



wwPDB EM Validation Summary Report ⓘ

Sep 27, 2023 – 05:40 PM EDT

PDB ID : 8HBD
EMDB ID : EMD-34619
Title : Cryo-EM structure of IRL1620-bound ETBR-Gi complex
Authors : Yuan, Q.; Ji, Y.; Jiang, Y.; Duan, J.; Xu, H.E.
Deposited on : 2022-10-28
Resolution : 2.99 Å(reported)

This is a wwPDB EM Validation Summary 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 : 0.0.1.dev50
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) : 2.35.1

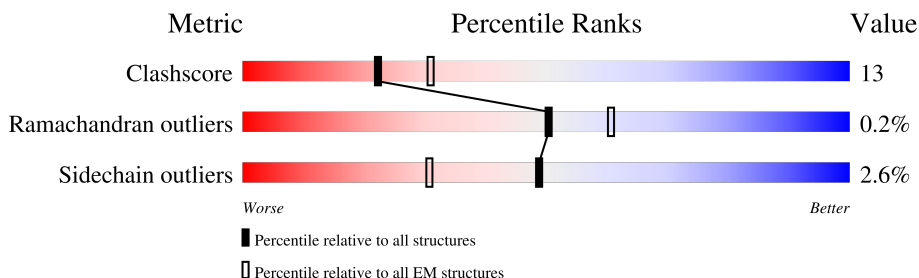
1 Overall quality at a glance i

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 2.99 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	A	354	
2	B	377	
3	G	71	
4	H	285	
5	L	15	
6	R	603	

2 Entry composition [i](#)

There are 6 unique types of molecules in this entry. The entry contains 8803 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	215	1717	1096	285	324	12	0	0

There are 2 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	203	ALA	GLY	conflict	UNP P63096
A	326	SER	ALA	conflict	UNP P63096

- 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	339	2540	1574	459	487	20	0	0

There are 38 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-10	MET	-	initiating methionine	UNP P62873
B	-9	HIS	-	expression tag	UNP P62873
B	-8	HIS	-	expression tag	UNP P62873
B	-7	HIS	-	expression tag	UNP P62873
B	-6	HIS	-	expression tag	UNP P62873
B	-5	HIS	-	expression tag	UNP P62873
B	-4	HIS	-	expression tag	UNP P62873
B	-3	GLY	-	expression tag	UNP P62873
B	-2	SER	-	expression tag	UNP P62873
B	-1	LEU	-	expression tag	UNP P62873
B	0	LEU	-	expression tag	UNP P62873
B	1	GLN	-	expression tag	UNP P62873
B	341	GLY	-	expression tag	UNP P62873
B	342	SER	-	expression tag	UNP P62873

Continued on next page...

Continued from previous page...

Chain	Residue	Modelled	Actual	Comment	Reference
B	343	SER	-	expression tag	UNP P62873
B	344	GLY	-	expression tag	UNP P62873
B	345	GLY	-	expression tag	UNP P62873
B	346	GLY	-	expression tag	UNP P62873
B	347	GLY	-	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	SER	-	expression tag	UNP P62873
B	355	GLY	-	expression tag	UNP P62873
B	356	VAL	-	expression tag	UNP P62873
B	357	SER	-	expression tag	UNP P62873
B	358	GLY	-	expression tag	UNP P62873
B	359	TRP	-	expression tag	UNP P62873
B	360	ARG	-	expression tag	UNP P62873
B	361	LEU	-	expression tag	UNP P62873
B	362	PHE	-	expression tag	UNP P62873
B	363	LYS	-	expression tag	UNP P62873
B	364	LYS	-	expression tag	UNP P62873
B	365	ILE	-	expression tag	UNP P62873
B	366	SER	-	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	G	56	404	255	72	74	3	0	0

- Molecule 4 is a protein called scFv16.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
4	H	233	1726	1103	286	327	10	0	0

- Molecule 5 is a protein called IRL1620.

Mol	Chain	Residues	Atoms				AltConf	Trace
			Total	C	N	O		
5	L	14	123	82	17	24	0	0

- Molecule 6 is a protein called Endothelin receptor type B,Endothelin receptor type B,Oplop horus-luciferin 2-monooxygenase catalytic subunit chimera.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
6	R	301	2293	1525	362	388	18	0	0

There are 47 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
R	-20	MET	-	initiating methionine	UNP P24530
R	-19	ASP	-	expression tag	UNP P24530
R	-18	SER	-	expression tag	UNP P24530
R	-17	LYS	-	expression tag	UNP P24530
R	-16	GLY	-	expression tag	UNP P24530
R	-15	SER	-	expression tag	UNP P24530
R	-14	SER	-	expression tag	UNP P24530
R	-13	GLN	-	expression tag	UNP P24530
R	-12	LYS	-	expression tag	UNP P24530
R	-11	GLY	-	expression tag	UNP P24530
R	-10	SER	-	expression tag	UNP P24530
R	-9	ARG	-	expression tag	UNP P24530
R	-8	LEU	-	expression tag	UNP P24530
R	-7	LEU	-	expression tag	UNP P24530
R	-6	LEU	-	expression tag	UNP P24530
R	-5	LEU	-	expression tag	UNP P24530
R	-4	LEU	-	expression tag	UNP P24530
R	-3	VAL	-	expression tag	UNP P24530
R	-2	VAL	-	expression tag	UNP P24530
R	-1	SER	-	expression tag	UNP P24530
R	0	ASN	-	expression tag	UNP P24530
R	1	LEU	-	expression tag	UNP P24530
R	2	LEU	-	expression tag	UNP P24530
R	3	LEU	-	expression tag	UNP P24530
R	4	CYS	-	expression tag	UNP P24530
R	5	GLN	-	expression tag	UNP P24530
R	6	GLY	-	expression tag	UNP P24530
R	7	VAL	-	expression tag	UNP P24530
R	8	VAL	-	expression tag	UNP P24530
R	9	SER	-	expression tag	UNP P24530

Continued on next page...

Continued from previous page...

