



# wwPDB X-ray Structure Validation Summary Report ⓘ

Jun 23, 2024 – 02:45 PM EDT

PDB ID : 5M2M  
Title : Complex between human TNF alpha and Llama VHH3  
Authors : Cambillau, C.; Spinelli, S.; Desmyter, A.; de Haard, H.  
Deposited on : 2016-10-13  
Resolution : 2.30 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](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  
Xtriage (Phenix) : 1.13  
EDS : 2.37.1  
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) : 2.37.1

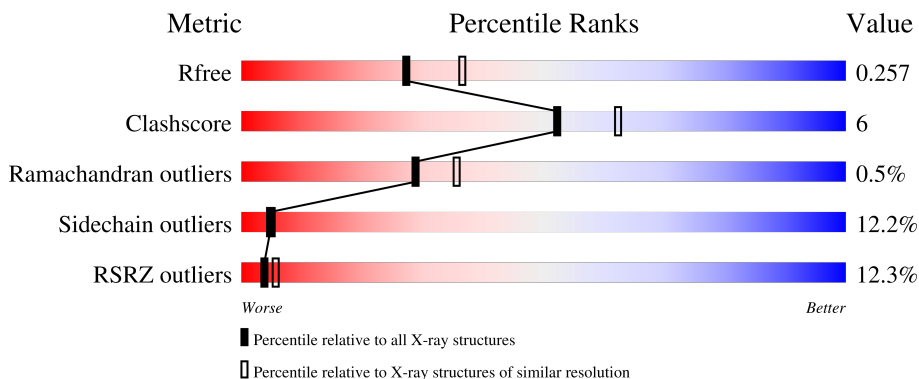
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*X-RAY DIFFRACTION*

The reported resolution of this entry is 2.30 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	5042 (2.30-2.30)
Clashscore	141614	5643 (2.30-2.30)
Ramachandran outliers	138981	5575 (2.30-2.30)
Sidechain outliers	138945	5575 (2.30-2.30)
RSRZ outliers	127900	4938 (2.30-2.30)

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	157	 8% 73% 18% . .
1	B	157	 13% 74% 18% . .
1	C	157	 11% 72% 18% 5% .
1	G	157	 17% 71% 20% . .
1	I	157	 18% 73% 17% 6% .

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Mol	Chain	Length	Quality of chain
1	M	157	<p>20% 67% 20% 7% 6%</p>
2	D	129	<p>5% 84% 12% ..</p>
2	E	129	<p>2% 84% 12% ..</p>
2	F	129	<p>2% 84% 12% ..</p>
2	H	129	<p>12% 82% 16% ..</p>
2	J	129	<p>15% 84% 12% ..</p>
2	N	129	<p>16% 85% 11% ..</p>

## 2 Entry composition [i](#)

There are 3 unique types of molecules in this entry. The entry contains 13516 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 Tumor necrosis factor.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	150	1149	736	197	214	2	0	0	0
1	B	151	1160	743	199	216	2	0	0	0
1	C	150	1154	740	198	214	2	0	0	0
1	G	150	1154	740	198	214	2	0	0	0
1	I	150	1157	740	198	217	2	0	0	0
1	M	148	1131	724	195	210	2	0	0	0

- Molecule 2 is a protein called Llama nanobody VHH3.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
2	D	127	984	612	173	196	3	0	0	0
2	E	127	984	612	173	196	3	0	0	0
2	F	127	984	612	173	196	3	0	0	0
2	H	127	984	612	173	196	3	0	0	0
2	J	127	984	612	173	196	3	0	0	0
2	N	127	980	611	170	196	3	0	0	0

