



# Full wwPDB X-ray Structure Validation Report ⓘ

Aug 8, 2020 – 08:05 AM BST

PDB ID : 4V00  
Title : Structural and functional characterization of a novel monotreme-specific protein from the milk of the platypus  
Authors : Enjapoori, A.K.; Newman, J.; Polekina, G.; Adams, T.E.; Sharp, J.A.; Peat, T.S.; Nicholas, K.R.  
Deposited on : 2014-09-10  
Resolution : 1.82 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](mailto: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.

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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 : 1.8.5 (274361), CSD as541be (2020)  
Xtriage (Phenix) : 1.13  
EDS : 2.13.1  
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) : 2.13.1

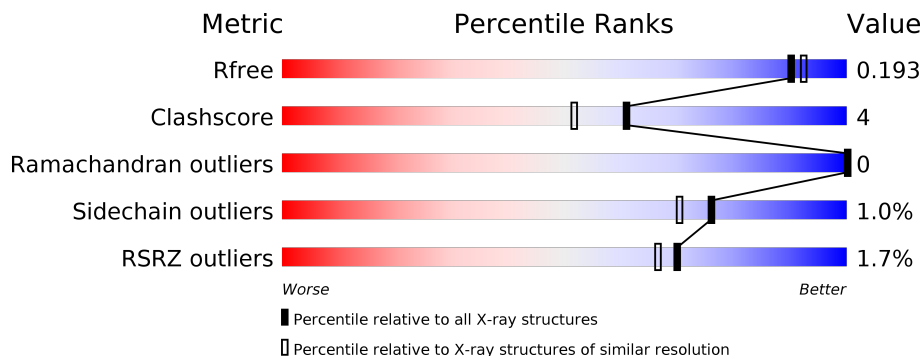
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*X-RAY DIFFRACTION*

The reported resolution of this entry is 1.82 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	7484 (1.84-1.80)
Clashscore	141614	8401 (1.84-1.80)
Ramachandran outliers	138981	8290 (1.84-1.80)
Sidechain outliers	138945	8290 (1.84-1.80)
RSRZ outliers	127900	7371 (1.84-1.80)

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 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	366	 2% 89% 6%
1	B	366	 2% 87% 7% 6%

## 2 Entry composition i

There are 4 unique types of molecules in this entry. The entry contains 6267 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 MONOTREME LACTATING PROTEIN.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	343	2903	1861	479	543	20	0	25	0
1	B	345	2870	1843	475	532	20	0	18	0

There are 46 discrepancies between the modelled and reference sequences:

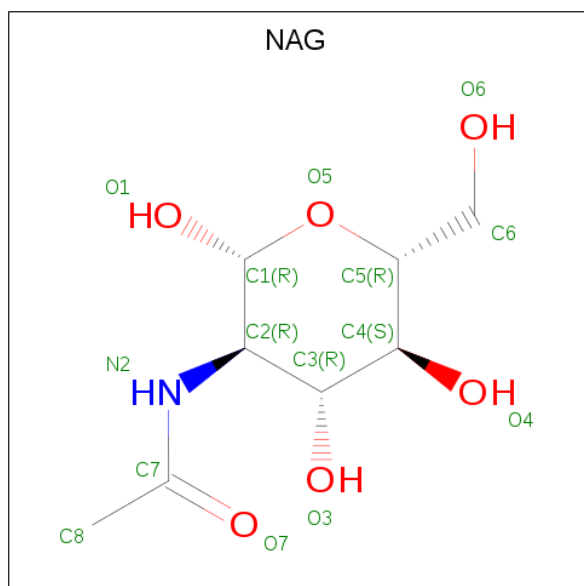
Chain	Residue	Modelled	Actual	Comment	Reference
A	344	VAL	-	expression tag	UNP F6UME2
A	345	GLU	-	expression tag	UNP F6UME2
A	346	VAL	-	expression tag	UNP F6UME2
A	347	GLN	-	expression tag	UNP F6UME2
A	348	VAL	-	expression tag	UNP F6UME2
A	349	GLU	-	expression tag	UNP F6UME2
A	350	LEU	-	expression tag	UNP F6UME2
A	351	ALA	-	expression tag	UNP F6UME2
A	352	LYS	-	expression tag	UNP F6UME2
A	353	LEU	-	expression tag	UNP F6UME2
A	354	GLY	-	expression tag	UNP F6UME2
A	355	ILE	-	expression tag	UNP F6UME2
A	356	PRO	-	expression tag	UNP F6UME2
A	357	THR	-	expression tag	UNP F6UME2
A	358	PRO	-	expression tag	UNP F6UME2
A	359	VAL	-	expression tag	UNP F6UME2
A	360	ASP	-	expression tag	UNP F6UME2
A	361	TYR	-	expression tag	UNP F6UME2
A	362	LYS	-	expression tag	UNP F6UME2
A	363	ASP	-	expression tag	UNP F6UME2
A	364	ASP	-	expression tag	UNP F6UME2
A	365	ASP	-	expression tag	UNP F6UME2
A	366	LYS	-	expression tag	UNP F6UME2
B	344	VAL	-	expression tag	UNP F6UME2
B	345	GLU	-	expression tag	UNP F6UME2