Chain	Residue	Modelled	Actual	Comment	Reference
R	10	ASP	-	expression tag	UNP P24530
R	11	TYR	-	expression tag	UNP P24530
R	12	LYS	-	expression tag	UNP P24530
R	13	ASP	-	expression tag	UNP P24530
R	14	ASP	-	expression tag	UNP P24530
R	15	ASP	-	expression tag	UNP P24530
R	16	ASP	-	expression tag	UNP P24530
R	17	VAL	-	expression tag	UNP P24530
R	18	ASP	-	expression tag	UNP P24530
R	19	HIS	-	expression tag	UNP P24530
R	20	HIS	-	expression tag	UNP P24530
R	21	HIS	-	expression tag	UNP P24530
R	22	HIS	-	expression tag	UNP P24530
R	23	HIS	-	expression tag	UNP P24530
R	24	HIS	-	expression tag	UNP P24530
R	25	HIS	-	expression tag	UNP P24530
R	26	HIS	-	expression tag	UNP P24530

PRO
TYR
GLU
GLY
ILE
ALA
VAL
PHE
ASP
GLY
LYS
LYS
ILE
THR
VAL
THR
GLY
THR
THR
LEU
TRP
ASN
GLY
ASN
LYS
ILE
ILE
ASP
GLU
ARG
LEU
ILE
THR
PRO
ASP
GLY
SER
MET
LEU
PHE
ARG
VAL
THR
THR
ILE
ASN
SER

4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	191493	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 ($e^-/\text{\AA}^2$)	50	Depositor
Minimum defocus (nm)	800	Depositor
Maximum defocus (nm)	18000	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	1.682	Depositor
Minimum map value	-0.014	Depositor
Average map value	0.001	Depositor
Map value standard deviation	0.026	Depositor
Recommended contour level	0.0553	Depositor
Map size (Å)	266.24, 266.24, 266.24	wwPDB
Map dimensions	256, 256, 256	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.04, 1.04, 1.04	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	0.27	0/1744	0.48	0/2338
2	B	0.27	0/2587	0.58	1/3514 (0.0%)
3	G	0.24	0/410	0.43	0/557
4	H	0.26	0/1770	0.49	0/2409
5	L	0.23	0/127	0.37	0/172
6	R	0.26	0/2346	0.50	0/3209
All	All	0.27	0/8984	0.52	1/12199 (0.0%)

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 (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed($^{\circ}$)	Ideal($^{\circ}$)
2	B	254	ASP	CB-CG-OD1	5.79	123.51	118.30

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
2	B	313	ASN	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	1717	0	1686	47	0
2	B	2540	0	2413	68	0
3	G	404	0	394	8	0
4	H	1726	0	1618	29	0
5	L	123	0	106	8	0
6	R	2293	0	2295	77	0
All	All	8803	0	8512	219	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 13.

The worst 5 of 219 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:A:296:TYR:CE1	1:A:297:GLU:OE2	1.91	1.20
1:A:296:TYR:C	1:A:296:TYR:HD1	1.49	1.12
1:A:296:TYR:C	1:A:296:TYR:CD1	2.24	1.00
6:R:96:ILE:HD12	6:R:162:LEU:HD21	1.45	0.97
6:R:96:ILE:CD1	6:R:162:LEU:CD2	2.46	0.94

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	207/354 (58%)	201 (97%)	6 (3%)	0	100 100

Continued on next page...

Continued from previous page...

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
2	B	337/377 (89%)	323 (96%)	13 (4%)	1 (0%)	41	76
3	G	54/71 (76%)	54 (100%)	0	0	100	100
4	H	229/285 (80%)	224 (98%)	5 (2%)	0	100	100
5	L	12/15 (80%)	12 (100%)	0	0	100	100
6	R	295/603 (49%)	275 (93%)	19 (6%)	1 (0%)	41	76
All	All	1134/1705 (66%)	1089 (96%)	43 (4%)	2 (0%)	50	82

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
6	R	208	ARG
2	B	215	GLU

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	184/306 (60%)	179 (97%)	5 (3%)	44	77
2	B	264/308 (86%)	256 (97%)	8 (3%)	41	75
3	G	38/58 (66%)	37 (97%)	1 (3%)	46	78
4	H	177/228 (78%)	175 (99%)	2 (1%)	73	90
5	L	12/13 (92%)	11 (92%)	1 (8%)	11	39
6	R	242/536 (45%)	235 (97%)	7 (3%)	42	76
All	All	917/1449 (63%)	893 (97%)	24 (3%)	49	78

5 of 24 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
4	H	229	CYS
6	R	90	CYS
5	L	14	PHE
6	R	175	LYS

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type
2	B	189	SER

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 8 such sidechains are listed below:

Mol	Chain	Res	Type
6	R	378	ASN
6	R	373	ASN
4	H	39	GLN
3	G	18	GLN
6	R	351	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.

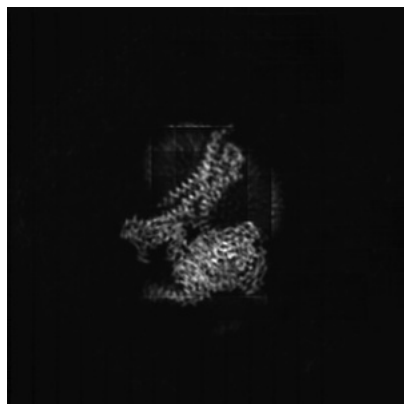
6 Map visualisation [i](#)

This section contains visualisations of the EMDB entry EMD-34619. These allow visual inspection of the internal detail of the map and identification of artifacts.

