



# wwPDB X-ray Structure Validation Summary Report ⓘ

Feb 13, 2017 – 04:14 pm GMT

PDB ID : 3FS4  
Title : Crystal structure determination of Ostrich hemoglobin at 2.2 Angstrom resolution  
Authors : Sundaresan, S.S.; Ramesh, P.; Sivakumar, K.; Ponnuswamy, M.N.  
Deposited on : 2009-01-09  
Resolution : 2.22 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

<http://wwpdb.org/validation/2016/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 : 1.7.2 (RC1), CSD as538be (2017)  
Xtriage (Phenix) : 1.9-1692  
EDS : trunk28620  
Percentile statistics : 20161228.v01 (using entries in the PDB archive December 28th 2016)  
Refmac : 5.8.0135  
CCP4 : 6.5.0  
Ideal geometry (proteins) : Engh & Huber (2001)  
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)  
Validation Pipeline (wwPDB-VP) : recalc28949

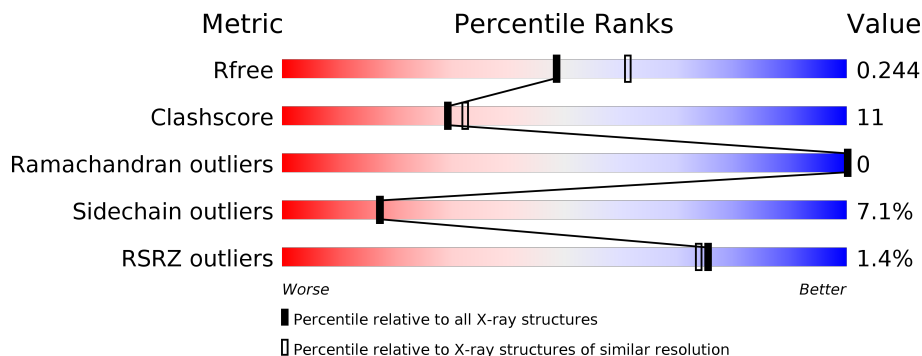
# 1 Overall quality at a glance ⓘ

The following experimental techniques were used to determine the structure:

*X-RAY DIFFRACTION*

The reported resolution of this entry is 2.22 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))
$R_{free}$	100719	4744 (2.24-2.20)
Clashscore	112137	5509 (2.24-2.20)
Ramachandran outliers	110173	5427 (2.24-2.20)
Sidechain outliers	110143	5428 (2.24-2.20)
RSRZ outliers	101464	4776 (2.24-2.20)

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. 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>3%</div> <div>82%</div> <div>16%</div> <div>.</div> </div>
1	C	141	<div> <div>%</div> <div>77%</div> <div>18%</div> <div>5%</div> </div>
2	B	146	<div> <div>%</div> <div>78%</div> <div>17%</div> <div>5%</div> </div>
2	D	146	<div> <div>%</div> <div>77%</div> <div>21%</div> <div>.</div> </div>

## 2 Entry composition [i](#)

There are 5 unique types of molecules in this entry. The entry contains 4935 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.

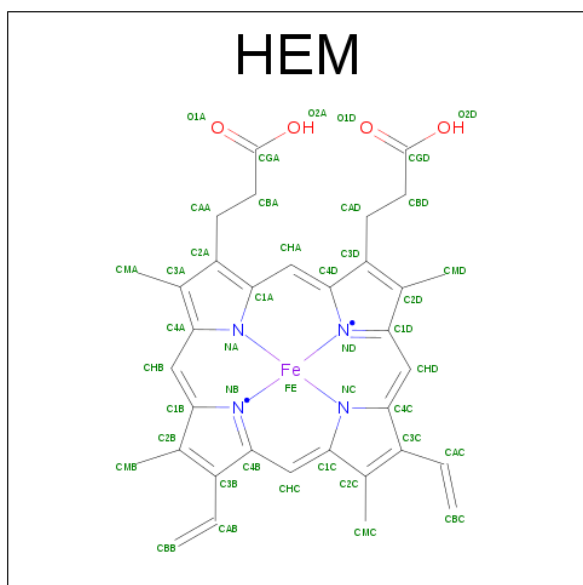
- Molecule 1 is a protein called Hemoglobin subunit alpha-A.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	141	Total	C	N	O	S	0	0	0
			1091	700	190	198	3			
1	C	141	Total	C	N	O	S	0	0	0
			1090	700	190	197	3			

- Molecule 2 is a protein called Hemoglobin subunit beta.

