Full wwPDB X-ray Structure Validation Report ⓘ

Aug 23, 2023 – 03:39 AM EDT

PDB ID	:	3EGS
Title	:	Crystal structure of the HIV-1 broadly neutralizing antibody 2F5 in complex with the gp41 scrambledFP-MPER scrHyb3K construct GIGAFGLLGFLAA GSKK-Ahx-K656NEQELLELDKWASLWN671 soaked in ammonium sulfate
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Deposited on	:	2008-09-11
Resolution	:	3.60 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*
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.

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

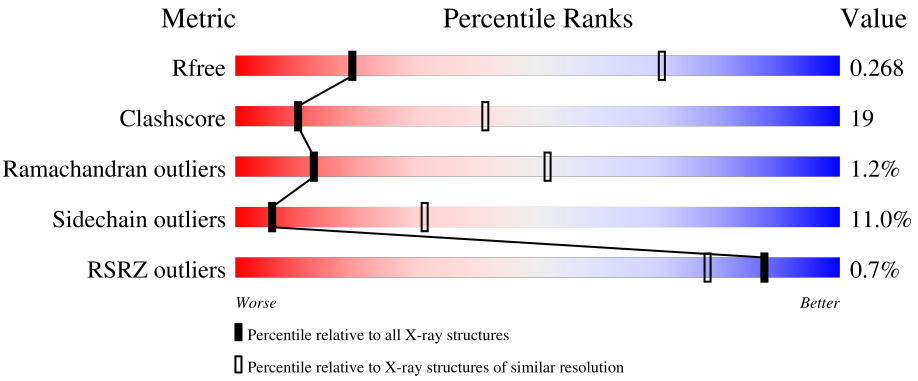
MolProbity	:	4.02b-467
Xtriage (Phenix)	:	1.13
EDS	:	2.35
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.35

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 3.60 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}	130704	1257 (3.70-3.50)
Clashscore	141614	1353 (3.70-3.50)
Ramachandran outliers	138981	1307 (3.70-3.50)
Sidechain outliers	138945	1307 (3.70-3.50)
RSRZ outliers	127900	1161 (3.70-3.50)

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 >=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 <=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	214	65% 29% 6%
2	B	235	% 59% 27% 5% • 8%
3	C	35	9% 9% • 80%

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 3319 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 2F5 Fab' light chain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	213	1637	1022	280	331	4	0	0	0

- Molecule 2 is a protein called 2F5 Fab' heavy chain.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
2	B	216	1623	1030	274	313	6	0	0	0

- Molecule 3 is a protein called gp41 scrFP-MPER construct.

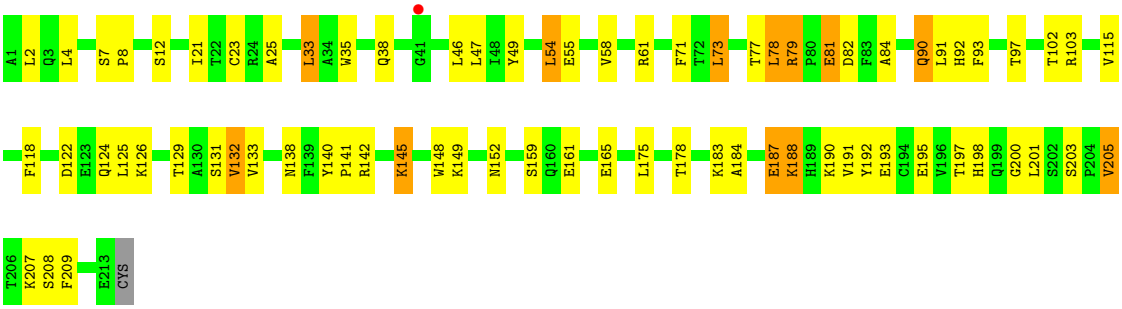
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace
			Total	C	N	O			
3	C	7	59	38	9	12	0	0	0

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.

