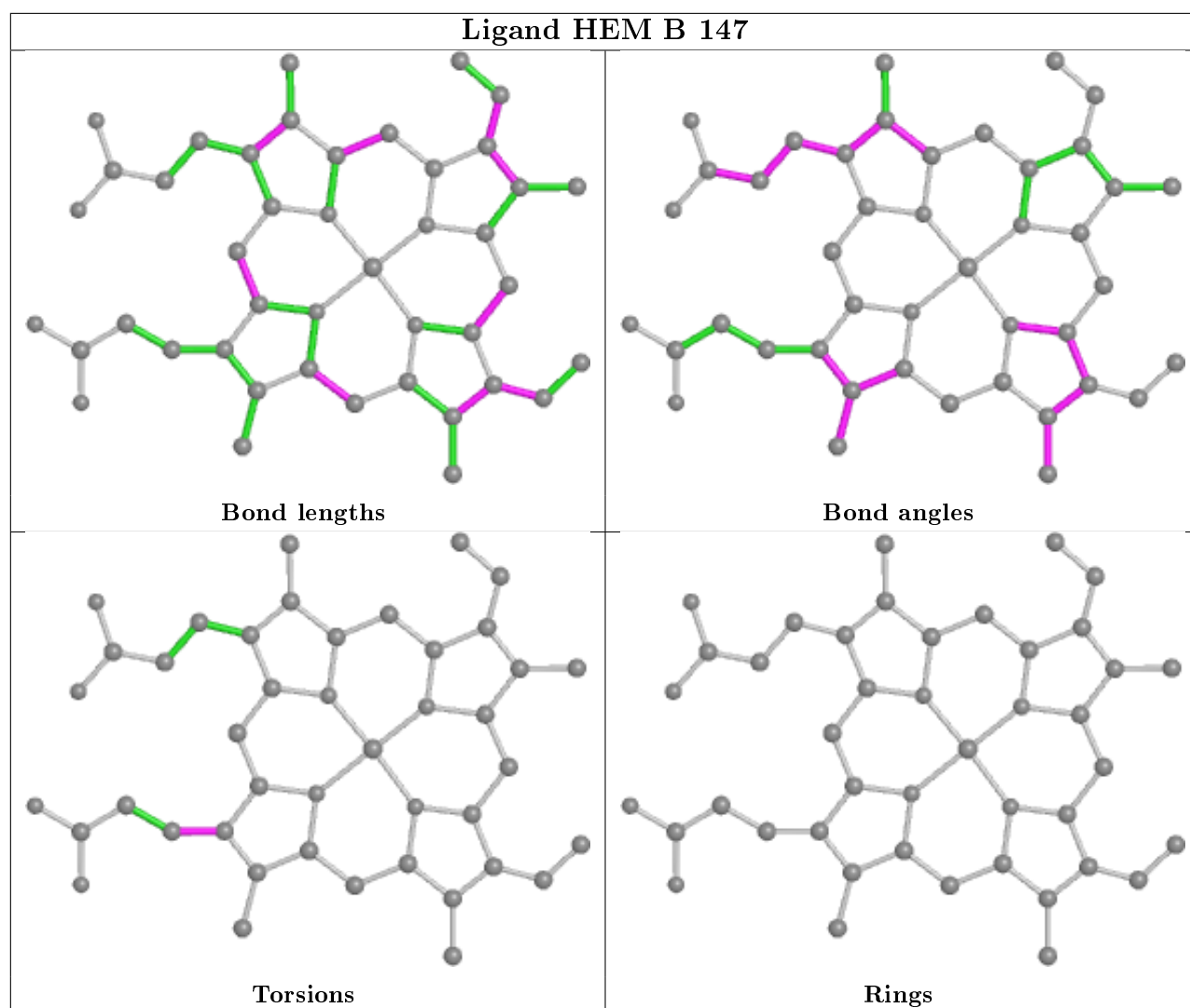
Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	C	142	HEM	7	0
3	A	142	HEM	9	0
4	B	148	CMO	5	0
3	D	147	HEM	7	0
3	B	147	HEM	7	0

