



wwPDB/EMDataBank EM Map/Model Validation Summary Report ⓘ

Nov 27, 2017 – 03:57 PM EST

PDB ID : 5M3L
EMDB ID: : EMD-3434
Title : Single-particle cryo-EM using alignment by classification (ABC): the structure of Lumbricus terrestris hemoglobin
Authors : Afanasyev, P.; Linnemayr-Seer, C.; Ravelli, R.B.G.; Matadeen, R.; De Carlo, S.; Alewijnse, B.; Portugal, R.V.; Pannu, N.S.; Schatz, M.; van Heel, M.
Deposited on : unknown
Resolution : 3.80 Å(reported)

This is a wwPDB/EMDataBank EM Map/Model Validation Summary Report for a publicly released PDB/EMDB entry.

We welcome your comments at validation@mail.wwpdb.org
A user guide is available at
<http://wwpdb.org/validation/2016/EMValidationReportHelp>
with specific help available everywhere you see the ⓘ symbol.

MolProbity : 4.02b-467
Mogul : 1.7.2 (RC1), CSD as538be (2017)
Percentile statistics : 20161228.v01 (using entries in the PDB archive December 28th 2016)
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et. al. (1996)
Validation Pipeline (wwPDB-VP) : rb-20030345

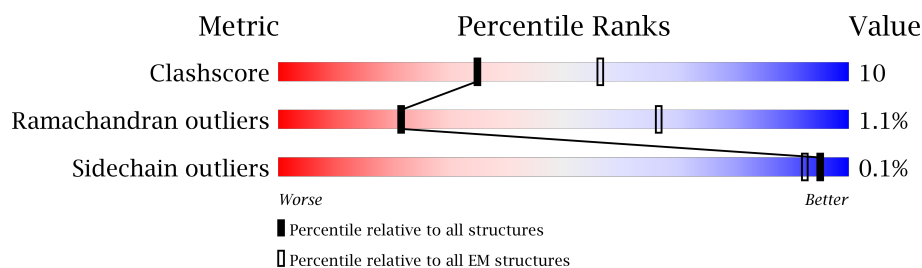
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 3.80 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)	EM structures (#Entries)
Clashscore	125131	1336
Ramachandran outliers	121729	1120
Sidechain outliers	121581	1026

The table below summarises the geometric issues observed across the polymeric chains. The red, orange, yellow and green segments on the bar indicate the fraction of residues that contain outliers for ≥ 3 , 2, 1 and 0 types of geometric quality criteria. 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	151	
1	E	151	
1	I	151	
2	B	145	
2	F	145	
2	J	145	
3	C	153	
3	G	153	
3	K	153	

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Mol	Chain	Length	Quality of chain
4	D	140	 85%15%
4	H	140	 81%17%•
4	L	140	 81%18%•
5	M	217	 82%17%•
6	N	220	 82%17%•
7	O	215	 81%19%

2 Entry composition

There are 8 unique types of molecules in this entry. The entry contains 19007 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, 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 Extracellular globin-4.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	147	Total	C	N	O	S	0	0
			1163	750	213	196	4		
1	E	147	Total	C	N	O	S	0	0
			1174	752	219	200	3		
1	I	147	Total	C	N	O	S	0	0
			1168	750	217	198	3		

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	78	LYS	ASP	conflict	UNP P13579
E	78	LYS	ASP	conflict	UNP P13579
I	78	LYS	ASP	conflict	UNP P13579

- Molecule 2 is a protein called Extracellular globin-2.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	145	Total	C	N	O	S	0	0
			1095	698	206	189	2		
2	F	145	Total	C	N	O	S	0	0
			1076	686	206	181	3		
2	J	144	Total	C	N	O	S	0	0
			1089	689	205	192	3		

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	66	ASP	GLU	conflict	UNP P02218
F	66	ASP	GLU	conflict	UNP P02218
J	66	ASP	GLU	conflict	UNP P02218

- Molecule 3 is a protein called Extracellular globin-3.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	C	149	Total	C	N	O	S	0	0
			1152	744	212	193	3		
3	G	149	Total	C	N	O	S	0	0
			1151	739	207	202	3		
3	K	149	Total	C	N	O	S	0	0
			1156	743	212	198	3		

