



Full wwPDB X-ray Structure Validation Report ⓘ

Mar 18, 2026 – 03:48 AM UTC

PDB ID : 4PCF / pdb_00004pcf
Title : Structure-based protein engineering of a monomeric triosephosphate isomerase
towards changing substrate specificity
Authors : Krause, M.; Neubauer, P.; Wierenga, R.K.
Deposited on : 2014-04-15
Resolution : 2.71 Å(reported)

This is a Full wwPDB X-ray Structure Validation 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 types of validation reports are described at

<http://www.wwpdb.org/validation/2017/FAQs#types>.

The following versions of software and data (see [references ⓘ](#)) were used in the production of this report:

MolProbity : 4-5-2 with Phenix2.0
Xtriage (Phenix) : 2.0
EDS : 3.0
Percentile statistics : 20250101.v01 (using entries in the PDB archive January 1st 2025)
CCP4 : 9.0.010 (Gargrove)
Density-Fitness : 1.0.12
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.49

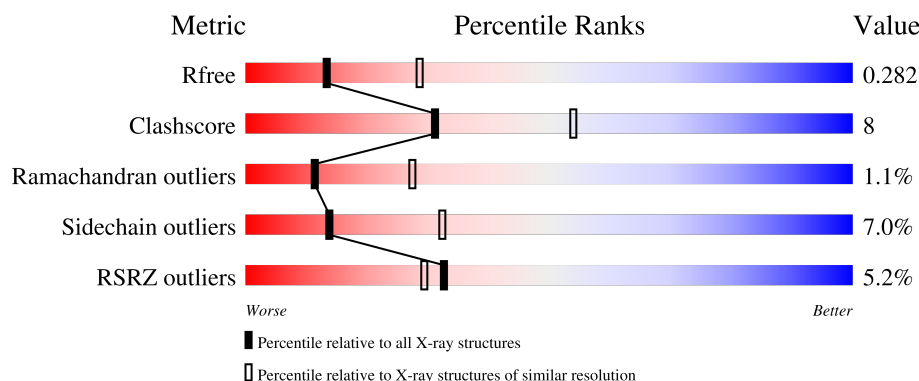
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.71 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}	180053	4348 (2.74-2.70)
Clashscore	190562	4665 (2.74-2.70)
Ramachandran outliers	187476	4584 (2.74-2.70)
Sidechain outliers	187428	4585 (2.74-2.70)
RSRZ outliers	180081	4348 (2.74-2.70)

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 $\leq 5\%$. 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	239	2% 82% 12% • •
1	B	239	7% 75% 20% • •
1	C	239	7% 80% 18% •

2 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 5545 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 Ma18-TIM.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
1	A	234	Total	C	N	O	S	0	0	0
			1787	1142	314	327	4			
1	B	236	Total	C	N	O	S	0	0	0
			1799	1149	315	331	4			
1	C	238	Total	C	N	O	S	0	0	0
			1812	1156	318	334	4			

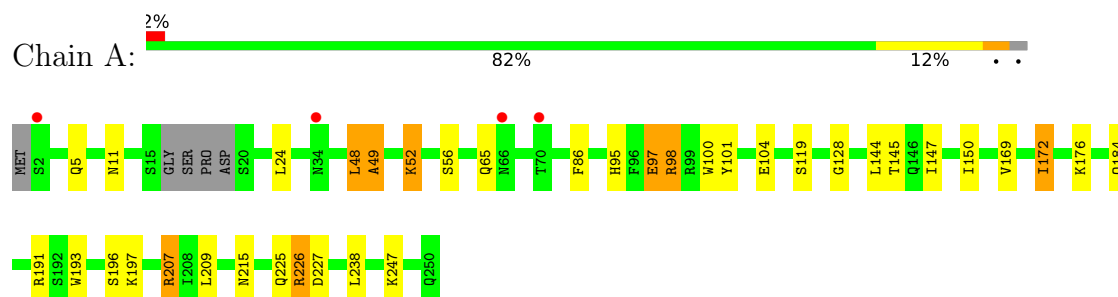
- Molecule 2 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
2	A	53	Total	O	0	0
			53	53		
2	B	47	Total	O	0	0
			47	47		
2	C	47	Total	O	0	0
			47	47		

