



# Full wwPDB X-ray Structure Validation Report ⓘ

May 13, 2024 – 12:18 PM EDT

PDB ID : 8SFV  
Title : High affinity nanobodies to GFP  
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Deposited on : 2023-04-11  
Resolution : 1.83 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.

The types of validation reports are described at

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

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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 : 2.36.2  
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) : 2.36.2

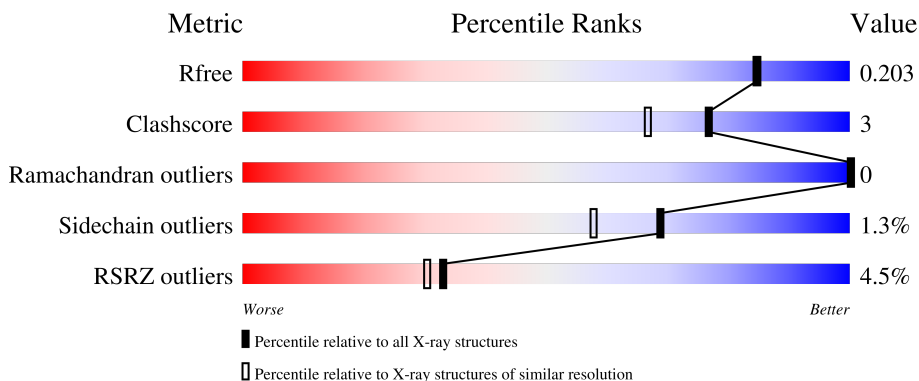
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*X-RAY DIFFRACTION*


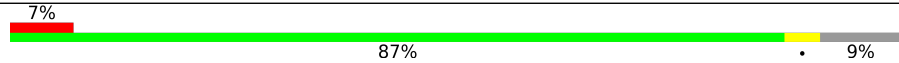
The reported resolution of this entry is 1.83 Å.

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	4003 (1.86-1.82)
Clashscore	141614	4233 (1.86-1.82)
Ramachandran outliers	138981	4185 (1.86-1.82)
Sidechain outliers	138945	4186 (1.86-1.82)
RSRZ outliers	127900	3957 (1.86-1.82)

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  $\geq 3$ , 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	253	
2	B	142	

## 2 Entry composition [i](#)

There are 6 unique types of molecules in this entry. The entry contains 5778 atoms, of which 2742 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 Green fluorescent protein.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
			Total	C	H	N	O	S			
1	A	228	3590	1163	1764	310	347	6	0	0	0

There are 22 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	ALA	-	insertion	UNP P42212
A	66	CR2	SER	chromophore	UNP P42212
A	66	CR2	TYR	chromophore	UNP P42212
A	66	CR2	GLY	chromophore	UNP P42212
A	72	ALA	SER	conflict	UNP P42212
A	177	HIS	GLN	conflict	UNP P42212
A	239	GLY	-	expression tag	UNP P42212
A	240	LEU	-	expression tag	UNP P42212
A	241	GLU	-	expression tag	UNP P42212
A	242	VAL	-	expression tag	UNP P42212
A	243	LEU	-	expression tag	UNP P42212
A	244	PHE	-	expression tag	UNP P42212
A	245	GLN	-	expression tag	UNP P42212
A	246	GLY	-	expression tag	UNP P42212
A	247	PRO	-	expression tag	UNP P42212
A	248	SER	-	expression tag	UNP P42212
A	249	HIS	-	expression tag	UNP P42212
A	250	HIS	-	expression tag	UNP P42212
A	251	HIS	-	expression tag	UNP P42212
A	252	HIS	-	expression tag	UNP P42212
A	253	HIS	-	expression tag	UNP P42212
A	254	HIS	-	expression tag	UNP P42212

- Molecule 2 is a protein called LaG19.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace	
			Total	C	H	N	O				S
2	B	129	1941	616	955	173	192	5	0	0	0

- 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
			Total	C	H	O		
3	A	1	14	3	8	3	0	0
3	B	1	13	3	7	3	0	0
3	B	1	14	3	8	3	0	0

- Molecule 4 is SULFATE ION (three-letter code: SO4) (formula: O<sub>4</sub>S).