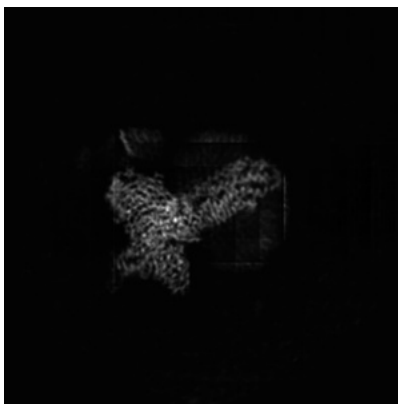
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections [i](#)

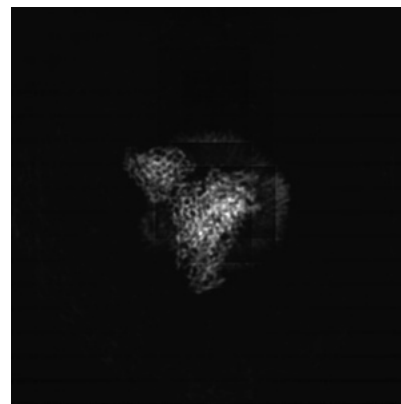
6.1.1 Primary map



X

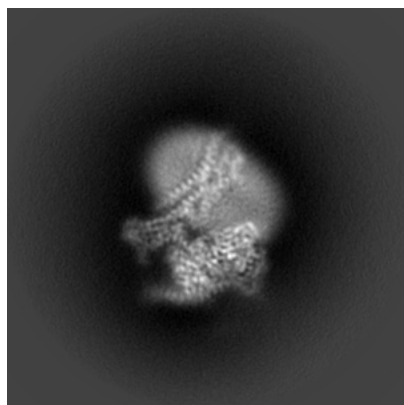


Y

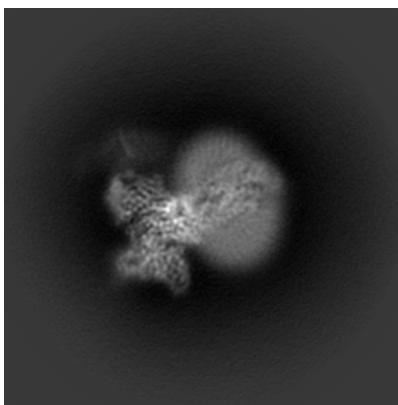


Z

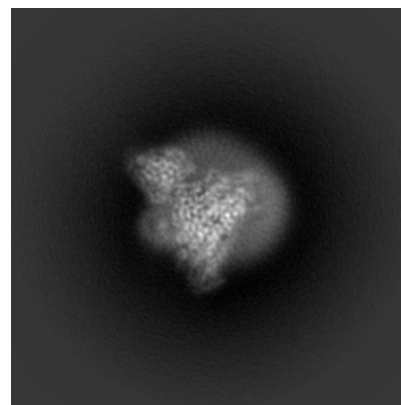
6.1.2 Raw map



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: 128

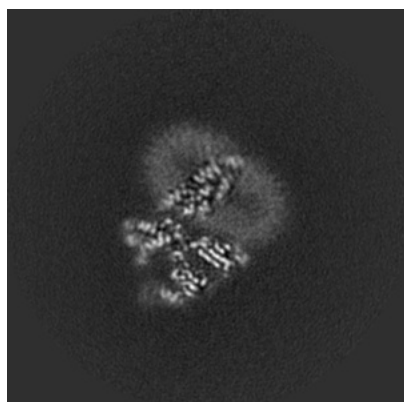


Y Index: 128

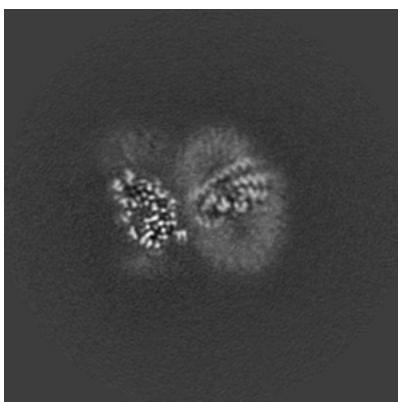


Z Index: 128

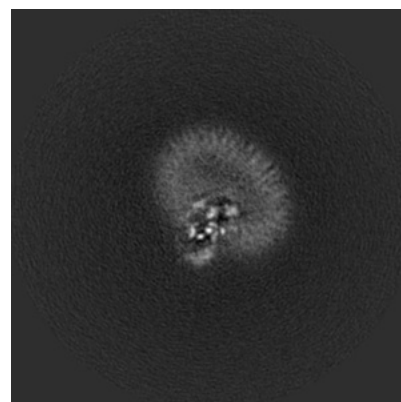
6.2.2 Raw map



X Index: 128



Y Index: 128

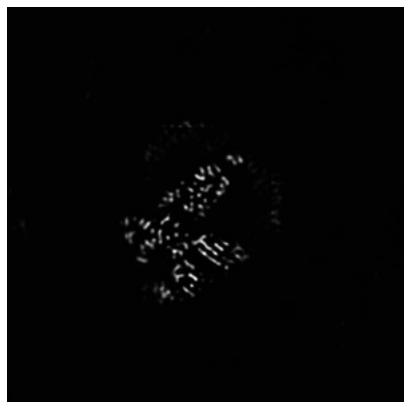


Z Index: 128

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: 127

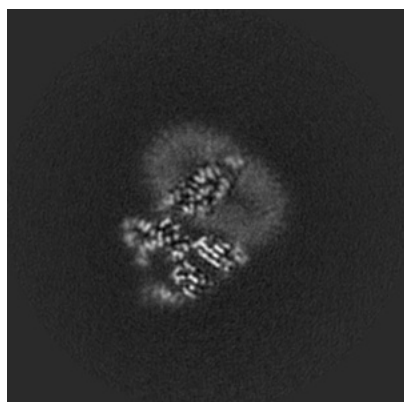


Y Index: 128

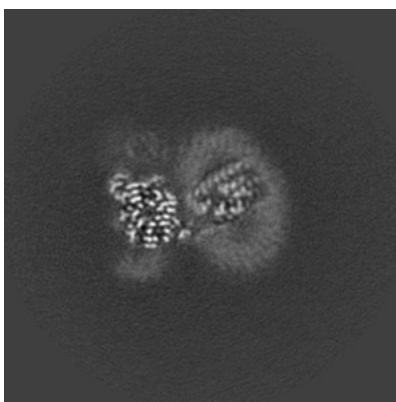


Z Index: 100

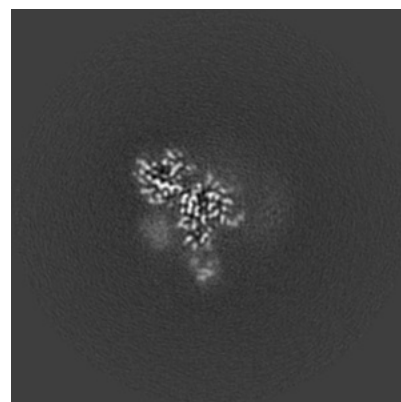
6.3.2 Raw map



X Index: 127



Y Index: 124



Z Index: 101

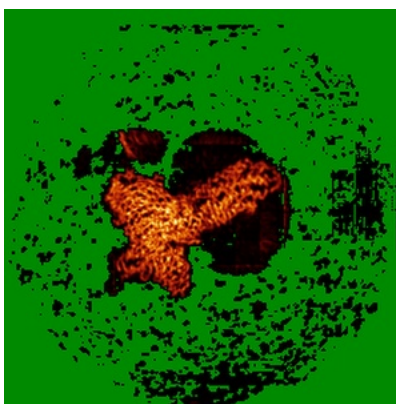
The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal standard-deviation projections (False-color) [i](#)

