wwPDB X-ray Structure Validation Summary Report (i)

Dec 16, 2023 – 10:17 pm GMT

PDB ID	:	2XH4
Title	:	Engineering the enolase active site pocket: Crystal structure of the S39A D321A mutant of yeast enolase 1
Authors	:	Schreier, B.; Hocker, B.
Deposited on	:	2010-06-09
Resolution	:	1.70 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 (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 (i)) were used in the production of this report:

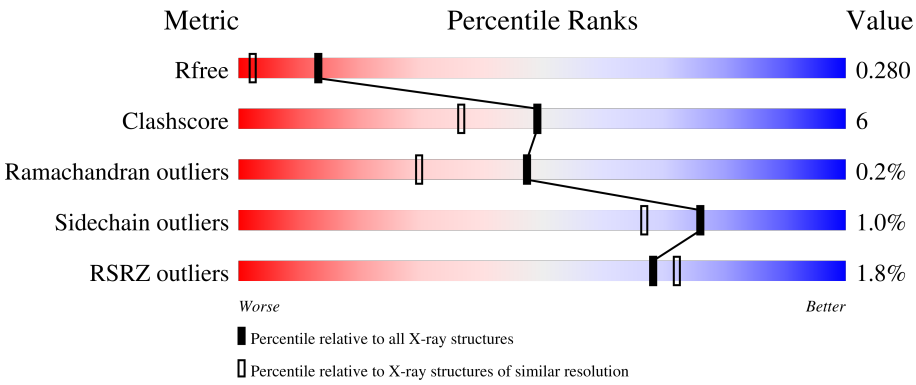
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
X-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	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
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	A	443	87% 12% •
1	B	443	3% 82% 11% • 6%
1	C	443	87% 12% •
1	D	443	4% 85% 9% 6%

2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 14463 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 ENOLASE 1.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	438	3278	2075	564	632	7	0	4	1
			Total	C	N	O	S			
1	B	415	3082	1955	533	590	4	0	5	1
			Total	C	N	O	S			
1	C	438	3278	2075	564	632	7	0	4	1
			Total	C	N	O	S			
1	D	415	3081	1954	532	591	4	0	6	1

There are 40 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	437	LEU	-	expression tag	UNP P00924
A	438	HIS	-	expression tag	UNP P00924
A	439	HIS	-	expression tag	UNP P00924
A	440	HIS	-	expression tag	UNP P00924
A	441	HIS	-	expression tag	UNP P00924
A	442	HIS	-	expression tag	UNP P00924
A	443	HIS	-	expression tag	UNP P00924
A	39	ALA	SER	engineered mutation	UNP P00924
A	241	ILE	VAL	conflict	UNP P00924
A	321	ALA	ASP	engineered mutation	UNP P00924
B	437	LEU	-	expression tag	UNP P00924
B	438	HIS	-	expression tag	UNP P00924
B	439	HIS	-	expression tag	UNP P00924
B	440	HIS	-	expression tag	UNP P00924
B	441	HIS	-	expression tag	UNP P00924
B	442	HIS	-	expression tag	UNP P00924
B	443	HIS	-	expression tag	UNP P00924
B	39	ALA	SER	engineered mutation	UNP P00924
B	241	ILE	VAL	conflict	UNP P00924
B	321	ALA	ASP	engineered mutation	UNP P00924
C	437	LEU	-	expression tag	UNP P00924