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

- Molecule 5 is SODIUM ION (three-letter code: NA) (formula: Na).

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
5	A	7	Total Na 7 7	0	0
5	B	4	Total Na 4 4	0	0

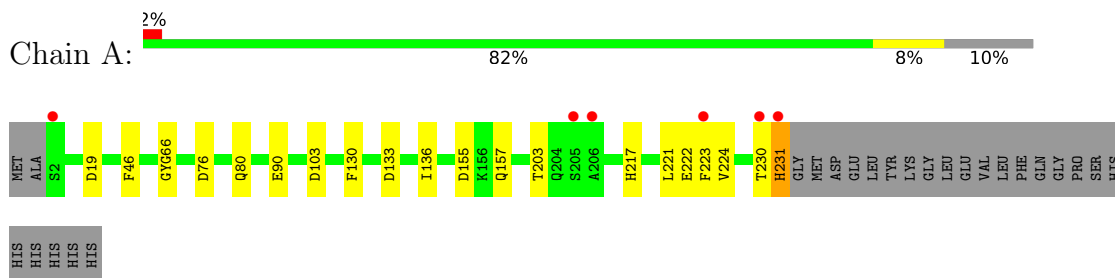
- Molecule 6 is water.

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
6	A	121	Total O 121 121	0	0
6	B	49	Total O 49 49	0	0

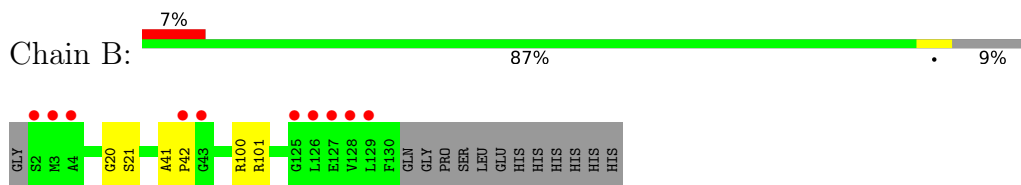
### 3 Residue-property plots [i](#)

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: Green fluorescent protein



- Molecule 2: LaG19



## 4 Data and refinement statistics

Property	Value	Source
Space group	I 41 2 2	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	111.25Å 111.25Å 193.80Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	41.25 – 1.83 41.25 – 1.80	Depositor EDS
% Data completeness (in resolution range)	96.5 (41.25-1.83) 95.8 (41.25-1.80)	Depositor EDS
$R_{merge}$	0.08	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	2.83 (at 1.81Å)	Xtrriage
Refinement program	PHENIX 1.19.2-4158	Depositor
R, $R_{free}$	0.183 , 0.206 0.180 , 0.203	Depositor DCC
$R_{free}$ test set	2000 reflections (3.70%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	31.3	Xtrriage
Anisotropy	0.029	Xtrriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.40 , 48.8	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.48$ , $\langle L^2 \rangle = 0.32$	Xtrriage
Estimated twinning fraction	No twinning to report.	Xtrriage
$F_o, F_c$ correlation	0.97	EDS
Total number of atoms	5778	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	50.0	wwPDB-VP

Xtrriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 3.02% 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

### 5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: NA, CR2, SO4, GOL

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	0.59	0/1850	0.72	0/2500
2	B	0.50	0/1004	0.71	0/1356
All	All	0.56	0/2854	0.72	0/3856

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

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	1826	1764	1764	12	0
2	B	986	955	955	5	0
3	A	6	8	8	1	0
3	B	12	15	16	4	0
4	A	10	0	0	0	0
4	B	15	0	0	0	0
5	A	7	0	0	0	0
5	B	4	0	0	0	0
6	A	121	0	0	1	1
6	B	49	0	0	0	0
All	All	3036	2742	2743	17	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 3.