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 ⓘ

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, 95th percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled ‘Q< 0.9’ lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2	OWAB(Å ²)	Q<0.9
1	A	141/141 (100%)	-0.10	1 (0%) 87 87	16, 28, 45, 57	2 (1%)
1	C	141/141 (100%)	-0.08	3 (2%) 63 61	20, 34, 46, 59	0
2	B	145/145 (100%)	0.11	3 (2%) 63 61	15, 31, 62, 79	1 (0%)
2	D	145/145 (100%)	0.58	17 (11%) 4 3	19, 43, 74, 94	2 (1%)
All	All	572/572 (100%)	0.13	24 (4%) 36 32	15, 33, 60, 94	5 (0%)

The worst 5 of 24 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	D	93	CYS	7.0
2	B	93	CYS	4.7
2	D	89	SER	4.3
2	B	2	MET	4.3
1	A	141	ARG	4.3

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 ⓘ

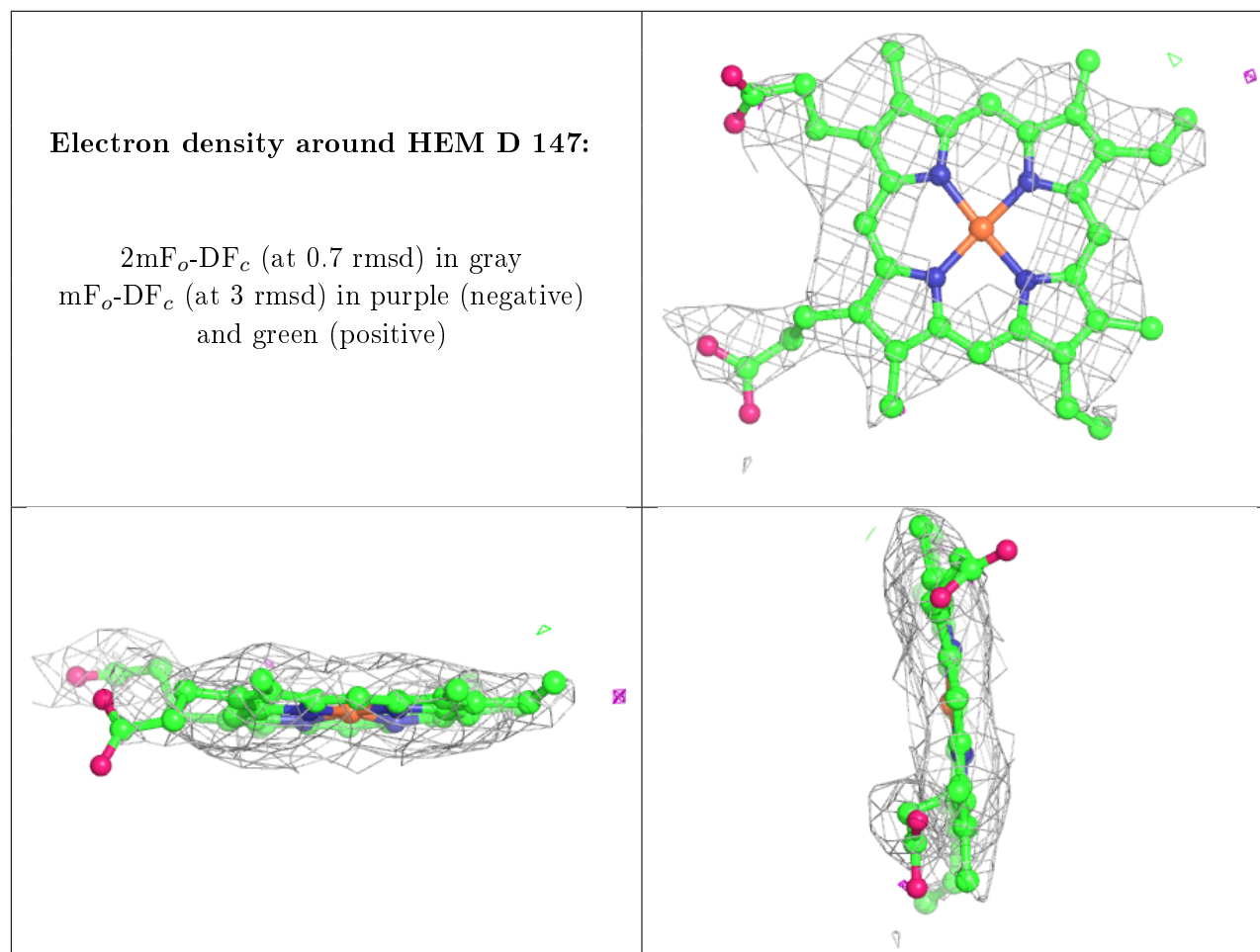
There are no carbohydrates in this entry.

