Full wwPDB EM Validation Report (i)

Nov 20, 2022 – 04:58 AM EST

PDB ID	:	7RKX
EMDB ID	:	EMD-24506
Title	:	Structure of US27-Gi-scFv16 in CL-state
Authors	:	Tsutsumi, N.; Jude, K.M.; Garcia, K.C.
Deposited on	:	2021-07-22
Resolution	:	3.10 Å(reported)

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 (i) 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 (i)) were used in the production of this report:

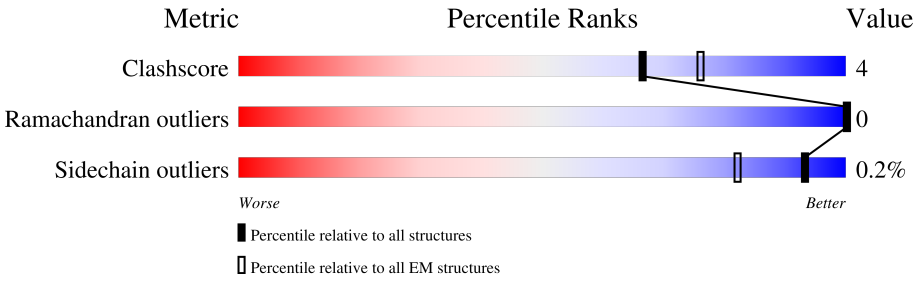
EMDB validation analysis	:	0.0.1.dev43
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.31.3

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

The reported resolution of this entry is 3.10 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)	EM structures (#Entries)
Clashscore	158937	4297
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826

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 <=5% The upper red bar (where present) indicates the fraction of residues that have poor fit to the EM map (all-atom inclusion < 40%). The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	353	57% 6% 37%
2	B	345	94% . .
3	C	67	79% 6% 15%
4	D	256	83% 8% 9%
5	R	382	9% 57% 15% 28%

2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 8559 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(i) subunit alpha-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	223	1759	1119	292	335	13	0	0

- 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
			Total	C	N	O	S		
2	B	338	2594	1601	465	507	21	0	0

There are 6 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-4	GLY	-	expression tag	UNP P62873
B	-3	PRO	-	expression tag	UNP P62873
B	-2	GLY	-	expression tag	UNP P62873
B	-1	SER	-	expression tag	UNP P62873
B	0	SER	-	expression tag	UNP P62873
B	1	GLY	-	expression tag	UNP P62873

- Molecule 3 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
3	C	57	435	273	77	82	3	0	0

- Molecule 4 is a protein called Antibody fragment scFv16.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
4	D	233	1780	1130	296	344	10	0	0

- Molecule 5 is a protein called G-protein coupled receptor homolog US27.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
5	R	275	1991	1320	326	333	12	0	0

There are 20 discrepancies between the modelled and reference sequences:

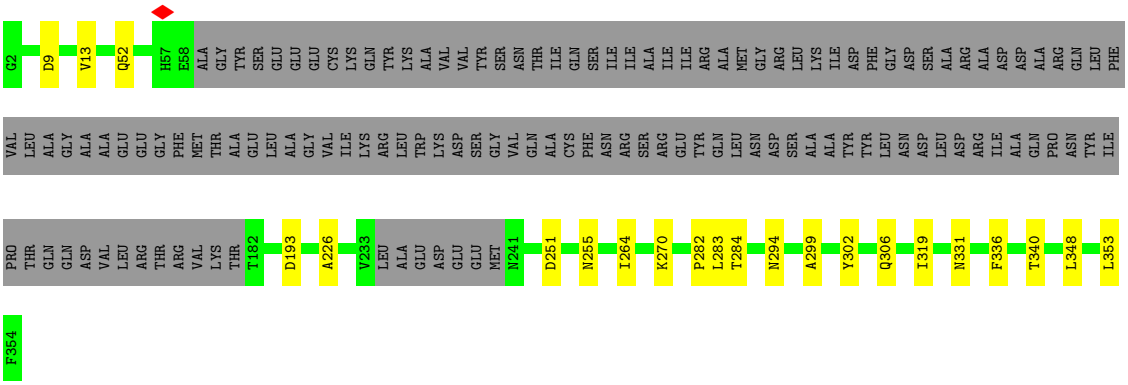
Chain	Residue	Modelled	Actual	Comment	Reference
R	-6	ASP	-	expression tag	UNP P09703
R	-5	TYR	-	expression tag	UNP P09703
R	-4	LYS	-	expression tag	UNP P09703
R	-3	ASP	-	expression tag	UNP P09703
R	-2	ASP	-	expression tag	UNP P09703
R	-1	ASP	-	expression tag	UNP P09703
R	0	ASP	-	expression tag	UNP P09703
R	363	SER	-	expression tag	UNP P09703
R	364	ARG	-	expression tag	UNP P09703
R	365	GLY	-	expression tag	UNP P09703
R	366	ALA	-	expression tag	UNP P09703
R	367	ALA	-	expression tag	UNP P09703
R	368	HIS	-	expression tag	UNP P09703
R	369	HIS	-	expression tag	UNP P09703
R	370	HIS	-	expression tag	UNP P09703
R	371	HIS	-	expression tag	UNP P09703
R	372	HIS	-	expression tag	UNP P09703
R	373	HIS	-	expression tag	UNP P09703
R	374	HIS	-	expression tag	UNP P09703
R	375	HIS	-	expression tag	UNP P09703

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 atom inclusion in map 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 diamond above a residue indicates a poor fit to the EM map for this residue (all-atom inclusion < 40%). 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(i) subunit alpha-1

Chain A: 57% 6% 37%



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

Chain B: 94%

• Molecule 3: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2

Chain C: 79% 6% 15%

• Molecule 4: Antibody fragment scFv16

Chain D: 83% 8% 9%

SER LEU GLU VAL LEU PHE GLN

• Molecule 5: G-protein coupled receptor homolog US27

Chain R:

ASP TYR LYS ASP ASP ASP ASP MET THR THR SER THR ASN ASN GLN THR LEU THR GLN VAL SER ASN MET THR ASN HIS T20 L21 T24 E25 T26 E31 Y32 L39 M40 V43 G44 T45 F46 L47 N48 R59 R60 K61 K62 S66 D67 T68 Y69 I70 C71 N72 L78

V81 P85 A91 S98 V101 V102 C103 S104 N107 A108 C109 I112 V118 L121 R128 W134 GLY VAL GLU LEU ASN ARG VAL ARG ASN ASN LYS ARG ALA T148 C149 W150 I156 L157 A158 L167 S170 H171 E175 C176 V177 G178 E183 THR

