



Full wwPDB EM Validation Report ⓘ

Nov 11, 2021 – 04:09 pm GMT

PDB ID : 7PIU
EMDB ID : EMD-13453
Title : Cryo-EM structure of the agonist setmelanotide bound to the active melanocortin-4 receptor (MC4R) in complex with the heterotrimeric Gs protein at 2.6 Å resolution.
Authors : Heyder, N.A.; Schmidt, A.; Kleinau, G.; Hilal, T.; Scheerer, P.
Deposited on : 2021-08-23
Resolution : 2.58 Å (reported)
Based on initial model : 7PIV

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

EMDB validation analysis : 0.0.0.dev97
MolProbity : 4.02b-467
Percentile statistics : 20191225.v01 (using entries in the PDB archive December 25th 2019)
Ideal geometry (proteins) : Engh & Huber (2001)
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP) : 2.23.2

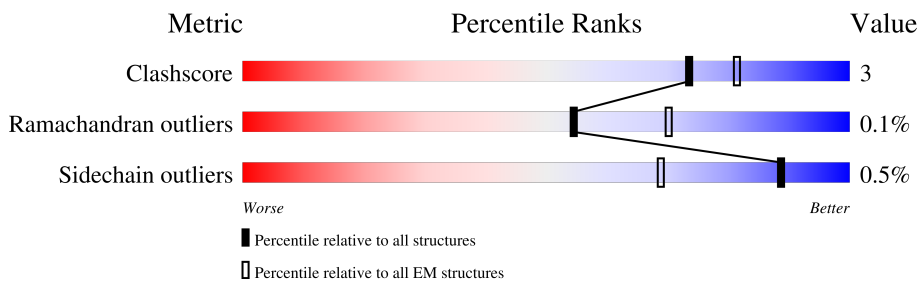
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 2.58 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 $\leq 5\%$. 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	R	346	
2	P	8	
3	A	380	
4	B	345	
5	G	71	
6	N	134	

2 Entry composition

There are 8 unique types of molecules in this entry. The entry contains 7788 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 Melanocortin receptor 4.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	R	259	2005	1340	312	330	23	3	0

There are 14 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
R	-7	ASP	-	expression tag	UNP P32245
R	-6	TYR	-	expression tag	UNP P32245
R	-5	LYS	-	expression tag	UNP P32245
R	-4	ASP	-	expression tag	UNP P32245
R	-3	ASP	-	expression tag	UNP P32245
R	-2	ASP	-	expression tag	UNP P32245
R	-1	ASP	-	expression tag	UNP P32245
R	0	LYS	-	expression tag	UNP P32245
R	333	LEU	-	expression tag	UNP P32245
R	334	GLU	-	expression tag	UNP P32245
R	335	VAL	-	expression tag	UNP P32245
R	336	LEU	-	expression tag	UNP P32245
R	337	PHE	-	expression tag	UNP P32245
R	338	GLN	-	expression tag	UNP P32245

- Molecule 2 is a protein called Setmelanotide (other names RM-493; BIM-22493; IRC-022493; Incivree).

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
2	P	8	74	47	17	8	2	0	0

- Molecule 3 is a protein called Isoform Gnas-2 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
3	A	189	1581	1012	297	266	6	1	0

- Molecule 4 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		
4	B	337	2629	1632	476	499	22	11	0

There are 6 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-4	GLY	-	expression tag	UNP P54311
B	-3	PRO	-	expression tag	UNP P54311
B	-2	GLY	-	expression tag	UNP P54311
B	-1	SER	-	expression tag	UNP P54311
B	0	SER	-	expression tag	UNP P54311
B	1	GLY	-	expression tag	UNP P54311

- Molecule 5 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		
5	G	55	421	266	74	78	3	1	0

- Molecule 6 is a protein called Camelid antibody fragment - nanobody 35.


Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
6	N	128	986	614	174	192	6	2	0

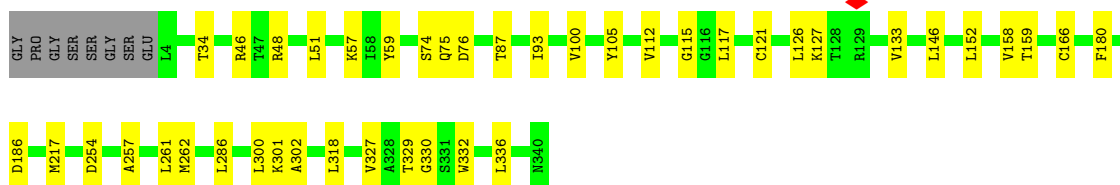
- Molecule 7 is CALCIUM ION (three-letter code: CA) (formula: Ca) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms		AltConf
			Total	Ca	
7	R	1	1	1	0

- Molecule 8 is water.