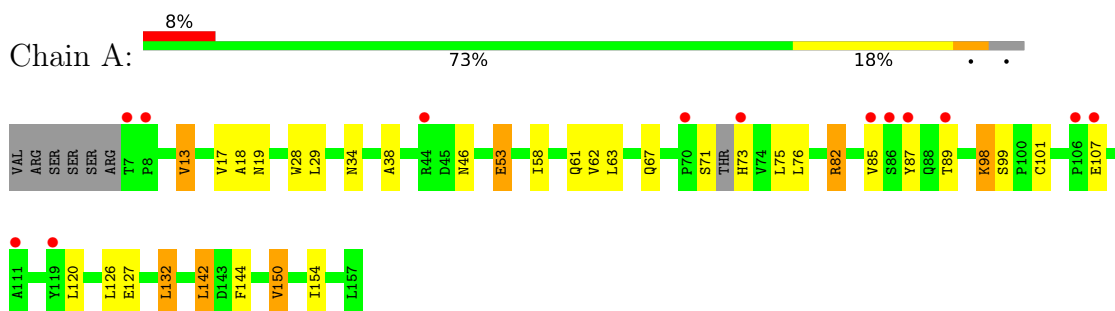
- Molecule 3 is water.

Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	A	92	Total O 92 92	0	0
3	B	93	Total O 93 93	0	0
3	C	94	Total O 94 94	0	0
3	D	78	Total O 78 78	0	0
3	E	88	Total O 88 88	0	0
3	F	86	Total O 86 86	0	0
3	G	36	Total O 36 36	0	0
3	H	23	Total O 23 23	0	0
3	I	36	Total O 36 36	0	0
3	J	31	Total O 31 31	0	0
3	M	32	Total O 32 32	0	0
3	N	22	Total O 22 22	0	0

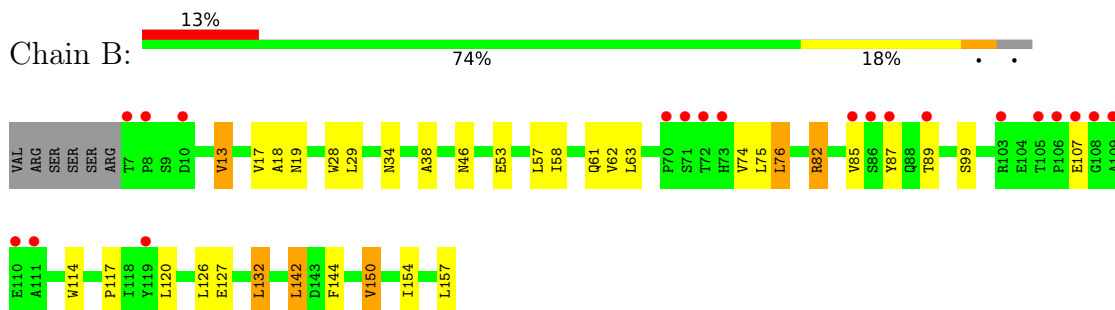
### 3 Residue-property plots [i](#)

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.