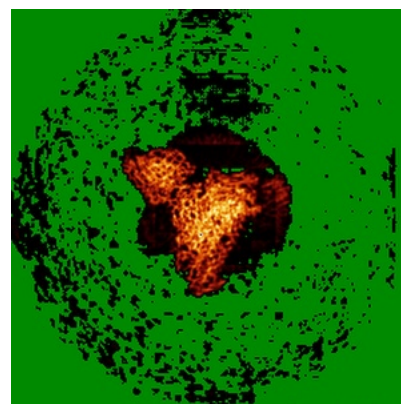
6.4.1 Primary map



X

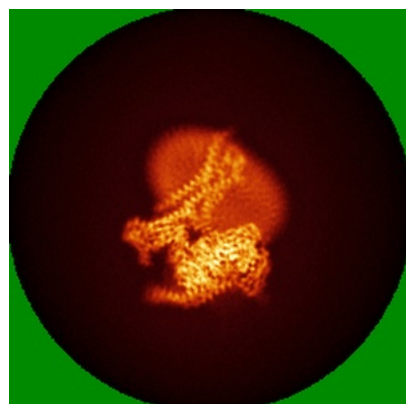


Y

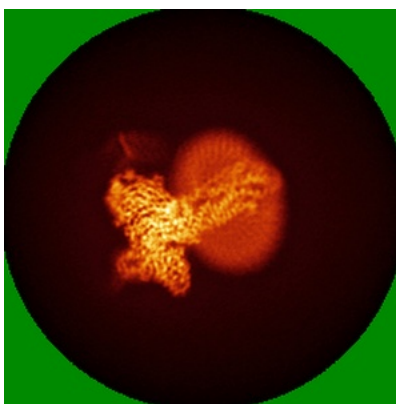


Z

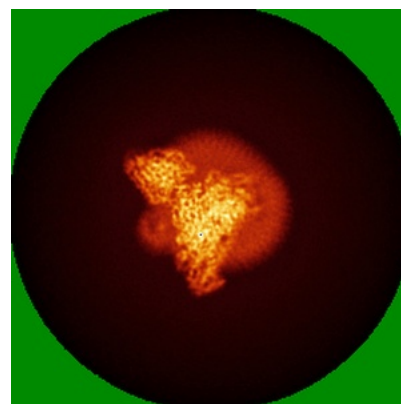
6.4.2 Raw map



X



Y

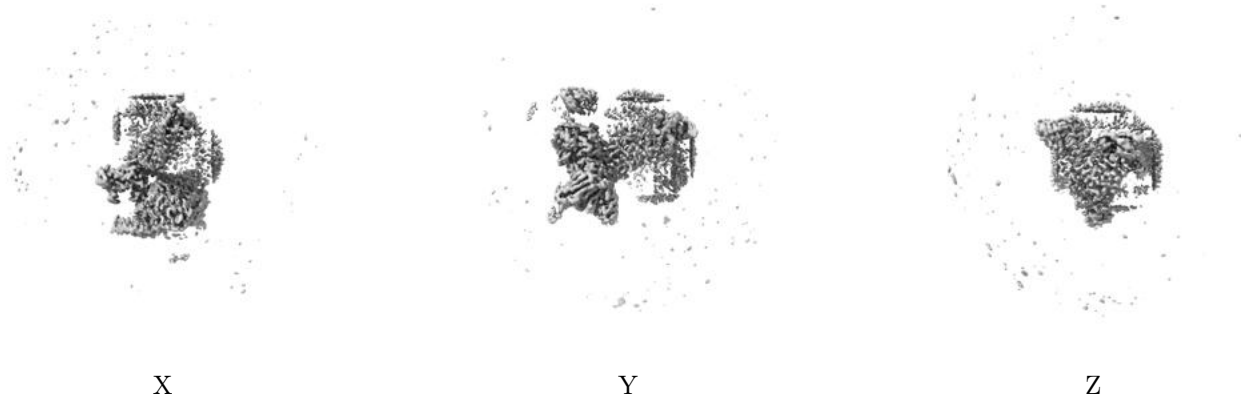


Z

The images above show the map standard deviation projections with false color in three orthogonal directions. Minimum values are shown in green, max in blue, and dark to light orange shades represent small to large values respectively.

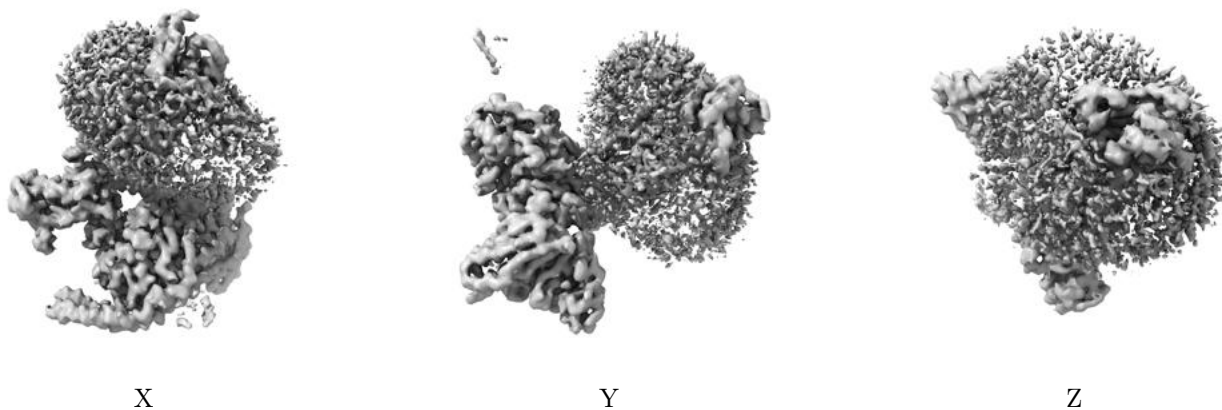
6.5 Orthogonal surface views [i](#)

6.5.1 Primary map



The images above show the 3D surface view of the map at the recommended contour level 0.0553. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.5.2 Raw map



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.