Mol	Chain	Residues	Atoms	AltConf
8	R	4	Total O 5 5	1
8	P	2	Total O 2 2	0
8	A	21	Total O 22 22	1
8	B	50	Total O 51 51	1
8	G	5	Total O 5 5	0
8	N	6	Total O 6 6	0

Chain B:  86% 12%




- Molecule 5: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2

Chain G:  75% 23%



- Molecule 6: Camelid antibody fragment - nanobody 35

Chain N:  90% 6%



4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	370621	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/\text{\AA}^2$)	40	Depositor
Minimum defocus (nm)	800	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	96000	Depositor
Image detector	FEI FALCON III (4k x 4k)	Depositor
Maximum map value	0.292	Depositor
Minimum map value	-0.131	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.006	Depositor
Recommended contour level	0.027	Depositor
Map size (Å)	232.96, 232.96, 232.96	wwPDB
Map dimensions	280, 280, 280	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.832, 0.832, 0.832	Depositor

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: DAL, CA, DPN

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	R	0.68	0/2056	0.71	0/2802
2	P	0.69	0/58	0.95	0/73
3	A	0.63	0/1614	0.67	0/2164
4	B	0.66	0/2709	0.74	0/3671
5	G	0.65	0/430	0.70	0/581
6	N	0.66	0/1012	0.71	0/1370
All	All	0.66	0/7879	0.71	0/10661

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	R	2005	0	2057	13	0
2	P	74	0	65	1	0
3	A	1581	0	1559	6	0
4	B	2629	0	2564	28	0
5	G	421	0	429	3	0
6	N	986	0	956	5	0
7	R	1	0	0	0	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
8	A	22	0	0	0	0
8	B	51	0	0	0	0
8	G	5	0	0	0	0
8	N	6	0	0	0	0
8	P	2	0	0	0	0
8	R	5	0	0	0	0
All	All	7788	0	7630	50	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 3.

All (50) 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:R:46:VAL:HG11	1:R:289:ILE:HD11	1.57	0.86
1:R:100[A]:GLU:HB2	1:R:125:ILE:HG21	1.56	0.86
4:B:46[B]:ARG:NH1	5:G:62:ARG:HH22	1.86	0.73
1:R:100[B]:GLU:HG2	1:R:125:ILE:HG21	1.78	0.66
4:B:152:LEU:HD11	4:B:158:VAL:HG23	1.77	0.66
4:B:93:ILE:HG12	4:B:133:VAL:HG11	1.79	0.64
4:B:34:THR:HG21	4:B:300:LEU:HB3	1.80	0.64
4:B:112:VAL:HG23	4:B:126:LEU:HD11	1.81	0.62
1:R:78:PRO:HB3	1:R:166:VAL:HG21	1.84	0.59
1:R:100[B]:GLU:CG	1:R:125:ILE:HG21	2.33	0.58
3:A:253:GLN:HG2	6:N:61:THR:HG22	1.86	0.56
4:B:51:LEU:HB2	4:B:336:LEU:HB2	1.87	0.56
6:N:37:VAL:HG12	6:N:47:TRP:HA	1.88	0.56
1:R:119:VAL:O	1:R:123:ASN:OD1	2.25	0.55
4:B:74:SER:OG	4:B:76:ASP:OD1	2.25	0.54
4:B:254:ASP:HB2	4:B:261:LEU:HD11	1.90	0.53
4:B:46[B]:ARG:HH11	5:G:62:ARG:HH22	1.54	0.52
4:B:75:GLN:NE2	4:B:100:VAL:O	2.43	0.52
3:A:220:TRP:HB2	4:B:117:LEU:HD21	1.91	0.52
4:B:87:THR:O	4:B:87:THR:HG22	2.11	0.51
4:B:318:LEU:HD12	4:B:329:THR:HG22	1.91	0.51
4:B:254:ASP:HB3	4:B:257:ALA:HB3	1.92	0.50
6:N:34:MET:HB3	6:N:79:LEU:HD22	1.93	0.50
4:B:46[B]:ARG:NH1	4:B:46[B]:ARG:HB3	2.27	0.49
3:A:214:ARG:NH1	4:B:186:ASP:OD1	2.46	0.49
1:R:189:ASP:N	1:R:189:ASP:OD1	2.45	0.49
4:B:48:ARG:HH21	5:G:62:ARG:HH21	1.60	0.48