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
C	49	GLU	ASP	conflict	UNP P11069
G	49	GLU	ASP	conflict	UNP P11069
K	49	GLU	ASP	conflict	UNP P11069

- Molecule 4 is a protein called Hemoglobin chain d1.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	D	140	Total	C	N	O	S	0	0
			1088	705	193	186	4		
4	H	140	Total	C	N	O	S	0	0
			1094	705	196	189	4		
4	L	140	Total	C	N	O	S	0	0
			1094	707	196	187	4		

- Molecule 5 is a protein called Hemoglobin linker chain L1.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	M	217	Total	C	N	O	S	0	0
			1679	1048	303	318	10		

- Molecule 6 is a protein called Extracellular hemoglobin linker L2 subunit.

Mol	Chain	Residues	Atoms					AltConf	Trace
6	N	220	Total	C	N	O	S	0	0
			1676	1043	314	309	10		

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
N	55	GLU	THR	conflict	UNP Q2I743

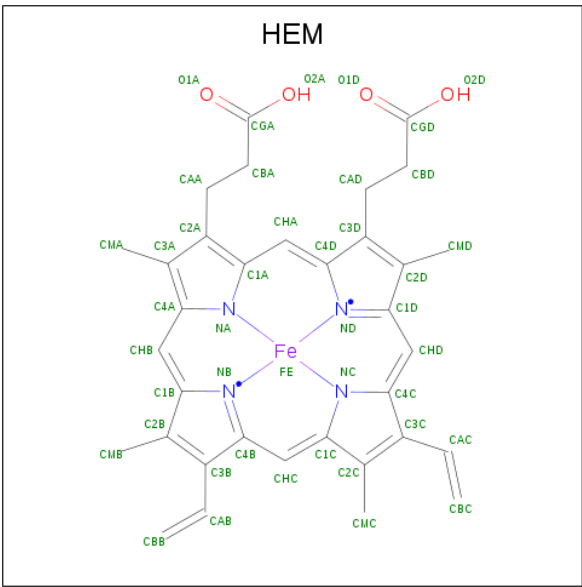
- Molecule 7 is a protein called Extracellular hemoglobin linker L3 subunit.

Mol	Chain	Residues	Atoms					AltConf	Trace
7	O	215	Total	C	N	O	S	0	0
			1636	1015	290	321	10		

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
O	17	ILE	LEU	conflict	UNP Q2I742
O	113	CYS	VAL	conflict	UNP Q2I742

- Molecule 8 is PROTOPORPHYRIN IX CONTAINING FE (three-letter code: HEM) (formula: $C_{34}H_{32}FeN_4O_4$).



Mol	Chain	Residues	Atoms					AltConf
8	A	1	Total	C	Fe	N	O	0
			43	34	1	4	4	
8	B	1	Total	C	Fe	N	O	0
			43	34	1	4	4	
8	C	1	Total	C	Fe	N	O	0
			43	34	1	4	4	
8	D	1	Total	C	Fe	N	O	0
			43	34	1	4	4	
8	E	1	Total	C	Fe	N	O	0
			43	34	1	4	4	
8	F	1	Total	C	Fe	N	O	0
			43	34	1	4	4	
8	G	1	Total	C	Fe	N	O	0
			43	34	1	4	4	

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Mol	Chain	Residues	Atoms					AltConf
8	H	1	Total 43	C 34	Fe 1	N 4	O 4	0
8	I	1	Total 43	C 34	Fe 1	N 4	O 4	0
8	J	1	Total 43	C 34	Fe 1	N 4	O 4	0
8	K	1	Total 43	C 34	Fe 1	N 4	O 4	0
8	L	1	Total 43	C 34	Fe 1	N 4	O 4	0

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. 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.

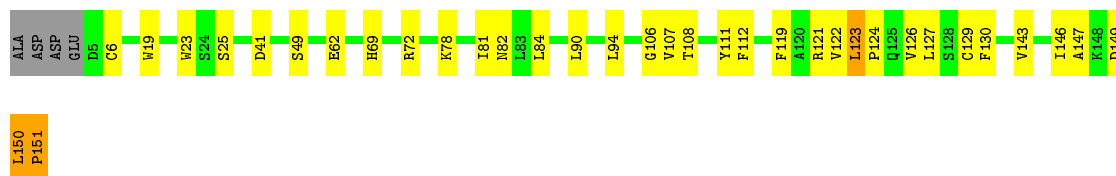
- Molecule 1: Extracellular globin-4

Chain A:  79% 17% . .