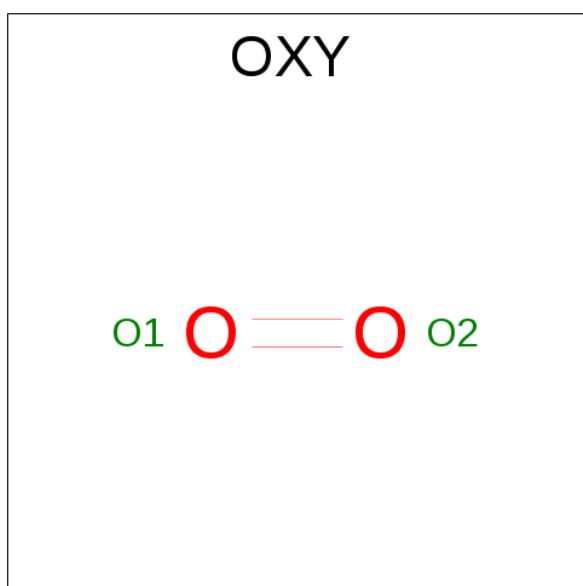
Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
2	B	146	Total	C	N	O	S	0	0	0
			1152	744	203	201	4			
2	D	146	Total	C	N	O	S	0	0	0
			1152	744	203	201	4			

- 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 43	C 34	Fe 1	N 4	O 4	0	0
3	B	1	Total 43	C 34	Fe 1	N 4	O 4	0	0
3	C	1	Total 43	C 34	Fe 1	N 4	O 4	0	0
3	D	1	Total 43	C 34	Fe 1	N 4	O 4	0	0

- Molecule 4 is OXYGEN MOLECULE (three-letter code: OXY) (formula: O<sub>2</sub>).



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

- Molecule 5 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	A	61	Total 61	O 61	0	0
5	B	83	Total 83	O 83	0	0

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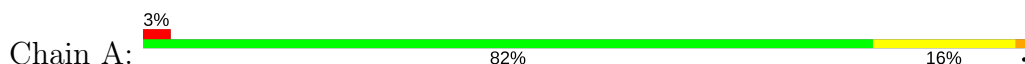
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Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
5	C	52	Total 52	O 52	0	0
5	D	74	Total 74	O 74	0	0

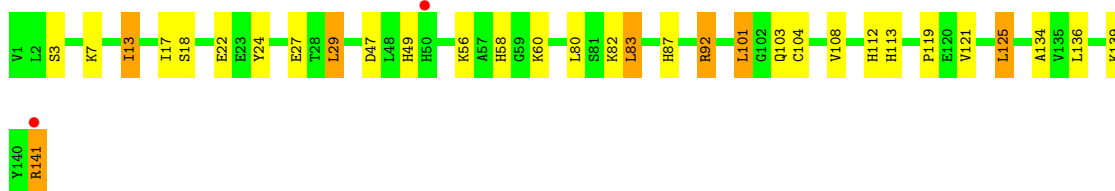
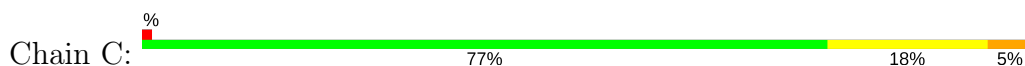
### 3 Residue-property plots [i](#)

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 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.

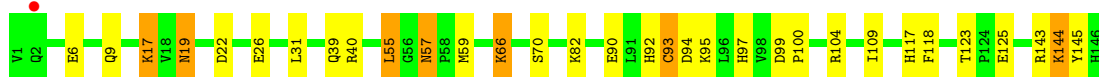
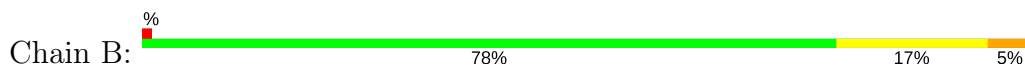
- Molecule 1: Hemoglobin subunit alpha-A



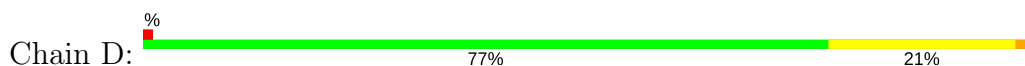
- Molecule 1: Hemoglobin subunit alpha-A