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, 95th percentile and maximum values of B factors of atoms in the group. The column labelled ‘Q< 0.9’ lists the number of atoms with occupancy less than 0.9.

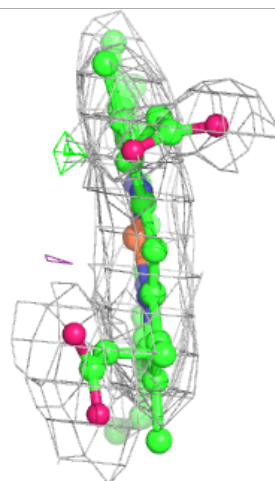
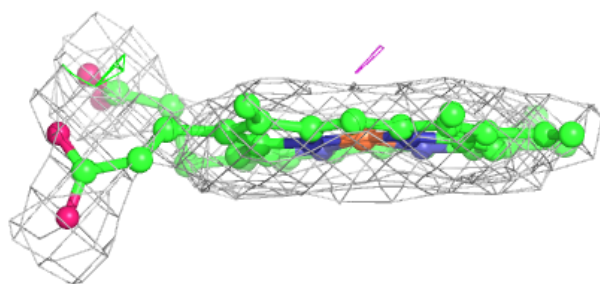
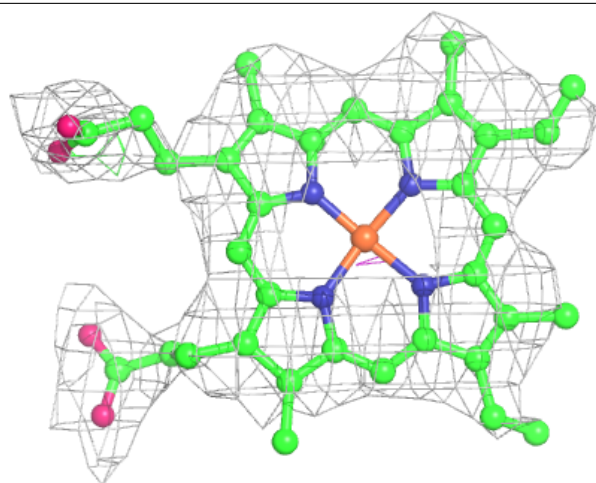
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(\AA^2)	Q<0.9
4	CMO	D	148	2/2	0.89	0.46	17,17,17,18	0
4	CMO	A	143	2/2	0.90	0.30	7,7,7,10	0
4	CMO	C	143	2/2	0.91	0.27	11,11,11,15	0
3	HEM	D	147	43/43	0.94	0.26	16,27,54,59	0
3	HEM	B	147	43/43	0.94	0.20	7,15,44,48	0
3	HEM	C	142	43/43	0.95	0.20	3,13,29,40	0
3	HEM	A	142	43/43	0.96	0.16	3,13,29,43	0
4	CMO	B	148	2/2	0.98	0.31	13,13,13,15	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.



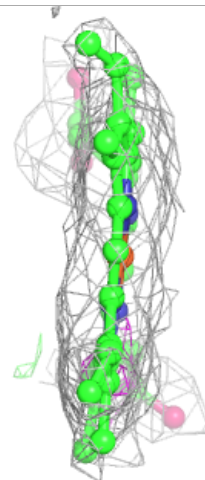
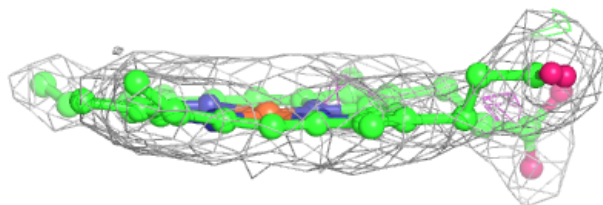
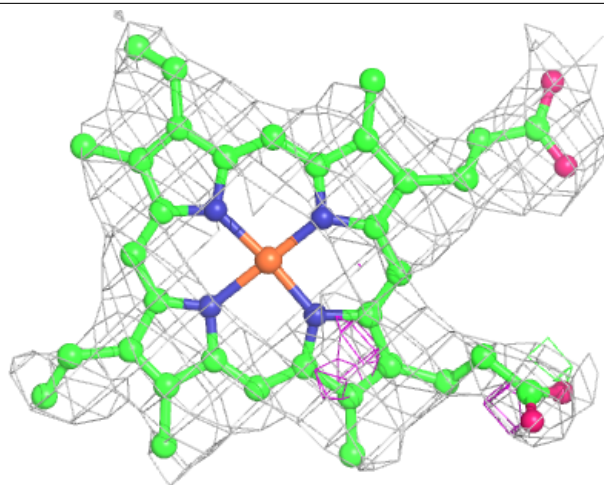
Electron density around HEM B 147:

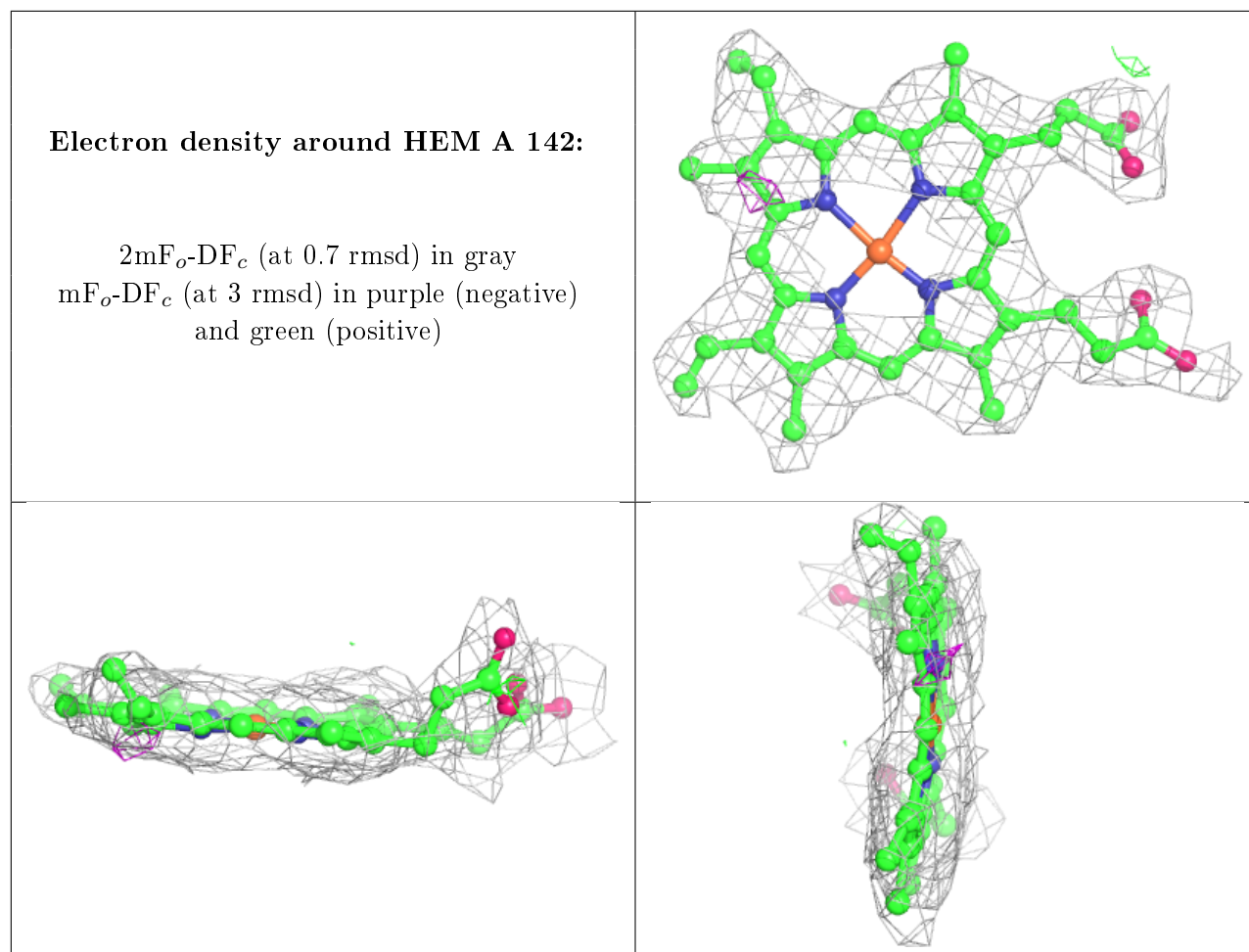
$2mF_o-DF_c$ (at 0.7 rmsd) in gray
 mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)



Electron density around HEM C 142:

$2mF_o-DF_c$ (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.