



wwPDB X-ray Structure Validation Summary Report ⓘ

May 23, 2016 – 04:17 PM EDT

PDB ID : 4TZG
Title : Crystal structure of eCGP123, an extremely thermostable green fluorescent protein
Authors : Close, D.W.; Don Paul, C.; Traore, D.A.K.; Wilce, M.C.J.; Prescott, M.; Bradbury, A.R.M.
Deposited on : 2014-07-10
Resolution : 2.10 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
<http://wwpdb.org/validation/2016/XrayValidationReportHelp>
with specific help available everywhere you see the ⓘ symbol.

The following versions of software and data (see [references ⓘ](#)) were used in the production of this report:

MolProbity : 4.02b-467
Mogul : 1.7.1 (RC1), CSD as537be (2016)
Xtriage (Phenix) : 1.9-1692
EDS : rb-20027674
Percentile statistics : 20151230.v01 (using entries in the PDB archive December 30th 2015)
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) : rb-20027674

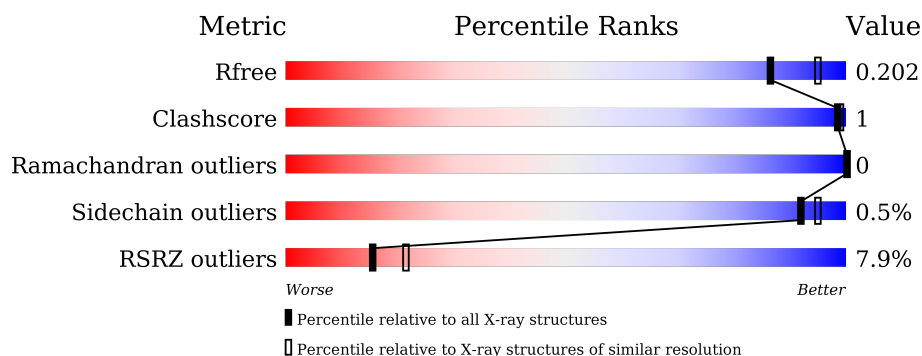
1 Overall quality at a glance i

The following experimental techniques were used to determine the structure:

X-RAY DIFFRACTION

The reported resolution of this entry is 2.10 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}	91344	3939 (2.10-2.10)
Clashscore	102246	4460 (2.10-2.10)
Ramachandran outliers	100387	4413 (2.10-2.10)
Sidechain outliers	100360	4414 (2.10-2.10)
RSRZ outliers	91569	3948 (2.10-2.10)

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 ≥ 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	245	<div> <div>5%</div> <div> <div></div> <div>85%</div> <div>•</div> <div>11%</div> </div> </div>
1	B	245	<div> <div>2%</div> <div> <div></div> <div>88%</div> <div>•</div> <div>10%</div> </div> </div>
1	C	245	<div> <div>4%</div> <div> <div></div> <div>88%</div> <div>•</div> <div>10%</div> </div> </div>
1	D	245	<div> <div>4%</div> <div> <div></div> <div>89%</div> <div>•</div> <div>10%</div> </div> </div>
1	E	245	<div> <div>9%</div> <div> <div></div> <div>87%</div> <div>•</div> <div>11%</div> </div> </div>
1	F	245	<div> <div>8%</div> <div> <div></div> <div>86%</div> <div>•</div> <div>10%</div> </div> </div>

Continued on next page...

Continued from previous page...

Mol	Chain	Length	Quality of chain
1	G	245	
1	H	245	

2 Entry composition

There are 2 unique types of molecules in this entry. The entry contains 29280 atoms, of which 13908 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 Fluorescent Protein.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
1	A	219	Total	C	H	N	O	S	0	0	0
			3523	1147	1734	298	333	11			
1	B	220	Total	C	H	N	O	S	0	0	0
			3542	1150	1747	299	335	11			
1	C	220	Total	C	H	N	O	S	0	0	0
			3542	1150	1747	299	335	11			
1	D	220	Total	C	H	N	O	S	0	0	0
			3541	1150	1746	299	335	11			
1	E	218	Total	C	H	N	O	S	0	0	0
			3509	1142	1727	297	332	11			
1	F	220	Total	C	H	N	O	S	0	0	0
			3541	1150	1746	299	335	11			
1	G	220	Total	C	H	N	O	S	0	0	0
			3514	1150	1719	299	335	11			
1	H	220	Total	C	H	N	O	S	0	0	0
			3537	1150	1742	299	335	11			

- Molecule 2 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
2	A	142	Total	O	0	0
			142	142		
2	B	160	Total	O	0	0
			160	160		
2	C	166	Total	O	0	0
			166	166		
2	D	120	Total	O	0	0
			120	120		
2	E	124	Total	O	0	0
			124	124		
2	F	126	Total	O	0	0
			126	126		

Continued on next page...