SER G186 W187 V190 F191 T194 K195 V196 N197 Y201 P204 I205 R214 I219 I220 N221 Y222 V223 G224 K225 W226 H227 M228 Q229 T230 L231 V236 A242 F253 I257 R258 L268 V271 C275 L276 Y277 V278 L282 R286 A287 N290 I293

K303 D304 M305 W306 T307 T308 L309 ARG VAL PHE ALA CYS CYS CYS VAL LYS GLN GLU ILE PRO TYR GLN ASP ILE ASP ILE GLU LEU GLN LYS ASP ILE GLN ARG ARG ALA LYS HIS THR LYS ARG THR HIS TYR ASP ARG LYS ASN ALA PRO MET GLU SER GLY GLU GLU GLU PHE LEU LEU

SER ARG GLY ALA ALA HIS HIS HIS HIS HIS HIS HIS HIS

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	268877	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION; Final per-particle CTF values were determined by cryoSPARC Local CTF Refinement.	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	73	Depositor
Minimum defocus (nm)	-800	Depositor
Maximum defocus (nm)	-2000	Depositor
Magnification	57624	Depositor
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	1.160	Depositor
Minimum map value	-0.756	Depositor
Average map value	0.001	Depositor
Map value standard deviation	0.028	Depositor
Recommended contour level	0.11	Depositor
Map size (Å)	260.31, 260.31, 260.31	wwPDB
Map dimensions	300, 300, 300	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.8677, 0.8677, 0.8677	Depositor

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	0.29	0/1788	0.50	1/2404 (0.0%)
2	B	0.32	0/2641	0.58	0/3580
3	C	0.28	0/441	0.48	0/596
4	D	0.34	0/1824	0.55	0/2474
5	R	0.28	0/2045	0.52	1/2812 (0.0%)
All	All	0.31	0/8739	0.54	2/11866 (0.0%)

There are no bond length outliers.

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	193	ASP	CB-CG-OD1	5.98	123.68	118.30
5	R	276	LEU	CA-CB-CG	5.30	127.49	115.30

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	1759	0	1715	13	0
2	B	2594	0	2495	9	0
3	C	435	0	445	3	0
4	D	1780	0	1714	11	0
5	R	1991	0	1768	33	0
All	All	8559	0	8137	66	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 4.

All (66) 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)
4:D:130:GLN:NE2	4:D:217:CYS:SG	2.56	0.78
2:B:230:ASN:ND2	2:B:246:ASP:OD1	2.26	0.68
2:B:227:SER:O	2:B:245:SER:OG	2.14	0.66
4:D:52:SER:O	4:D:72:ARG:NH1	2.29	0.65
5:R:21:LEU:HB2	5:R:24:THR:HG22	1.82	0.62
4:D:190:ARG:NH2	4:D:211:ASP:OD2	2.34	0.61
5:R:103:CYS:SG	5:R:107:ASN:ND2	2.75	0.59
1:A:52:GLN:OE1	1:A:331:ASN:ND2	2.35	0.59
5:R:26:ILE:HD11	5:R:276:LEU:HD11	1.85	0.59
1:A:353:LEU:O	5:R:229:GLN:NE2	2.37	0.58
1:A:226:ALA:HB1	1:A:270:LYS:HD2	1.85	0.58
5:R:201:TYR:OH	5:R:242:ALA:O	2.23	0.57
1:A:251:ASP:OD1	1:A:255:ASN:ND2	2.39	0.56
1:A:282:PRO:HB3	1:A:294:ASN:HD21	1.70	0.56
3:C:18:GLN:NE2	3:C:22:GLU:OE2	2.28	0.55
5:R:67:ASP:O	5:R:70:ILE:HG22	2.07	0.55
4:D:52:SER:OG	4:D:53:SER:N	2.41	0.54
4:D:12:VAL:HG11	4:D:86:LEU:HD12	1.90	0.54
5:R:197:ASN:HD21	5:R:253:PHE:HB2	1.73	0.53
5:R:44:GLY:O	5:R:48:ASN:ND2	2.41	0.53
5:R:205:ILE:HD13	5:R:242:ALA:HB2	1.91	0.53
5:R:118:VAL:HG13	5:R:204:PRO:HG3	1.90	0.52
4:D:94:TYR:O	4:D:114:GLY:HA2	2.10	0.52
1:A:282:PRO:HB2	1:A:284:THR:HG22	1.93	0.51
2:B:4:LEU:HD21	2:B:8:ARG:HH21	1.76	0.50
5:R:258:ARG:HB3	5:R:268:LEU:HD12	1.94	0.49
2:B:247:ASP:OD1	2:B:247:ASP:N	2.43	0.49
3:C:62:ARG:NH1	3:C:62:ARG:HA	2.29	0.48
4:D:232:LYS:NZ	4:D:234:GLU:OE1	2.47	0.48
2:B:146:LEU:HD11	2:B:159:THR:HB	1.95	0.48
2:B:245:SER:OG	2:B:246:ASP:N	2.46	0.47
5:R:66:SER:HA	5:R:69:TYR:CD2	2.49	0.47
5:R:128:ARG:HE	5:R:230:THR:HG21	1.78	0.47
5:R:303:LYS:O	5:R:307:THR:HG23	2.16	0.46
3:C:58:GLU:N	3:C:58:GLU:OE1	2.49	0.46
2:B:165:THR:HG22	2:B:181:THR:HG22	1.97	0.45
5:R:20:THR:HG23	5:R:31:GLU:HG2	1.98	0.45