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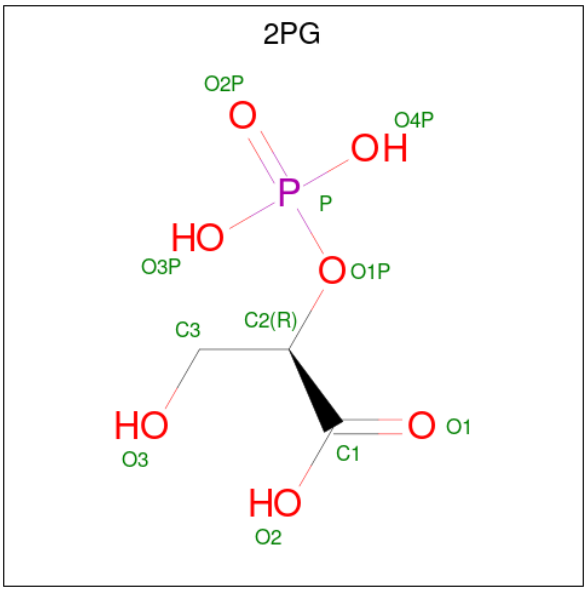
Chain	Residue	Modelled	Actual	Comment	Reference
C	438	HIS	-	expression tag	UNP P00924
C	439	HIS	-	expression tag	UNP P00924
C	440	HIS	-	expression tag	UNP P00924
C	441	HIS	-	expression tag	UNP P00924
C	442	HIS	-	expression tag	UNP P00924
C	443	HIS	-	expression tag	UNP P00924
C	39	ALA	SER	engineered mutation	UNP P00924
C	241	ILE	VAL	conflict	UNP P00924
C	321	ALA	ASP	engineered mutation	UNP P00924
D	437	LEU	-	expression tag	UNP P00924
D	438	HIS	-	expression tag	UNP P00924
D	439	HIS	-	expression tag	UNP P00924
D	440	HIS	-	expression tag	UNP P00924
D	441	HIS	-	expression tag	UNP P00924
D	442	HIS	-	expression tag	UNP P00924
D	443	HIS	-	expression tag	UNP P00924
D	39	ALA	SER	engineered mutation	UNP P00924
D	241	ILE	VAL	conflict	UNP P00924
D	321	ALA	ASP	engineered mutation	UNP P00924

- Molecule 2 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	Mg		
2	A	1	1	1	0	0
2	B	3	3	3	0	0
2	C	1	1	1	0	0
2	D	2	2	2	0	0

- Molecule 3 is 2-PHOSPHOGLYCERIC ACID (three-letter code: 2PG) (formula: $C_3H_7O_7P$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	O	P		
3	A	1	11	3	7	1	0	0
			Total	C	O	P		
3	B	1	11	3	7	1	0	0
			Total	C	O	P		
3	C	1	11	3	7	1	0	0
			Total	C	O	P		
3	D	1	11	3	7	1	0	0

- Molecule 4 is water.

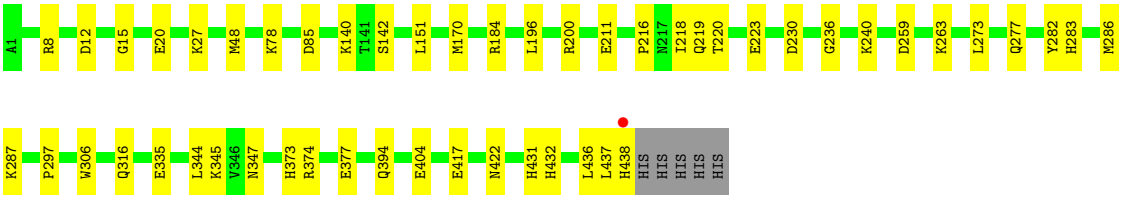
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
4	A	508	508	508	0	0
			Total	O		
4	B	377	377	377	0	0
			Total	O		
4	C	492	492	492	0	0
			Total	O		
4	D	316	316	316	0	0

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.