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
3:A:280:GLN:NE2	3:A:350:THR:O	2.47	0.48
1:R:287:TYR:O	1:R:291:ILE:HG12	2.13	0.47
6:N:37:VAL:HG23	6:N:95:TYR:HB2	1.97	0.45
1:R:96:SER:O	1:R:100[B]:GLU:HB2	2.17	0.45
4:B:57:LYS:HB2	4:B:332:TRP:HA	1.97	0.45
4:B:34:THR:HG22	4:B:34:THR:O	2.17	0.44
4:B:262:MET:SD	4:B:302:ALA:HB2	2.57	0.44
4:B:330:GLY:HA2	4:B:336:LEU:HD23	1.99	0.43
4:B:286:LEU:HD22	4:B:327:VAL:HG11	2.00	0.42
6:N:67:ARG:NH2	6:N:90:ASP:OD2	2.52	0.42
3:A:374:LEU:HB3	3:A:380:LEU:HB2	2.00	0.42
1:R:298:ASP:HB2	1:R:299:PRO:HD3	2.01	0.42
4:B:46[B]:ARG:NH1	4:B:46[B]:ARG:CB	2.83	0.42
4:B:166:CYS:HB2	4:B:180:PHE:HB2	2.01	0.42
4:B:301:LYS:O	4:B:302:ALA:HB3	2.20	0.42
1:R:293:CYS:HA	1:R:296:ILE:HD12	2.02	0.41
2:P:1:ARG:HD2	2:P:1:ARG:HA	1.90	0.41
4:B:115:GLY:HA3	4:B:146[B]:LEU:HD23	2.02	0.41
3:A:210:VAL:HG11	3:A:220:TRP:CZ3	2.56	0.41
4:B:121:CYS:HB2	4:B:146[A]:LEU:HD21	2.02	0.41
4:B:146[B]:LEU:CD1	4:B:159:THR:HB	2.51	0.40
1:R:259:ALA:N	1:R:260:PRO:CD	2.85	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	R	256/346 (74%)	251 (98%)	5 (2%)	0	100	100
2	P	4/8 (50%)	4 (100%)	0	0	100	100
3	A	178/380 (47%)	171 (96%)	7 (4%)	0	100	100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
4	B	346/345 (100%)	327 (94%)	18 (5%)	1 (0%)	41	62
5	G	54/71 (76%)	52 (96%)	2 (4%)	0	100	100
6	N	128/134 (96%)	121 (94%)	7 (6%)	0	100	100
All	All	966/1284 (75%)	926 (96%)	39 (4%)	1 (0%)	54	73

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
4	B	127	LYS

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	R	220/309 (71%)	220 (100%)	0	100	100
2	P	6/6 (100%)	6 (100%)	0	100	100
3	A	163/341 (48%)	163 (100%)	0	100	100
4	B	282/285 (99%)	279 (99%)	3 (1%)	73	88
5	G	43/58 (74%)	42 (98%)	1 (2%)	50	73
6	N	107/112 (96%)	107 (100%)	0	100	100
All	All	821/1111 (74%)	817 (100%)	4 (0%)	89	96

All (4) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
4	B	59	TYR
4	B	105	TYR
4	B	217	MET
5	G	21	MET

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
2	P	4	HIS

5.3.3 RNA [i](#)

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains [i](#)

2 non-standard protein/DNA/RNA residues are modelled in this entry.

There are no bond length outliers.

There are no bond angle outliers.

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 monosaccharides in this entry.

5.6 Ligand geometry [i](#)

Of 1 ligands modelled in this entry, 1 is monoatomic - leaving 0 for Mogul analysis.

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

5.7 Other polymers [i](#)

There are no such residues in this entry.

5.8 Polymer linkage issues

There are no chain breaks in this entry.

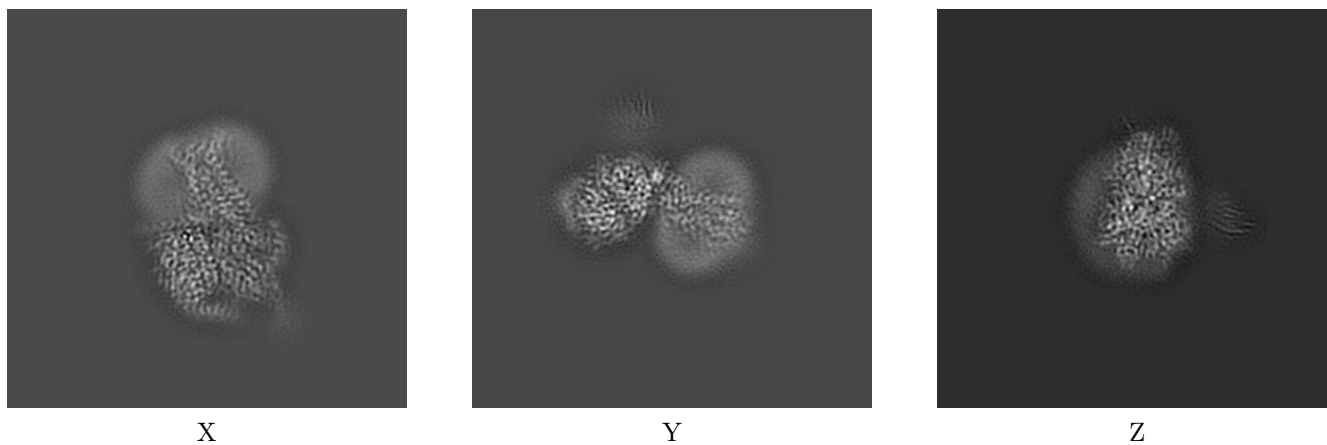
6 Map visualisation [i](#)

This section contains visualisations of the EMDB entry EMD-13453. These allow visual inspection of the internal detail of the map and identification of artifacts.