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
5:R:220:ILE:HA	5:R:224:GLY:HA2	1.98	0.45
5:R:175:GLU:HG3	5:R:178:GLY:H	1.82	0.44
1:A:336:PHE:O	1:A:340:THR:HG23	2.17	0.44
1:A:348:LEU:HD11	5:R:219:ILE:HG13	1.98	0.43
5:R:40:MET:HA	5:R:43:VAL:HG12	1.99	0.43
5:R:45:THR:HA	5:R:48:ASN:HD22	1.83	0.43
5:R:231:LEU:HD23	5:R:231:LEU:HA	1.88	0.43
5:R:68:THR:O	5:R:72:ASN:ND2	2.49	0.43
1:A:264:ILE:HB	1:A:319:ILE:HD13	2.01	0.42
2:B:76:ASP:OD1	2:B:76:ASP:N	2.53	0.42
5:R:236:VAL:HG13	5:R:293:ILE:HD12	2.02	0.42
1:A:283:LEU:HD22	1:A:299:ALA:HB1	2.02	0.42
5:R:78:LEU:O	5:R:81:VAL:HG22	2.19	0.42
5:R:26:ILE:HG21	5:R:176:CYS:HB2	2.01	0.42
4:D:99:SER:HB2	4:D:107:PRO:HB3	2.02	0.42
4:D:13:GLN:HG3	4:D:14:PRO:HD2	2.02	0.42
5:R:81:VAL:O	5:R:85:PRO:HD2	2.20	0.41
4:D:215:TYR:O	4:D:230:GLY:HA2	2.20	0.41
1:A:348:LEU:HD22	5:R:223:VAL:HG21	2.01	0.41
4:D:91:THR:HG23	4:D:118:THR:HA	2.01	0.41
5:R:121:LEU:HD23	5:R:121:LEU:HA	1.93	0.41
5:R:282:LEU:HD23	5:R:282:LEU:HA	1.88	0.41
5:R:286:ARG:HG3	5:R:287:ALA:N	2.35	0.41
1:A:9:ASP:O	1:A:13:VAL:HG23	2.21	0.40
2:B:26:ALA:HB2	2:B:259:GLN:HE22	1.86	0.40
5:R:214:ARG:HA	5:R:214:ARG:HD2	1.91	0.40
1:A:302:TYR:O	1:A:306:GLN:HG2	2.22	0.40
5:R:109:CYS:O	5:R:112:ILE:HG22	2.22	0.40
5:R:101:VAL:O	5:R:104:SER:HB2	2.22	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 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	217/353 (62%)	208 (96%)	9 (4%)	0	100	100
2	B	336/345 (97%)	325 (97%)	11 (3%)	0	100	100
3	C	55/67 (82%)	55 (100%)	0	0	100	100
4	D	229/256 (90%)	221 (96%)	8 (4%)	0	100	100
5	R	269/382 (70%)	254 (94%)	15 (6%)	0	100	100
All	All	1106/1403 (79%)	1063 (96%)	43 (4%)	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 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	189/304 (62%)	189 (100%)	0	100	100
2	B	279/285 (98%)	279 (100%)	0	100	100
3	C	45/55 (82%)	45 (100%)	0	100	100
4	D	195/208 (94%)	195 (100%)	0	100	100
5	R	172/344 (50%)	170 (99%)	2 (1%)	71	88
All	All	880/1196 (74%)	878 (100%)	2 (0%)	93	97

All (2) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
5	R	222	TYR
5	R	290	ASN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (8) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	52	GLN
1	A	269	ASN
1	A	294	ASN

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Mol	Chain	Res	Type
1	A	331	ASN
2	B	17	GLN
2	B	32	GLN
5	R	48	ASN
5	R	197	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 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)

There are no chain breaks in this entry.

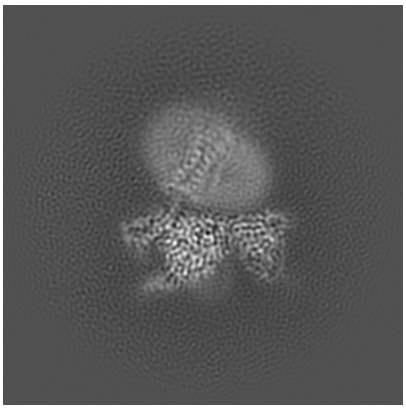
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-24506. These allow visual inspection of the internal detail of the map and identification of artifacts.

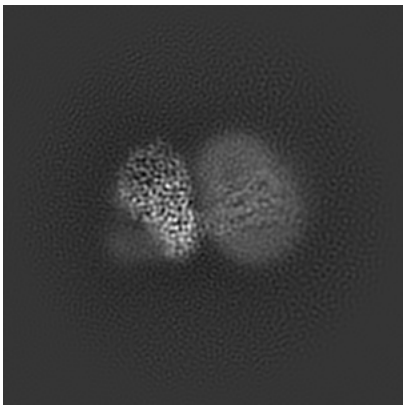
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections (i)

6.1.1 Primary map



X



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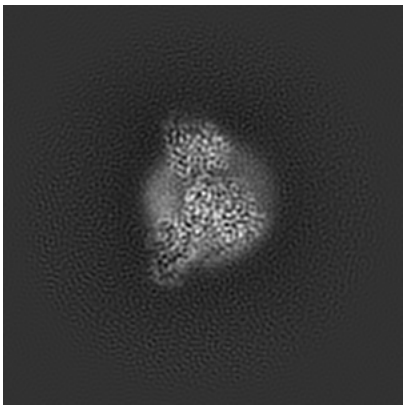
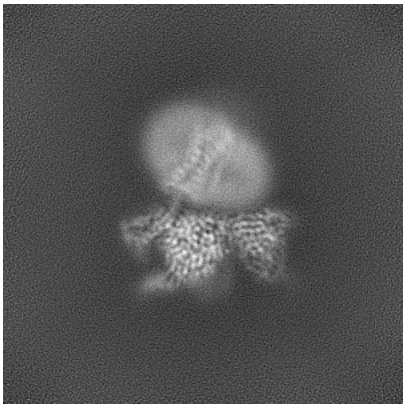
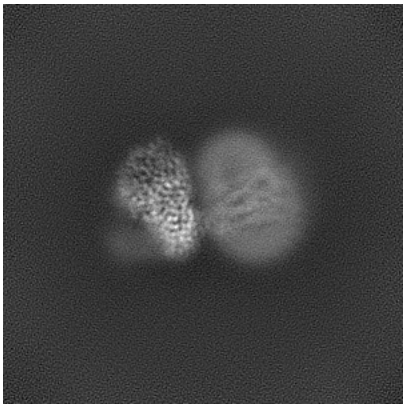


Z

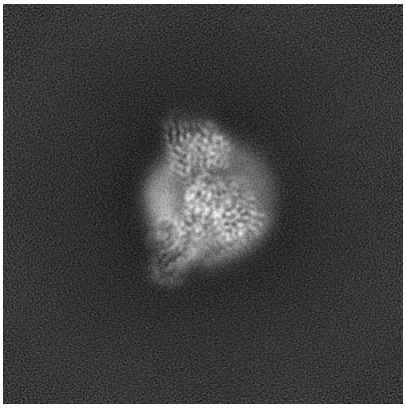
6.1.2 Raw map



X



Y

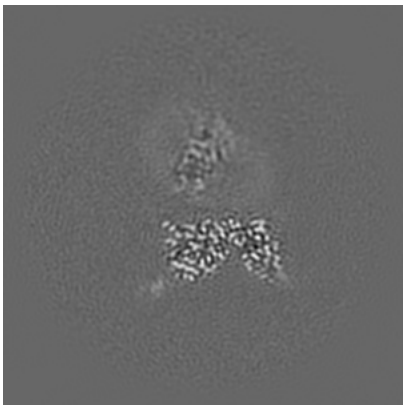


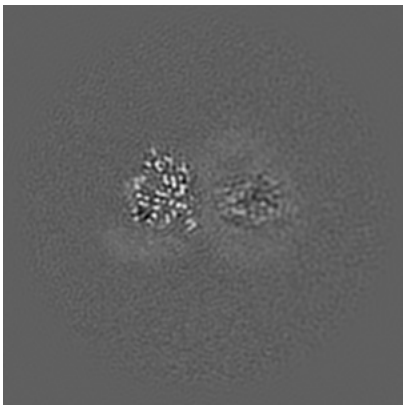
Z

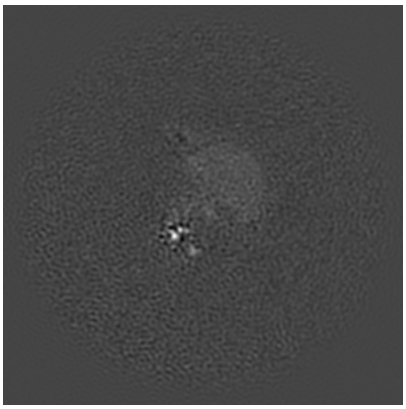
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