- Molecule 1: Extracellular globin-4

Chain E:  74% 21% . .




- Molecule 1: Extracellular globin-4

Chain I:  81% 14% . .




- Molecule 2: Extracellular globin-2

Chain B:  86% 14%

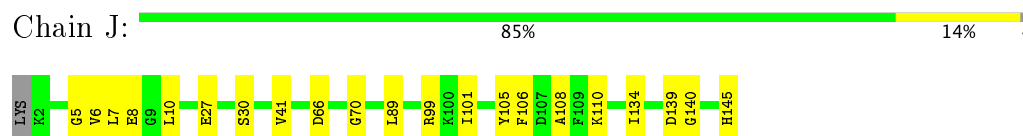


- Molecule 2: Extracellular globin-2

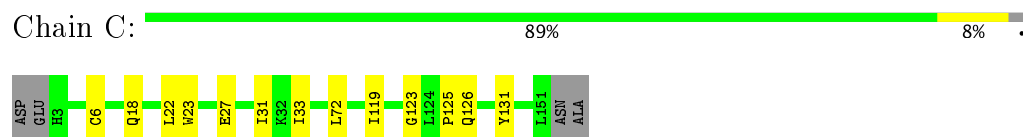
Chain F:  86% 14%



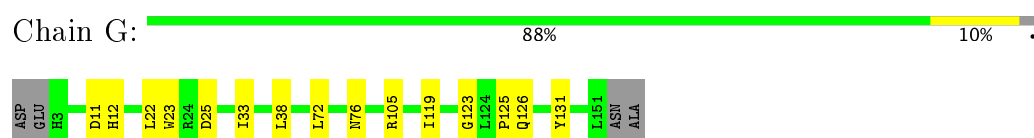
- Molecule 2: Extracellular globin-2



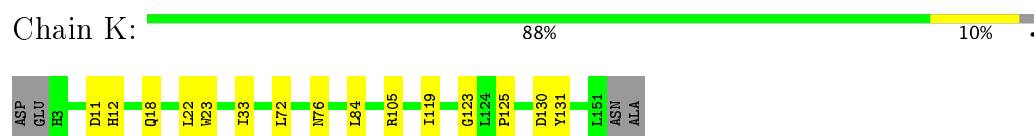
- Molecule 3: Extracellular globin-3



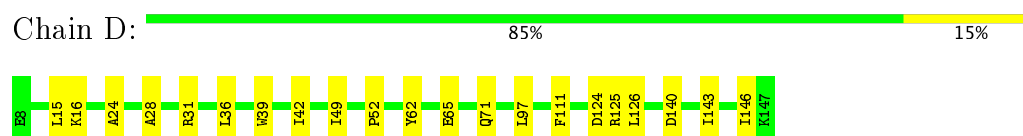
- Molecule 3: Extracellular globin-3



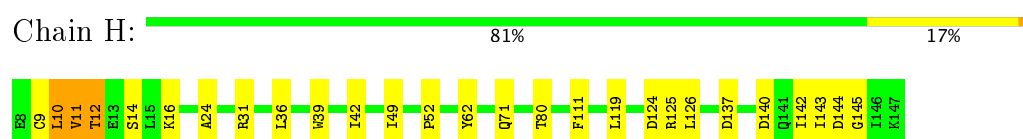
- Molecule 3: Extracellular globin-3



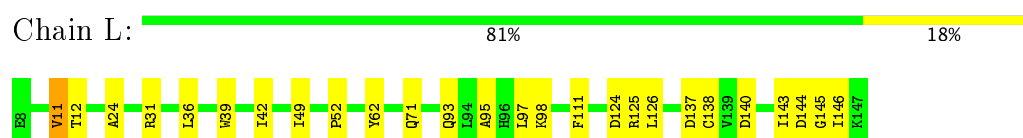
- Molecule 4: Hemoglobin chain d1



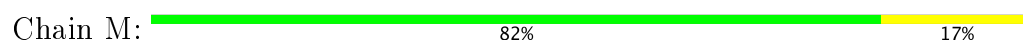
- Molecule 4: Hemoglobin chain d1

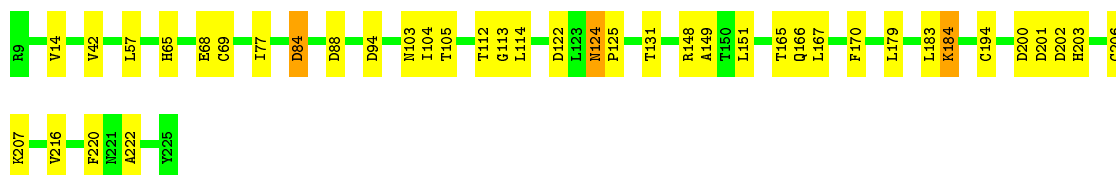


- Molecule 4: Hemoglobin chain d1



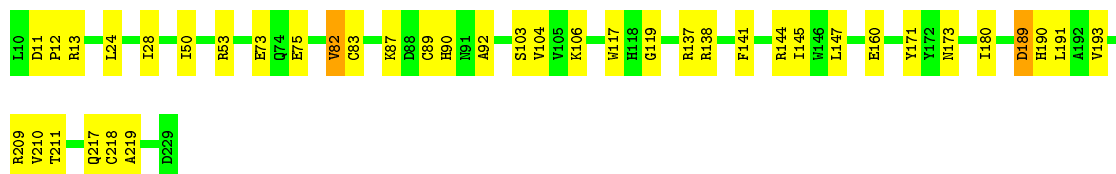
- Molecule 5: Hemoglobin linker chain L1





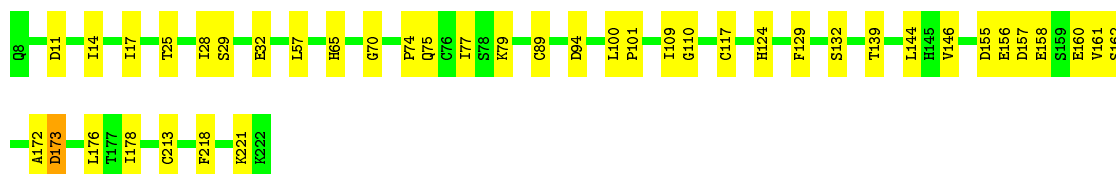
- Molecule 6: Extracellular hemoglobin linker L2 subunit

Chain N: 82% 17%



- Molecule 7: Extracellular hemoglobin linker L3 subunit

Chain O: 81% 19%



4 Experimental information

Property	Value	Source
Reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, D6	Depositor
Number of particles used	75000	Depositor