- 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, $\alpha$ , $\beta$ , $\gamma$	80.79Å 81.57Å 101.45Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	27.62 – 2.22 27.62 – 2.22	Depositor EDS
% Data completeness (in resolution range)	99.2 (27.62-2.22) 99.2 (27.62-2.22)	Depositor EDS
$R_{merge}$	(Not available)	Depositor
$R_{sym}$	0.06	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	1.97 (at 2.22Å)	Xtriage
Refinement program	REFMAC 5.2.0019	Depositor
R, $R_{free}$	0.216 , 0.256 0.224 , 0.244	Depositor DCC
$R_{free}$ test set	1695 reflections (5.35%)	DCC
Wilson B-factor (Å <sup>2</sup> )	31.0	Xtriage
Anisotropy	0.124	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.34 , 48.3	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.49$ , $\langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	0.000 for k,h,-l	Xtriage
$F_o, F_c$ correlation	0.95	EDS
Total number of atoms	4935	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	44.0	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 44.67 % 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 1.4753e-04. The detected translational NCS is most likely also responsible for the elevated intensity ratio.*

<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: HEM, OXY

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	0.73	0/1116	0.73	0/1510
1	C	0.71	0/1115	0.77	2/1510 (0.1%)
2	B	0.83	1/1180 (0.1%)	0.75	0/1600
2	D	0.77	0/1180	0.77	0/1600
All	All	0.76	1/4591 (0.0%)	0.75	2/6220 (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
1	A	0	1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	93	CYS	CB-SG	5.16	1.91	1.82

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	C	101	LEU	CA-CB-CG	5.95	128.98	115.30
1	C	141	ARG	N-CA-C	5.54	125.96	111.00

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	17	ILE	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	1091	0	1106	18	0
1	C	1090	0	1106	34	0
2	B	1152	0	1166	27	0
2	D	1152	0	1166	25	0
3	A	43	0	30	1	0
3	B	43	0	30	2	0
3	C	43	0	30	2	0
3	D	43	0	30	3	0
4	A	2	0	0	0	0
4	B	2	0	0	0	0
4	C	2	0	0	0	0
4	D	2	0	0	1	0
5	A	61	0	0	2	0
5	B	83	0	0	4	0
5	C	52	0	0	2	0
5	D	74	0	0	4	0
All	All	4935	0	4664	101	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 11.

The worst 5 of 101 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:26:GLU:OE1	2:B:117:HIS:HE1	1.55	0.88
1:A:141:ARG:HG2	5:C:412:HOH:O	1.80	0.82
2:B:26:GLU:OE1	2:B:117:HIS:CE1	2.33	0.81
1:C:134:ALA:HB1	1:C:141:ARG:HH12	1.51	0.76
2:B:93:CYS:SG	2:B:145:TYR:CE2	2.78	0.76

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 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%)	132 (95%)	7 (5%)	0	100	100
1	C	139/141 (99%)	137 (99%)	2 (1%)	0	100	100
2	B	144/146 (99%)	141 (98%)	3 (2%)	0	100	100
2	D	144/146 (99%)	141 (98%)	3 (2%)	0	100	100
All	All	566/574 (99%)	551 (97%)	15 (3%)	0	100	100

There are no Ramachandran outliers to report.

### 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 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	118/118 (100%)	111 (94%)	7 (6%)	23	25
1	C	118/118 (100%)	109 (92%)	9 (8%)	15	15
2	B	121/121 (100%)	111 (92%)	10 (8%)	13	12
2	D	121/121 (100%)	113 (93%)	8 (7%)	19	20
All	All	478/478 (100%)	444 (93%)	34 (7%)	17	17

5 of 34 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	104	ARG
1	C	29	LEU
2	D	90	GLU

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Mol	Chain	Res	Type
1	C	13	ILE
1	A	92	ARG

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 17 such sidechains are listed below:

Mol	Chain	Res	Type
2	B	117	HIS
2	B	146	HIS
2	D	39	GLN
2	B	57	ASN
2	D	57	ASN

### 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	$\# Z  > 2$
3	HEM	A	150	1,4	28,50,50	2.04	7 (25%)	17,82,82	2.29	9 (52%)