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Chain	Residue	Modelled	Actual	Comment	Reference
B	346	VAL	-	expression tag	UNP F6UME2
B	347	GLN	-	expression tag	UNP F6UME2
B	348	VAL	-	expression tag	UNP F6UME2
B	349	GLU	-	expression tag	UNP F6UME2
B	350	LEU	-	expression tag	UNP F6UME2
B	351	ALA	-	expression tag	UNP F6UME2
B	352	LYS	-	expression tag	UNP F6UME2
B	353	LEU	-	expression tag	UNP F6UME2
B	354	GLY	-	expression tag	UNP F6UME2
B	355	ILE	-	expression tag	UNP F6UME2
B	356	PRO	-	expression tag	UNP F6UME2
B	357	THR	-	expression tag	UNP F6UME2
B	358	PRO	-	expression tag	UNP F6UME2
B	359	VAL	-	expression tag	UNP F6UME2
B	360	ASP	-	expression tag	UNP F6UME2
B	361	TYR	-	expression tag	UNP F6UME2
B	362	LYS	-	expression tag	UNP F6UME2
B	363	ASP	-	expression tag	UNP F6UME2
B	364	ASP	-	expression tag	UNP F6UME2
B	365	ASP	-	expression tag	UNP F6UME2
B	366	LYS	-	expression tag	UNP F6UME2

- Molecule 2 is 2-acetamido-2-deoxy-beta-D-glucopyranose (three-letter code: NAG) (formula: C<sub>8</sub>H<sub>15</sub>NO<sub>6</sub>).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
2	A	1	Total	C	N	O	0	0
			14	8	1	5		
2	B	1	Total	C	N	O	0	0
			14	8	1	5		

- Molecule 3 is GLYCEROL (three-letter code: GOL) (formula: C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
3	A	1	Total	C	O	0	0
			6	3	3		
3	A	1	Total	C	O	0	0
			6	3	3		
3	B	1	Total	C	O	0	0
			6	3	3		

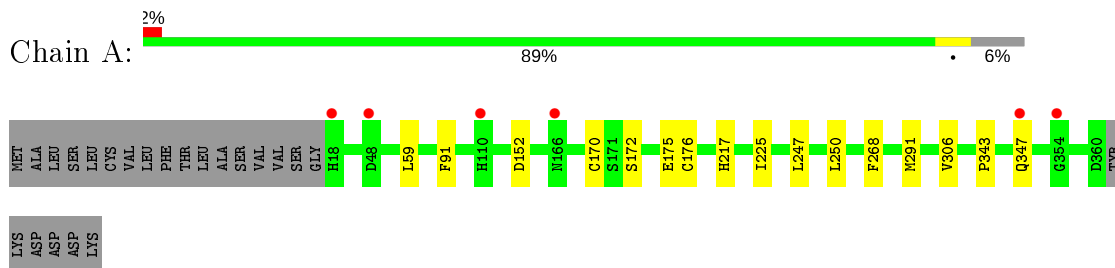
- Molecule 4 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
4	A	226	Total	O	0	0
			226	226		
4	B	222	Total	O	0	0
			222	222		