No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections [i](#)

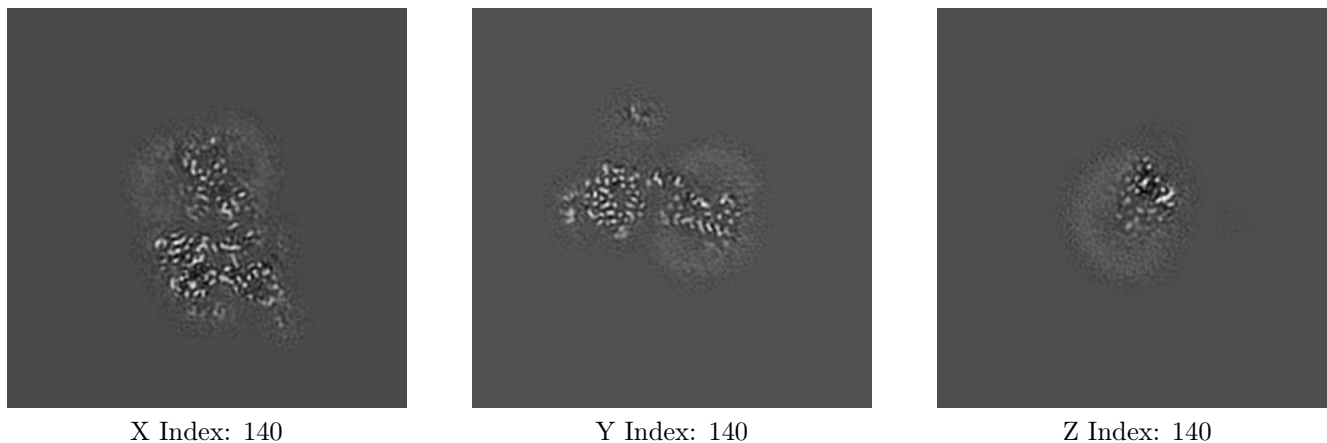
6.1.1 Primary map



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

6.2 Central slices [i](#)

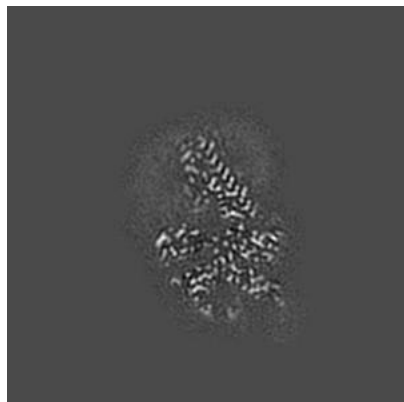
6.2.1 Primary map



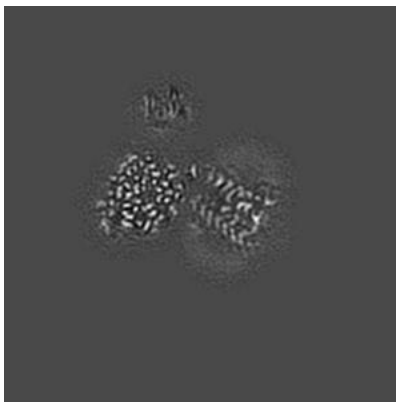
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices [i](#)

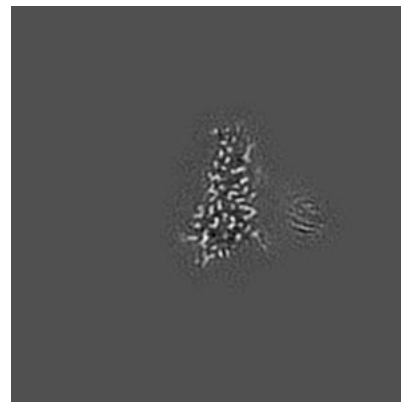
6.3.1 Primary map



X Index: 147



Y Index: 130



Z Index: 113

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 0.027. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