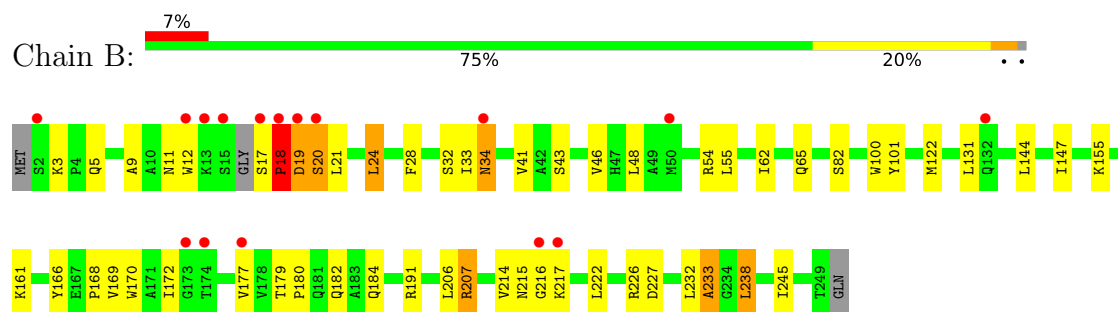
3 Residue-property plots [i](#)

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.

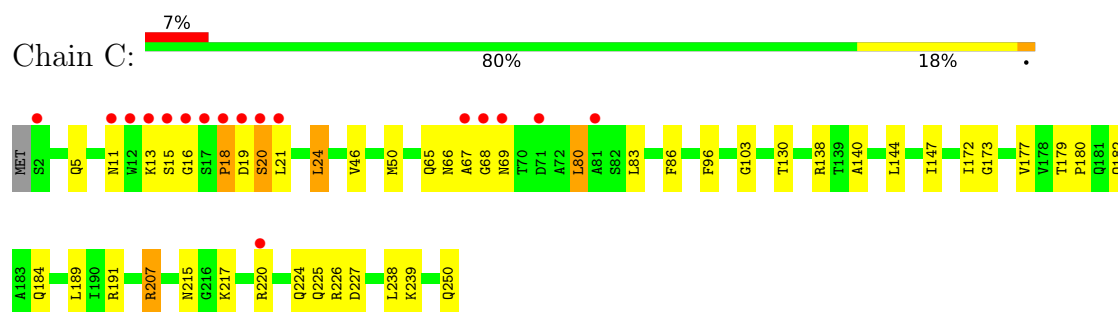
• Molecule 1: Ma18-TIM



• Molecule 1: Ma18-TIM



• Molecule 1: Ma18-TIM



4 Data and refinement statistics

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α , β , γ	92.92Å 49.42Å 95.51Å 90.00° 118.50° 90.00°	Depositor
Resolution (Å)	83.94 – 2.71 83.94 – 2.71	Depositor EDS
% Data completeness (in resolution range)	99.4 (83.94-2.71) 99.5 (83.94-2.71)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	1.75 (at 2.73Å)	Xtriage
Refinement program	REFMAC 5.8.0049	Depositor
R, R_{free}	0.235 , 0.283 0.237 , 0.282	Depositor DCC
R_{free} test set	1078 reflections (5.10%)	wwPDB-VP
Wilson B-factor (Å ²)	19.3	Xtriage
Anisotropy	0.162	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.35 , 29.2	EDS
L-test for twinning ²	$\langle L \rangle = 0.46$, $\langle L^2 \rangle = 0.29$	Xtriage
Estimated twinning fraction	0.037 for -h-l,k,h 0.037 for l,k,-h-l 0.034 for h,-k,-h-l 0.048 for -h-l,-k,l 0.038 for l,-k,h	Xtriage
F_o, F_c correlation	0.87	EDS
Total number of atoms	5545	wwPDB-VP
Average B, all atoms (Å ²)	17.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.17% 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

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	0.72	0/1821	0.95	2/2471 (0.1%)
1	B	0.75	0/1834	0.98	4/2490 (0.2%)
1	C	0.73	0/1848	0.94	1/2510 (0.0%)
All	All	0.73	0/5503	0.96	7/7471 (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	1
1	C	0	1
All	All	0	2

There are no bond length outliers.