All (17) 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)
2:B:20:GLY:HA2	3:B:202:GOL:H32	1.81	0.62
1:A:130:PHE:HE1	1:A:136:ILE:HD13	1.65	0.59
1:A:230:THR:O	1:A:231:HIS:HB3	2.02	0.59
1:A:221:LEU:HD21	1:A:223:PHE:CE2	2.42	0.55
1:A:130:PHE:CE1	1:A:136:ILE:HD13	2.44	0.50
2:B:41:ALA:HB1	2:B:42:PRO:HD2	1.93	0.50
2:B:20:GLY:CA	3:B:202:GOL:H11	2.42	0.50
1:A:221:LEU:HG	1:A:222:GLU:N	2.30	0.46
2:B:21:SER:H	3:B:202:GOL:H11	1.81	0.45
1:A:76:ASP:H	3:A:301:GOL:H2	1.82	0.45
1:A:46:PHE:O	1:A:217:HIS:HB2	2.18	0.43
1:A:133:ASP:HB3	6:A:440:HOH:O	2.19	0.42
1:A:103:ASP:OD1	1:A:130:PHE:HA	2.21	0.41
1:A:90:GLU:H	1:A:90:GLU:CD	2.23	0.41
2:B:101:ARG:HH12	3:B:201:GOL:C2	2.32	0.41
1:A:155:ASP:OD1	1:A:157:GLN:HB2	2.21	0.41
1:A:203:THR:HG22	1:A:224:VAL:HG13	2.02	0.41

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)
6:A:467:HOH:O	6:A:516:HOH:O[10_555]	2.08	0.12

## 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	223/253 (88%)	220 (99%)	3 (1%)	0	100	100
2	B	127/142 (89%)	126 (99%)	1 (1%)	0	100	100
All	All	350/395 (89%)	346 (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	198/219 (90%)	195 (98%)	3 (2%)	65	52
2	B	103/114 (90%)	102 (99%)	1 (1%)	76	68
All	All	301/333 (90%)	297 (99%)	4 (1%)	69	58

All (4) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	19	ASP
1	A	80	GLN
1	A	231	HIS
2	B	100	ARG

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

Mol	Chain	Res	Type
2	B	17	GLN

### 5.3.3 RNA [i](#)

There are no RNA molecules in this entry.

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

1 non-standard protein/DNA/RNA residue is 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	CR2	A	66	5,1	20,20,21	2.64	7 (35%)	25,27,29	3.17	7 (28%)

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	CR2	A	66	5,1	-	1/6/25/26	0/2/2/2

All (7) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	66	CR2	CA2-C2	6.35	1.54	1.48
1	A	66	CR2	C1-N3	6.01	1.46	1.37
1	A	66	CR2	C1-N2	4.60	1.40	1.32
1	A	66	CR2	CG2-CB2	2.89	1.52	1.46
1	A	66	CR2	CA2-N2	2.62	1.44	1.38
1	A	66	CR2	C2-N3	2.49	1.45	1.39
1	A	66	CR2	CB2-CA2	-2.25	1.33	1.35

All (7) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	A	66	CR2	CA2-C2-N3	9.42	107.82	103.37
1	A	66	CR2	C2-N3-C1	-8.23	103.97	107.99
1	A	66	CR2	O2-C2-CA2	-6.74	127.18	130.96
1	A	66	CR2	C1-CA1-N1	-3.31	105.54	112.85
1	A	66	CR2	O3-C3-CA3	-3.00	117.33	126.39
1	A	66	CR2	C2-CA2-N2	-2.71	107.03	108.93
1	A	66	CR2	N3-C1-N2	2.29	114.50	111.76

There are no chirality outliers.

All (1) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	A	66	CR2	C3-CA3-N3-C2

There are no ring outliers.

No monomer is involved in short contacts.

## 5.5 Carbohydrates [i](#)

There are no monosaccharides in this entry.