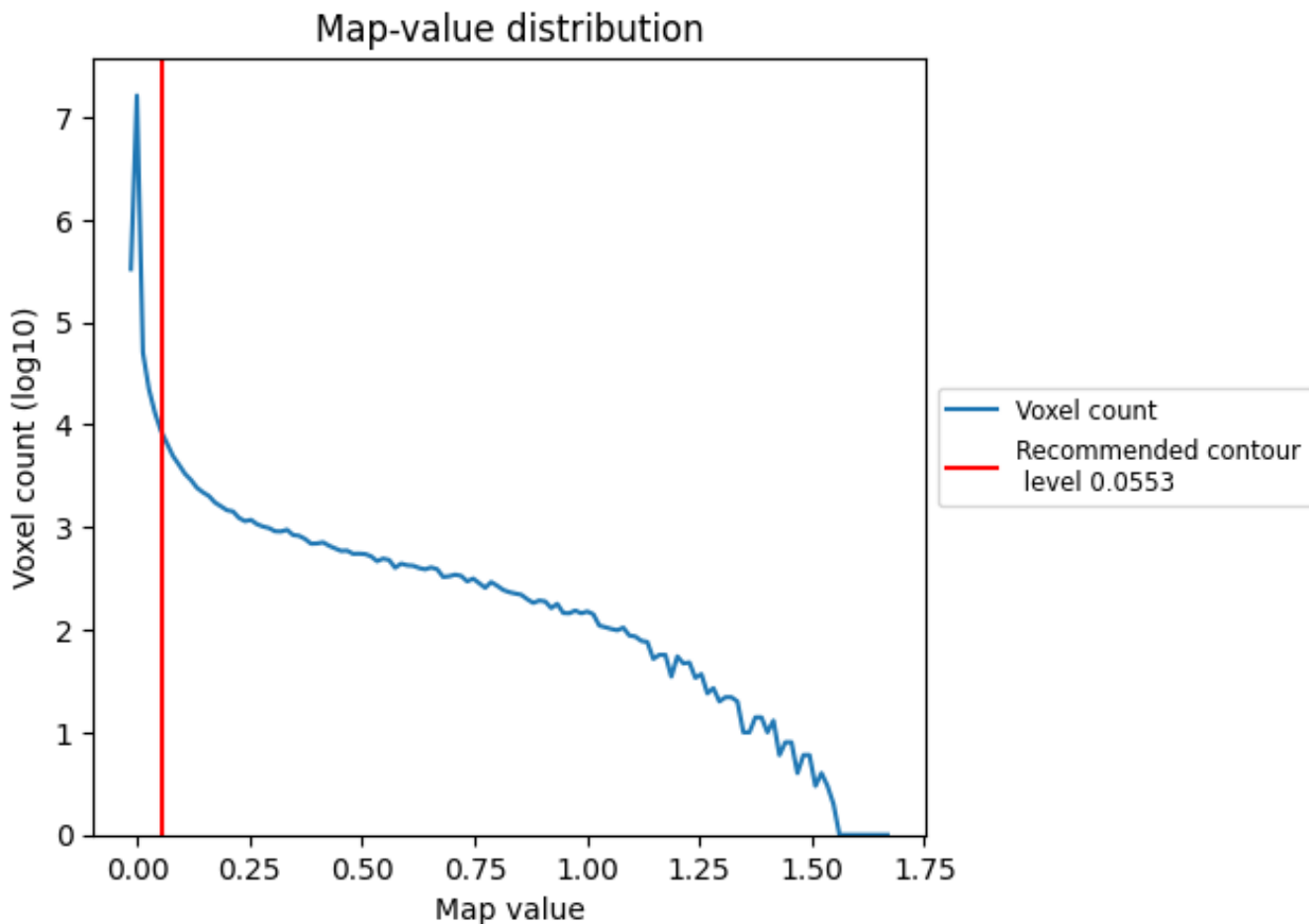
6.6 Mask visualisation [i](#)