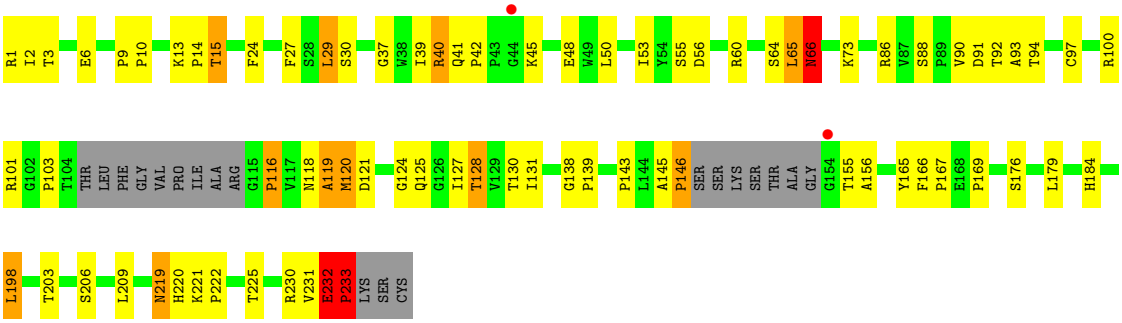
- Molecule 1: 2F5 Fab' light chain

Chain A:



- Molecule 2: 2F5 Fab' heavy chain

Chain B:



- Molecule 3: gp41 scrFP-MPER construct

Chain C:

4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 21 21 21	Depositor
Cell constants a, b, c, α, β, γ	63.80Å 75.90Å 93.50Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	16.73 – 3.60 16.73 – 3.60	Depositor EDS
% Data completeness (in resolution range)	87.2 (16.73-3.60) 87.2 (16.73-3.60)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	0.28	Depositor
$< I/\sigma(I) >$ [1]	3.37 (at 3.59Å)	Xtriage
Refinement program	CNS	Depositor
R, R_{free}	0.235 , 0.249 0.236 , 0.268	Depositor DCC
R_{free} test set	270 reflections (5.03%)	wwPDB-VP
Wilson B-factor (Å^2)	29.7	Xtriage
Anisotropy	0.286	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.34 , 14.3	EDS
L-test for twinning[2]	$< \|L\| > = 0.45$, $< L^2 > = 0.28$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.83	EDS
Total number of atoms	3319	wwPDB-VP
Average B, all atoms (Å^2)	23.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 5.12% of the height of the origin peak. No significant pseudotranslation is detected.*

[1] Intensities estimated from amplitudes.

[2] Theoretical values of $< |L| >$, $< L^2 >$ 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

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	0.41	0/1674	0.75	0/2276
2	B	0.42	0/1662	0.85	4/2273 (0.2%)
3	C	0.66	0/60	0.94	0/80
All	All	0.42	0/3396	0.81	4/4629 (0.1%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand.A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
2	B	0	1

There are no bond length outliers.

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	B	232	GLU	N-CA-C	-6.29	94.00	111.00
2	B	233	PRO	N-CA-C	-6.24	95.88	112.10
2	B	66	ASN	N-CA-C	5.63	126.19	111.00
2	B	120	MET	N-CA-C	-5.23	96.88	111.00

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
2	B	232	GLU	Mainchain

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	1637	0	1583	60	0
2	B	1623	0	1630	72	0
3	C	59	0	53	7	0
All	All	3319	0	3266	123	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 19.

All (123) 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:1:ARG:CG	2:B:2:ILE:H	1.53	1.15
2:B:1:ARG:HG3	2:B:2:ILE:N	1.72	1.05
2:B:1:ARG:HG3	2:B:2:ILE:H	0.86	1.02
1:A:103:ARG:HH12	1:A:142:ARG:HD2	1.24	1.01
1:A:149:LYS:HB2	1:A:193:GLU:HG3	1.40	1.01
2:B:232:GLU:HB2	2:B:233:PRO:CD	1.96	0.95
1:A:138:ASN:HD21	2:B:184:HIS:HE1	1.12	0.91
1:A:103:ARG:HH12	1:A:142:ARG:CD	1.83	0.91
2:B:232:GLU:HB2	2:B:233:PRO:HD3	1.56	0.86
1:A:138:ASN:HD21	2:B:184:HIS:CE1	1.95	0.85
1:A:103:ARG:NH1	1:A:142:ARG:HD2	1.94	0.82
1:A:90:GLN:HE22	1:A:93:PHE:H	1.33	0.76
1:A:125:LEU:O	1:A:183:LYS:HD3	1.86	0.75
2:B:14:PRO:O	2:B:15:THR:HB	1.87	0.75
2:B:40:ARG:HD3	2:B:48:GLU:OE1	1.87	0.75
2:B:56:ASP:OD2	3:C:6:LYS:NZ	2.19	0.74
1:A:198:HIS:CD2	1:A:200:GLY:H	2.05	0.74
2:B:103:PRO:HG2	3:C:7:TRP:CZ3	2.24	0.73
2:B:1:ARG:CG	2:B:2:ILE:N	2.29	0.72
1:A:138:ASN:ND2	2:B:184:HIS:HE1	1.87	0.71
1:A:149:LYS:HB2	1:A:193:GLU:CG	2.18	0.71
2:B:143:PRO:HB3	2:B:231:VAL:HG22	1.71	0.71
2:B:139:PRO:HB3	2:B:165:TYR:HB3	1.76	0.68
2:B:64:SER:O	2:B:65:LEU:HD13	1.92	0.67