Resolution determination method	FSC 1/2 BIT CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING ONLY; Unsupervised MSA classification of amplitude spectra throughout the full data set ("Full data set CTF correction")	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/\text{\AA}^2$)	6	Depositor
Minimum defocus (nm)	1000	Depositor
Maximum defocus (nm)	1200	Depositor
Magnification	59000	Depositor
Image detector	FEI FALCON II (4k x 4k)	Depositor

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	
		RMSZ	$\# Z > 2$	RMSZ	$\# Z > 2$
1	A	0.50	0/1191	0.72	0/1614
1	E	0.55	1/1202 (0.1%)	0.98	3/1626 (0.2%)
1	I	0.47	0/1196	0.68	0/1622
2	B	0.45	0/1122	0.64	0/1520
2	F	0.44	0/1102	0.65	0/1495
2	J	0.42	0/1116	0.63	0/1512
3	C	0.43	0/1177	0.63	0/1592
3	G	0.43	0/1176	0.64	0/1593
3	K	0.41	0/1181	0.61	0/1597
4	D	0.44	0/1118	0.64	0/1518
4	H	0.45	0/1124	0.63	0/1526
4	L	0.44	0/1123	0.63	0/1522
5	M	0.67	0/1716	0.85	3/2328 (0.1%)
6	N	0.56	0/1707	0.77	0/2314
7	O	0.57	0/1671	0.77	1/2261 (0.0%)
All	All	0.50	1/18922 (0.0%)	0.71	7/25640 (0.0%)

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
7	O	0	1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	E	151	PRO	N-CD	7.66	1.58	1.47

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

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	E	150	LEU	C-N-CD	-21.19	73.97	120.60
5	M	84	ASP	CB-CG-OD1	6.40	124.06	118.30
5	M	148	ARG	NE-CZ-NH1	5.74	123.17	120.30
1	E	106	GLY	N-CA-C	5.44	126.69	113.10
1	E	151	PRO	CA-N-CD	-5.35	104.01	111.50