All (7) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	B	215	ASN	N-CA-C	8.34	123.54	113.12
1	A	172	ILE	N-CA-C	6.61	117.39	107.80
1	B	33	ILE	CA-C-N	6.12	128.39	120.44
1	B	33	ILE	C-N-CA	6.12	128.39	120.44
1	C	13	LYS	N-CA-C	5.88	123.33	110.80
1	B	233	ALA	N-CA-C	5.78	123.11	110.80
1	A	48	LEU	N-CA-C	-5.58	101.38	109.59

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	B	18	PRO	Peptide

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Mol	Chain	Res	Type	Group
1	C	68	GLY	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	1787	0	1815	26	0
1	B	1799	0	1823	41	0
1	C	1812	0	1835	29	0
2	A	53	0	0	3	0
2	B	47	0	0	1	0
2	C	47	0	0	1	0
All	All	5545	0	5473	89	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 8.

All (89) 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:166:TYR:OH	1:B:170:TRP:HZ3	1.49	0.96
1:B:9:ALA:O	1:B:232:LEU:O	1.86	0.94
1:B:216:GLY:O	2:B:321:HOH:O	1.89	0.90
1:B:166:TYR:OH	1:B:170:TRP:CZ3	2.22	0.90
1:C:67:ALA:HB1	1:C:80:LEU:HD21	1.57	0.86
1:A:100:TRP:HA	1:B:82:SER:HB3	1.66	0.78
1:B:166:TYR:HH	1:B:170:TRP:HZ3	0.77	0.75
1:B:17:SER:N	1:B:18:PRO:HD3	2.04	0.72
1:B:207:ARG:HG3	1:B:207:ARG:HH11	1.55	0.71
1:A:207:ARG:HH11	1:A:207:ARG:HG3	1.57	0.70
1:B:5:GLN:O	1:B:207:ARG:HD3	1.90	0.70
1:C:5:GLN:OE1	1:C:207:ARG:NH2	2.24	0.69
1:B:12:TRP:HB2	1:B:238:LEU:HD13	1.74	0.68
1:A:48:LEU:O	1:A:49:ALA:HB2	1.91	0.68
1:A:5:GLN:O	1:A:207:ARG:HD3	1.92	0.68
1:A:52:LYS:HD3	1:C:173:GLY:O	1.94	0.67

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:A:322:HOH:O	1:C:103:GLY:HA2	1.95	0.66
1:A:48:LEU:O	1:A:49:ALA:CB	2.45	0.65
1:B:191:ARG:HD2	1:B:227:ASP:OD1	2.00	0.61
1:A:184:GLN:HB2	1:A:225:GLN:HG2	1.83	0.60
1:C:138:ARG:HD2	2:C:303:HOH:O	2.00	0.60
1:C:16:GLY:O	1:C:18:PRO:HD3	2.03	0.59
1:A:98:ARG:HD2	1:A:104:GLU:OE2	2.03	0.58
1:A:193:TRP:CE2	1:A:197:LYS:HD2	2.39	0.58
1:C:96:PHE:CZ	1:C:172:ILE:HD13	2.39	0.58
1:A:97:GLU:O	1:A:101:TYR:HB2	2.05	0.56
1:B:207:ARG:HG3	1:B:207:ARG:NH1	2.21	0.55
1:C:226:ARG:HG2	1:C:227:ASP:N	2.21	0.55
1:A:207:ARG:HG3	1:A:207:ARG:NH1	2.22	0.55
1:C:184:GLN:HB2	1:C:225:GLN:HG2	1.89	0.55
1:C:65:GLN:O	1:C:66:ASN:CG	2.51	0.54
1:B:214:VAL:HG13	1:B:245:ILE:CD1	2.37	0.54
1:A:11:ASN:OD1	1:A:65:GLN:NE2	2.41	0.54
1:B:214:VAL:HG13	1:B:245:ILE:HD12	1.90	0.53
1:B:122:MET:HE2	1:B:161:LYS:HD3	1.89	0.53
1:B:55:LEU:HD23	1:B:62:ILE:CD1	2.39	0.52
1:B:17:SER:HB3	1:C:20:SER:HB2	1.91	0.52
1:B:179:THR:OG1	1:B:180:PRO:HD2	2.09	0.52
1:C:191:ARG:HH21	1:C:227:ASP:HA	1.75	0.52
1:C:179:THR:OG1	1:C:180:PRO:HD2	2.09	0.52
1:C:21:LEU:HD23	1:C:50:MET:HE3	1.91	0.51
1:B:24:LEU:CD2	1:B:28:PHE:CZ	2.94	0.51
1:A:98:ARG:HG3	1:A:104:GLU:CD	2.36	0.50
1:A:65:GLN:O	1:A:95:HIS:HE1	1.94	0.50
1:A:191:ARG:HH21	1:A:227:ASP:HA	1.76	0.50
1:B:19:ASP:O	1:B:20:SER:HB3	2.11	0.50
1:C:184:GLN:OE1	1:C:226:ARG:O	2.30	0.49
1:A:207:ARG:NH1	2:A:319:HOH:O	2.45	0.49
1:C:19:ASP:O	1:C:20:SER:CB	2.60	0.49
1:C:11:ASN:OD1	1:C:65:GLN:NE2	2.46	0.49
1:C:144:LEU:HA	1:C:147:ILE:HG22	1.96	0.48
1:A:169:VAL:HA	1:A:172:ILE:HD12	1.96	0.48
1:A:184:GLN:OE1	1:A:226:ARG:O	2.32	0.47
1:C:215:ASN:OD1	1:C:215:ASN:C	2.58	0.47
1:B:184:GLN:OE1	1:B:226:ARG:O	2.33	0.47
1:C:21:LEU:HB3	1:C:50:MET:HE3	1.96	0.47
1:C:18:PRO:HG2	1:C:24:LEU:HD11	1.97	0.46

