

Full wwPDB X-ray Structure Validation Report (i)

Oct 18, 2023 – 03:20 AM EDT

PDB ID	:	2YYM
Title	:	Crystal structure of the mutant of HpaB (T198I, A276G, and R466H) com-
		plexed with FAD and 4-hydroxyphenylacetate
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Deposited on		
Resolution	:	1.70 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 (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:

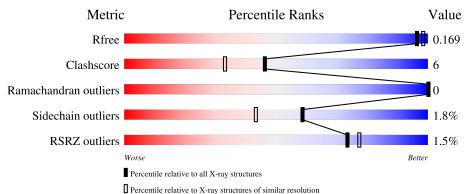
Xtriage (Phenix) EDS buster-report Percentile statistics Refmac CCP4 Ideal geometry (proteins) Ideal geometry (DNA, RNA)	:::::::::::::::::::::::::::::::::::::::	20191225.v01 (using entries in the PDB archive December 25th 2019) 5.8.0158 7.0.044 (Gargrove) Engh & Huber (2001) Parkinson et al. (1996)
Ideal geometry (DNA, RNA) Validation Pipeline (wwPDB-VP)		Parkinson et al. (1996) 2.36

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure: $X\text{-}RAY\;DIFFRACTION$

The reported resolution of this entry is 1.70 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	$egin{array}{c} { m Whole \ archive} \ (\#{ m Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries,\ resolution\ range}({ m \AA}))$
R _{free}	130704	4298 (1.70-1.70)
Clashscore	141614	4695 (1.70-1.70)
Ramachandran outliers	138981	4610 (1.70-1.70)
Sidechain outliers	138945	4610 (1.70-1.70)
RSRZ outliers	127900	4222 (1.70-1.70)

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 <=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	А	481	% 8 4%	15%	•



2YYM

2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 4348 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.

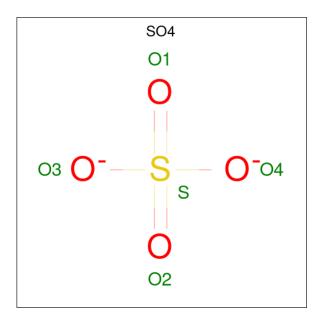
• Molecule 1 is a protein called 4-hydroxyphenylacetate-3-hydroxylase.

Mol	Chain	Residues	Atoms			ZeroOcc	AltConf	Trace		
1	Λ	476	Total	С	Ν	0	\mathbf{S}	0	0	0
1	A	470	3793	2436	652	690	15	0	0	0

There are 3 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
А	198	ILE	THR	engineered mutation	UNP Q5SJP8
А	276	GLY	ALA	engineered mutation	UNP Q5SJP8
А	466	HIS	ARG	engineered mutation	UNP Q5SJP8

• Molecule 2 is SULFATE ION (three-letter code: SO4) (formula: O₄S).

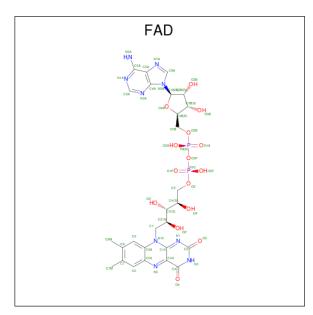


Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	А	1	$\begin{array}{ccc} \text{Total} & \text{O} & \text{S} \\ 5 & 4 & 1 \end{array}$	0	0
2	А	1	$\begin{array}{ccc} \text{Total} & \text{O} & \text{S} \\ 5 & 4 & 1 \end{array}$	0	0



Mo	Chain	Residues	Ato	\mathbf{ms}		ZeroOcc	AltConf
2	А	1	Total 5	0 4	S 1	0	0

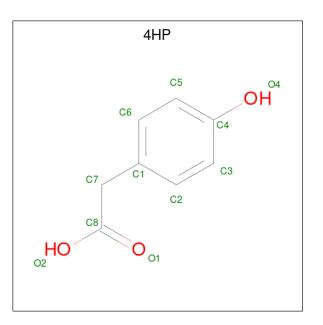
• Molecule 3 is FLAVIN-ADENINE DINUCLEOTIDE (three-letter code: FAD) (formula: $C_{27}H_{33}N_9O_{15}P_2$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	
3	А	1	Total 53	С 27				0	0

• Molecule 4 is 4-HYDROXYPHENYLACETATE (three-letter code: 4HP) (formula: $C_8H_8O_3$).





Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	1	Total C O 11 8 3	0	0

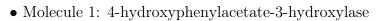
• Molecule 5 is water.

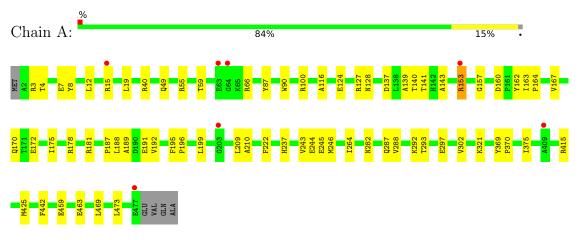
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	А	476	Total O 476 476	0	0



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.







4 Data and refinement statistics (i)

Property	Value	Source
Space group	I 2 2 2	Depositor
Cell constants	91.41Å 98.84Å 130.35Å	Depositor
a, b, c, α , β , γ	90.00° 90.00° 90.00°	Depositor
Resolution (Å)	26.18 - 1.70	Depositor
Resolution (A)	26.18 - 1.70	EDS
% Data completeness	(Not available) $(26.18-1.70)$	Depositor
(in resolution range)	$90.1\ (26.18-1.70)$	EDS
R _{merge}	0.05	Depositor
R _{sym}	(Not available)	Depositor
$< I/\sigma(I) > 1$	$15.05 (at 1.70 \text{\AA})$	Xtriage
Refinement program	CNS 1.1	Depositor
R, R_{free}	0.170 , 0.188	Depositor
It, Itfree	0.167 , 0.169	DCC
R_{free} test set	2960 reflections (5.06%)	wwPDB-VP
Wilson B-factor $(Å^2)$	16.6	Xtriage
Anisotropy	0.509	Xtriage
Bulk solvent $k_{sol}(e/Å^3)$, $B_{sol}(Å^2)$	0.35 , 45.3	EDS
L-test for twinning ²	$ < L >=0.48, < L^2>=0.31$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o, F_c correlation	0.96	EDS
Total number of atoms	4348	wwPDB-VP
Average B, all atoms $(Å^2)$	21.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 3.62% of the height of the origin peak. No significant pseudotranslation is detected.

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.



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: FAD, SO4, $4\mathrm{HP}$

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	ol Chain Bond lengths				angles
	Chain	RMSZ	# Z > 5	RMSZ	# Z > 5
1	А	0.30	0/3888	0.57	0/5272

There are no bond length outliers.

There are no bond angle outliers.

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	А	3793	0	3757	48	0
2	А	15	0	0	0	0
3	А	53	0	31	0	0
4	А	11	0	6	0	0
5	А	476	0	0	4	1
All	All	4348	0	3794	48	1

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 6.

All (48) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.