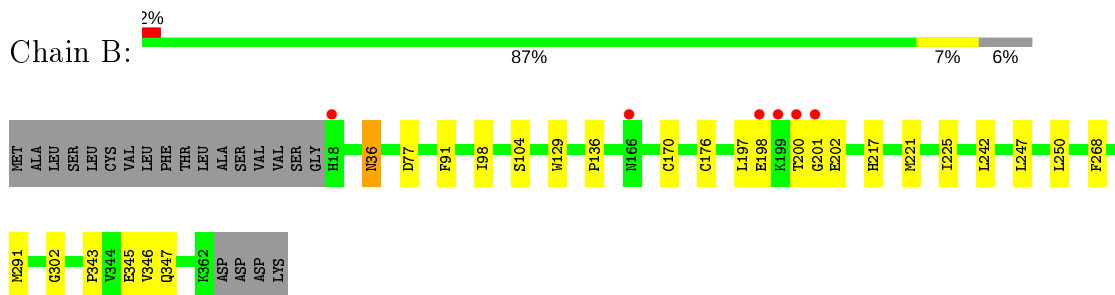
### 3 Residue-property plots [i](#)

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: MONOTREME LACTATING PROTEIN



- Molecule 1: MONOTREME LACTATING PROTEIN



## 4 Data and refinement statistics

Property	Value	Source
Space group	P 1	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	57.15Å 59.72Å 63.07Å 80.15° 82.98° 89.27°	Depositor
Resolution (Å)	61.68 – 1.82 46.75 – 1.82	Depositor EDS
% Data completeness (in resolution range)	99.4 (61.68-1.82) 99.5 (46.75-1.82)	Depositor EDS
$R_{merge}$	0.09	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.12 (at 1.82Å)	Xtrriage
Refinement program	REFMAC 5.8.0073	Depositor
R, $R_{free}$	0.166 , 0.188 0.177 , 0.193	Depositor DCC
$R_{free}$ test set	3676 reflections (5.05%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	20.4	Xtrriage
Anisotropy	0.123	Xtrriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.35 , 42.9	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.49$ , $\langle L^2 \rangle = 0.32$	Xtrriage
Estimated twinning fraction	No twinning to report.	Xtrriage
$F_o, F_c$ correlation	0.96	EDS
Total number of atoms	6267	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	21.0	wwPDB-VP

Xtrriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 6.49% of the height of the origin peak. No significant pseudotranslation is detected.*

<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: GOL, NAG

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	0.45	0/2976	0.58	0/4044
1	B	0.45	0/2943	0.57	0/4000
All	All	0.45	0/5919	0.57	0/8044

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	A	2903	0	2815	24	0
1	B	2870	0	2790	38	0
2	A	14	0	13	0	0
2	B	14	0	13	0	0
3	A	12	0	16	0	0
3	B	6	0	8	0	0
4	A	226	0	0	2	0
4	B	222	0	0	3	0
All	All	6267	0	5655	52	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 4.



