



Full wwPDB EM Validation Report ⓘ

May 31, 2026 – 12:08 AM JST

PDB ID : 9V1H / pdb_00009v1h
EMDB ID : EMD-64693
Title : Cryo-EM structure of Gi-coupled NPFF2R in complex with GUB08248
Authors : Zhao, L.; Li, X.; Li, S.; Yuan, Q.
Deposited on : 2025-05-19
Resolution : 2.85 Å(reported)
Based on initial model : .

This is a Full wwPDB EM 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/EMValidationReportHelp>
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:

EMDB validation analysis : **FAILED**
MolProbity : 4-5-2 with Phenix2.0
Percentile statistics : 20250101.v01 (using entries in the PDB archive January 1st 2025)
EM percentile statistics : **NOT EXECUTED**
MapQ : **FAILED**
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.49

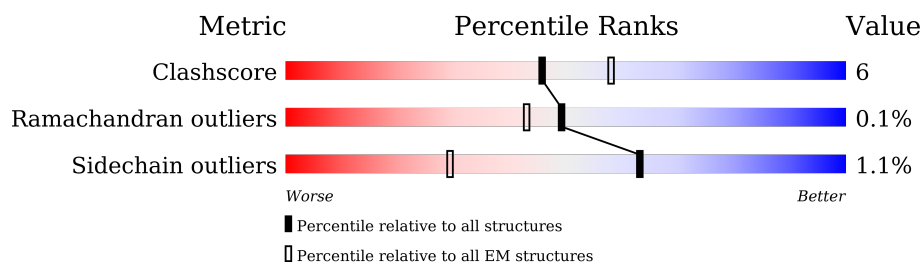
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 2.85 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)	EM structures (#Entries)
Clashscore	229148	23984
Ramachandran outliers	224038	23583
Sidechain outliers	223484	23102

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the 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 $\leq 5\%$

Mol	Chain	Length	Quality of chain
1	A	361	
2	B	371	
3	E	247	
4	G	70	
5	L	11	
6	R	420	

2 Entry composition

There are 6 unique types of molecules in this entry. The entry contains 8890 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, 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 Guanine nucleotide-binding protein G(s) subunit alpha-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	208	Total	C	N	O	S	0	0
			1709	1080	311	310	8		

- Molecule 2 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	335	Total	C	N	O	S	0	0
			2537	1571	457	488	21		

There are 32 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	1	MET	-	initiating methionine	UNP P62873
B	2	GLY	-	expression tag	UNP P62873
B	3	SER	-	expression tag	UNP P62873
B	4	LEU	-	expression tag	UNP P62873
B	5	LEU	-	expression tag	UNP P62873
B	6	GLN	-	expression tag	UNP P62873
B	346	GLY	-	expression tag	UNP P62873
B	347	SER	-	expression tag	UNP P62873
B	348	SER	-	expression tag	UNP P62873
B	349	GLY	-	expression tag	UNP P62873
B	350	GLY	-	expression tag	UNP P62873
B	351	GLY	-	expression tag	UNP P62873
B	352	GLY	-	expression tag	UNP P62873
B	353	SER	-	expression tag	UNP P62873
B	354	GLY	-	expression tag	UNP P62873
B	355	GLY	-	expression tag	UNP P62873
B	356	GLY	-	expression tag	UNP P62873
B	357	GLY	-	expression tag	UNP P62873
B	358	SER	-	expression tag	UNP P62873
B	359	SER	-	expression tag	UNP P62873

Continued on next page...

Continued from previous page...

Chain	Residue	Modelled	Actual	Comment	Reference
B	360	GLY	-	expression tag	UNP P62873
B	361	VAL	-	expression tag	UNP P62873
B	362	SER	-	expression tag	UNP P62873
B	363	GLY	-	expression tag	UNP P62873
B	364	TRP	-	expression tag	UNP P62873
B	365	ARG	-	expression tag	UNP P62873
B	366	LEU	-	expression tag	UNP P62873
B	367	PHE	-	expression tag	UNP P62873
B	368	LYS	-	expression tag	UNP P62873
B	369	LYS	-	expression tag	UNP P62873
B	370	ILE	-	expression tag	UNP P62873
B	371	SER	-	expression tag	UNP P62873

- Molecule 3 is a protein called scFv16.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	E	228	Total	C	N	O	S	0	0
			1698	1085	280	323	10		

- Molecule 4 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	G	55	Total	C	N	O	S	0	0
			409	258	75	73	3		

- Molecule 5 is a protein called GUB08248.