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
7	O	139	THR	Peptide

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	1163	0	1129	27	0
1	E	1174	0	1152	87	0
1	I	1168	0	1130	29	0
2	B	1095	0	1047	12	0
2	F	1076	0	1027	24	0
2	J	1089	0	1032	13	0
3	C	1152	0	1159	9	0
3	G	1151	0	1149	11	0
3	K	1156	0	1163	10	0
4	D	1088	0	1043	17	0
4	H	1094	0	1048	20	0
4	L	1094	0	1060	16	0
5	M	1679	0	1564	27	0
6	N	1676	0	1592	37	0
7	O	1636	0	1494	31	0
8	A	43	0	30	3	0
8	B	43	0	30	4	0
8	C	43	0	30	3	0
8	D	43	0	30	3	0
8	E	43	0	30	2	0
8	F	43	0	30	6	0
8	G	43	0	30	5	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
8	H	43	0	30	2	0
8	I	43	0	30	3	0
8	J	43	0	30	4	0
8	K	43	0	30	5	0
8	L	43	0	30	2	0
All	All	19007	0	18149	370	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 10.

The worst 5 of 370 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:E:107:VAL:CG2	1:E:151:PRO:HB3	1.15	1.63
1:E:150:LEU:HD12	1:E:151:PRO:CD	1.31	1.59
1:E:112:PHE:CE2	1:E:146:ILE:CD1	1.90	1.53
1:E:107:VAL:CG2	1:E:151:PRO:CB	1.93	1.46
1:E:107:VAL:HG21	1:E:151:PRO:CB	1.47	1.43

There are no symmetry-related clashes.

5.3 Torsion angles ⓘ

5.3.1 Protein backbone ⓘ

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

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	145/151 (96%)	131 (90%)	12 (8%)	2 (1%)	13	54
1	E	145/151 (96%)	131 (90%)	12 (8%)	2 (1%)	13	54
1	I	145/151 (96%)	130 (90%)	12 (8%)	3 (2%)	8	47
2	B	143/145 (99%)	133 (93%)	9 (6%)	1 (1%)	25	68
2	F	143/145 (99%)	132 (92%)	10 (7%)	1 (1%)	25	68

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
2	J	142/145 (98%)	132 (93%)	9 (6%)	1 (1%)	25	68
3	C	147/153 (96%)	137 (93%)	9 (6%)	1 (1%)	25	68
3	G	147/153 (96%)	139 (95%)	8 (5%)	0	100	100
3	K	147/153 (96%)	138 (94%)	9 (6%)	0	100	100
4	D	138/140 (99%)	127 (92%)	11 (8%)	0	100	100
4	H	138/140 (99%)	125 (91%)	10 (7%)	3 (2%)	8	46
4	L	138/140 (99%)	127 (92%)	10 (7%)	1 (1%)	25	68
5	M	215/217 (99%)	190 (88%)	19 (9%)	6 (3%)	6	42
6	N	218/220 (99%)	192 (88%)	22 (10%)	4 (2%)	10	50
7	O	213/215 (99%)	198 (93%)	13 (6%)	2 (1%)	20	62
All	All	2364/2419 (98%)	2162 (92%)	175 (7%)	27 (1%)	21	60

5 of 27 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	I	125	GLN
4	L	11	VAL
4	H	10	LEU
4	H	12	THR
5	M	184	LYS

5.3.2 Protein sidechains ⓘ

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

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	116/134 (87%)	116 (100%)	0	100	100
1	E	121/134 (90%)	121 (100%)	0	100	100
1	I	117/134 (87%)	117 (100%)	0	100	100
2	B	101/117 (86%)	101 (100%)	0	100	100
2	F	97/117 (83%)	97 (100%)	0	100	100
2	J	103/117 (88%)	103 (100%)	0	100	100

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
3	C	114/131 (87%)	114 (100%)	0	100	100
3	G	118/131 (90%)	118 (100%)	0	100	100
3	K	117/131 (89%)	117 (100%)	0	100	100
4	D	109/121 (90%)	109 (100%)	0	100	100
4	H	111/121 (92%)	111 (100%)	0	100	100
4	L	111/121 (92%)	111 (100%)	0	100	100
5	M	176/195 (90%)	176 (100%)	0	100	100
6	N	172/193 (89%)	172 (100%)	0	100	100
7	O	172/193 (89%)	171 (99%)	1 (1%)	89	95
All	All	1855/2090 (89%)	1854 (100%)	1 (0%)	95	98

All (1) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
7	O	173	ASP

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 24 such sidechains are listed below:

Mol	Chain	Res	Type
2	F	120	GLN
1	I	82	ASN
5	M	203	HIS
4	H	129	HIS
1	I	75	ASN

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

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

5.5 Carbohydrates ⓘ

There are no carbohydrates in this entry.