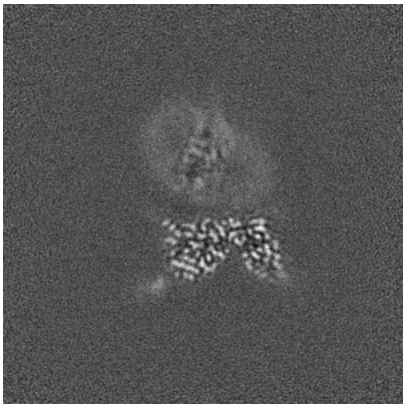
6.2.1 Primary map

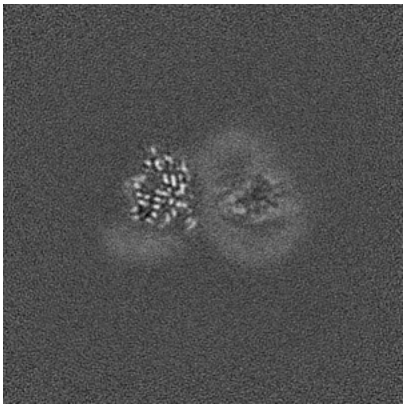

X Index: 150

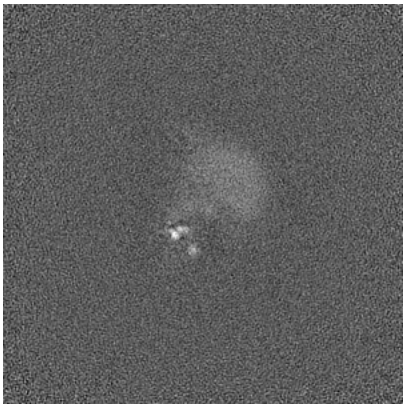

Y Index: 150


Z Index: 150

6.2.2 Raw map


X Index: 150

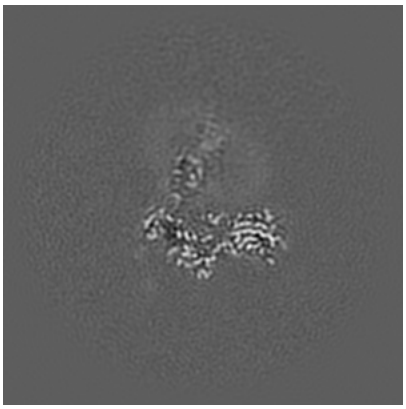

Y Index: 150

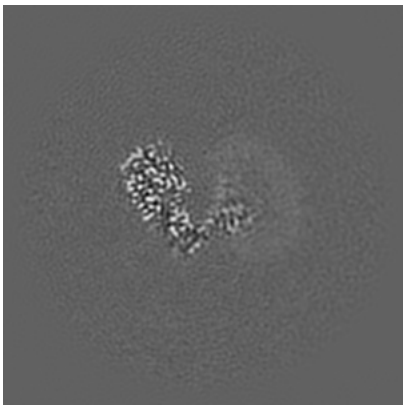

Z Index: 150

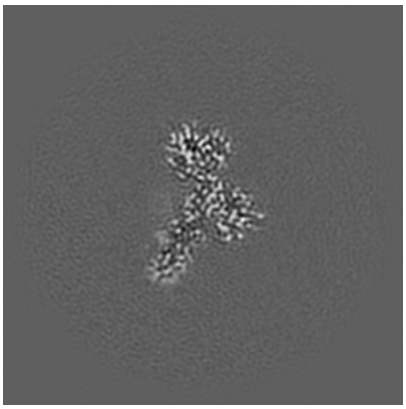
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

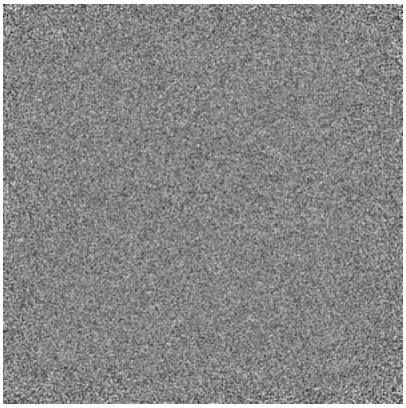
6.3.1 Primary map

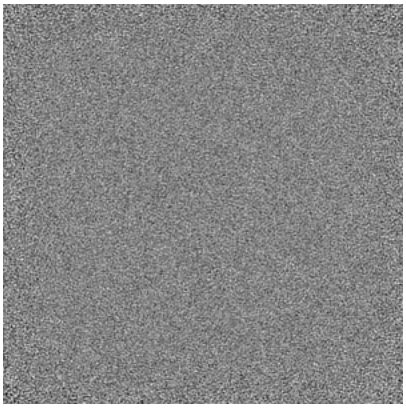

X Index: 139

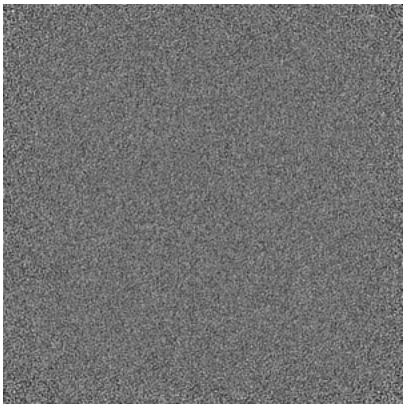

Y Index: 132


Z Index: 124

6.3.2 Raw map


X Index: 0


Y Index: 0


Z Index: 0

The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal surface views (i)

6.4.1 Primary map

X

Y

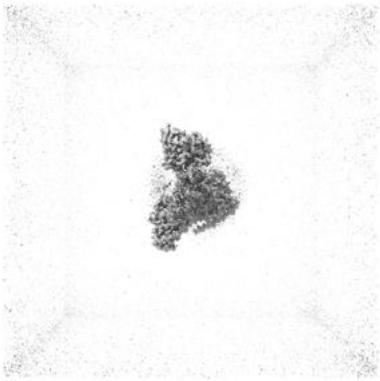
Z

The images above show the 3D surface view of the map at the recommended contour level 0.11. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.4.2 Raw map

X

Y



Z

These images show the 3D surface of the raw map. The raw map's contour level was selected so that its surface encloses the same volume as the primary map does at its recommended contour level.