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z  > 2
4	OXY	A	151	3	1,1,1	0.39	0	0,0,0	0.00	-
3	HEM	B	150	2,4	28,50,50	2.34	11 (39%)	17,82,82	1.79	5 (29%)
4	OXY	B	151	3	1,1,1	0.32	0	0,0,0	0.00	-
3	HEM	C	150	1,4	28,50,50	2.26	7 (25%)	17,82,82	2.87	8 (47%)
4	OXY	C	151	3	1,1,1	0.26	0	0,0,0	0.00	-
3	HEM	D	150	2,4	28,50,50	2.27	7 (25%)	17,82,82	1.75	4 (23%)
4	OXY	D	151	3	1,1,1	0.36	0	0,0,0	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	150	1,4	-	0/6/54/54	0/0/8/8
4	OXY	A	151	3	-	0/0/0/0	0/0/0/0
3	HEM	B	150	2,4	-	0/6/54/54	0/0/8/8
4	OXY	B	151	3	-	0/0/0/0	0/0/0/0
3	HEM	C	150	1,4	-	0/6/54/54	0/0/8/8
4	OXY	C	151	3	-	0/0/0/0	0/0/0/0
3	HEM	D	150	2,4	-	0/6/54/54	0/0/8/8
4	OXY	D	151	3	-	0/0/0/0	0/0/0/0

The worst 5 of 32 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	D	150	HEM	C3B-C2B	-5.12	1.33	1.40
3	C	150	HEM	C3C-C2C	-4.96	1.33	1.40
3	C	150	HEM	C3B-C2B	-4.78	1.34	1.40
3	B	150	HEM	C3C-C2C	-4.38	1.34	1.40
3	A	150	HEM	C3B-C2B	-4.24	1.34	1.40

The worst 5 of 26 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
3	C	150	HEM	CAA-CBA-CGA	-6.92	100.84	112.66
3	A	150	HEM	CBD-CAD-C3D	-5.36	102.25	112.47
3	C	150	HEM	CBD-CAD-C3D	-5.22	102.50	112.47
3	D	150	HEM	CAD-CBD-CGD	-3.65	106.42	112.66
3	C	150	HEM	CMA-C3A-C4A	-3.56	122.99	128.46

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

5 monomers are involved in 9 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	A	150	HEM	1	0
3	B	150	HEM	2	0
3	C	150	HEM	2	0
3	D	150	HEM	3	0
4	D	151	OXY	1	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.

## 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<sup>th</sup> 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 <sup>2</sup> )	Q<0.9
1	A	141/141 (100%)	-0.10	4 (2%) 53 51	28, 45, 64, 74	0
1	C	141/141 (100%)	-0.08	2 (1%) 75 74	32, 45, 65, 74	0
2	B	146/146 (100%)	-0.17	1 (0%) 87 86	30, 40, 49, 59	0
2	D	146/146 (100%)	-0.21	1 (0%) 87 86	33, 41, 54, 58	0
All	All	574/574 (100%)	-0.14	8 (1%) 75 74	28, 42, 61, 74	0

The worst 5 of 8 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	C	141	ARG	6.4
2	D	87	GLN	2.9
1	A	22	GLU	2.6
2	B	2	GLN	2.6
1	A	50	HIS	2.2

### 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 carbohydrates 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. LLDF column lists the quality of electron density of the group with respect to its neighbouring residues in protein, DNA or RNA chains. The B-factors column lists the minimum, median, 95<sup>th</sup> 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	LLDF	B-factors( $\text{\AA}^2$ )	Q<0.9
3	HEM	B	150	43/43	0.94	0.13	0.42	32,38,55,58	0
4	OXY	C	151	2/2	0.99	0.12	0.33	39,39,39,46	0
3	HEM	D	150	43/43	0.94	0.13	0.23	35,42,58,64	0
3	HEM	C	150	43/43	0.96	0.12	0.00	28,39,52,59	0
4	OXY	D	151	2/2	0.99	0.11	-0.07	40,40,40,46	0
3	HEM	A	150	43/43	0.96	0.10	-0.60	35,40,49,54	0
4	OXY	B	151	2/2	1.00	0.08	-1.53	39,39,39,43	0
4	OXY	A	151	2/2	0.99	0.07	-2.00	36,36,36,43	0

## 6.5 Other polymers [i](#)

There are no such residues in this entry.