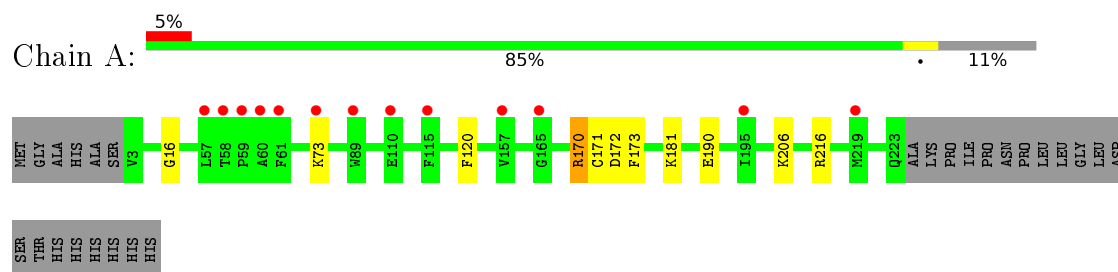
Continued from previous page...

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
2	G	94	Total	O	0	0
			94	94		
2	H	99	Total	O	0	0
			99	99		

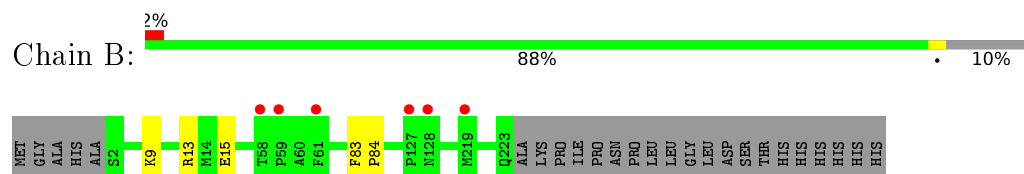
3 Residue-property plots [i](#)

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of errors 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.

