



# Full wwPDB X-ray Structure Validation Report ⓘ

Feb 12, 2017 – 09:16 pm GMT

PDB ID : 3U0N  
Title : Crystal structure of the engineered fluorescent protein mRuby, crystal form 2  
Authors : Akerboom, J.; Looger, L.L.; Schreiter, E.R.  
Deposited on : 2011-09-28  
Resolution : 1.60 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

<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

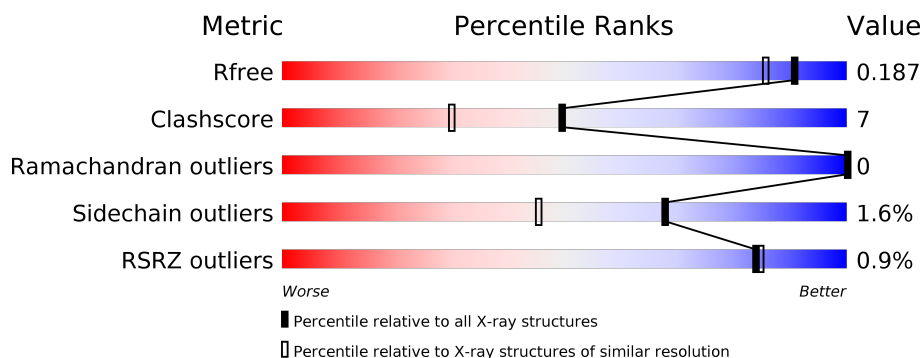
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

## *X-RAY DIFFRACTION*

The reported resolution of this entry is 1.60 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	2696 (1.60-1.60)
Clashscore	112137	2967 (1.60-1.60)
Ramachandran outliers	110173	2887 (1.60-1.60)
Sidechain outliers	110143	2886 (1.60-1.60)
RSRZ outliers	101464	2714 (1.60-1.60)

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	230	<div> <div></div> <div>82%</div> <div>13%</div> <div>..</div> </div>

## 2 Entry composition [i](#)

There are 3 unique types of molecules in this entry. The entry contains 2057 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.

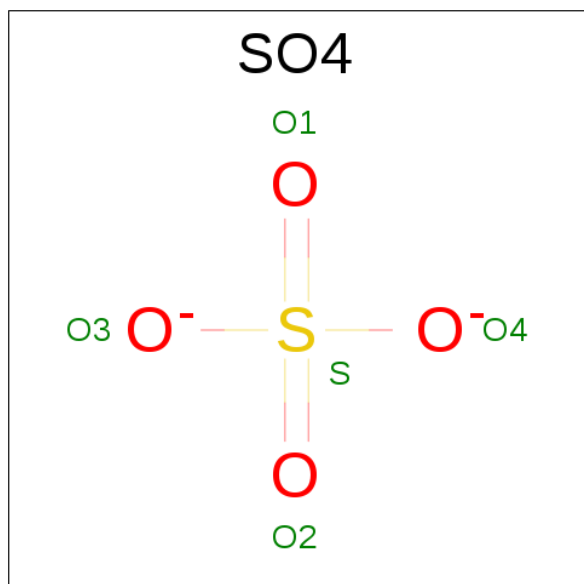
- Molecule 1 is a protein called mRuby.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	222	1852	1185	315	332	20	0	21	0

There are 9 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-4	ASP	-	EXPRESSION TAG	PDB 3U0N
A	-3	ARG	-	EXPRESSION TAG	PDB 3U0N
A	-2	TRP	-	EXPRESSION TAG	PDB 3U0N
A	-1	GLY	-	EXPRESSION TAG	PDB 3U0N
A	0	SER	-	EXPRESSION TAG	PDB 3U0N
A	64	NRQ	MET	CHROMOPHORE	PDB 3U0N
A	64	NRQ	TYR	CHROMOPHORE	PDB 3U0N
A	64	NRQ	GLY	CHROMOPHORE	PDB 3U0N
A	196	SER	ASP	ENGINEERED MUTATION	PDB 3U0N

- Molecule 2 is SULFATE ION (three-letter code: SO<sub>4</sub>) (formula: O<sub>4</sub>S).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
2	A	1	Total	O	S	0	0
			5	4	1		
2	A	1	Total	O	S	0	0
			5	4	1		