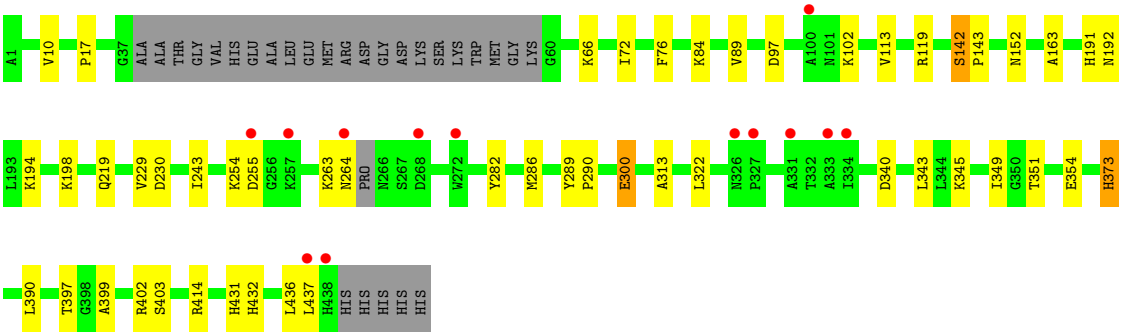
• Molecule 1: ENOLASE 1

Chain A:



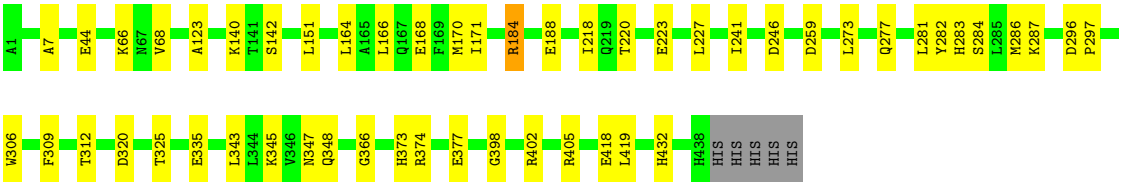
• Molecule 1: ENOLASE 1

Chain B:



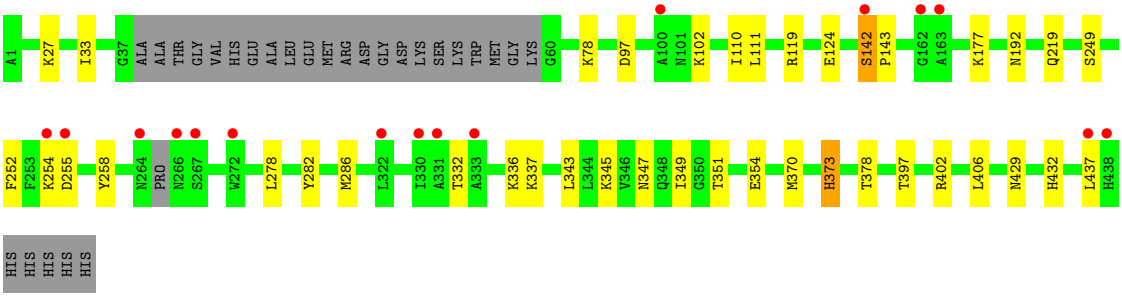
• Molecule 1: ENOLASE 1

Chain C:



• Molecule 1: ENOLASE 1

Chain D:


A1
K27
I33
G37
ALA
ALA
THR
GLY
VAL
HIS
GLU
ALA
LEU
GLU
MET
ARG
ASP
GLY
ASP
LYS
SER
LYS
TRP
MET
GLY
LYS
G60
K78
D97
A100
N101
K102
I110
L111
R119
E124
S142
P143
G162
A163
K177
N192
Q219
S249
F252
F253
K254
D255
Y258
N264
PRO
N266
S267
W272
L278
Y282
M286
L322
I330
A331
T332
A333
K336
K337
L343
L344
K345
V346
N347
G348
I349
G350
T351
E354
M370
H373
T378
T397
R402
L406
N429
H432
L437
H438
HIS
HIS
HIS
HIS
HIS