Mol	Chain	Residues	Atoms				AltConf	Trace
5	L	11	Total	C	N	O	0	1
			82	50	21	11		

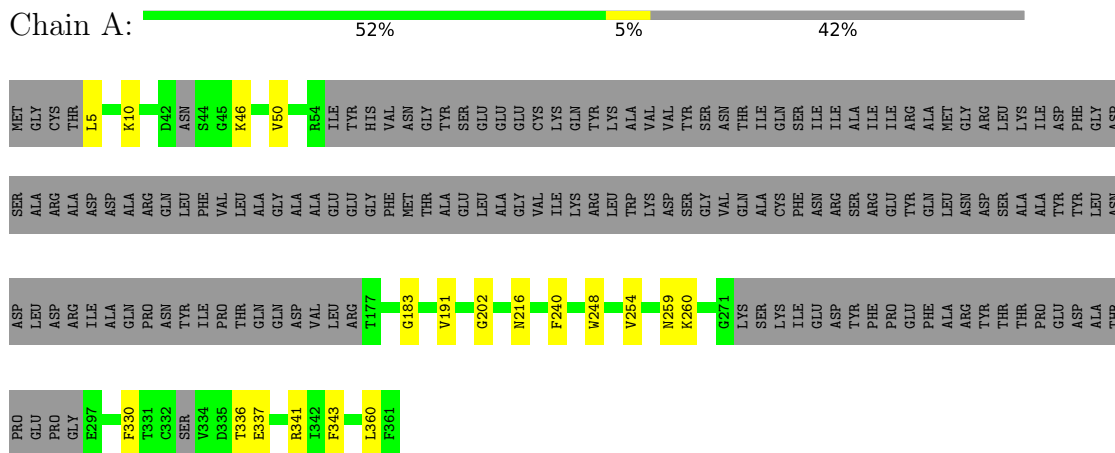
- Molecule 6 is a protein called Neuropeptide FF receptor 2.

Mol	Chain	Residues	Atoms					AltConf	Trace
6	R	303	Total	C	N	O	S	0	0
			2455	1645	389	401	20		

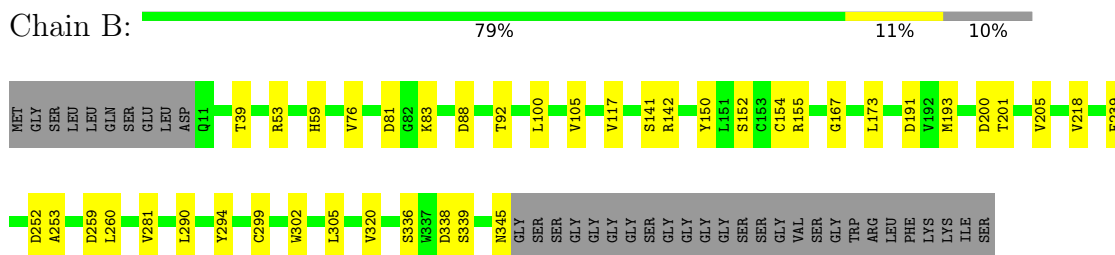
3 Residue-property plots [i](#)

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. 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. 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.

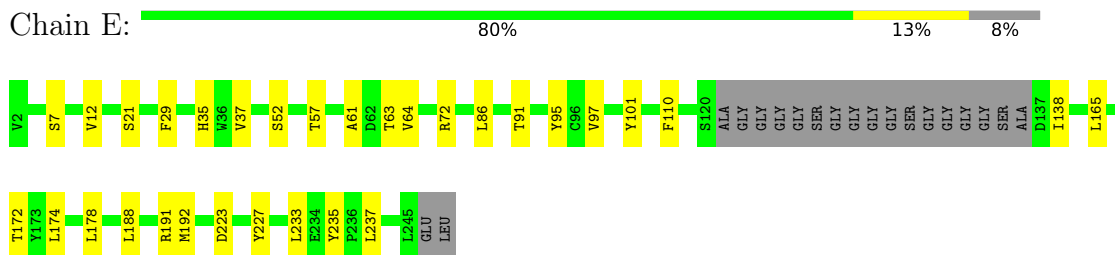
- Molecule 1: Guanine nucleotide-binding protein G(s) subunit alpha-1



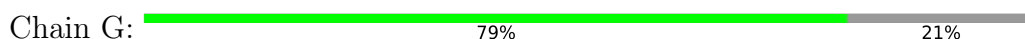
- Molecule 2: Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1