5.6 Ligand geometry

12 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
8	HEM	A	201	1	28,50,50	1.00	2 (7%)	17,82,82	1.56	3 (17%)
8	HEM	B	201	2	28,50,50	1.12	2 (7%)	17,82,82	1.54	3 (17%)
8	HEM	C	201	3	28,50,50	1.17	3 (10%)	17,82,82	1.35	1 (5%)
8	HEM	D	201	4	28,50,50	0.91	1 (3%)	17,82,82	1.82	4 (23%)
8	HEM	E	201	1	28,50,50	0.91	1 (3%)	17,82,82	1.23	1 (5%)
8	HEM	F	201	2	28,50,50	1.01	1 (3%)	17,82,82	1.83	6 (35%)
8	HEM	G	201	3	28,50,50	1.01	2 (7%)	17,82,82	1.45	3 (17%)
8	HEM	H	201	4	28,50,50	0.89	1 (3%)	17,82,82	1.45	2 (11%)
8	HEM	I	201	1	28,50,50	0.93	1 (3%)	17,82,82	1.39	2 (11%)
8	HEM	J	201	2	28,50,50	0.71	0	17,82,82	1.55	2 (11%)
8	HEM	K	201	3	28,50,50	0.96	1 (3%)	17,82,82	1.37	2 (11%)
8	HEM	L	201	4	28,50,50	0.92	1 (3%)	17,82,82	1.89	6 (35%)

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
8	HEM	A	201	1	-	0/6/54/54	0/0/8/8
8	HEM	B	201	2	-	0/6/54/54	0/0/8/8
8	HEM	C	201	3	-	0/6/54/54	0/0/8/8
8	HEM	D	201	4	-	0/6/54/54	0/0/8/8
8	HEM	E	201	1	-	0/6/54/54	0/0/8/8
8	HEM	F	201	2	-	0/6/54/54	0/0/8/8
8	HEM	G	201	3	-	0/6/54/54	0/0/8/8
8	HEM	H	201	4	-	0/6/54/54	0/0/8/8
8	HEM	I	201	1	-	0/6/54/54	0/0/8/8
8	HEM	J	201	2	-	0/6/54/54	0/0/8/8

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Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
8	HEM	K	201	3	-	0/6/54/54	0/0/8/8
8	HEM	L	201	4	-	0/6/54/54	0/0/8/8

The worst 5 of 16 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
8	C	201	HEM	C3B-C2B	-3.35	1.35	1.40
8	B	201	HEM	C3B-C2B	-3.06	1.36	1.40
8	G	201	HEM	C3B-C2B	-3.02	1.36	1.40
8	K	201	HEM	C3B-C2B	-2.66	1.36	1.40
8	D	201	HEM	C3B-C2B	-2.65	1.36	1.40

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

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
8	A	201	HEM	CAD-CBD-CGD	-4.01	105.80	112.66
8	C	201	HEM	CAA-CBA-CGA	-3.83	106.11	112.66
8	I	201	HEM	CAD-CBD-CGD	-3.83	106.12	112.66
8	L	201	HEM	CAD-CBD-CGD	-3.55	106.60	112.66
8	D	201	HEM	CAA-CBA-CGA	-3.33	106.98	112.66

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

12 monomers are involved in 42 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
8	A	201	HEM	3	0
8	B	201	HEM	4	0
8	C	201	HEM	3	0
8	D	201	HEM	3	0
8	E	201	HEM	2	0
8	F	201	HEM	6	0
8	G	201	HEM	5	0
8	H	201	HEM	2	0
8	I	201	HEM	3	0
8	J	201	HEM	4	0
8	K	201	HEM	5	0
8	L	201	HEM	2	0

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.