Atom-1	Atom-2	Interatomic	Clash
		distance (Å)	overlap (Å)
1:A:153:ARG:HH11	1:A:153:ARG:HB3	1.39	0.85
1:A:153:ARG:HH12	1:A:157:GLY:HA3	1.42	0.84
1:A:153:ARG:NH1	1:A:157:GLY:HA3	2.02	0.72
1:A:49:GLN:O	1:A:55:ARG:HD3	1.93	0.67
1:A:459:GLU:O	1:A:463:GLU:HG3	1.95	0.66
1:A:163:ILE:HB	1:A:164:PRO:HD3	1.78	0.64
1:A:199:LEU:HD23	1:A:199:LEU:H	1.63	0.64
1:A:153:ARG:HB3	1:A:153:ARG:NH1	2.13	0.64
1:A:3:ARG:HD2	1:A:137:ASP:O	1.97	0.63
1:A:282:ASN:HD22	1:A:282:ASN:H	1.48	0.60
1:A:55:ARG:HG2	5:A:1386:HOH:O	2.04	0.58
1:A:188:LEU:HD22	1:A:188:LEU:N	2.21	0.56
1:A:19:LEU:HD21	1:A:187:PRO:HB2	1.90	0.54
1:A:116:ALA:HB3	1:A:127:ARG:NH1	2.25	0.51
1:A:55:ARG:NE	1:A:66:ARG:HD3	2.27	0.50
1:A:143:ALA:HB1	1:A:181:ARG:HB3	1.94	0.49
1:A:4:THR:OG1	1:A:7:GLU:HG3	2.12	0.49
1:A:170:GLN:HG2	1:A:175:ILE:HG22	1.95	0.48
1:A:167:VAL:CG2	1:A:209:LEU:HD21	2.44	0.48
1:A:55:ARG:HD2	1:A:59:THR:OG1	2.14	0.48
1:A:141:THR:HG23	1:A:192:VAL:HG23	1.96	0.47
1:A:160:ASP:HB3	1:A:163:ILE:HG13	1.96	0.47
1:A:153:ARG:HH12	1:A:157:GLY:CA	2.23	0.47
1:A:282:ASN:H	1:A:282:ASN:ND2	2.13	0.46
1:A:473:LEU:HD22	1:A:473:LEU:N	2.30	0.46
1:A:369:TYR:HB3	1:A:370:PRO:HD3	1.96	0.46
1:A:321:LYS:HB3	1:A:375:ILE:HG23	1.98	0.46
1:A:210:ALA:HB3	1:A:264:ILE:HB	1.98	0.46
1:A:40:ARG:HD3	5:A:1332:HOH:O	2.17	0.45
1:A:19:LEU:HD23	1:A:222:PHE:HB2	1.98	0.45
1:A:302:VAL:HG21	1:A:425:MET:SD	2.56	0.45
1:A:237:HIS:HD2	5:A:1018:HOH:O	2.00	0.45
1:A:293:THR:O	1:A:297:GLU:HG3	2.17	0.44
1:A:8:TYR:CZ	1:A:12:LEU:HD11	2.52	0.44
1:A:15:ARG:HG3	1:A:15:ARG:HH21	1.81	0.44
1:A:140:THR:HG22	1:A:191:GLU:HB2	2.00	0.44
1:A:199:LEU:HD23	1:A:199:LEU:N	2.31	0.43
1:A:243:VAL:O	1:A:243:VAL:HG12	2.18	0.43
1:A:87:TYR:CD2	1:A:287:GLN:HA	2.54	0.43
1:A:245:GLU:O	1:A:246:MET:HB3	2.18	0.43
1:A:124:GLU:HG3	1:A:128:ASN:ND2	2.34	0.43
1:A:139:ALA:O	1:A:189:ALA:HB1	2.19	0.42



Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:162:TYR:HB3	1:A:178:ARG:HG3	2.01	0.42
1:A:181:ARG:HD3	1:A:181:ARG:HA	1.86	0.42
1:A:195:PHE:HB3	1:A:196:PRO:CD	2.49	0.42
1:A:288:VAL:O	1:A:292:LYS:HG2	2.20	0.42
1:A:469:LEU:O	1:A:473:LEU:HD23	2.21	0.41
1:A:127:ARG:HD2	5:A:1366:HOH:O	2.20	0.40

All (1) symmetry-related close contacts are listed below. The label for Atom-2 includes the symmetry operator and encoded unit-cell translations to be applied.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
5:A:1268:HOH:O	5:A:1268:HOH:O[3_555]	1.71	0.49

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	А	474/481~(98%)	462 (98%)	12 (2%)	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	А	390/394~(99%)	383~(98%)	7 (2%)	59 43	

All (7) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	90	TRP
1	А	100	ARG
1	А	153	ARG
1	А	172	GLU
1	А	244	GLU
1	А	415	ARG
1	А	442	PHE

Sometimes side chains can be flipped to improve hydrogen bonding and reduce clashes. All (4) such side chains are listed below:

Mol	Chain	Res	Type
1	А	94	ASN
1	А	136	GLN
1	А	237	HIS
1	А	282	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 monosaccharides in this entry.