- Molecule 3: scFv16



- Molecule 4: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2

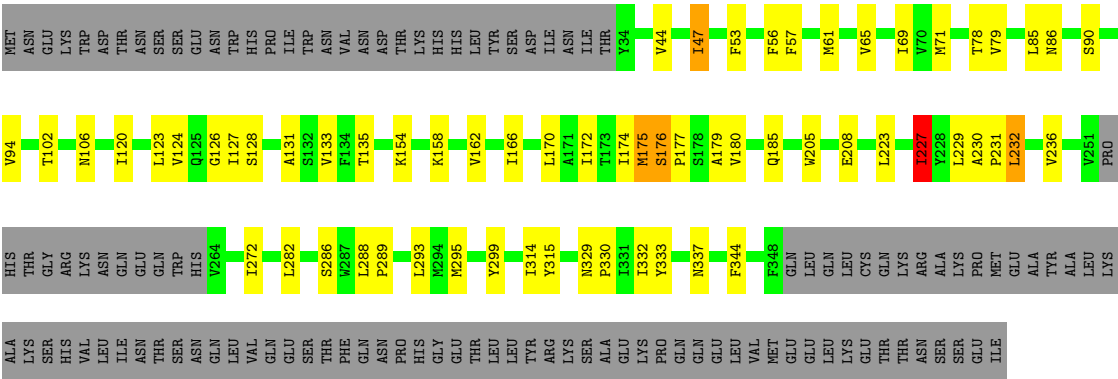




• Molecule 5: GUB08248



• Molecule 6: Neuropeptide FF receptor 2



4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	324707	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	TFS KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/\text{\AA}^2$)	50	Depositor
Minimum defocus (nm)	8000	Depositor
Maximum defocus (nm)	18000	Depositor
Magnification	Not provided	
Image detector	FEI FALCON IV (4k x 4k)	Depositor

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	0.09	0/1734	0.23	0/2324
2	B	0.09	0/2584	0.23	0/3508
3	E	0.08	0/1742	0.22	0/2371
4	G	0.11	0/415	0.19	0/561
5	L	0.41	0/83	0.70	0/108
6	R	0.27	1/2522 (0.0%)	0.52	0/3433
All	All	0.16	1/9080 (0.0%)	0.34	0/12305

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
6	R	227	ILE	N-CA	5.28	1.51	1.46

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	1709	0	1715	10	0
2	B	2537	0	2430	24	0
3	E	1698	0	1594	25	0
4	G	409	0	414	0	0
5	L	82	0	90	3	0
6	R	2455	0	2539	49	0

Continued on next page...

Continued from previous page...

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
All	All	8890	0	8782	109	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.

All (109) 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)
6:R:69:ILE:CD1	6:R:344:PHE:HB3	1.76	1.14
3:E:191:ARG:O	3:E:192:MET:HG2	1.56	1.05
6:R:69:ILE:HD13	6:R:344:PHE:HB3	1.05	1.04
6:R:295:MET:CE	6:R:299:TYR:HE2	1.78	0.96
6:R:295:MET:HE3	6:R:299:TYR:CE2	2.05	0.91
6:R:295:MET:CE	6:R:299:TYR:CE2	2.55	0.89
6:R:295:MET:HE1	6:R:299:TYR:HE2	1.41	0.83
3:E:191:ARG:O	3:E:192:MET:CG	2.26	0.83
3:E:172:THR:HB	3:E:192:MET:SD	2.23	0.79
6:R:295:MET:HE1	6:R:299:TYR:CE2	2.23	0.71
3:E:172:THR:HG21	3:E:192:MET:SD	2.31	0.71
6:R:71:MET:SD	6:R:85:LEU:HD22	2.33	0.68
6:R:69:ILE:HD13	6:R:344:PHE:CB	2.02	0.67
6:R:333:TYR:O	6:R:337:ASN:ND2	2.28	0.66
1:A:259:ASN:OD1	1:A:260:LYS:N	2.28	0.66
3:E:172:THR:CG2	3:E:192:MET:SD	2.84	0.66
3:E:172:THR:CB	3:E:192:MET:SD	2.83	0.66
6:R:69:ILE:CD1	6:R:344:PHE:CB	2.67	0.65
2:B:150:TYR:O	2:B:167:GLY:N	2.29	0.65
6:R:90:SER:OG	6:R:128:SER:O	2.15	0.65
3:E:63:THR:HG23	3:E:64:VAL:HG13	1.79	0.65
2:B:39:THR:HG21	2:B:305:LEU:O	1.99	0.63
5:L:9:ARG:HB2	5:L:10:PHE:CD2	2.34	0.62
3:E:29:PHE:O	3:E:72:ARG:NH2	2.32	0.62
1:A:330:PHE:O	1:A:341:ARG:NH1	2.32	0.62
1:A:5:LEU:O	1:A:10:LYS:NZ	2.34	0.60
3:E:52:SER:OG	3:E:57:THR:OG1	2.13	0.60
3:E:191:ARG:C	3:E:192:MET:HG2	2.26	0.60
1:A:46:LYS:O	1:A:50:VAL:HG22	2.04	0.58
2:B:150:TYR:OH	2:B:191:ASP:OD2	2.15	0.58
3:E:223:ASP:OD1	3:E:227:TYR:OH	2.20	0.57
3:E:165:LEU:HA	3:E:233:LEU:HD11	1.89	0.55
2:B:88:ASP:O	2:B:92:THR:N	2.40	0.53