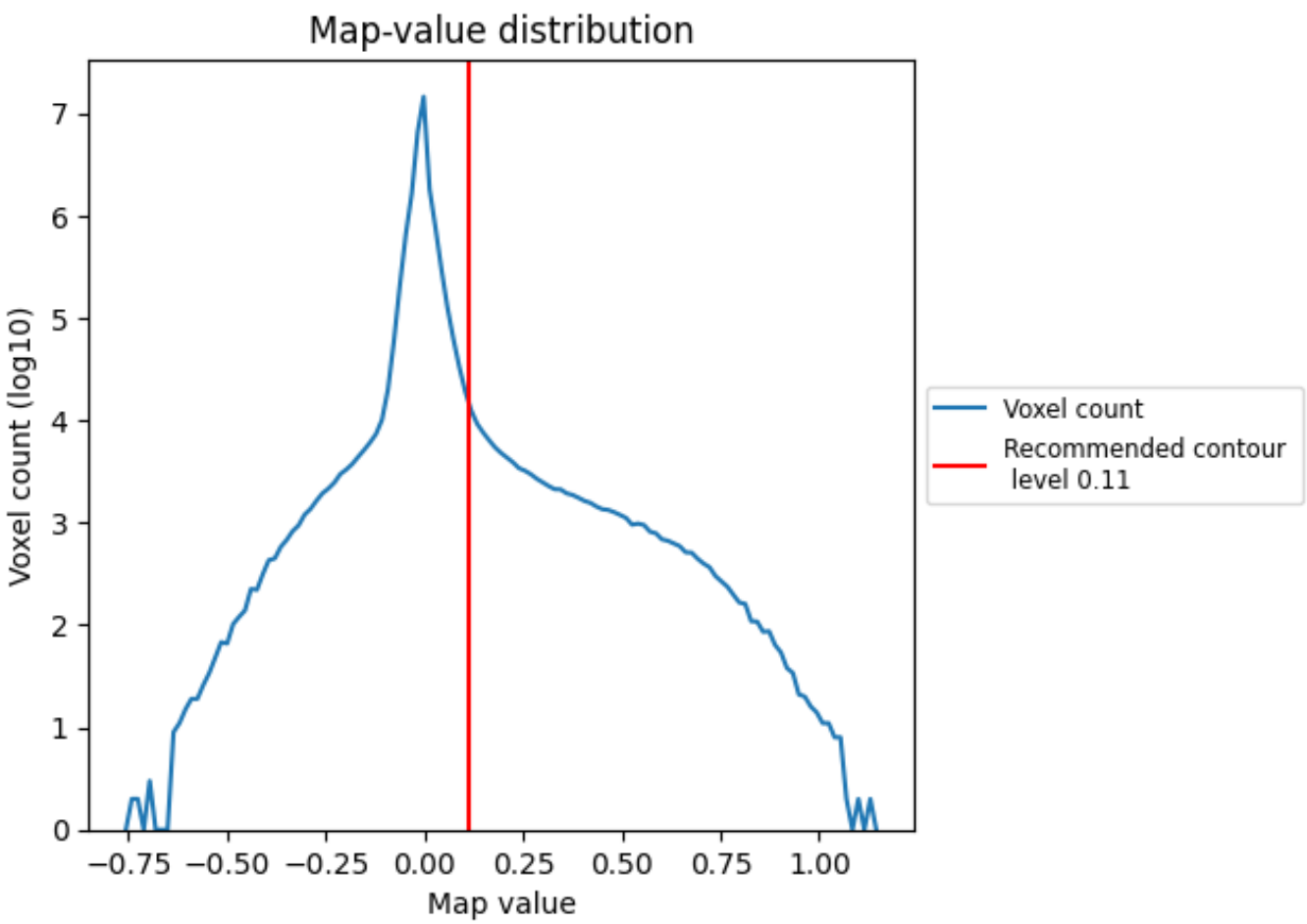
6.5 Mask visualisation (i)

This section was not generated. No masks/segmentation were deposited.

7 Map analysis (i)

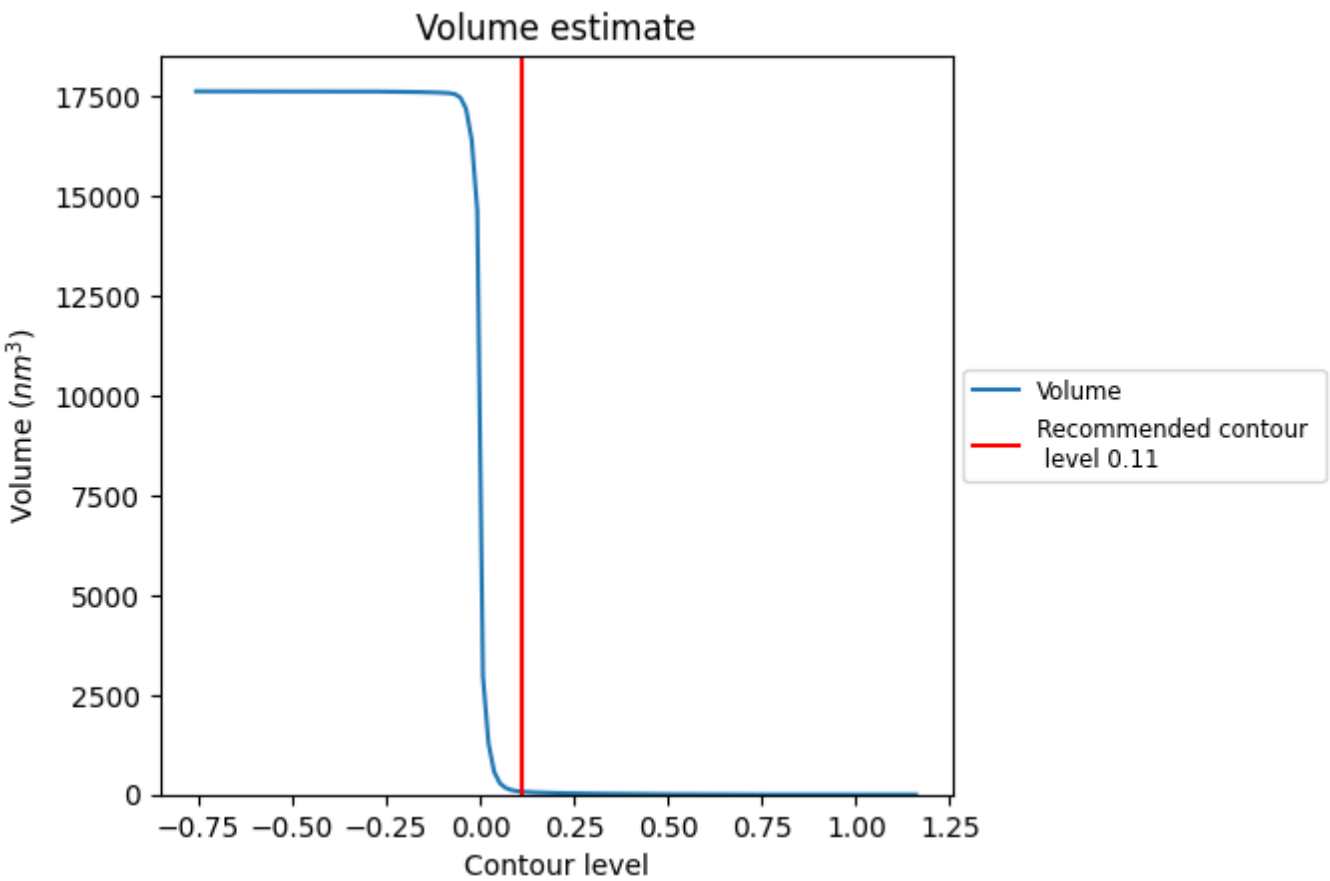
This section contains the results of statistical analysis of the map.