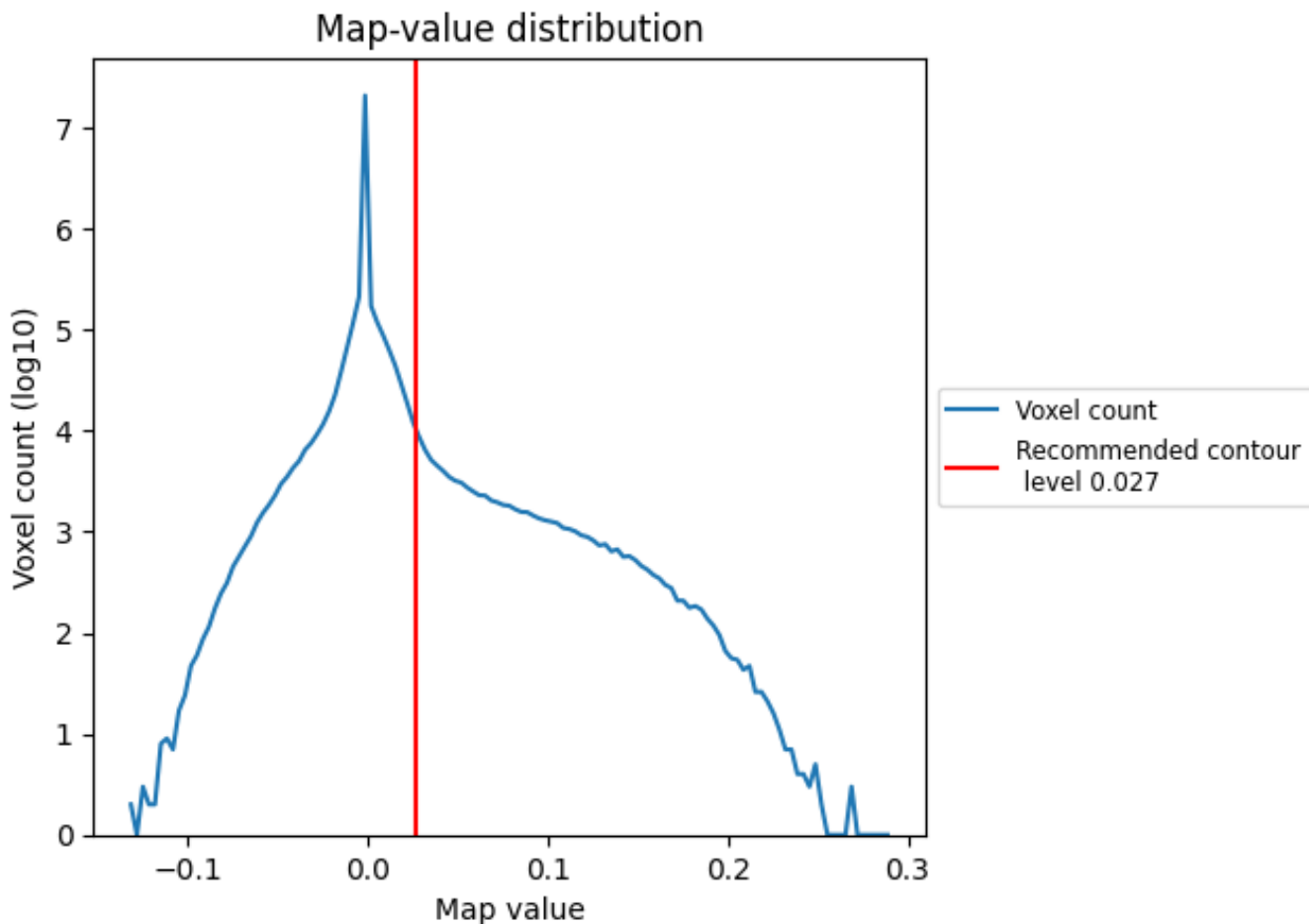
6.5 Mask visualisation

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

7 Map analysis [i](#)

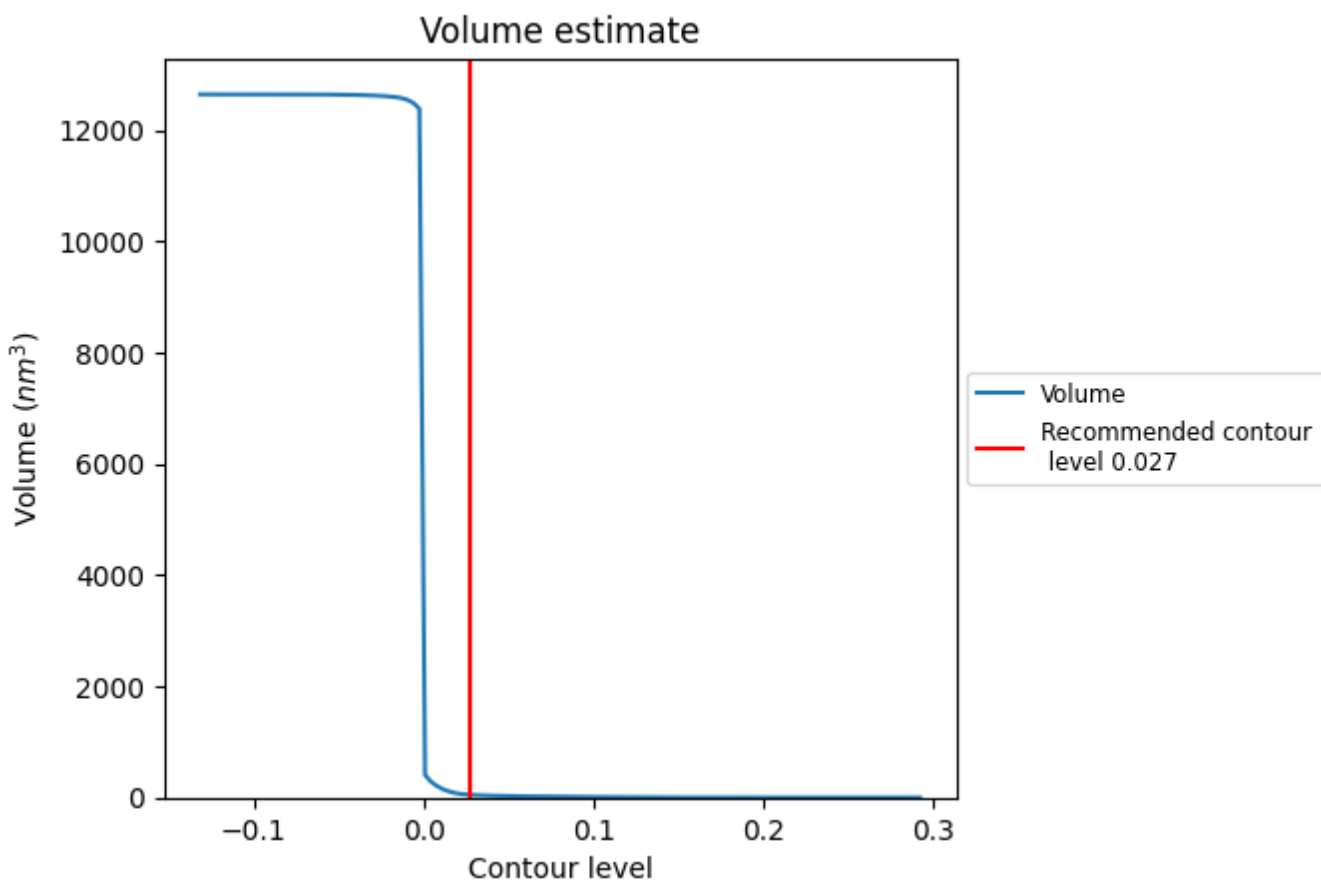
This section contains the results of statistical analysis of the map.