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:201:LEU:HD13	1:A:205:VAL:CG2	2.25	0.67
1:A:79:ARG:HH11	1:A:79:ARG:HG2	1.60	0.66
1:A:90:GLN:NE2	1:A:92:HIS:H	1.94	0.65
2:B:88:SER:OG	2:B:90:VAL:HG22	1.97	0.64
1:A:201:LEU:HD13	1:A:205:VAL:HG23	1.80	0.63
1:A:161:GLU:OE2	1:A:175:LEU:HD11	1.98	0.63
2:B:100:ARG:HD2	2:B:120:MET:HE1	1.81	0.62
2:B:103:PRO:HD3	3:C:7:TRP:CZ2	2.34	0.62
2:B:145:ALA:HB2	2:B:231:VAL:CG1	2.29	0.62
1:A:54:LEU:CD2	1:A:58:VAL:HB	2.29	0.62
2:B:206:SER:HA	2:B:209:LEU:HD12	1.81	0.61
1:A:61:ARG:NE	1:A:79:ARG:HG3	2.16	0.60
2:B:103:PRO:HG2	3:C:7:TRP:CH2	2.37	0.59
2:B:232:GLU:CB	2:B:233:PRO:CD	2.71	0.59
1:A:49:TYR:CE1	2:B:116:PRO:HB3	2.36	0.59
1:A:61:ARG:CZ	1:A:79:ARG:HG3	2.32	0.59
2:B:42:PRO:HB2	2:B:45:LYS:HG3	1.85	0.59
1:A:184:ALA:O	1:A:188:LYS:HG2	2.01	0.59
2:B:138:GLY:HA3	2:B:225:THR:OG1	2.04	0.58
2:B:100:ARG:HB2	2:B:120:MET:CE	2.34	0.57
2:B:145:ALA:HB1	2:B:146:PRO:HD2	1.86	0.57
2:B:232:GLU:HB2	2:B:233:PRO:HD2	1.85	0.57
2:B:100:ARG:HB2	2:B:120:MET:HE2	1.86	0.57
1:A:159:SER:HA	1:A:178:THR:O	2.05	0.57
2:B:2:ILE:HG23	2:B:27:PHE:CD2	2.40	0.57
1:A:55:GLU:CD	2:B:101:ARG:HH12	2.08	0.56
1:A:115:VAL:HG12	1:A:207:LYS:HG3	1.85	0.56
1:A:138:ASN:ND2	2:B:184:HIS:CE1	2.69	0.56
1:A:81:GLU:H	1:A:81:GLU:CD	2.10	0.55
2:B:60:ARG:NE	3:C:3:GLU:OE2	2.32	0.55
2:B:118:ASN:O	2:B:119:ALA:HB2	2.07	0.55
1:A:132:VAL:HG22	1:A:148:TRP:CH2	2.42	0.55
2:B:15:THR:O	2:B:86:ARG:HA	2.07	0.55
2:B:2:ILE:HG23	2:B:27:PHE:HD2	1.72	0.54
1:A:12:SER:OG	1:A:140:TYR:OH	2.24	0.54
2:B:198:LEU:HD12	2:B:198:LEU:C	2.28	0.53
2:B:40:ARG:HD2	2:B:50:LEU:HD21	1.89	0.53
2:B:39:ILE:HD12	2:B:39:ILE:N	2.24	0.53
1:A:2:LEU:HD22	1:A:90:GLN:HG2	1.91	0.53
1:A:54:LEU:HD22	1:A:58:VAL:HB	1.91	0.51
2:B:24:PHE:CZ	2:B:29:LEU:HD22	2.45	0.51