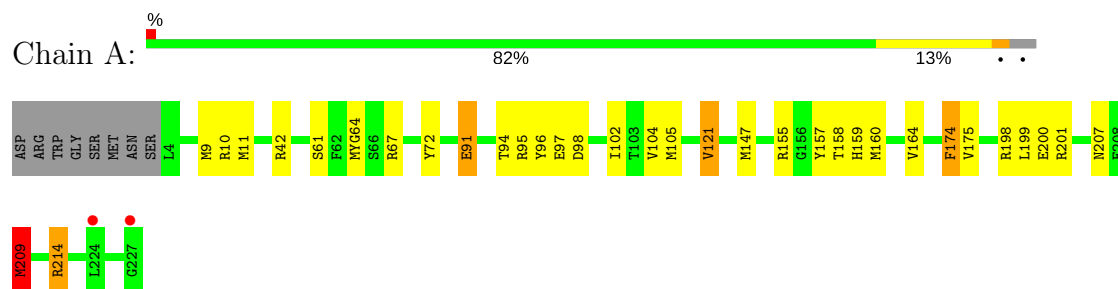
- Molecule 3 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
3	A	195	Total	O	0	4
			195	195		

### 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: mRuby



## 4 Data and refinement statistics

Property	Value	Source
Space group	I 2 2 2	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	67.27Å 82.52Å 88.91Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	27.89 – 1.60 27.89 – 1.60	Depositor EDS
% Data completeness (in resolution range)	99.8 (27.89-1.60) 99.8 (27.89-1.60)	Depositor EDS
$R_{merge}$	(Not available)	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	4.81 (at 1.60Å)	Xtriage
Refinement program	REFMAC 5.6.0111	Depositor
R, $R_{free}$	0.154 , 0.179 0.165 , 0.187	Depositor DCC
$R_{free}$ test set	1673 reflections (5.35%)	DCC
Wilson B-factor (Å <sup>2</sup> )	11.3	Xtriage
Anisotropy	0.346	Xtriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.42 , 47.8	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.49$ , $\langle L^2 \rangle = 0.32$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
$F_o, F_c$ correlation	0.96	EDS
Total number of atoms	2057	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	14.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 7.33% 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: NRQ, SO4

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	1.50	15/1922 (0.8%)	1.36	18/2582 (0.7%)

All (15) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	164	VAL	CB-CG2	-7.55	1.36	1.52
1	A	97	GLU	CB-CG	-6.96	1.39	1.52
1	A	91[A]	GLU	CD-OE2	6.69	1.33	1.25
1	A	91[B]	GLU	CD-OE2	6.69	1.33	1.25
1	A	201	ARG	CG-CD	-6.66	1.35	1.51
1	A	174	PHE	CD1-CE1	-6.21	1.26	1.39
1	A	97	GLU	CG-CD	6.08	1.61	1.51
1	A	175	VAL	CB-CG1	-6.03	1.40	1.52
1	A	61	SER	CA-CB	-6.00	1.44	1.52
1	A	91[A]	GLU	CG-CD	6.00	1.60	1.51
1	A	91[B]	GLU	CG-CD	6.00	1.60	1.51
1	A	72	TYR	CG-CD2	5.90	1.46	1.39
1	A	95	ARG	CG-CD	-5.57	1.38	1.51
1	A	121[A]	VAL	CB-CG1	5.56	1.64	1.52
1	A	121[B]	VAL	CB-CG1	5.56	1.64	1.52

All (18) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	209[A]	MET	CG-SD-CE	9.57	115.51	100.20
1	A	209[B]	MET	CG-SD-CE	9.57	115.51	100.20
1	A	42	ARG	NE-CZ-NH1	8.69	124.64	120.30
1	A	214[A]	ARG	NE-CZ-NH1	8.63	124.62	120.30
1	A	214[B]	ARG	NE-CZ-NH1	8.63	124.62	120.30
1	A	98	ASP	CB-CG-OD1	8.15	125.63	118.30