All (52) 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:343[B]:PRO:HB3	1:B:291[B]:MET:SD	1.75	1.25
1:A:291[B]:MET:SD	1:B:343:PRO:HB3	2.06	0.95
1:B:302:GLY:HA3	1:B:346[B]:VAL:HG21	1.52	0.88
1:A:343[B]:PRO:CB	1:B:291[B]:MET:SD	2.63	0.86
1:A:172:SER:OG	1:A:175[B]:GLU:HG3	1.79	0.82
1:B:225[B]:ILE:HG12	1:B:247[B]:LEU:HD21	1.60	0.82
1:A:291[B]:MET:SD	1:B:343:PRO:CB	2.74	0.75
1:B:346[B]:VAL:HG23	4:B:2212:HOH:O	1.88	0.74
1:A:343[B]:PRO:HB3	1:B:291[B]:MET:CG	2.21	0.70
1:B:225[A]:ILE:HD12	1:B:247[A]:LEU:HD22	1.78	0.66
1:A:291[B]:MET:HG2	4:A:2196:HOH:O	1.94	0.66
1:B:302:GLY:CA	1:B:346[B]:VAL:HG21	2.26	0.64
1:B:91[B]:PHE:CE2	1:B:217:HIS:ND1	2.66	0.63
1:B:225[B]:ILE:HG12	1:B:247[B]:LEU:CD2	2.29	0.60
1:B:198:GLU:N	1:B:198:GLU:OE1	2.34	0.59
1:A:91[B]:PHE:CE2	1:A:217:HIS:ND1	2.71	0.58
1:B:170[A]:CYS:HB2	1:B:176:CYS:SG	2.43	0.58
1:B:36:ASN:HD22	1:B:36:ASN:H	1.54	0.55
1:B:98[B]:ILE:HG22	1:B:104:SER:HB3	1.89	0.54
1:A:343[B]:PRO:CB	1:B:291[B]:MET:HG3	2.38	0.54
1:A:250[B]:LEU:C	1:A:250[B]:LEU:HD23	2.28	0.53
1:B:91[B]:PHE:CE2	1:B:217:HIS:CG	2.96	0.53
1:A:225:ILE:HD12	1:A:247[A]:LEU:HD22	1.89	0.53
1:A:343[B]:PRO:CB	1:B:291[B]:MET:CG	2.85	0.53
1:A:170[B]:CYS:HB2	1:A:176:CYS:SG	2.48	0.52
1:B:302:GLY:HA3	1:B:346[B]:VAL:CG2	2.32	0.52
1:A:91[B]:PHE:CE2	1:A:217:HIS:CG	2.98	0.52
1:B:346[B]:VAL:HG22	4:B:2210:HOH:O	2.09	0.51
1:A:343[B]:PRO:HG3	1:B:291[B]:MET:HG3	1.93	0.51
1:B:250[A]:LEU:C	1:B:250[A]:LEU:HD23	2.31	0.50
1:B:91[B]:PHE:CE2	1:B:217:HIS:CE1	3.00	0.50
1:A:91[B]:PHE:CE2	1:A:217:HIS:CE1	3.00	0.49
1:A:291[B]:MET:HG3	1:B:343:PRO:CB	2.42	0.49
1:A:306[A]:VAL:CG2	1:A:347[A]:GLN:HG3	2.43	0.49
1:B:345[B]:GLU:OE2	1:B:347:GLN:NE2	2.47	0.48
1:B:221:MET:O	1:B:225[B]:ILE:HG13	2.14	0.48
1:B:200:THR:HG21	1:B:202:GLU:CD	2.35	0.47
1:A:291[A]:MET:HG2	4:A:2190:HOH:O	2.15	0.47
1:A:306[A]:VAL:HG22	1:A:347[A]:GLN:HG3	1.99	0.45
1:A:343[B]:PRO:HB3	1:B:291[B]:MET:HG3	1.93	0.45

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:B:221:MET:HG3	1:B:250[B]:LEU:HD23	1.99	0.44
1:A:91[B]:PHE:HE2	1:A:217:HIS:ND1	2.12	0.44
1:B:36:ASN:HD22	1:B:36:ASN:N	2.16	0.44
1:B:170[A]:CYS:CB	1:B:176:CYS:SG	3.05	0.43
1:B:91[B]:PHE:HE2	1:B:217:HIS:ND1	2.14	0.43
1:A:59[B]:LEU:HD23	1:A:59[B]:LEU:HA	1.87	0.42
1:B:91[B]:PHE:CD2	1:B:136:PRO:HB3	2.54	0.42
1:B:200:THR:CG2	1:B:202:GLU:HG3	2.50	0.42
1:B:225[B]:ILE:CG1	1:B:247[B]:LEU:HD21	2.39	0.41
1:A:170[B]:CYS:CB	1:A:176:CYS:SG	3.07	0.41
1:B:197:LEU:O	1:B:201:GLY:N	2.53	0.41
1:B:129:TRP:HD1	4:B:2068:HOH:O	2.04	0.41

There are no symmetry-related clashes.