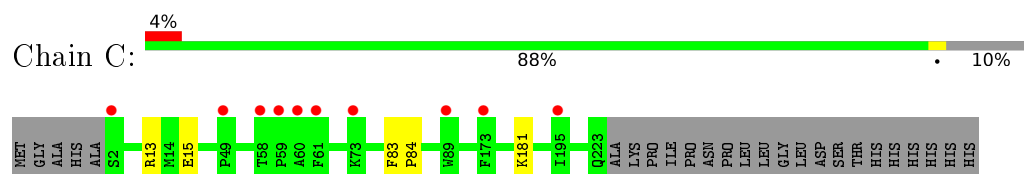
• Molecule 1: Fluorescent Protein



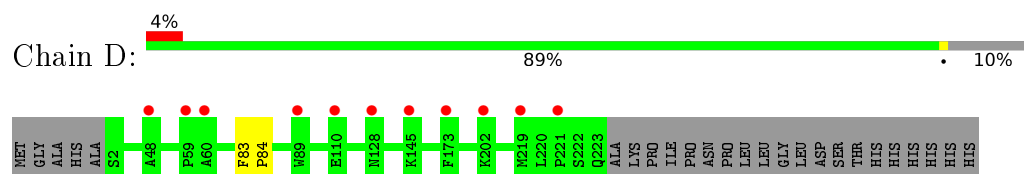
• Molecule 1: Fluorescent Protein



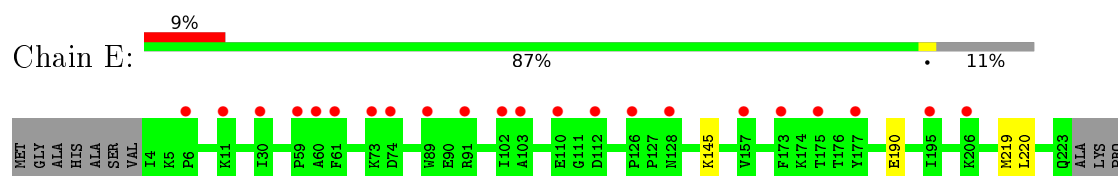
• Molecule 1: Fluorescent Protein



• Molecule 1: Fluorescent Protein

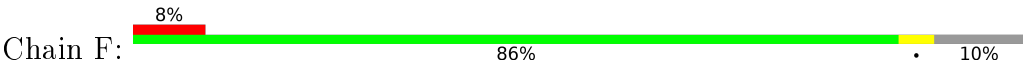


• Molecule 1: Fluorescent Protein



ILE
PRO
ASN
PRO
LEU
LEU
GLY
ASP
SER
THR
HIS
HIS
HIS
HIS
HIS

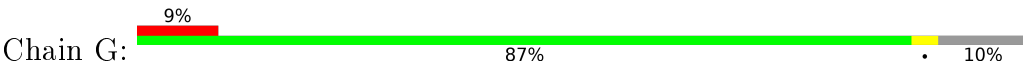
● Molecule 1: Fluorescent Protein



MET
GLY
ALA
HIS
HIS
S2
V3
D41
T58
P69
A60
F61
R66
P72
K73
D74
F83
P84
W89
E90
R91
A103
G111
H128
V157
G171
D172
F173
K174
T175
R184
L185
K202
R208
L209
Q223
ALA
LYS
PRO
PRO
ILE
PRO
ASN
PRO
LEU
LEU

GLY
LEU
ASP
SER
THR
HIS
HIS
HIS
HIS

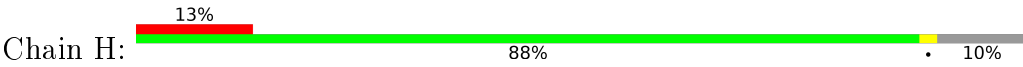
● Molecule 1: Fluorescent Protein



MET
GLY
ALA
ALA
V3
I4
K5
P6
E26
I30
G31
K32
K45
P69
A60
F61
QYG62
N65
K73
W89
E110
P127
H128
E144
K145
Y157
F173
Y177
R184
E190
K202
Y217
S218
M219
L220
P221
S222
Q223
ALA
LYS
PRO
PRO
ILE

PRO
ASN
PRO
LEU
LEU
GLY
ASP
SER
THR
HIS
HIS
HIS
HIS
HIS

● Molecule 1: Fluorescent Protein



MET
GLY
ALA
HIS
HIS
S2
V3
I4
K5
P6
K22
I30
Y34
V44
P49
L57
T58
P59
A60
F61
K73
D74
F83
P84
W89
E90
R91
I100
C101
I102
A103
E110
G111
Y116
P126
W139
Y152
V157
F173
K174
T175
T176
Y177

D182
V183
R184
L185
K202
S222
Q223
ALA
LYS
PRO
PRO
ILE
PRO
ASN
PRO
PRO
LEU
LEU
GLY
LEU
ASP
SER
THR
HIS
HIS
HIS
HIS

4 Data and refinement statistics

Property	Value	Source
Space group	P 1	Depositor
Cell constants a, b, c, α , β , γ	74.63 Å 75.38 Å 84.51 Å 90.96° 89.82° 104.03°	Depositor
Resolution (Å)	29.85 – 2.10 29.85 – 2.10	Depositor EDS
% Data completeness (in resolution range)	94.4 (29.85-2.10) 93.9 (29.85-2.10)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$\langle I/\sigma(I) \rangle$ ¹	2.23 (at 2.10 Å)	Xtriage
Refinement program	PHENIX (phenix.refine: 1.9_1685)	Depositor
R, R_{free}	0.167 , 0.203 0.168 , 0.202	Depositor DCC
R_{free} test set	4882 reflections (4.96%)	DCC
Wilson B-factor (Å ²)	33.6	Xtriage
Anisotropy	0.549	Xtriage
Bulk solvent k_{sol} (e/Å ³), B_{sol} (Å ²)	0.41 , 47.1	EDS
L-test for twinning ²	$\langle L \rangle = 0.50$, $\langle L^2 \rangle = 0.33$	Xtriage
Estimated twinning fraction	0.015 for -h,-k,l 0.001 for -k,-h,-l 0.006 for k,h,-l	Xtriage
F_o, F_c correlation	0.96	EDS
Total number of atoms	29280	wwPDB-VP
Average B, all atoms (Å ²)	45.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 12.10% of the height of the origin peak. No significant pseudotranslation is detected.*

¹Intensities estimated from amplitudes.