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.

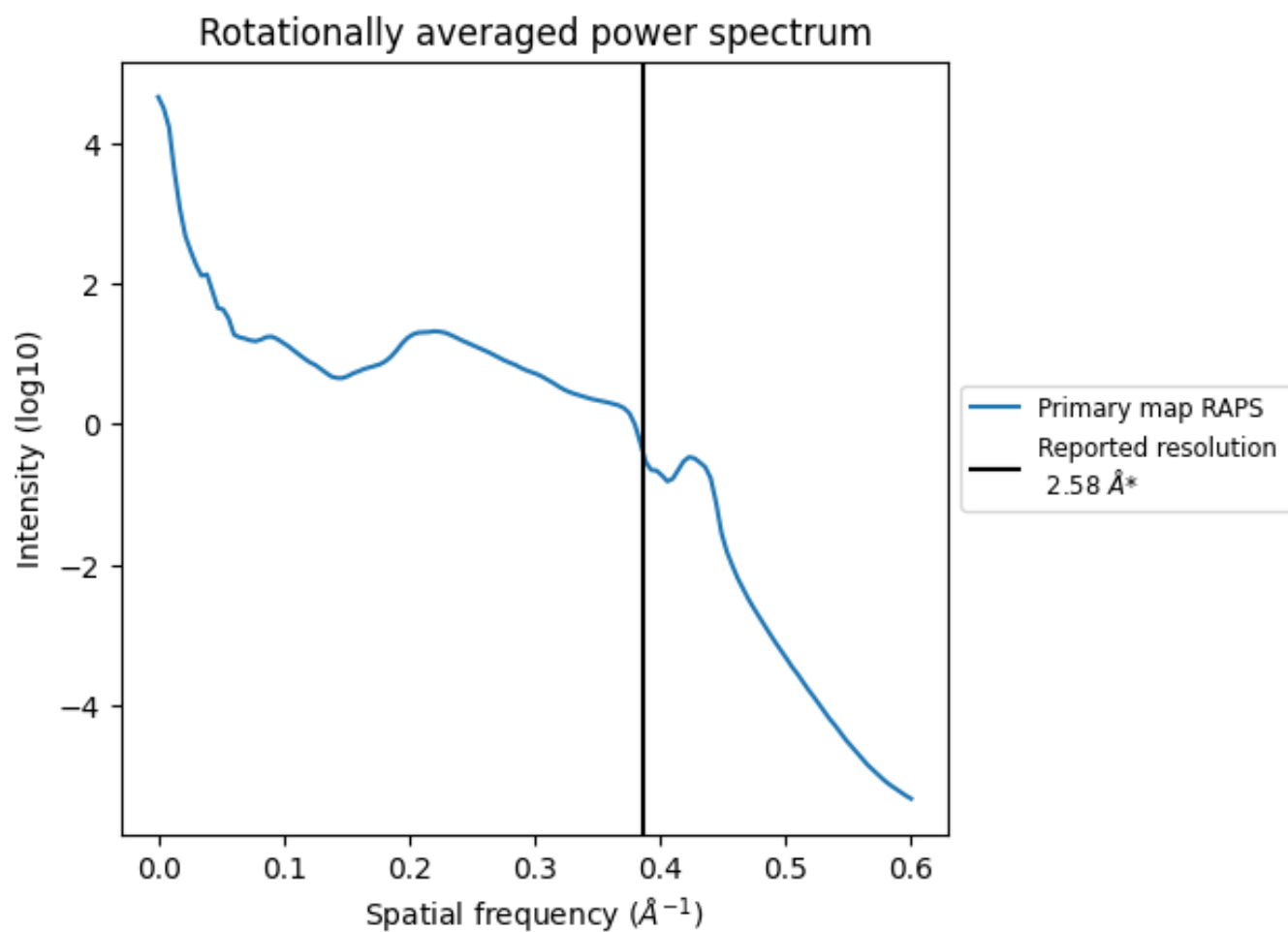
7.2 Volume estimate [\(i\)](#)



The volume at the recommended contour level is 50 nm³; this corresponds to an approximate mass of 45 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](#)