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Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	98	ASP	CB-CG-OD2	-7.63	111.44	118.30
1	A	72	TYR	CB-CG-CD2	6.25	124.75	121.00
1	A	95	ARG	CG-CD-NE	-6.17	98.85	111.80
1	A	96	TYR	CB-CG-CD2	-6.09	117.34	121.00
1	A	42	ARG	NE-CZ-NH2	-5.98	117.31	120.30
1	A	9	MET	CG-SD-CE	5.66	109.25	100.20
1	A	201	ARG	CA-CB-CG	-5.55	101.19	113.40
1	A	214[A]	ARG	NE-CZ-NH2	-5.38	117.61	120.30
1	A	214[B]	ARG	NE-CZ-NH2	-5.38	117.61	120.30
1	A	155	ARG	NE-CZ-NH1	5.29	122.95	120.30
1	A	10	ARG	NE-CZ-NH1	-5.19	117.71	120.30
1	A	157	TYR	CB-CG-CD2	-5.01	117.99	121.00

There are no chirality outliers.

There are no planarity outliers.

## 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	1852	0	1852	24	0
2	A	10	0	0	0	0
3	A	195	0	0	7	1
All	All	2057	0	1852	25	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 7.

All (25) 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:64[B]:NRQ:OH	1:A:158[B]:THR:HG21	1.73	0.88
1:A:207[A]:ASN:OD1	3:A:347:HOH:O	1.90	0.87
1:A:64[A]:NRQ:HE1	1:A:199:LEU:HB2	1.57	0.85
1:A:207[A]:ASN:OD1	1:A:209[A]:MET:HG3	1.85	0.75
1:A:91[A]:GLU:HG2	3:A:290:HOH:O	1.87	0.73

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:64[A]:NRQ:CE1	1:A:199:LEU:HB2	2.18	0.73
1:A:67:ARG:HH21	1:A:147[A]:MET:CE	2.04	0.70
1:A:67:ARG:NH2	1:A:147[A]:MET:CE	2.58	0.65
1:A:102[B]:ILE:HD11	1:A:121[B]:VAL:CG1	2.30	0.62
1:A:158[A]:THR:CG2	1:A:160:MET:HG3	2.30	0.62
1:A:159:HIS:CE1	3:A:315:HOH:O	2.54	0.59
1:A:64[A]:NRQ:CE1	1:A:199:LEU:HD22	2.33	0.59
1:A:104:VAL:HG22	1:A:121[A]:VAL:HG12	1.87	0.55
1:A:67:ARG:NH2	1:A:147[A]:MET:HE2	2.23	0.54
1:A:200:GLU:OE1	1:A:214[B]:ARG:NE	2.42	0.53
1:A:207[A]:ASN:CG	1:A:209[A]:MET:HG3	2.30	0.51
1:A:64[A]:NRQ:CZ	1:A:199:LEU:HB2	2.43	0.48
1:A:67:ARG:NH2	1:A:147[A]:MET:HE1	2.30	0.47
1:A:94:THR:HA	1:A:174:PHE:HB3	1.97	0.45
1:A:64[A]:NRQ:OH	1:A:199:LEU:HB2	2.17	0.45
1:A:207[A]:ASN:CG	3:A:347:HOH:O	2.50	0.44
1:A:105:MET:HE3	3:A:290:HOH:O	2.19	0.41
1:A:105:MET:CE	3:A:290:HOH:O	2.69	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)
3:A:263:HOH:O	3:A:296:HOH:O[8_555]	2.15	0.05

## 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	237/230 (103%)	235 (99%)	2 (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	203/190 (107%)	198 (98%)	5 (2%)	53 25

All (5) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	11[A]	MET
1	A	11[B]	MET
1	A	198	ARG
1	A	209[A]	MET
1	A	209[B]	MET

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

### 5.3.3 RNA [i](#)

There are no RNA molecules in this entry.