²Theoretical values of $\langle |L| \rangle$, $\langle L^2 \rangle$ for acentric reflections are 0.5, 0.375 respectively for untwinned datasets, and 0.333, 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: CRQ

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	0.28	0/1811	0.46	0/2441
1	B	0.27	0/1817	0.47	0/2449
1	C	0.28	0/1817	0.46	0/2449
1	D	0.27	0/1817	0.46	0/2449
1	E	0.26	0/1804	0.45	0/2431
1	F	0.27	0/1817	0.45	0/2449
1	G	0.25	0/1817	0.46	1/2449 (0.0%)
1	H	0.25	0/1817	0.43	0/2449
All	All	0.27	0/14517	0.45	1/19566 (0.0%)

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	G	218	SER	C-N-CA	7.10	139.44	121.70

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	1789	1734	1743	6	0
1	B	1795	1747	1748	3	0

Continued on next page...

Continued from previous page...

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	C	1795	1747	1748	3	0
1	D	1795	1746	1748	1	0
1	E	1782	1727	1734	1	0
1	F	1795	1746	1748	7	0
1	G	1795	1719	1748	3	0
1	H	1795	1742	1747	2	0
2	A	142	0	0	2	0
2	B	160	0	0	1	0
2	C	166	0	0	1	0
2	D	120	0	0	0	0
2	E	124	0	0	0	0
2	F	126	0	0	2	0
2	G	94	0	0	1	0
2	H	99	0	0	0	0
All	All	15372	13908	13964	26	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 1.

The worst 5 of 26 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:145:LYS:NZ	1:G:190:GLU:OE2	2.29	0.66
1:C:181:LYS:NZ	2:C:301:HOH:O	2.32	0.61
1:F:41:ASP:OD1	1:F:208:ARG:NH1	2.34	0.60
1:C:13:ARG:NH1	1:C:15:GLU:OE2	2.35	0.59
1:A:73:LYS:NZ	2:A:301:HOH:O	2.36	0.58

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	216/245 (88%)	216 (100%)	0	0	100	100
1	B	217/245 (89%)	217 (100%)	0	0	100	100
1	C	217/245 (89%)	217 (100%)	0	0	100	100
1	D	217/245 (89%)	216 (100%)	1 (0%)	0	100	100
1	E	215/245 (88%)	214 (100%)	1 (0%)	0	100	100
1	F	217/245 (89%)	216 (100%)	1 (0%)	0	100	100
1	G	217/245 (89%)	215 (99%)	2 (1%)	0	100	100
1	H	217/245 (89%)	215 (99%)	2 (1%)	0	100	100
All	All	1733/1960 (88%)	1726 (100%)	7 (0%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2 Protein sidechains ⓘ

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	190/211 (90%)	188 (99%)	2 (1%)	80	85
1	B	191/211 (90%)	191 (100%)	0	100	100
1	C	191/211 (90%)	191 (100%)	0	100	100
1	D	191/211 (90%)	191 (100%)	0	100	100
1	E	189/211 (90%)	187 (99%)	2 (1%)	80	85
1	F	191/211 (90%)	191 (100%)	0	100	100
1	G	191/211 (90%)	190 (100%)	1 (0%)	92	95
1	H	191/211 (90%)	189 (99%)	2 (1%)	82	87
All	All	1525/1688 (90%)	1518 (100%)	7 (0%)	92	95

5 of 7 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	E	220	LEU
1	H	116	TYR

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type
1	G	30	ILE
1	A	181	LYS
1	H	100	ILE