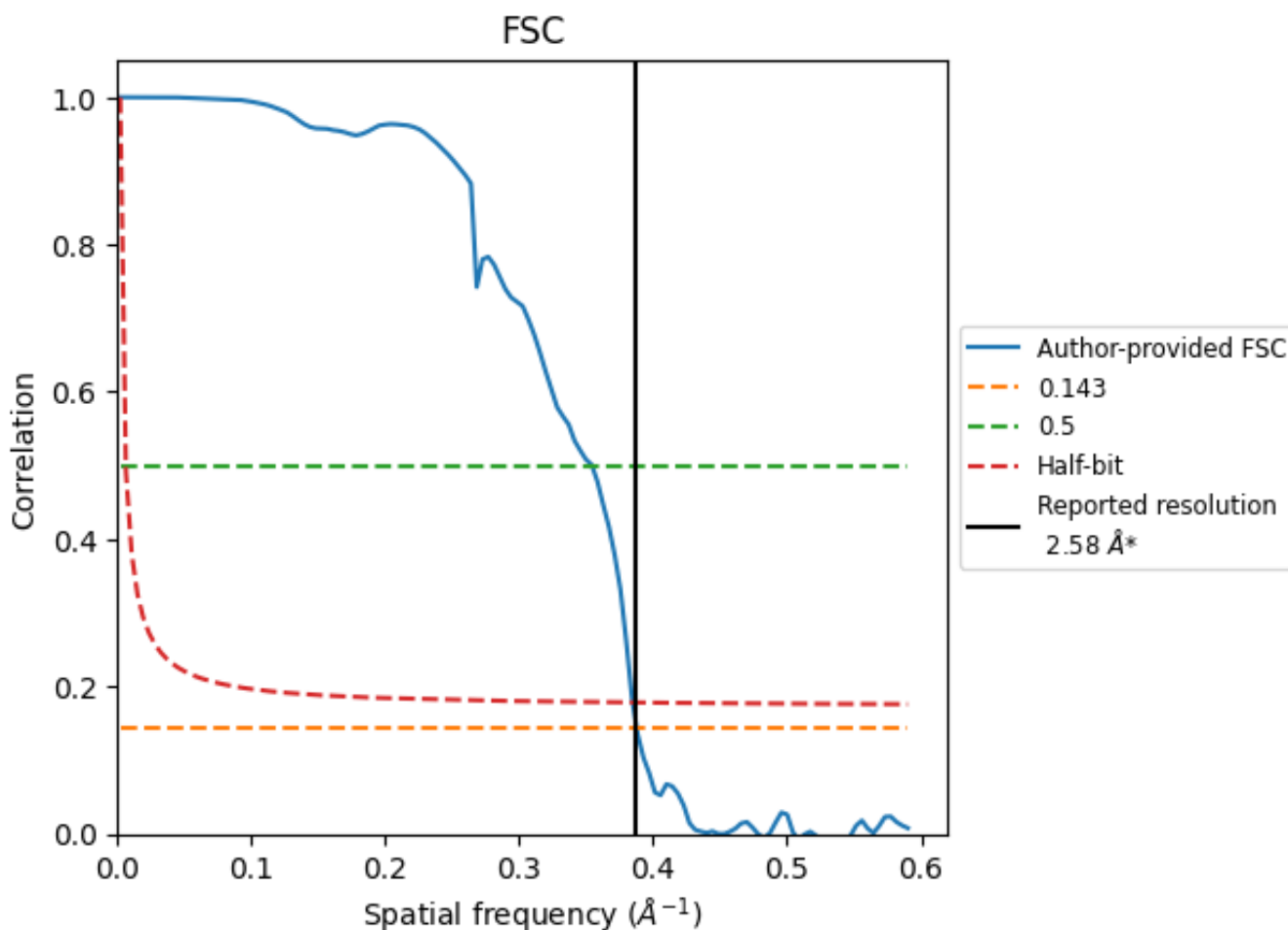


*Reported resolution corresponds to spatial frequency of 0.388\AA^{-1}

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 0.388 Å⁻¹

8.2 Resolution estimates [i](#)

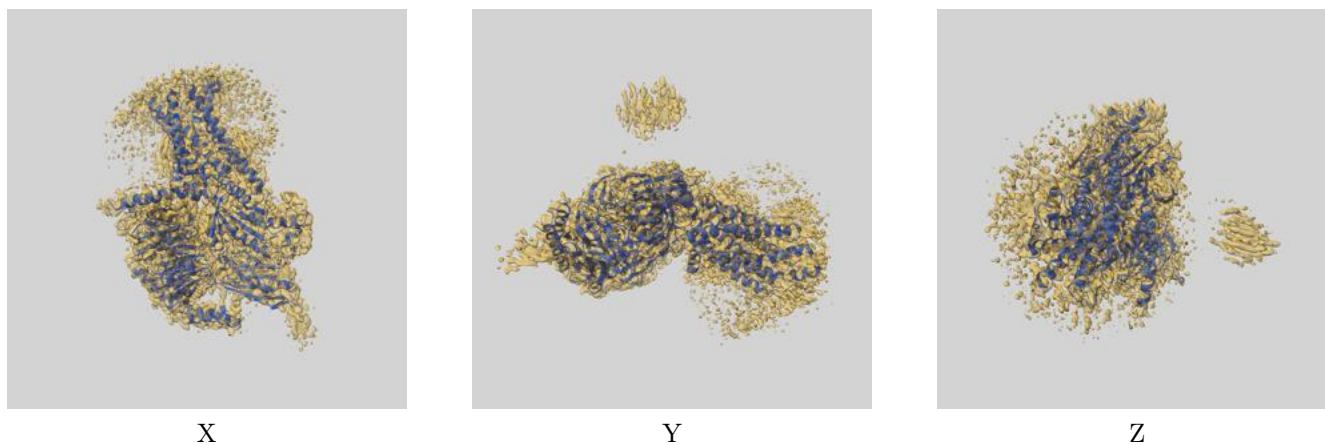
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	2.58	-	-
Author-provided FSC curve	2.58	2.82	2.60
Unmasked-calculated*	-	-	-