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

7 Map analysis [i](#)

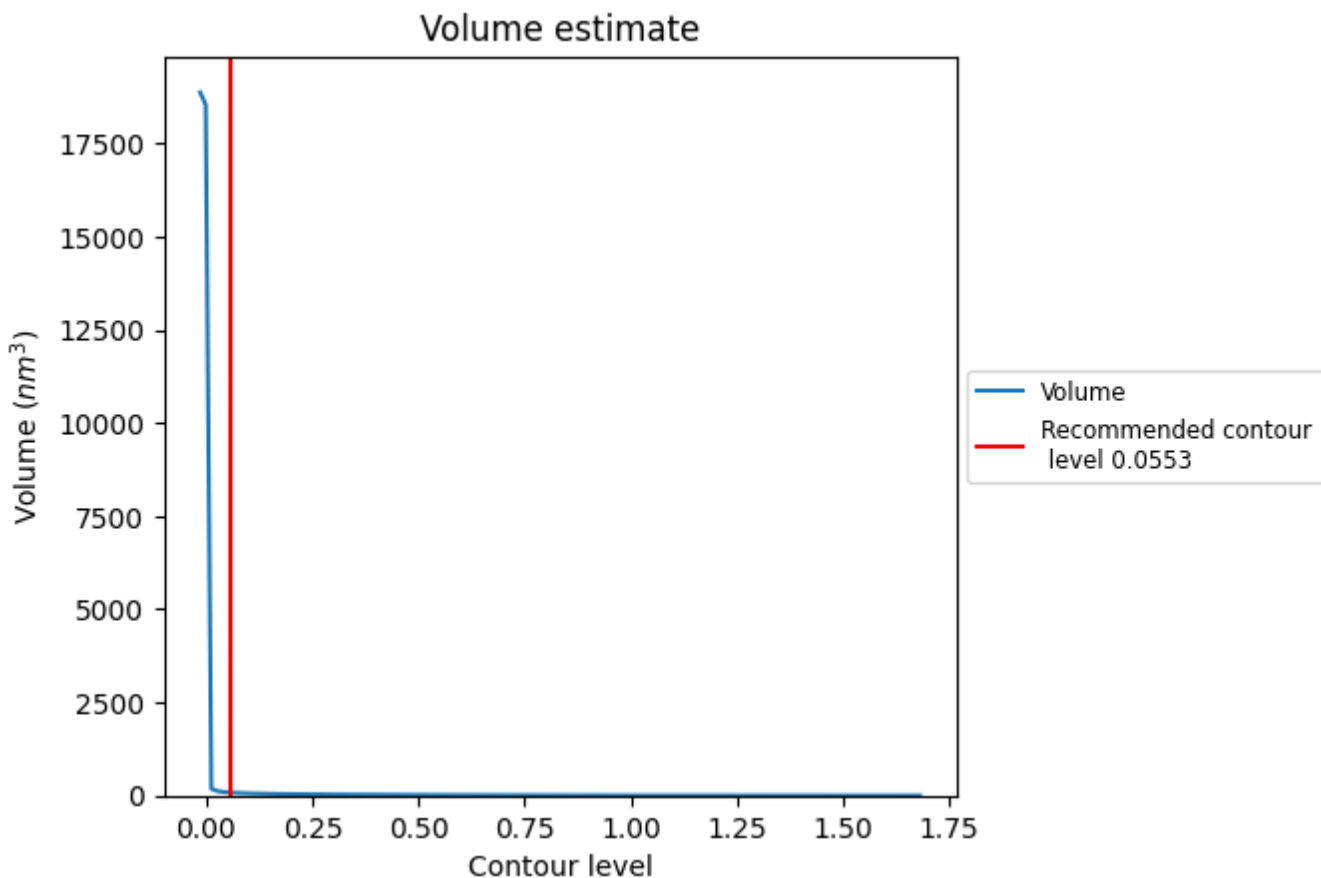
This section contains the results of statistical analysis of the map.

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.

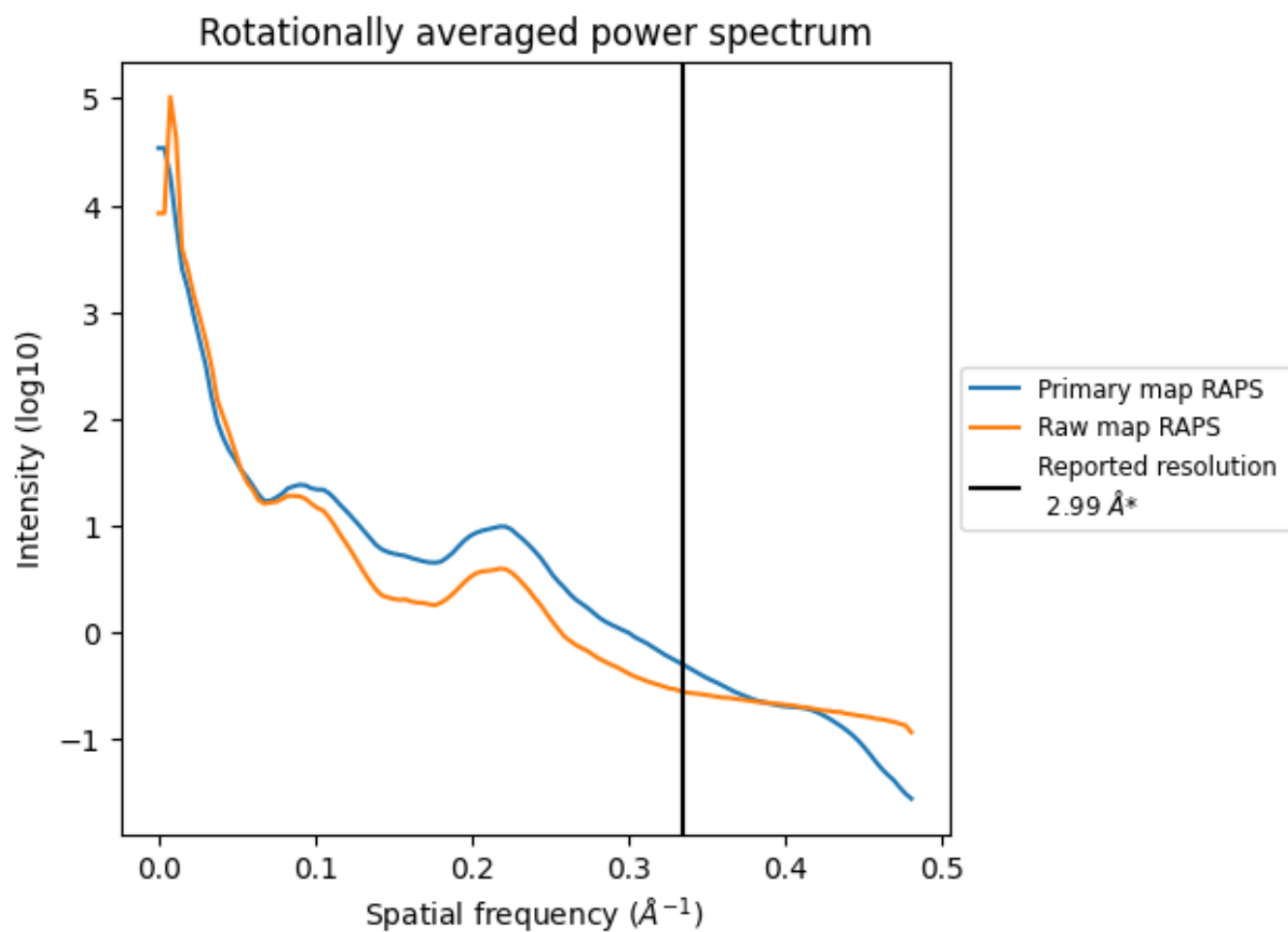
7.2 Volume estimate [i](#)