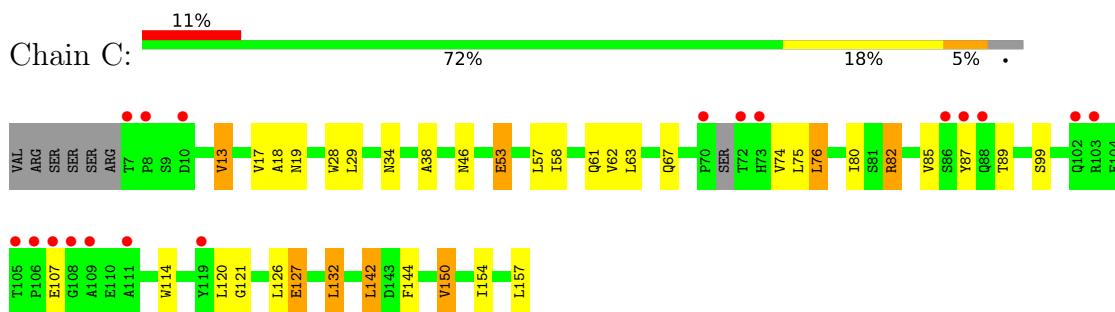
- Molecule 1: Tumor necrosis factor



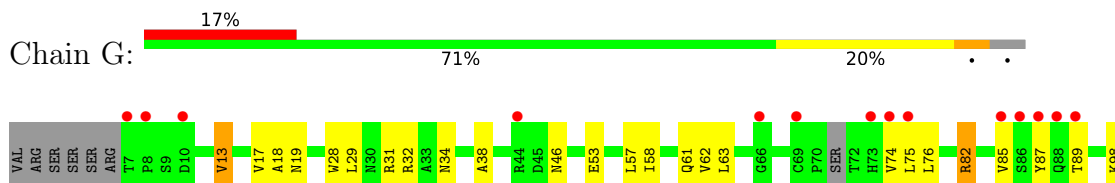
- Molecule 1: Tumor necrosis factor

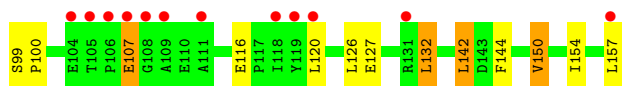


- Molecule 1: Tumor necrosis factor

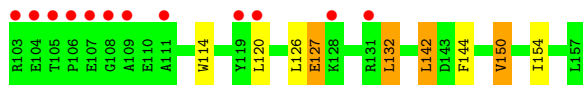


- Molecule 1: Tumor necrosis factor





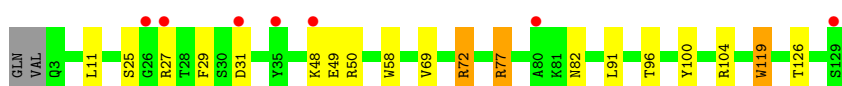
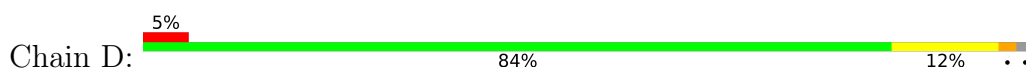
- Molecule 1: Tumor necrosis factor



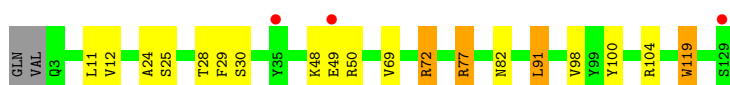
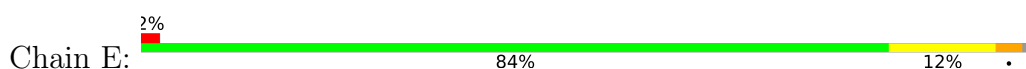
- Molecule 1: Tumor necrosis factor