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:98:ARG:HA	1:A:98:ARG:HD3	1.68	0.46
1:A:144:LEU:HA	1:A:147:ILE:HG22	1.98	0.46
1:B:5:GLN:O	1:B:207:ARG:CD	2.61	0.46
1:B:34:ASN:OD1	1:B:34:ASN:N	2.48	0.46
1:B:100:TRP:HZ3	1:C:86:PHE:CD1	2.34	0.46
1:A:215:ASN:OD1	1:A:215:ASN:C	2.59	0.45
1:A:193:TRP:NE1	1:A:197:LYS:HD2	2.31	0.45
1:C:179:THR:HG22	1:C:182:GLN:OE1	2.17	0.45
1:B:24:LEU:HD23	1:B:28:PHE:CZ	2.52	0.45
1:B:144:LEU:HA	1:B:147:ILE:HG22	1.98	0.44
1:B:11:ASN:OD1	1:B:65:GLN:NE2	2.49	0.44
1:B:166:TYR:OH	1:B:170:TRP:CH2	2.67	0.44
1:B:179:THR:HG22	1:B:182:GLN:OE1	2.17	0.44
1:B:168:PRO:HA	1:B:170:TRP:CZ3	2.53	0.44
1:B:122:MET:CE	1:B:161:LYS:HD3	2.46	0.44
1:A:5:GLN:O	1:A:207:ARG:CD	2.63	0.43
1:B:207:ARG:HH11	1:B:207:ARG:CG	2.29	0.43
1:B:100:TRP:CZ3	1:C:86:PHE:CD1	3.07	0.43
1:B:101:TYR:CE1	1:C:83:LEU:HD23	2.54	0.43
1:B:169:VAL:HA	1:B:172:ILE:HD12	2.01	0.42
1:B:17:SER:CB	1:C:20:SER:HB2	2.50	0.42
1:B:131:LEU:HA	1:B:170:TRP:HB2	2.00	0.42
1:B:17:SER:N	1:B:18:PRO:CD	2.81	0.42
1:C:140:ALA:HB2	1:C:189:LEU:HD11	2.02	0.41
1:B:41:VAL:HG13	1:B:238:LEU:HD11	2.02	0.41
1:A:145:THR:HB	2:A:321:HOH:O	2.20	0.41
1:C:69:ASN:O	1:C:80:LEU:HD22	2.20	0.41
1:C:220:ARG:O	1:C:224:GLN:HG3	2.21	0.41
1:A:52:LYS:HE2	1:A:86:PHE:O	2.21	0.41
1:B:179:THR:HG22	1:B:182:GLN:CD	2.46	0.41
1:A:100:TRP:HH2	1:A:128:GLY:O	2.03	0.40
1:B:191:ARG:NH2	1:B:206:LEU:O	2.53	0.40