*Resolution estimate based on FSC curve calculated by comparison of deposited half-maps.

9 Map-model fit [i](#)

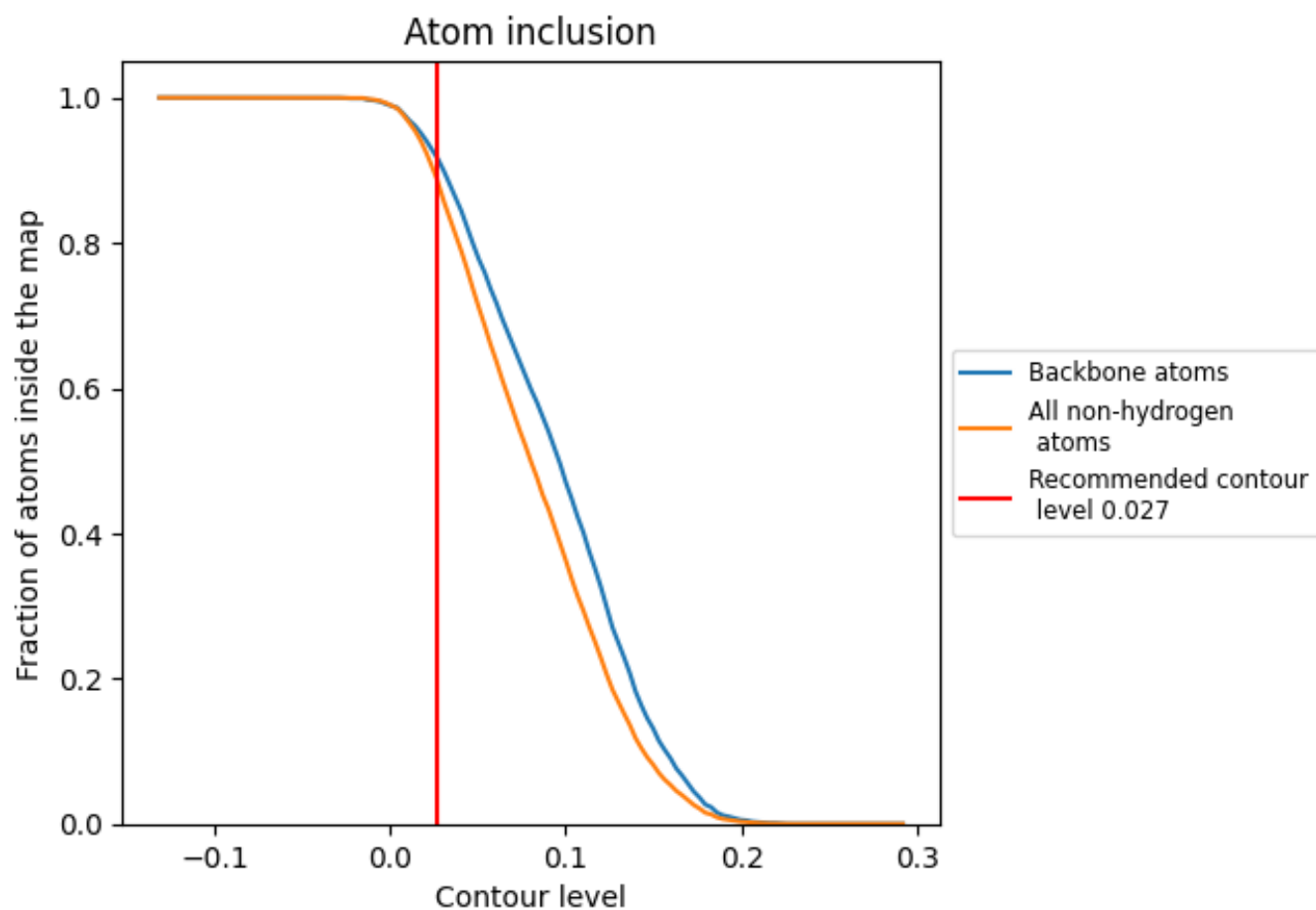
This section contains information regarding the fit between EMDB map EMD-13453 and PDB model 7PIU. Per-residue inclusion information can be found in section 3 on page 6.

9.1 Map-model overlay [i](#)



The images above show the 3D surface view of the map at the recommended contour level 0.027 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 Atom inclusion [i](#)



At the recommended contour level, 92% of all backbone atoms, 89% of all non-hydrogen atoms, are inside the map.