7.1 Map-value distribution (i)



The map-value distribution is plotted in 128 intervals along the x-axis. The y-axis is logarithmic. A spike in this graph at zero usually indicates that the volume has been masked.

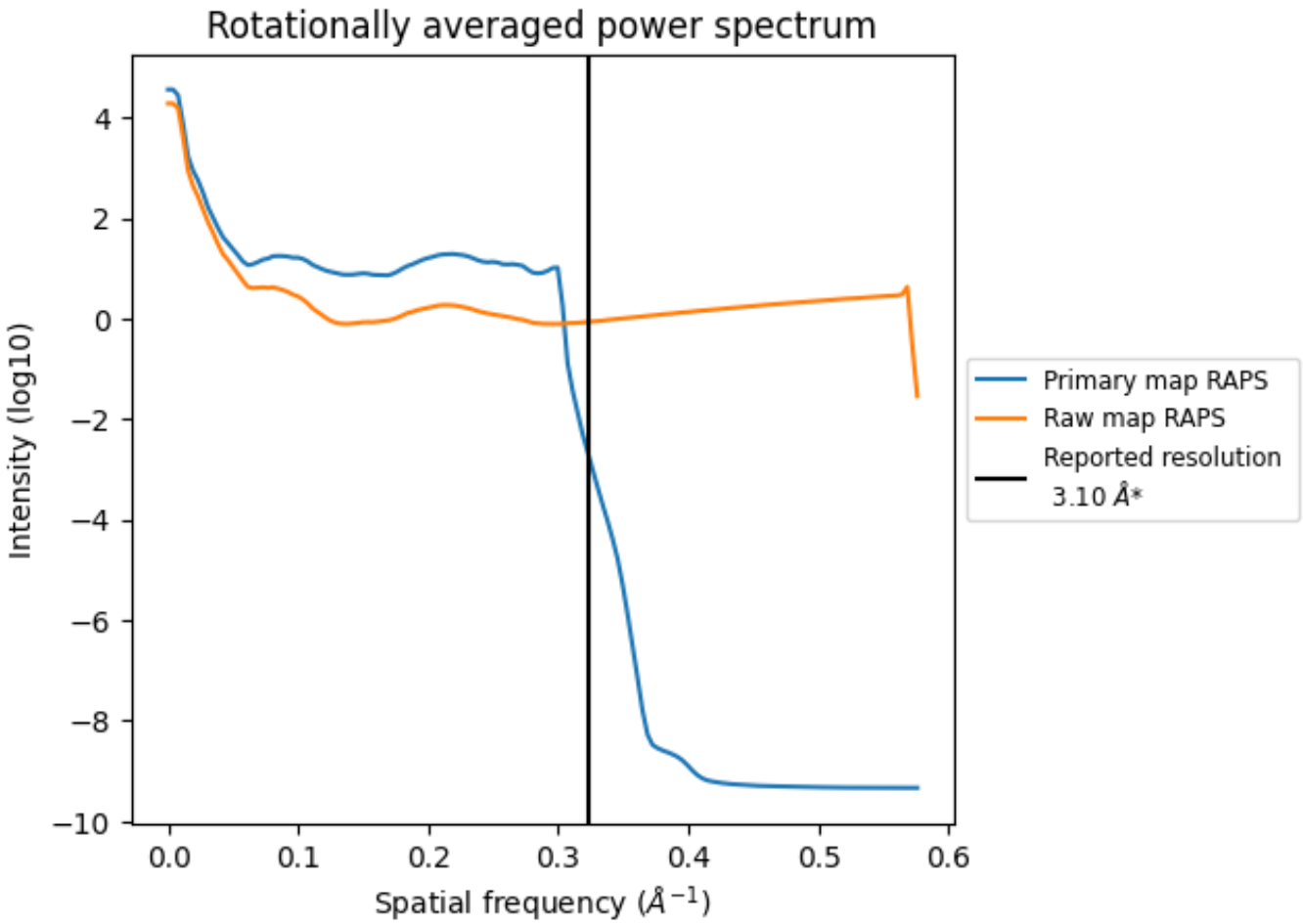
7.2 Volume estimate (i)



The volume at the recommended contour level is 74 nm^3; this corresponds to an approximate mass of 67 kDa.

The volume estimate graph shows how the enclosed volume varies with the contour level. The recommended contour level is shown as a vertical line and the intersection between the line and the curve gives the volume of the enclosed surface at the given level.