## 5.6 Ligand geometry [i](#)

Of 19 ligands modelled in this entry, 11 are monoatomic - leaving 8 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	GOL	B	202	-	5,5,5	0.76	0	5,5,5	0.59	0
4	SO4	B	203	-	4,4,4	0.20	0	6,6,6	0.12	0
3	GOL	A	301	-	5,5,5	0.76	0	5,5,5	0.62	0
4	SO4	A	303	-	4,4,4	0.15	0	6,6,6	0.09	0
4	SO4	A	302	-	4,4,4	0.15	0	6,6,6	0.07	0
4	SO4	B	204	-	4,4,4	0.19	0	6,6,6	0.15	0
4	SO4	B	205	-	4,4,4	0.16	0	6,6,6	0.15	0
3	GOL	B	201	-	5,5,5	0.79	0	5,5,5	0.98	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
3	GOL	B	202	-	-	4/4/4/4	-

*Continued from previous page...*

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
3	GOL	B	201	-	-	1/4/4/4	-
3	GOL	A	301	-	-	4/4/4/4	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (9) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	B	202	GOL	C1-C2-C3-O3
3	B	202	GOL	O2-C2-C3-O3
3	A	301	GOL	O1-C1-C2-C3
3	A	301	GOL	C1-C2-C3-O3
3	B	201	GOL	O1-C1-C2-C3
3	B	202	GOL	O1-C1-C2-C3
3	B	202	GOL	O1-C1-C2-O2
3	A	301	GOL	O1-C1-C2-O2
3	A	301	GOL	O2-C2-C3-O3

There are no ring outliers.

3 monomers are involved in 5 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
3	B	202	GOL	3	0
3	A	301	GOL	1	0
3	B	201	GOL	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, 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	227/253 (89%)	0.27	6 (2%) 56 53	27, 39, 68, 101	0
2	B	129/142 (90%)	0.35	10 (7%) 13 12	33, 45, 77, 107	0
All	All	356/395 (90%)	0.30	16 (4%) 33 30	27, 42, 76, 107	0

All (16) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	B	126	LEU	6.7
2	B	2	SER	4.3
2	B	4	ALA	4.2
1	A	231	HIS	4.1
2	B	125	GLY	3.7
2	B	127	GLU	3.6
2	B	129	LEU	3.4
1	A	230	THR	2.9
2	B	43	GLY	2.8
1	A	2	SER	2.6
2	B	128	VAL	2.5
2	B	3	MET	2.5
1	A	223	PHE	2.1
1	A	206	ALA	2.0
2	B	42	PRO	2.0
1	A	205	SER	2.0

### 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. 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(Å <sup>2</sup> )	Q<0.9
1	CR2	A	66	19/20	0.97	0.21	28,34,41,44	0

### 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(Å <sup>2</sup> )	Q<0.9
3	GOL	B	202	6/6	0.65	0.18	70,85,97,104	0
4	SO4	A	303	5/5	0.72	0.37	95,107,139,165	0
4	SO4	B	203	5/5	0.73	0.30	83,92,102,171	0
4	SO4	B	204	5/5	0.81	0.28	96,101,105,140	0
5	NA	B	209	1/1	0.83	0.11	58,58,58,58	0
4	SO4	A	302	5/5	0.84	0.19	86,108,139,163	0
4	SO4	B	205	5/5	0.89	0.16	75,79,103,121	0
5	NA	B	207	1/1	0.91	0.40	67,67,67,67	0
5	NA	B	206	1/1	0.92	0.22	55,55,55,55	0
3	GOL	B	201	6/6	0.94	0.26	71,85,99,106	0
5	NA	A	306	1/1	0.94	0.10	57,57,57,57	0
5	NA	A	310	1/1	0.94	0.14	46,46,46,46	0
3	GOL	A	301	6/6	0.95	0.15	46,70,91,91	0
5	NA	B	208	1/1	0.96	0.28	61,61,61,61	0
5	NA	A	305	1/1	0.97	0.08	53,53,53,53	0
5	NA	A	308	1/1	0.98	0.15	40,40,40,40	0
5	NA	A	304	1/1	0.98	0.11	37,37,37,37	0
5	NA	A	307	1/1	0.98	0.21	31,31,31,31	1
5	NA	A	309	1/1	1.00	0.13	41,41,41,41	0

### 6.5 Other polymers [i](#)

There are no such residues in this entry.