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:B:103:PRO:CD	3:C:7:TRP:CZ2	2.94	0.51
2:B:92:THR:HG23	2:B:130:THR:HA	1.93	0.50
2:B:166:PHE:CE1	2:B:167:PRO:HB3	2.47	0.50
1:A:79:ARG:HH11	1:A:79:ARG:CG	2.25	0.49
1:A:192:TYR:O	1:A:208:SER:HA	2.11	0.49
1:A:33:LEU:HD22	1:A:71:PHE:CG	2.47	0.48
1:A:38:GLN:O	1:A:84:ALA:HB1	2.14	0.48
1:A:78:LEU:HD22	1:A:82:ASP:HB2	1.95	0.48
2:B:100:ARG:CB	2:B:120:MET:HE2	2.44	0.48
1:A:195:GLU:HA	1:A:205:VAL:O	2.13	0.47
1:A:46:LEU:HD22	2:B:121:ASP:HA	1.97	0.47
2:B:40:ARG:HB3	2:B:50:LEU:HD11	1.96	0.47
1:A:77:THR:O	1:A:77:THR:HG23	2.15	0.47
1:A:198:HIS:HD2	1:A:200:GLY:H	1.57	0.46
2:B:156:ALA:CB	2:B:209:LEU:HD11	2.45	0.46
1:A:7:SER:HA	1:A:8:PRO:C	2.35	0.46
2:B:221:LYS:N	2:B:222:PRO:CD	2.79	0.46
2:B:100:ARG:HG3	2:B:120:MET:HE2	1.98	0.46
2:B:221:LYS:H	2:B:221:LYS:HG2	1.51	0.46
1:A:124:GLN:NE2	1:A:131:SER:H	2.14	0.46
1:A:141:PRO:O	1:A:198:HIS:HE1	1.99	0.46
2:B:127:ILE:HG23	2:B:127:ILE:O	2.16	0.46
2:B:9:PRO:HA	2:B:10:PRO:HD3	1.82	0.45
1:A:4:LEU:HD13	1:A:23:CYS:SG	2.57	0.45
1:A:132:VAL:HG22	1:A:148:TRP:CZ3	2.52	0.45
2:B:100:ARG:CG	2:B:120:MET:HE2	2.47	0.44
1:A:55:GLU:OE2	2:B:101:ARG:NH1	2.50	0.44
2:B:143:PRO:CB	2:B:231:VAL:HG22	2.44	0.44
1:A:124:GLN:HE22	1:A:131:SER:H	1.66	0.44
1:A:21:ILE:HG21	1:A:102:THR:HG21	1.99	0.43
1:A:49:TYR:CD1	2:B:116:PRO:HA	2.53	0.43
1:A:79:ARG:CG	1:A:79:ARG:NH1	2.79	0.43
2:B:6:GLU:OE2	2:B:124:GLY:HA3	2.18	0.43
2:B:53:ILE:CD1	2:B:73:LYS:HG2	2.48	0.43
2:B:206:SER:HA	2:B:209:LEU:CD1	2.46	0.42
2:B:91:ASP:C	2:B:131:ILE:HD13	2.40	0.42
2:B:167:PRO:HD2	2:B:222:PRO:HB3	2.00	0.42
1:A:93:PHE:CE1	3:C:4:LEU:HD23	2.55	0.42
2:B:41:GLN:O	2:B:93:ALA:HB1	2.20	0.42
2:B:92:THR:N	2:B:131:ILE:HD13	2.34	0.42
1:A:79:ARG:HG2	1:A:79:ARG:NH1	2.32	0.42