Continued on next page...

Continued from previous page...

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
2:B:152:SER:OG	2:B:193:MET:O	2.14	0.53
2:B:59:HIS:O	2:B:339:SER:OG	2.27	0.53
2:B:100:LEU:CD1	2:B:105:VAL:HG21	2.39	0.53
6:R:172:ILE:O	6:R:176:SER:OG	2.25	0.52
6:R:86:ASN:OD1	6:R:135:THR:OG1	2.09	0.52
6:R:295:MET:HE3	6:R:299:TYR:CD2	2.44	0.52
2:B:92:THR:HG22	2:B:92:THR:O	2.10	0.51
2:B:81:ASP:OD2	2:B:83:LYS:NZ	2.41	0.51
6:R:44:VAL:HA	6:R:47:ILE:HD12	1.93	0.51
6:R:61:MET:O	6:R:65:VAL:HG23	2.12	0.50
6:R:102:THR:O	6:R:106:ASN:ND2	2.44	0.50
2:B:141:SER:O	2:B:142:ARG:NH1	2.45	0.49
2:B:200:ASP:O	2:B:201:THR:OG1	2.24	0.49
1:A:216:ASN:ND2	1:A:248:TRP:O	2.42	0.49
2:B:294:TYR:OH	2:B:302:TRP:NE1	2.43	0.49
1:A:183:GLY:O	1:A:202:GLY:N	2.42	0.49
3:E:61:ALA:HB3	3:E:64:VAL:HG22	1.94	0.48
6:R:177:PRO:O	6:R:180:VAL:HB	2.13	0.48
1:A:191:VAL:HG11	1:A:343:PHE:HB2	1.96	0.48
2:B:173:LEU:CD1	2:B:218:VAL:HG13	2.44	0.48
2:B:299:CYS:SG	2:B:320:VAL:HG21	2.54	0.48
6:R:185:GLN:O	6:R:205:TRP:N	2.43	0.48
6:R:230:ALA:HB3	6:R:231:PRO:HD3	1.96	0.48
6:R:282:LEU:O	6:R:286:SER:OG	2.25	0.48
6:R:123:LEU:HD13	6:R:179:ALA:HB2	1.96	0.47
6:R:71:MET:SD	6:R:85:LEU:CD2	3.01	0.47
2:B:336:SER:OG	2:B:338:ASP:OD1	2.33	0.47
6:R:176:SER:H	6:R:177:PRO:CD	2.27	0.47
6:R:133:VAL:HG21	6:R:227:ILE:HG23	1.96	0.47
2:B:154:CYS:O	2:B:155:ARG:NH1	2.47	0.47
6:R:293:LEU:HD11	6:R:314:ILE:HG22	1.97	0.47
3:E:174:LEU:O	3:E:191:ARG:N	2.49	0.46
3:E:178:LEU:HB2	3:E:188:LEU:HD11	1.98	0.46
6:R:175:MET:HB3	6:R:175:MET:HE2	1.58	0.46
3:E:37:VAL:O	3:E:95:TYR:N	2.49	0.45
6:R:131:ALA:O	6:R:135:THR:OG1	2.34	0.45
2:B:281:VAL:HG13	2:B:290:LEU:HD11	1.97	0.45
6:R:127:ILE:HD13	6:R:172:ILE:HG12	1.99	0.45
3:E:35:HIS:NE2	3:E:101:TYR:OH	2.46	0.45
6:R:126:GLY:HA3	6:R:175:MET:HE2	1.99	0.45
2:B:76:VAL:HG21	2:B:117:VAL:HG21	1.98	0.45

Continued on next page...

Continued from previous page...