The volume at the recommended contour level is 81 nm^3 ; this corresponds to an approximate mass of 74 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

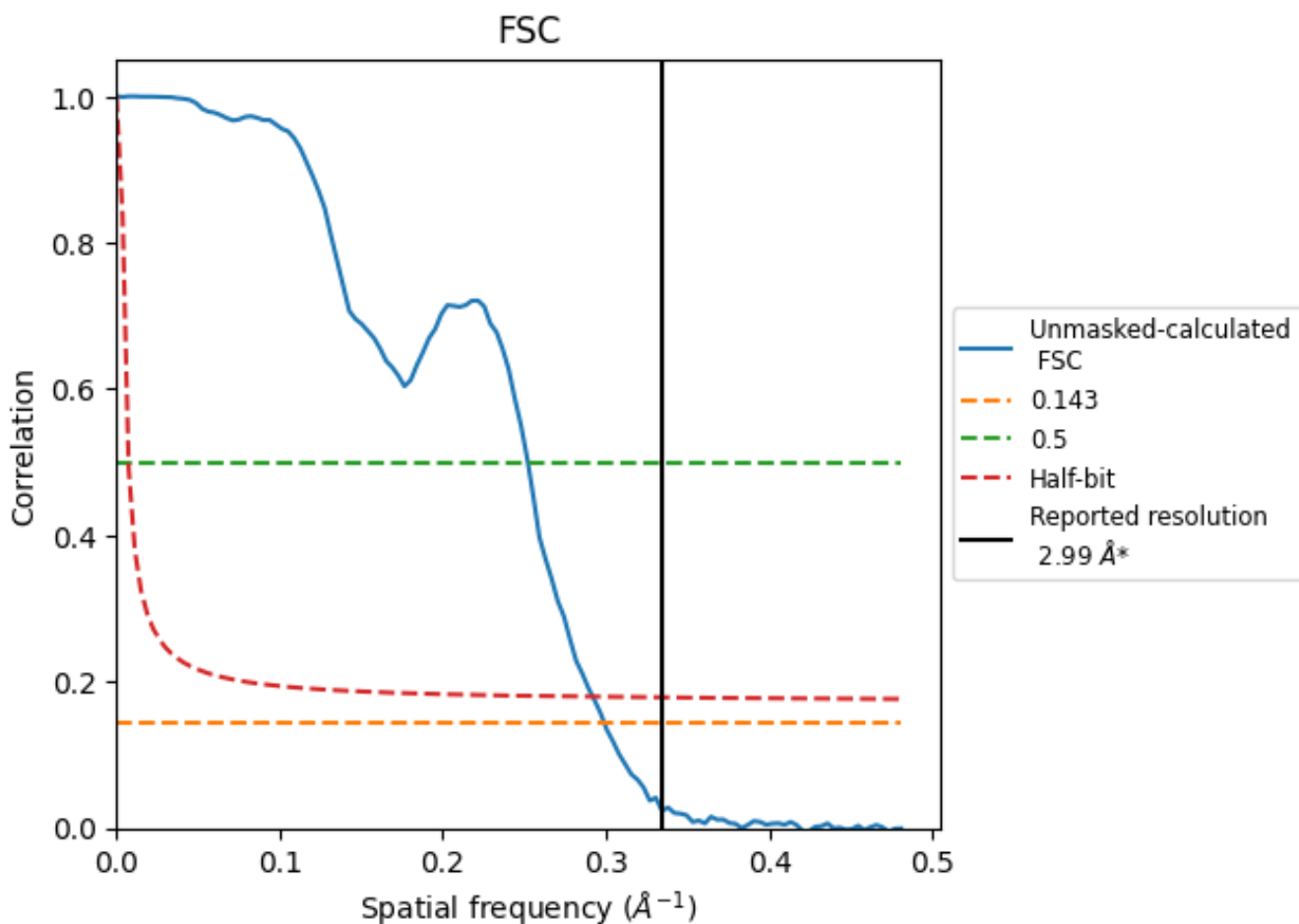


*Reported resolution corresponds to spatial frequency of 0.334 \AA^{-1}

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 0.334 Å⁻¹

8.2 Resolution estimates [i](#)

Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	2.99	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	3.34	3.97	3.43

*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 3.34 differs from the reported value 2.99 by more than 10 %

9 Map-model fit [i](#)

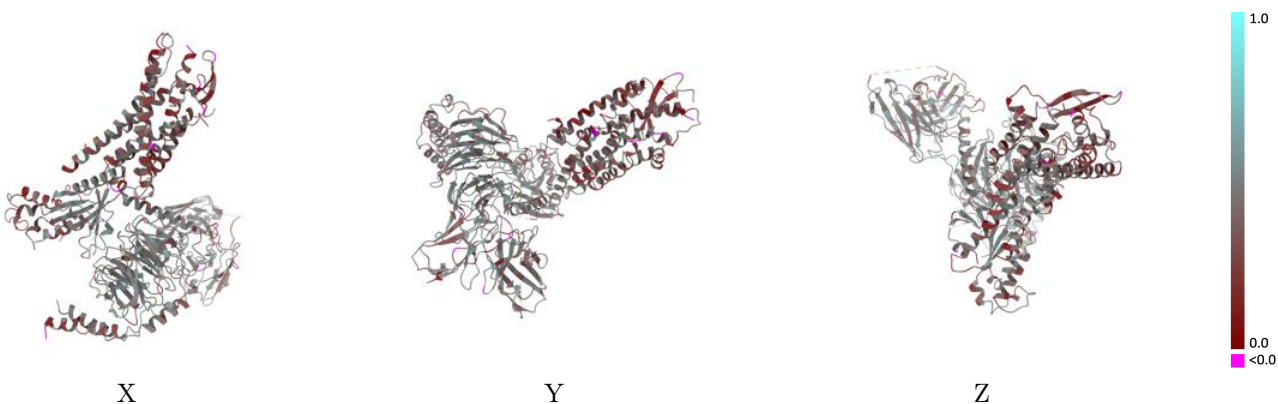
This section contains information regarding the fit between EMDB map EMD-34619 and PDB model 8HBD. Per-residue inclusion information can be found in section 3 on page 7.