## 5.4 Non-standard residues in protein, DNA, RNA chains [i](#)

2 non-standard protein/DNA/RNA residues 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	$\# Z  > 2$
1	NRQ	A	64[A]	-	23,24,25	3.61	7 (30%)	25,32,34	2.83	7 (28%)
1	NRQ	A	64[B]	-	23,24,25	3.88	7 (30%)	25,32,34	4.68	8 (32%)

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
1	NRQ	A	64[A]	-	-	0/9/31/32	0/2/2/2
1	NRQ	A	64[B]	-	-	0/9/31/32	0/2/2/2

All (14) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	64[B]	NRQ	CA2-C2	-10.24	1.38	1.48
1	A	64[A]	NRQ	CA2-C2	-10.24	1.38	1.48
1	A	64[B]	NRQ	CA3-N3	-2.65	1.41	1.47
1	A	64[A]	NRQ	CA3-N3	-2.65	1.41	1.47
1	A	64[B]	NRQ	C2-N3	-2.61	1.33	1.39
1	A	64[A]	NRQ	C2-N3	-2.61	1.33	1.39
1	A	64[B]	NRQ	CA2-N2	-2.00	1.34	1.38
1	A	64[A]	NRQ	CA2-N2	-2.00	1.34	1.38
1	A	64[B]	NRQ	O2-C2	2.54	1.28	1.23
1	A	64[A]	NRQ	O2-C2	2.54	1.28	1.23
1	A	64[B]	NRQ	CA1-N1	4.71	1.39	1.27
1	A	64[A]	NRQ	CA1-N1	4.71	1.39	1.27
1	A	64[A]	NRQ	CB2-CA2	11.54	1.45	1.35
1	A	64[B]	NRQ	CB2-CA2	13.60	1.47	1.35

All (15) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	64[B]	NRQ	CB2-CA2-N2	-14.21	107.27	128.79
1	A	64[A]	NRQ	CG2-CB2-CA2	-8.63	120.17	130.19
1	A	64[B]	NRQ	O3-C3-CA3	-2.87	116.90	126.38
1	A	64[A]	NRQ	O3-C3-CA3	-2.87	116.90	126.38
1	A	64[B]	NRQ	CD2-CG2-CB2	-2.56	112.44	121.23
1	A	64[B]	NRQ	CD2-CE2-CZ	-2.41	117.14	119.88
1	A	64[B]	NRQ	CG2-CB2-CA2	-2.22	127.61	130.19
1	A	64[A]	NRQ	CE1-CD1-CG2	-2.19	118.41	121.28
1	A	64[A]	NRQ	CB2-CA2-C2	-2.11	119.67	122.32
1	A	64[A]	NRQ	CD1-CG2-CD2	2.81	121.86	117.63
1	A	64[B]	NRQ	C2-CA2-N2	3.87	111.75	108.93
1	A	64[A]	NRQ	C2-CA2-N2	3.87	111.75	108.93
1	A	64[B]	NRQ	CA2-C2-N3	8.24	106.97	103.30
1	A	64[A]	NRQ	CA2-C2-N3	8.24	106.97	103.30
1	A	64[B]	NRQ	CB2-CA2-C2	14.65	140.75	122.32

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

2 monomers are involved in 7 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	A	64[A]	NRQ	6	0
1	A	64[B]	NRQ	1	0

## 5.5 Carbohydrates [i](#)

There are no carbohydrates in this entry.

## 5.6 Ligand geometry [i](#)

2 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	$\# Z  > 2$
2	SO4	A	228	-	4,4,4	0.80	0	6,6,6	0.48	0
2	SO4	A	229	-	4,4,4	0.51	0	6,6,6	0.50	0

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
2	SO4	A	228	-	-	0/0/0/0	0/0/0/0
2	SO4	A	229	-	-	0/0/0/0	0/0/0/0

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

## 5.7 Other polymers

There are no such residues in this entry.

## 5.8 Polymer linkage issues

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	221/230 (96%)	-0.32	2 (0%) 84 85	6, 10, 25, 47	0

All (2) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	227	GLY	3.1
1	A	224	LEU	2.8

### 6.2 Non-standard residues in protein, DNA, RNA chains [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(Å <sup>2</sup> )	Q<0.9
1	NRQ	A	64[B]	23/24	0.95	0.13	-	11,14,17,18	8
1	NRQ	A	64[A]	23/24	0.95	0.13	-	11,15,25,30	8

### 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
2	SO4	A	228	5/5	0.99	0.06	-0.76	11,12,14,17	0
2	SO4	A	229	5/5	0.98	0.12	-	46,46,50,53	0

## 6.5 Other polymers [i](#)

There are no such residues in this entry.