4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1	Depositor
Cell constants a, b, c, α, β, γ	62.42Å 62.61Å 100.95Å 87.95° 76.07° 76.43°	Depositor
Resolution (Å)	31.69 – 1.70 31.68 – 1.70	Depositor EDS
% Data completeness (in resolution range)	92.8 (31.69-1.70) 88.1 (31.68-1.70)	Depositor EDS
R_{merge}	0.21	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	2.25 (at 1.70Å)	Xtriage
Refinement program	REFMAC 5.2.0019	Depositor
R, R_{free}	0.233 , 0.279 0.234 , 0.280	Depositor DCC
R_{free} test set	7011 reflections (5.02%)	wwPDB-VP
Wilson B-factor (Å^2)	15.8	Xtriage
Anisotropy	0.109	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.37 , 184.9	EDS
L-test for twinning[2]	$< \|L\| > = 0.47$, $< L^2 > = 0.29$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.96	EDS
Total number of atoms	14463	wwPDB-VP
Average B, all atoms (Å^2)	20.0	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 89.29 % 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 3.6016e-08. The detected translational NCS is most likely also responsible for the elevated intensity ratio.*

[1]Intensities estimated from amplitudes.

[2]Theoretical values of $< |L| >$, $< L^2 >$ 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: MG, 2PG

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	0.80	0/3350	0.83	4/4540 (0.1%)
1	B	0.71	1/3151 (0.0%)	0.74	1/4275 (0.0%)
1	C	0.79	0/3350	0.84	6/4540 (0.1%)
1	D	0.69	0/3153	0.73	0/4279
All	All	0.75	1/13004 (0.0%)	0.79	11/17634 (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	D	0	1
All	All	0	2

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	300	GLU	C-N	-5.16	1.22	1.34

The worst 5 of 11 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	C	184	ARG	NE-CZ-NH1	8.50	124.55	120.30
1	C	320	ASP	CB-CG-OD2	6.68	124.31	118.30
1	C	227	LEU	CA-CB-CG	6.48	130.21	115.30
1	A	12	ASP	CB-CG-OD1	6.13	123.82	118.30
1	C	184	ARG	NE-CZ-NH2	-6.12	117.24	120.30

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	B	142	SER	Peptide
1	D	142	SER	Peptide

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	A	3278	0	3272	41	0
1	B	3082	0	3054	42	0
1	C	3278	0	3272	36	0
1	D	3081	0	3046	32	0
2	A	1	0	0	0	0
2	B	3	0	0	0	0
2	C	1	0	0	0	0
2	D	2	0	0	0	0
3	A	11	0	4	1	0
3	B	11	0	4	0	0
3	C	11	0	4	1	0
3	D	11	0	4	0	0
4	A	508	0	0	11	1
4	B	377	0	0	10	0
4	C	492	0	0	7	1
4	D	316	0	0	10	0
All	All	14463	0	12660	149	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.

The worst 5 of 149 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:A:287:LYS:HE2	4:A:2393:HOH:O	1.36	1.19
1:B:119[A]:ARG:NH2	4:B:2139:HOH:O	1.76	1.19
1:A:140:LYS:NZ	1:A:438:HIS:N	1.94	1.14

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:B:119[B]:ARG:CZ	4:B:2138:HOH:O	2.08	1.02
1:A:140:LYS:HZ3	1:A:438:HIS:N	1.56	1.00

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)
4:A:2375:HOH:O	4:C:2034:HOH:O[1_565]	1.88	0.32

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	440/443 (99%)	429 (98%)	11 (2%)	0	100	100
1	B	414/443 (94%)	402 (97%)	11 (3%)	1 (0%)	47	30
1	C	440/443 (99%)	433 (98%)	6 (1%)	1 (0%)	47	30
1	D	415/443 (94%)	401 (97%)	13 (3%)	1 (0%)	47	30
All	All	1709/1772 (96%)	1665 (97%)	41 (2%)	3 (0%)	47	30

All (3) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	402	ARG
1	C	402	ARG
1	D	402	ARG

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	339/349 (97%)	334 (98%)	5 (2%)	65	51
1	B	313/349 (90%)	311 (99%)	2 (1%)	86	80
1	C	339/349 (97%)	336 (99%)	3 (1%)	78	70
1	D	313/349 (90%)	310 (99%)	3 (1%)	76	67
All	All	1304/1396 (93%)	1291 (99%)	13 (1%)	76	67

5 of 13 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	C	335	GLU
1	C	347	ASN
1	D	406	LEU
1	D	192	ASN
1	D	373	HIS

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 15 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	159	HIS
1	D	192	ASN
1	B	191	HIS
1	D	432	HIS
1	D	80	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)

Of 11 ligands modelled in this entry, 7 are monoatomic - leaving 4 for Mogul analysis.