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
6:R:158:LYS:O	6:R:162:VAL:HG23	2.17	0.45
1:A:240:PHE:CZ	1:A:254:VAL:HG21	2.53	0.44
6:R:69:ILE:HD11	6:R:344:PHE:HB3	1.87	0.44
3:E:191:ARG:C	3:E:192:MET:CG	2.90	0.43
2:B:39:THR:HG21	2:B:305:LEU:C	2.43	0.43
6:R:56:PHE:O	6:R:57:PHE:C	2.61	0.43
6:R:232:LEU:O	6:R:236:VAL:HG23	2.17	0.43
1:A:360:LEU:HG	6:R:272:ILE:HG23	2.01	0.43
3:E:97:VAL:HG13	3:E:110:PHE:O	2.19	0.43
3:E:174:LEU:HB3	3:E:192:MET:HB3	2.00	0.43
5:L:10:PHE:CE2	6:R:175:MET:HE1	2.53	0.43
2:B:205:VAL:HG22	2:B:239:PHE:CE2	2.54	0.43
6:R:176:SER:H	6:R:177:PRO:HD2	1.84	0.43
6:R:123:LEU:CD1	6:R:179:ALA:HB2	2.49	0.42
3:E:12:VAL:HG11	3:E:86:LEU:CD1	2.49	0.42
3:E:7:SER:OG	3:E:21:SER:OG	2.36	0.42
6:R:166:ILE:O	6:R:166:ILE:HG22	2.18	0.42
2:B:281:VAL:CG1	2:B:290:LEU:HD11	2.49	0.42
3:E:91:THR:HG23	3:E:91:THR:O	2.20	0.42
2:B:53:ARG:NE	2:B:345:ASN:OD1	2.44	0.42
6:R:78:THR:HG22	6:R:79:VAL:N	2.35	0.42
6:R:170:LEU:O	6:R:174:ILE:HG13	2.20	0.42
6:R:120:ILE:O	6:R:124:VAL:HG23	2.19	0.41
6:R:329:ASN:N	6:R:330:PRO:HD2	2.35	0.41
6:R:288:LEU:N	6:R:289:PRO:CD	2.83	0.41
5:L:6:PRO:O	5:L:7:VAL:C	2.64	0.41
6:R:223:LEU:HD23	6:R:223:LEU:HA	1.82	0.41
2:B:252:ASP:O	2:B:253:ALA:HB3	2.21	0.41
6:R:229:LEU:HD23	6:R:229:LEU:HA	1.91	0.41
2:B:259:ASP:C	2:B:260:LEU:HD22	2.46	0.41
6:R:232:LEU:C	6:R:232:LEU:HD12	2.45	0.41
3:E:138:ILE:N	3:E:138:ILE:HD12	2.36	0.40
3:E:235:TYR:O	3:E:237:LEU:N	2.53	0.40
1:A:336:THR:OG1	1:A:337:GLU:N	2.55	0.40
6:R:90:SER:O	6:R:94:VAL:HG23	2.21	0.40

There are no symmetry-related clashes.

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 PDB entries followed by that with respect to all EM entries.

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	198/361 (55%)	197 (100%)	1 (0%)	0	100	100
2	B	333/371 (90%)	332 (100%)	1 (0%)	0	100	100
3	E	224/247 (91%)	220 (98%)	4 (2%)	0	100	100
4	G	53/70 (76%)	53 (100%)	0	0	100	100
5	L	9/11 (82%)	4 (44%)	5 (56%)	0	100	100
6	R	299/420 (71%)	289 (97%)	9 (3%)	1 (0%)	36	54
All	All	1116/1480 (75%)	1095 (98%)	20 (2%)	1 (0%)	49	68

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
6	R	176	SER

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 PDB entries followed by that with respect to all EM entries.

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	186/314 (59%)	186 (100%)	0	100	100
2	B	268/302 (89%)	268 (100%)	0	100	100
3	E	177/198 (89%)	177 (100%)	0	100	100
4	G	40/57 (70%)	40 (100%)	0	100	100
5	L	8/8 (100%)	7 (88%)	1 (12%)	4	8
6	R	271/382 (71%)	262 (97%)	9 (3%)	33	58

Continued on next page...

Continued from previous page...

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
All	All	950/1261 (75%)	940 (99%)	10 (1%)	63	81

All (10) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
5	L	9	ARG
6	R	47	ILE
6	R	53	PHE
6	R	154	LYS
6	R	175	MET
6	R	208	GLU
6	R	227	ILE
6	R	232	LEU
6	R	315	TYR
6	R	332	ILE

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (3) such sidechains are listed below:

Mol	Chain	Res	Type
2	B	235	ASN
6	R	41	GLN
6	R	73	ASN

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 oligosaccharides in this entry.

5.6 Ligand geometry [i](#)

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.