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

5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains ⓘ

8 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	CRQ	A	62	1	24,25,26	2.96	9 (37%)	24,34,36	2.14	7 (29%)
1	CRQ	B	62	1	24,25,26	2.89	8 (33%)	24,34,36	1.97	6 (25%)
1	CRQ	C	62	1	24,25,26	2.94	7 (29%)	24,34,36	1.88	6 (25%)
1	CRQ	D	62	1	24,25,26	2.95	7 (29%)	24,34,36	2.07	7 (29%)
1	CRQ	E	62	1	24,25,26	3.00	9 (37%)	24,34,36	2.07	6 (25%)
1	CRQ	F	62	1	24,25,26	3.26	9 (37%)	24,34,36	2.15	4 (16%)
1	CRQ	G	62	1	24,25,26	3.07	9 (37%)	24,34,36	2.23	5 (20%)
1	CRQ	H	62	1	24,25,26	3.10	8 (33%)	24,34,36	2.31	6 (25%)

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	CRQ	A	62	1	-	0/10/32/33	0/2/2/2
1	CRQ	B	62	1	-	0/10/32/33	0/2/2/2
1	CRQ	C	62	1	-	0/10/32/33	0/2/2/2
1	CRQ	D	62	1	-	0/10/32/33	0/2/2/2
1	CRQ	E	62	1	-	0/10/32/33	0/2/2/2
1	CRQ	F	62	1	-	0/10/32/33	0/2/2/2
1	CRQ	G	62	1	-	0/10/32/33	0/2/2/2
1	CRQ	H	62	1	-	0/10/32/33	0/2/2/2

The worst 5 of 66 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	62	CRQ	CA3-N3	-3.80	1.39	1.47
1	D	62	CRQ	CA3-N3	-3.49	1.39	1.47
1	H	62	CRQ	CA3-N3	-3.49	1.39	1.47
1	C	62	CRQ	CA3-N3	-3.47	1.39	1.47
1	G	62	CRQ	CA3-N3	-3.46	1.39	1.47

The worst 5 of 47 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
1	H	62	CRQ	O2-C2-CA2	-6.40	127.29	130.97
1	A	62	CRQ	O2-C2-CA2	-6.29	127.36	130.97
1	G	62	CRQ	O2-C2-CA2	-6.27	127.36	130.97
1	F	62	CRQ	O2-C2-CA2	-6.11	127.46	130.97
1	D	62	CRQ	O2-C2-CA2	-6.04	127.50	130.97

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

5.5 Carbohydrates [i](#)

There are no carbohydrates 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](#)

There are no chain breaks in this entry.

6 Fit of model and data ⓘ

6.1 Protein, DNA and RNA chains ⓘ

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	218/245 (88%)	0.30	13 (5%) 25 33	27, 36, 50, 65	0
1	B	219/245 (89%)	0.17	6 (2%) 58 65	24, 33, 46, 57	0
1	C	219/245 (89%)	0.28	10 (4%) 36 45	25, 34, 47, 70	0
1	D	219/245 (89%)	0.30	11 (5%) 32 41	26, 38, 51, 64	0
1	E	217/245 (88%)	0.57	22 (10%) 9 12	29, 42, 58, 71	0
1	F	219/245 (89%)	0.59	20 (9%) 11 16	27, 42, 62, 89	0
1	G	219/245 (89%)	0.69	23 (10%) 8 11	33, 46, 64, 82	0
1	H	219/245 (89%)	0.93	33 (15%) 3 4	30, 49, 67, 83	0
All	All	1749/1960 (89%)	0.48	138 (7%) 15 21	24, 40, 59, 89	0

The worst 5 of 138 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	F	2	SER	5.7
1	H	89	TRP	5.4
1	H	60	ALA	5.3
1	H	3	VAL	5.1
1	G	184	ARG	4.4

6.2 Non-standard residues in protein, DNA, RNA chains ⓘ

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, 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	LLDF	B-factors(\AA^2)	Q<0.9
1	CRQ	D	62	24/25	0.93	0.29	-	26,29,36,36	0
1	CRQ	H	62	24/25	0.92	0.42	-	39,44,58,58	0
1	CRQ	A	62	24/25	0.93	0.28	-	26,29,35,35	0
1	CRQ	B	62	24/25	0.93	0.25	-	22,27,33,34	0
1	CRQ	C	62	24/25	0.95	0.29	-	25,26,33,34	0
1	CRQ	E	62	24/25	0.93	0.35	-	30,36,48,48	0
1	CRQ	F	62	24/25	0.95	0.39	-	30,35,44,47	0
1	CRQ	G	62	24/25	0.91	0.38	-	37,40,51,56	0

6.3 Carbohydrates [i](#)

There are no carbohydrates 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.