5.6 Ligand geometry (i)

5 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	l Type Chain Res		Link	Bo	ond leng	ths	Bond angles			
	туре	Unam	nes		Counts	RMSZ	# Z >2	Counts	RMSZ	# Z >2
3	FAD	А	500	-	$53,\!58,\!58$	1.20	3 (5%)	68,89,89	3.08	29 (42%)
2	SO4	А	601	-	4,4,4	1.01	0	6,6,6	1.58	1 (16%)
2	SO4	А	600	-	4,4,4	1.03	0	6,6,6	1.57	1 (16%)
4	4HP	А	550	-	11,11,11	1.11	0	14,14,14	0.83	0
2	SO4	А	602	-	4,4,4	1.02	0	6,6,6	1.56	1 (16%)

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	FAD	А	500	-	-	7/30/50/50	0/6/6/6
4	4HP	А	550	-	-	0/4/4/4	0/1/1/1

All (3) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Ζ	Observed(Å)	Ideal(Å)
3	А	500	FAD	C4X-N5	5.22	1.40	1.30
3	А	500	FAD	C10-N1	3.80	1.41	1.33
3	А	500	FAD	O4B-C1B	2.09	1.44	1.41

All (32) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
3	А	500	FAD	N3A-C2A-N1A	-10.59	112.13	128.68
3	А	500	FAD	O2-C2-N3	6.89	132.04	118.65
3	А	500	FAD	C5X-N5-C4X	6.80	129.38	118.07
3	А	500	FAD	C9A-C5X-N5	-6.29	115.60	122.43
3	А	500	FAD	C10-N1-C2	5.96	128.82	116.90
3	А	500	FAD	N3-C2-N1	-5.90	107.81	119.38
3	А	500	FAD	C9-C9A-N10	5.78	129.65	121.84
3	А	500	FAD	C9A-N10-C10	5.66	129.58	120.77
3	А	500	FAD	C4-C4X-N5	5.28	125.75	118.23
3	А	500	FAD	C2A-N1A-C6A	4.80	126.96	118.75
3	А	500	FAD	C8M-C8-C7	4.76	130.49	120.74



Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
3	А	500	FAD	C10-C4X-N5	-4.51	115.29	124.86
3	А	500	FAD	C8M-C8-C9	-3.96	112.17	119.49
3	А	500	FAD	C7M-C7-C6	-3.86	112.35	119.49
2	А	601	SO4	O4-S-O3	3.62	124.52	109.06
2	А	600	SO4	O4-S-O3	3.61	124.47	109.06
2	А	602	SO4	O4-S-O3	3.59	124.40	109.06
3	А	500	FAD	C6-C5X-C9A	3.52	123.92	118.94
3	А	500	FAD	C5X-C9A-N10	-3.30	114.55	117.95
3	А	500	FAD	O2'-C2'-C3'	3.18	116.83	109.10
3	А	500	FAD	N10-C10-N1	2.97	126.89	118.35
3	А	500	FAD	C4X-C10-N1	-2.91	117.98	124.73
3	А	500	FAD	O2-C2-N1	-2.56	117.59	121.83
3	А	500	FAD	O4B-C1B-C2B	-2.55	103.19	106.93
3	А	500	FAD	C9-C9A-C5X	-2.33	115.71	120.11
3	А	500	FAD	C7M-C7-C8	2.13	125.11	120.74
3	А	500	FAD	O2'-C2'-C1'	-2.12	104.67	109.80
3	А	500	FAD	C9A-C9-C8	2.11	123.54	119.30
3	А	500	FAD	C4X-C4-N3	2.08	118.48	113.19
3	А	500	FAD	C5X-C6-C7	-2.05	116.95	120.71
3	А	500	FAD	C2B-C3B-C4B	-2.00	98.75	102.64
3	А	500	FAD	O5B-C5B-C4B	-2.00	102.10	108.99

There are no chirality outliers.