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	2PG	A	1440	2	9,10,10	1.50	3 (33%)	11,14,14	2.36	4 (36%)
3	2PG	B	1440	2	9,10,10	0.95	0	11,14,14	1.05	1 (9%)
3	2PG	D	1440	2	9,10,10	1.01	0	11,14,14	0.89	0
3	2PG	C	1440	2	9,10,10	2.77	3 (33%)	11,14,14	2.24	6 (54%)

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	2PG	A	1440	2	-	2/11/11/11	-
3	2PG	B	1440	2	-	0/11/11/11	-
3	2PG	D	1440	2	-	0/11/11/11	-
3	2PG	C	1440	2	-	1/11/11/11	-

The worst 5 of 6 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	C	1440	2PG	C2-C1	6.39	1.58	1.52
3	C	1440	2PG	C3-C2	3.68	1.60	1.52
3	C	1440	2PG	P-O3P	-2.54	1.45	1.54
3	A	1440	2PG	P-O1P	2.35	1.63	1.59
3	A	1440	2PG	O1-C1	2.32	1.29	1.22

The worst 5 of 11 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	A	1440	2PG	O4P-P-O2P	-4.54	92.91	110.68
3	A	1440	2PG	O3-C3-C2	4.15	122.29	111.73

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	C	1440	2PG	O4P-P-O2P	3.87	125.83	110.68
3	C	1440	2PG	O2-C1-C2	3.71	123.63	113.03
3	A	1440	2PG	O2-C1-O1	3.03	130.96	124.09

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	A	1440	2PG	C1-C2-C3-O3
3	A	1440	2PG	O1P-C2-C3-O3
3	C	1440	2PG	C3-C2-O1P-P

There are no ring outliers.

2 monomers are involved in 2 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	A	1440	2PG	1	0
3	C	1440	2PG	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, 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	438/443 (98%)	-0.44	1 (0%)	95	95	7, 15, 26, 35	0
1	B	415/443 (93%)	0.02	13 (3%)	49	53	9, 21, 37, 46	0
1	C	438/443 (98%)	-0.48	0	100	100	6, 15, 26, 35	0
1	D	415/443 (93%)	0.04	16 (3%)	39	44	9, 21, 37, 48	0
All	All	1706/1772 (96%)	-0.22	30 (1%)	68	72	6, 18, 34, 48	0

The worst 5 of 30 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	B	438	HIS	10.1
1	D	264	ASN	4.4
1	D	438	HIS	4.3
1	B	255	ASP	4.2
1	D	437	LEU	3.9

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, 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(Å^2)	Q<0.9
2	MG	B	1441	1/1	0.91	0.27	27,27,27,27	0
2	MG	B	1442	1/1	0.95	0.34	16,16,16,16	0
2	MG	C	1439	1/1	0.97	0.12	2,2,2,2	0
2	MG	D	1439	1/1	0.97	0.17	8,8,8,8	0
3	2PG	B	1440	11/11	0.97	0.08	17,19,20,21	0
3	2PG	D	1440	11/11	0.97	0.06	15,17,19,20	0
3	2PG	A	1440	11/11	0.98	0.05	2,9,11,12	0
2	MG	B	1439	1/1	0.98	0.21	7,7,7,7	0
3	2PG	C	1440	11/11	0.98	0.06	2,4,12,13	0
2	MG	D	1441	1/1	0.98	0.23	8,8,8,8	0
2	MG	A	1439	1/1	0.99	0.11	2,2,2,2	0

6.5 Other polymers (i)

There are no such residues in this entry.