7.3 Rotationally averaged power spectrum (i)

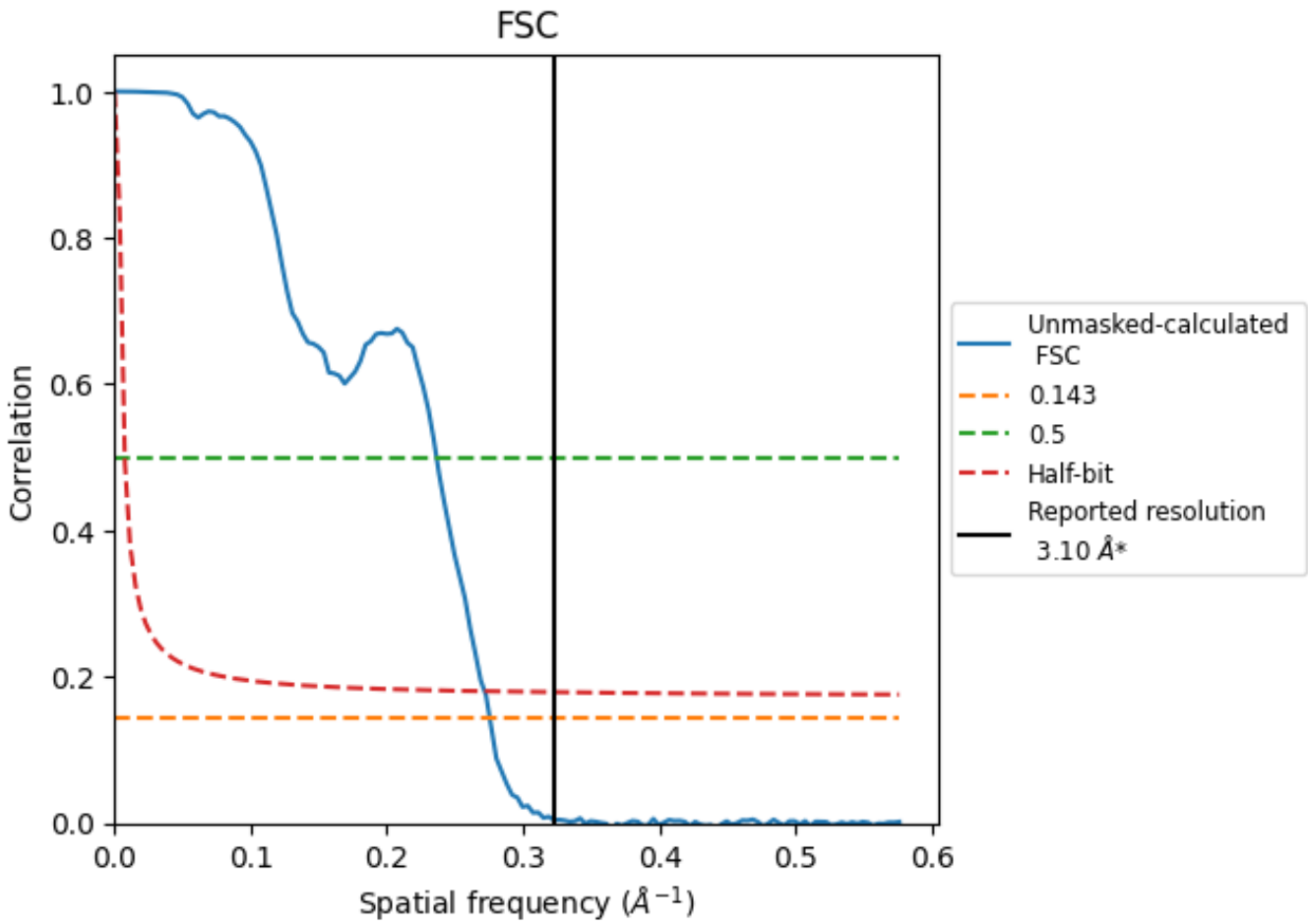


*Reported resolution corresponds to spatial frequency of 0.323 Å^{-1}

8 Fourier-Shell correlation (i)

Fourier-Shell Correlation (FSC) is the most commonly used method to estimate the resolution of single-particle and subtomogram-averaged maps. The shape of the curve depends on the imposed symmetry, mask and whether or not the two 3D reconstructions used were processed from a common reference. The reported resolution is shown as a black line. A curve is displayed for the half-bit criterion in addition to lines showing the 0.143 gold standard cut-off and 0.5 cut-off.

8.1 FSC (i)



*Reported resolution corresponds to spatial frequency of 0.323 Å^{-1}

8.2 Resolution estimates (i)

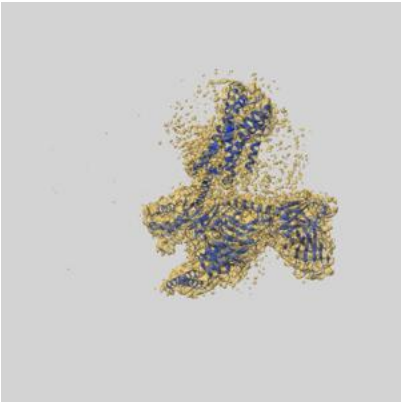
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.10	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	3.63	4.23	3.68

*Resolution estimate based on FSC curve calculated by comparison of deposited half-maps. The value from deposited half-maps intersecting FSC 0.143 CUT-OFF 3.63 differs from the reported value 3.1 by more than 10 %

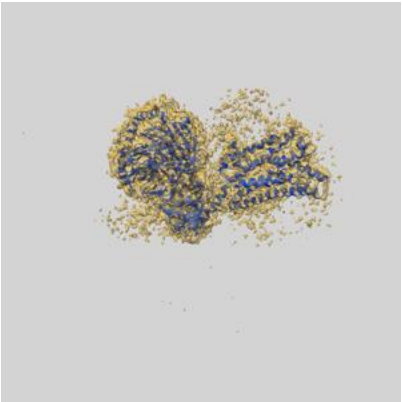
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-24506 and PDB model 7RKX. Per-residue inclusion information can be found in section 3 on page 5.

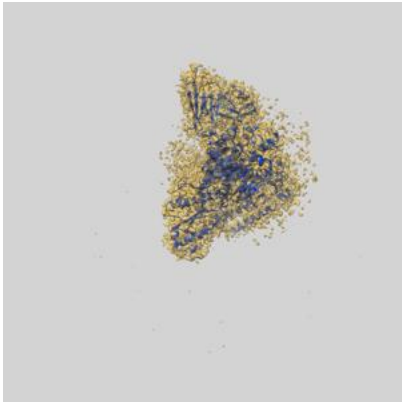
9.1 Map-model overlay (i)



X



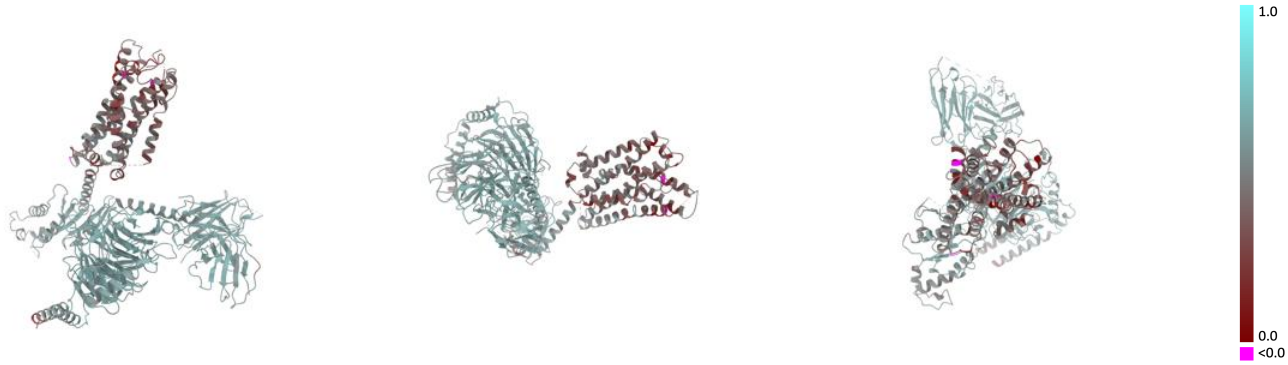
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.11 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