## 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	366/366 (100%)	363 (99%)	3 (1%)	0	100	100
1	B	361/366 (99%)	360 (100%)	1 (0%)	0	100	100
All	All	727/732 (99%)	723 (99%)	4 (1%)	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	A	317/313 (101%)	314 (99%)	3 (1%)	78	74
1	B	311/313 (99%)	307 (99%)	4 (1%)	69	61
All	All	628/626 (100%)	621 (99%)	7 (1%)	76	67

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

Mol	Chain	Res	Type
1	A	152[A]	ASP
1	A	152[B]	ASP
1	A	268	PHE
1	B	36	ASN
1	B	77	ASP
1	B	242	LEU
1	B	268	PHE

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	B	36	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	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z  > 2	Counts	RMSZ	# Z  > 2
3	GOL	B	1363	-	5,5,5	0.21	0	5,5,5	0.44	0
2	NAG	A	400	1	14,14,15	0.52	0	17,19,21	1.22	1 (5%)
3	GOL	A	1362	-	5,5,5	0.30	0	5,5,5	0.51	0
3	GOL	A	1361	-	5,5,5	0.39	0	5,5,5	1.04	0
2	NAG	B	400	1	14,14,15	0.53	0	17,19,21	1.19	3 (17%)

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	GOL	B	1363	-	-	1/4/4/4	-
2	NAG	A	400	1	-	0/6/23/26	0/1/1/1
3	GOL	A	1362	-	-	2/4/4/4	-
3	GOL	A	1361	-	-	0/4/4/4	-
2	NAG	B	400	1	-	0/6/23/26	0/1/1/1

There are no bond length outliers.

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	A	400	NAG	C1-O5-C5	2.78	115.96	112.19
2	B	400	NAG	C1-O5-C5	2.58	115.68	112.19
2	B	400	NAG	O5-C5-C4	-2.39	105.01	110.83
2	B	400	NAG	O7-C7-C8	-2.02	118.30	122.06

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	A	1362	GOL	C1-C2-C3-O3
3	A	1362	GOL	O2-C2-C3-O3

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Mol	Chain	Res	Type	Atoms
3	B	1363	GOL	O1-C1-C2-O2

There are no ring outliers.

No monomer is involved in short contacts.

## 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	343/366 (93%)	-0.17	6 (1%) 70 66	11, 18, 36, 51	0
1	B	345/366 (94%)	-0.18	6 (1%) 70 66	11, 20, 33, 65	0
All	All	688/732 (93%)	-0.18	12 (1%) 70 66	11, 19, 36, 65	0

All (12) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	48[A]	ASP	3.6
1	A	354	GLY	3.3
1	B	200	THR	3.0
1	B	199	LYS	2.9
1	A	18	HIS	2.7
1	B	198	GLU	2.5
1	B	18	HIS	2.4
1	A	166	ASN	2.4
1	A	347[A]	GLN	2.2
1	A	110	HIS	2.1
1	B	201	GLY	2.1
1	B	166	ASN	2.1

### 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<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	B-factors( $\text{\AA}^2$ )	Q<0.9
2	NAG	A	400	14/15	0.66	0.26	34,43,45,46	0
2	NAG	B	400	14/15	0.80	0.14	39,43,47,49	0
3	GOL	A	1362	6/6	0.92	0.16	28,32,35,38	0
3	GOL	B	1363	6/6	0.92	0.15	29,32,34,37	0
3	GOL	A	1361	6/6	0.94	0.16	22,31,38,47	0

## 6.5 Other polymers [i](#)

There are no such residues in this entry.