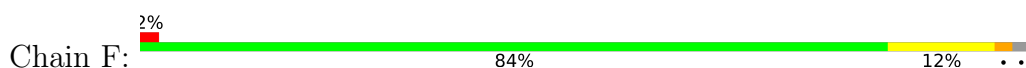
- Molecule 2: Llama nanobody VHH3



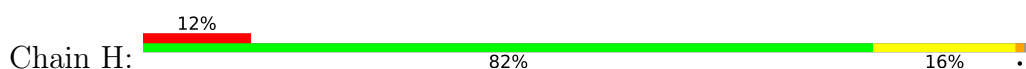
- Molecule 2: Llama nanobody VHH3

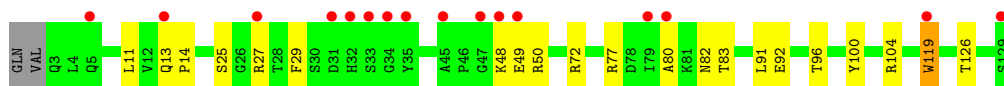


- Molecule 2: Llama nanobody VHH3

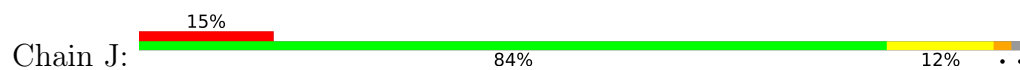


- Molecule 2: Llama nanobody VHH3

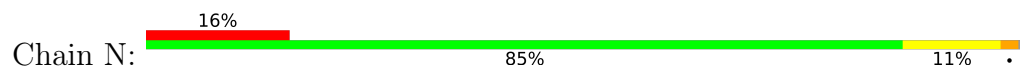




- Molecule 2: Llama nanobody VHH3



- Molecule 2: Llama nanobody VHH3





## 4 Data and refinement statistics i

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants a, b, c, $\alpha$ , $\beta$ , $\gamma$	145.38Å 83.84Å 150.06Å 90.00° 128.77° 90.00°	Depositor
Resolution (Å)	29.59 – 2.30 29.59 – 2.30	Depositor EDS
% Data completeness (in resolution range)	100.0 (29.59-2.30) 100.0 (29.59-2.30)	Depositor EDS
$R_{merge}$	0.09	Depositor
$R_{sym}$	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ <sup>1</sup>	3.96 (at 2.31Å)	Xtrriage
Refinement program	BUSTER 2.10.1	Depositor
R, $R_{free}$	0.211 , 0.247 0.222 , 0.257	Depositor DCC
$R_{free}$ test set	3229 reflections (5.16%)	wwPDB-VP
Wilson B-factor (Å <sup>2</sup> )	25.7	Xtrriage
Anisotropy	0.300	Xtrriage
Bulk solvent $k_{sol}$ (e/Å <sup>3</sup> ), $B_{sol}$ (Å <sup>2</sup> )	0.33 , 67.8	EDS
L-test for twinning <sup>2</sup>	$\langle  L  \rangle = 0.47$ , $\langle L^2 \rangle = 0.30$	Xtrriage
Estimated twinning fraction	0.000 for 1/2*h+3/2*k, 1/2*h-1/2*k, -h-k-l 0.000 for 1/2*h-3/2*k, -1/2*h-1/2*k, -h+k-l	Xtrriage
$F_o, F_c$ correlation	0.95	EDS
Total number of atoms	13516	wwPDB-VP
Average B, all atoms (Å <sup>2</sup> )	45.0	wwPDB-VP

Xtrriage's analysis on translational NCS is as follows: *The analyses of the Patterson function reveals a significant off-origin peak that is 89.13 % 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 3.8166e-08. The detected translational NCS is most likely also responsible for the elevated intensity ratio.*

<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](#)

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	0/1175	0.73	0/1603
1	B	0.50	0/1187	0.73	0/1620
1	C	0.49	0/1180	0.75	0/1609
1	G	0.42	0/1180	0.71	0/1609
1	I	0.42	0/1184	0.72	0/1616
1	M	0.46	0/1154	0.71	0/1571
2	D	0.41	0/1007	0.68	0/1366
2	E	0.39	0/1007	0.68	0/1366
2	F	0.40	0/1007	0.67	0/1366
2	H	0.37	0/1007	0.62	0/1366
2	J	0.37	0/1007	0.63	0/1366
2	N	0.37	0/1003	0.62	0/1361
All	All	0.43	0/13098	0.69	0/17819

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	1149	0	1128	21	0
1	B	1160	0	1147	20	0
1	C	1154	0	1141	21	0
1	G	1154	0	1141	19	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	I	1157	0	1139	19	0
1	M	1131	0	1112	26	0
2	D	984	0	922	6	0
2	E	984	0	922	7	0
2	F	984	0	922	6	0
2	H	984	0	922	5	0
2	J	984	0	922	6	0
2	N	980	0	915	6	0
3	A	92	0	0	2	0
3	B	93	0	0	0	0
3	C	94	0	0	0	0
3	D	78	0	0	0	0
3	E	88	0	0	0	0
3	F	86	0	0	0	0
3	G	36	0	0	0	0
3	H	23	0	0	0	0
3	I	36	0	0	0	0
3	J	31	0	0	0	0
3	M	32	0	0	0	0
3	N	22	0	0	0	0
All	All	13516	0	12333	139	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 6.

The worst 5 of 139 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:G:28:TRP:H	1:G:46:ASN:HD21	1.20	0.90
1:A:28:TRP:H	1:A:46:ASN:HD21	1.19	0.90
1:C:28:TRP:H	1:C:46:ASN:HD21	1.19	0.90
1:I:28:TRP:H	1:I:46:ASN:HD21	1.20	0.89
1:B:28:TRP:H	1:B:46:ASN:HD21	1.19	0.89