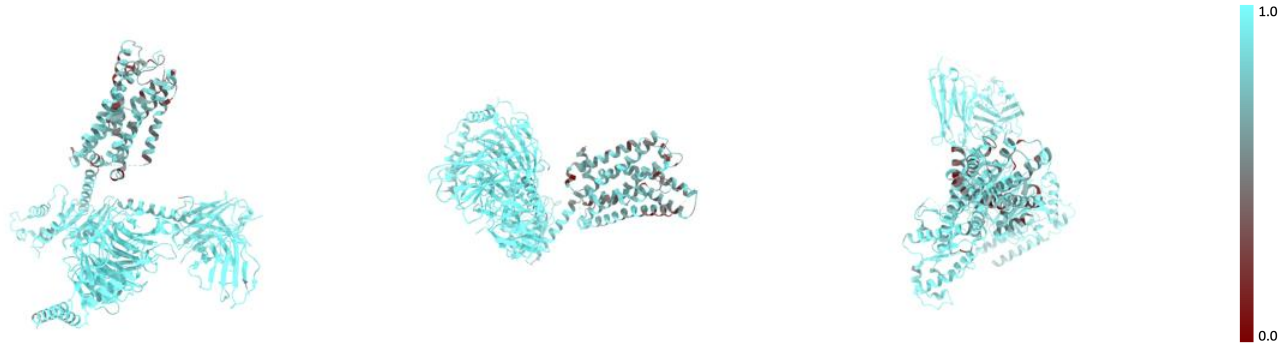
9.2 Q-score mapped to coordinate model (i)



X Y Z

The images above show the model with each residue coloured according its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions at which atoms can be resolved. Low Q-score values may therefore be expected for many entries.

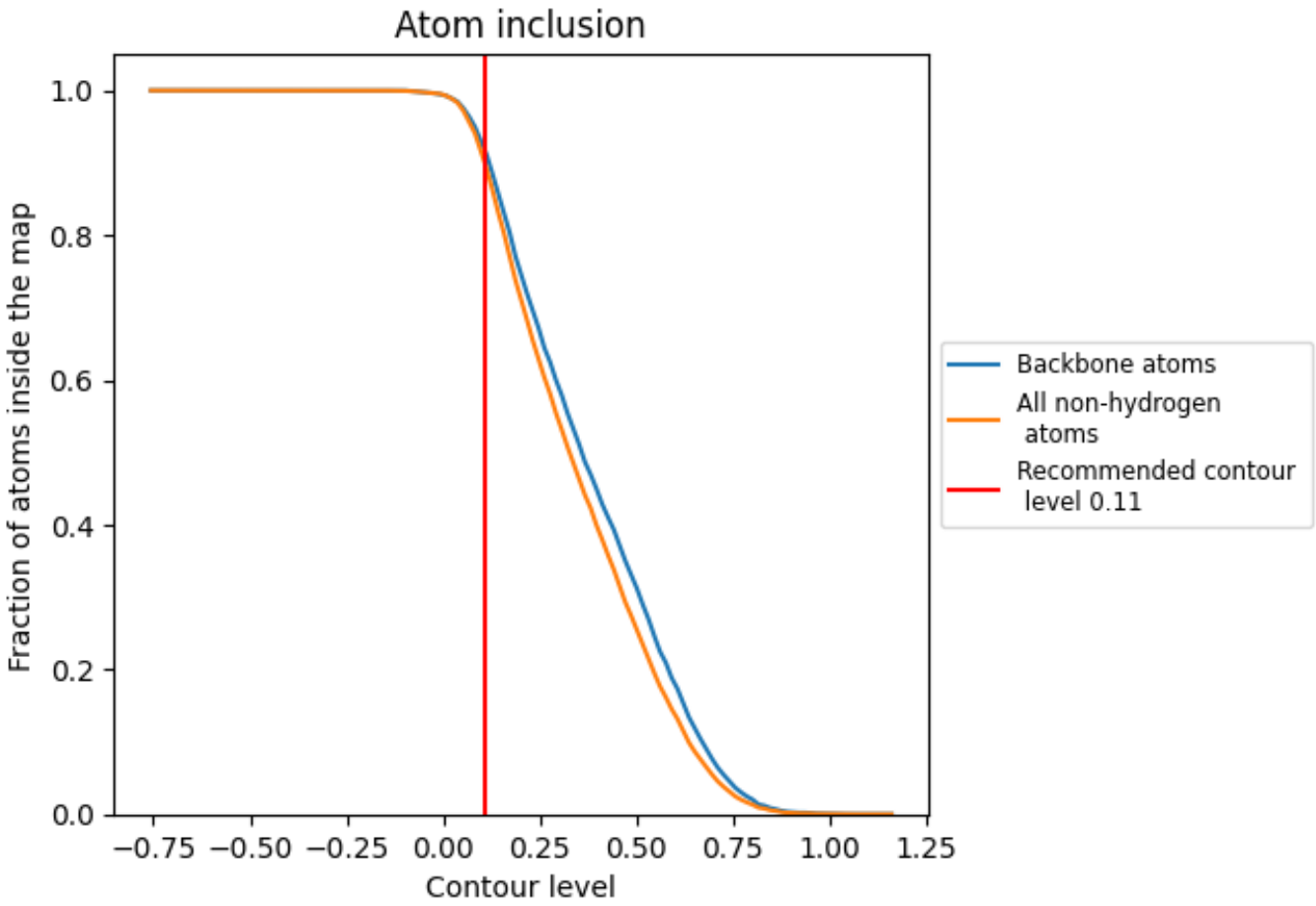
9.3 Atom inclusion mapped to coordinate model (i)



X Y Z

The images above show the model with each residue coloured according to its atom inclusion. This shows to what extent they are inside the map at the recommended contour level (0.11).

9.4 Atom inclusion (i)



At the recommended contour level, 91% of all backbone atoms, 90% of all non-hydrogen atoms, are inside the map.

9.5 Map-model fit summary (i)

The table lists the average atom inclusion at the recommended contour level (0.11) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.8963	0.5290
A	0.9245	0.5340
B	0.9720	0.5950
C	0.9136	0.5400
D	0.9690	0.5900
R	0.7049	0.3830