9.1 Map-model overlay [i](#)



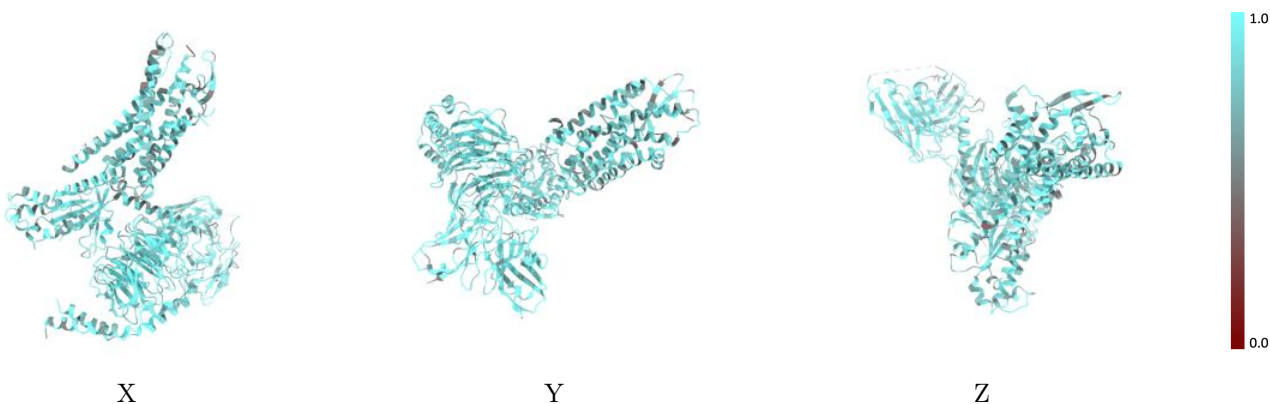
The images above show the 3D surface view of the map at the recommended contour level 0.0553 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

9.2 Q-score mapped to coordinate model [\(i\)](#)



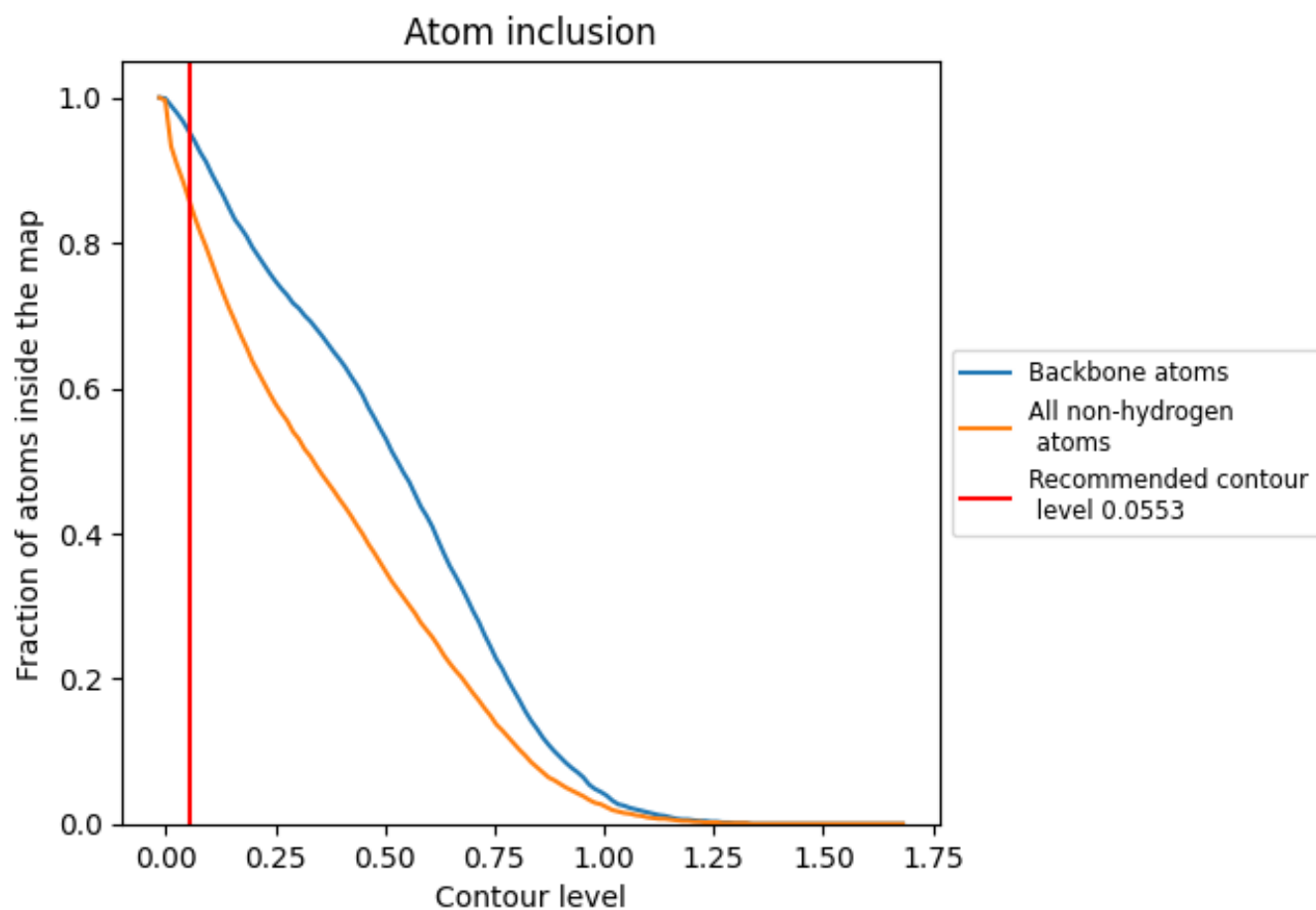
The images above show the model with each residue coloured according to its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions 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 [\(i\)](#)



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 (0.0553).















9.4 Atom inclusion [i](#)



At the recommended contour level, 95% of all backbone atoms, 86% 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 (0.0553) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	 0.8550	 0.4120
A	 0.8520	 0.4170
B	 0.8940	 0.4600
G	 0.8400	 0.4180
H	 0.8820	 0.4360
L	 0.8020	 0.3140
R	 0.7970	 0.3410