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	230/239 (96%)	225 (98%)	4 (2%)	1 (0%)	30	52
1	B	232/239 (97%)	219 (94%)	9 (4%)	4 (2%)	7	17
1	C	236/239 (99%)	224 (95%)	9 (4%)	3 (1%)	9	23
All	All	698/717 (97%)	668 (96%)	22 (3%)	8 (1%)	11	27

All (8) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	49	ALA
1	B	18	PRO
1	B	20	SER
1	C	15	SER
1	C	18	PRO
1	B	19	ASP
1	C	20	SER
1	B	233	ALA

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	185/189 (98%)	171 (92%)	14 (8%)	12	29
1	B	187/189 (99%)	172 (92%)	15 (8%)	11	26
1	C	188/189 (100%)	178 (95%)	10 (5%)	20	44
All	All	560/567 (99%)	521 (93%)	39 (7%)	14	32

All (39) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	24	LEU

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Mol	Chain	Res	Type
1	A	52	LYS
1	A	56	SER
1	A	97	GLU
1	A	98	ARG
1	A	119	SER
1	A	150	ILE
1	A	176	LYS
1	A	196	SER
1	A	207	ARG
1	A	209	LEU
1	A	226	ARG
1	A	238	LEU
1	A	247	LYS
1	B	3	LYS
1	B	21	LEU
1	B	24	LEU
1	B	32	SER
1	B	34	ASN
1	B	43	SER
1	B	46	VAL
1	B	48	LEU
1	B	54	ARG
1	B	155	LYS
1	B	177	VAL
1	B	207	ARG
1	B	217	LYS
1	B	222	LEU
1	B	238	LEU
1	C	24	LEU
1	C	46	VAL
1	C	80	LEU
1	C	130	THR
1	C	177	VAL
1	C	207	ARG
1	C	217	LYS
1	C	238	LEU
1	C	239	LYS
1	C	250	GLN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (3) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	95	HIS
1	B	187	HIS
1	C	66	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 oligosaccharides in this entry.

5.6 Ligand geometry [i](#)

There are no ligands in this entry.

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	234/239 (97%)	0.25	4 (1%) 69 66	8, 15, 29, 41	0
1	B	236/239 (98%)	0.49	16 (6%) 23 21	9, 16, 40, 84	0
1	C	238/239 (99%)	0.42	17 (7%) 22 19	7, 15, 37, 75	0
All	All	708/717 (98%)	0.39	37 (5%) 33 29	7, 15, 36, 84	0

All (37) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	C	12	TRP	5.8
1	C	2	SER	5.5
1	C	18	PRO	5.5
1	B	18	PRO	5.3
1	B	19	ASP	4.8
1	B	12	TRP	4.5
1	A	2	SER	4.4
1	C	19	ASP	4.3
1	B	17	SER	3.9
1	B	177	VAL	3.8
1	B	173	GLY	3.6
1	C	68	GLY	3.6
1	B	2	SER	3.4
1	C	69	ASN	3.3
1	B	15	SER	3.3
1	C	13	LYS	3.3
1	B	20	SER	3.0
1	B	174	THR	2.9
1	B	34	ASN	2.9
1	C	16	GLY	2.9
1	A	70	THR	2.9
1	C	11	ASN	2.7
1	C	15	SER	2.6

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Mol	Chain	Res	Type	RSRZ
1	C	71	ASP	2.5
1	C	67	ALA	2.5
1	C	81	ALA	2.4
1	C	20	SER	2.3
1	B	217	LYS	2.3
1	C	17	SER	2.3
1	C	220	ARG	2.3
1	B	50	MET	2.2
1	A	34	ASN	2.2
1	C	21	LEU	2.2
1	B	132	GLN	2.1
1	B	13	LYS	2.0
1	B	216	GLY	2.0
1	A	66	ASN	2.0

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 oligosaccharides in this entry.

6.4 Ligands ⓘ

There are no ligands in this entry.

6.5 Other polymers ⓘ

There are no such residues in this entry.