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:118:PHE:HD2	1:A:133:VAL:HG22	1.84	0.42
1:A:47:LEU:HA	1:A:58:VAL:HG21	2.01	0.42
1:A:192:TYR:HB2	1:A:209:PHE:CE2	2.55	0.42
2:B:86:ARG:O	2:B:86:ARG:HG3	2.20	0.42
2:B:37:GLY:O	2:B:97:CYS:HA	2.20	0.41
1:A:91:LEU:HD12	1:A:91:LEU:N	2.36	0.41
2:B:94:THR:HA	2:B:128:THR:HA	2.02	0.41
1:A:2:LEU:HD13	1:A:93:PHE:CD2	2.56	0.41
2:B:219:ASN:HD22	2:B:220:HIS:N	2.19	0.41
2:B:145:ALA:HA	2:B:146:PRO:HD3	1.89	0.40
2:B:155:THR:O	2:B:155:THR:HG23	2.21	0.40
1:A:35:TRP:CE2	1:A:73:LEU:HB2	2.56	0.40
1:A:4:LEU:CD2	1:A:25:ALA:HB2	2.52	0.40
2:B:92:THR:HA	2:B:131:ILE:HD12	2.03	0.40
1:A:145:LYS:HB3	1:A:197:THR:HB	2.04	0.40
1:A:183:LYS:O	1:A:187:GLU:HG2	2.21	0.40
2:B:6:GLU:HG3	2:B:97:CYS:SG	2.62	0.40

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	211/214 (99%)	203 (96%)	8 (4%)	0	100 100
2	B	210/235 (89%)	192 (91%)	13 (6%)	5 (2%)	6 37
3	C	5/35 (14%)	5 (100%)	0	0	100 100
All	All	426/484 (88%)	400 (94%)	21 (5%)	5 (1%)	13 51

All (5) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
2	B	232	GLU
2	B	15	THR
2	B	66	ASN
2	B	119	ALA
2	B	116	PRO

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	186/187 (100%)	165 (89%)	21 (11%)	6 30
2	B	189/204 (93%)	169 (89%)	20 (11%)	6 32
3	C	6/25 (24%)	5 (83%)	1 (17%)	2 14
All	All	381/416 (92%)	339 (89%)	42 (11%)	6 31

All (42) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	33	LEU
1	A	54	LEU
1	A	73	LEU
1	A	78	LEU
1	A	79	ARG
1	A	81	GLU
1	A	90	GLN
1	A	97	THR
1	A	122	ASP
1	A	126	LYS
1	A	129	THR
1	A	132	VAL
1	A	145	LYS
1	A	152	ASN
1	A	165	GLU
1	A	187	GLU
1	A	188	LYS
1	A	190	LYS

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Mol	Chain	Res	Type
1	A	191	VAL
1	A	203	SER
1	A	205	VAL
2	B	3	THR
2	B	13	LYS
2	B	29	LEU
2	B	30	SER
2	B	40	ARG
2	B	55	SER
2	B	65	LEU
2	B	66	ASN
2	B	125	GLN
2	B	128	THR
2	B	146	PRO
2	B	169	PRO
2	B	176	SER
2	B	179	LEU
2	B	198	LEU
2	B	203	THR
2	B	219	ASN
2	B	230	ARG
2	B	232	GLU
2	B	233	PRO
3	C	3	GLU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (14) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	38	GLN
1	A	90	GLN
1	A	92	HIS
1	A	124	GLN
1	A	138	ASN
1	A	147	GLN
1	A	152	ASN
1	A	198	HIS
1	A	199	GLN
2	B	16	GLN
2	B	41	GLN
2	B	184	HIS
2	B	212	GLN
2	B	219	ASN

5.3.3 RNA

There are no RNA molecules in this entry.

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

There are no non-standard protein/DNA/RNA residues in this entry.

5.5 Carbohydrates

There are no monosaccharides in this entry.

5.6 Ligand geometry

There are no ligands in this entry.

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

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, 95^{th} 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	213/214 (99%)	-0.26	1 (0%)	91	83	11, 20, 32, 51	0
2	B	216/235 (91%)	-0.10	2 (0%)	84	73	11, 24, 41, 50	0
3	C	7/35 (20%)	0.52	0	100	100	32, 36, 44, 45	0
All	All	436/484 (90%)	-0.17	3 (0%)	87	78	11, 22, 39, 51	0

All (3) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
2	B	44	GLY	2.4
2	B	154	GLY	2.2
1	A	41	GLY	2.2

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

There are no non-standard protein/DNA/RNA residues in this entry.

6.3 Carbohydrates

There are no monosaccharides in this entry.

6.4 Ligands

There are no ligands in this entry.

6.5 Other polymers

There are no such residues in this entry.