All (7) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	А	500	FAD	C2'-C1'-N10-C10
3	А	500	FAD	C5B-O5B-PA-O3P
3	А	500	FAD	PA-O3P-P-O2P
3	А	500	FAD	N10-C1'-C2'-C3'
3	А	500	FAD	C4'-C5'-O5'-P
3	А	500	FAD	PA-O3P-P-O1P
3	А	500	FAD	C5B-O5B-PA-O1A

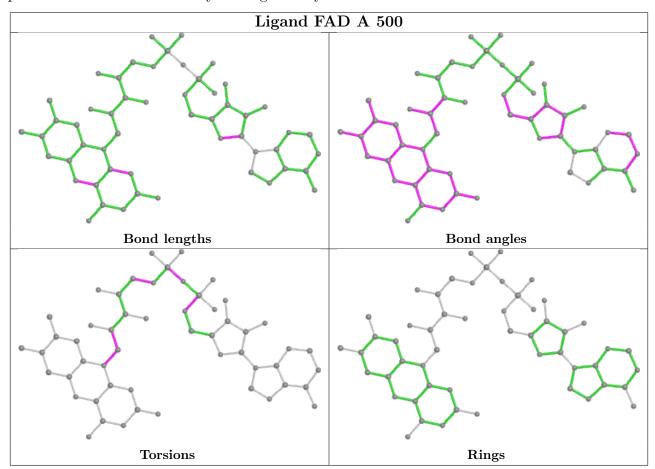
There are no ring outliers.

No monomer is involved in short contacts.

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	<RSRZ $>$	#RSRZ>2		$\mathbf{OWAB}(\mathbf{A}^2)$	Q < 0.9
1	А	476/481 (98%)	-0.19	7 (1%)	73 77	11, 18, 29, 37	0

All (7) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	А	63	GLU	3.6
1	А	203	GLY	3.4
1	А	477	GLU	2.8
1	А	64	GLY	2.7
1	А	153	ARG	2.1
1	А	15	ARG	2.1
1	А	409	ALA	2.0

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)

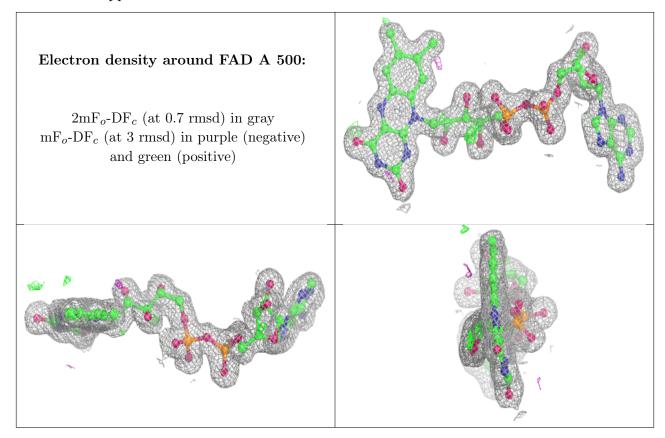
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95^{th} percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9'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

Mol	Type	Chain	Res	Atoms	RSCC	RSR	$B-factors(Å^2)$	Q<0.9
2	SO4	А	602	5/5	0.77	0.23	83,84,84,85	0



Continued from provide page								
Mol	Type	Chain	Res	Atoms	RSCC	RSR	${f B} ext{-factors}({ m \AA}^2)$	Q<0.9
2	SO4	А	601	5/5	0.80	0.17	76,77,78,78	0
2	SO4	А	600	5/5	0.87	0.17	89,89,90,90	0
4	4HP	А	550	11/11	0.94	0.11	18,21,26,32	0
3	FAD	А	500	53/53	0.98	0.05	12,15,19,20	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.