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	146/157 (93%)	137 (94%)	8 (6%)	1 (1%)	22	26
1	B	149/157 (95%)	142 (95%)	6 (4%)	1 (1%)	22	26
1	C	146/157 (93%)	139 (95%)	6 (4%)	1 (1%)	22	26
1	G	146/157 (93%)	137 (94%)	7 (5%)	2 (1%)	11	11
1	I	148/157 (94%)	138 (93%)	8 (5%)	2 (1%)	11	11
1	M	140/157 (89%)	132 (94%)	7 (5%)	1 (1%)	22	26
2	D	125/129 (97%)	123 (98%)	2 (2%)	0	100	100
2	E	125/129 (97%)	124 (99%)	1 (1%)	0	100	100
2	F	125/129 (97%)	123 (98%)	2 (2%)	0	100	100
2	H	125/129 (97%)	122 (98%)	3 (2%)	0	100	100
2	J	125/129 (97%)	123 (98%)	2 (2%)	0	100	100
2	N	125/129 (97%)	121 (97%)	4 (3%)	0	100	100
All	All	1625/1716 (95%)	1561 (96%)	56 (3%)	8 (0%)	29	35

5 of 8 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	107	GLU
1	B	107	GLU
1	I	71	SER
1	C	107	GLU
1	G	107	GLU

### 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	121/133 (91%)	105 (87%)	16 (13%)	4	4
1	B	123/133 (92%)	109 (89%)	14 (11%)	5	6
1	C	122/133 (92%)	107 (88%)	15 (12%)	4	5
1	G	122/133 (92%)	105 (86%)	17 (14%)	3	3
1	I	123/133 (92%)	106 (86%)	17 (14%)	3	3
1	M	119/133 (90%)	103 (87%)	16 (13%)	4	4
2	D	103/105 (98%)	91 (88%)	12 (12%)	5	6
2	E	103/105 (98%)	91 (88%)	12 (12%)	5	6
2	F	103/105 (98%)	92 (89%)	11 (11%)	6	7
2	H	103/105 (98%)	90 (87%)	13 (13%)	4	4
2	J	103/105 (98%)	92 (89%)	11 (11%)	6	7
2	N	102/105 (97%)	92 (90%)	10 (10%)	8	9
All	All	1347/1428 (94%)	1183 (88%)	164 (12%)	5	5

5 of 164 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	I	75	LEU
1	M	75	LEU
1	I	87	TYR
2	J	48	LYS
1	M	127	GLU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 30 such sidechains are listed below:

Mol	Chain	Res	Type
2	F	32	HIS
1	M	61	GLN
1	G	46	ASN
2	N	5	GLN
2	J	5	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](#)

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](#)

There are no ligands in this entry.

## 5.7 Other polymers [i](#)

There are no such residues in this entry.

## 5.8 Polymer linkage issues [i](#)

The following chains have linkage breaks:

Mol	Chain	Number of breaks
1	M	1

All chain breaks are listed below:

Model	Chain	Residue-1	Atom-1	Residue-2	Atom-2	Distance (Å)
1	M	106:PRO	C	107:GLU	N	2.95

## 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	150/157 (95%)	0.38	13 (8%) 10 14	8, 19, 82, 96	0
1	B	151/157 (96%)	0.54	20 (13%) 3 4	8, 18, 85, 107	0
1	C	150/157 (95%)	0.55	18 (12%) 4 6	9, 20, 85, 106	0
1	G	150/157 (95%)	1.10	26 (17%) 1 1	31, 52, 144, 199	0
1	I	150/157 (95%)	1.02	28 (18%) 1 1	34, 50, 134, 184	0
1	M	148/157 (94%)	1.16	32 (21%) 0 1	35, 53, 170, 207	0
2	D	127/129 (98%)	0.01	7 (5%) 25 31	9, 25, 59, 83	0
2	E	127/129 (98%)	0.16	3 (2%) 59 66	9, 29, 57, 107	0
2	F	127/129 (98%)	-0.03	3 (2%) 59 66	9, 28, 54, 86	0
2	H	127/129 (98%)	0.77	16 (12%) 3 5	34, 50, 86, 104	0
2	J	127/129 (98%)	0.95	19 (14%) 2 3	35, 54, 77, 111	0
2	N	127/129 (98%)	1.04	20 (15%) 2 2	32, 58, 89, 149	0
All	All	1661/1716 (96%)	0.65	205 (12%) 4 6	8, 43, 98, 207	0

The worst 5 of 205 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	G	87	TYR	16.7
1	I	87	TYR	12.9
1	M	87	TYR	12.5
1	B	87	TYR	12.3
1	C	87	TYR	11.6

### 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](#)

There are no ligands in this entry.

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

There are no such residues in this entry.