wwPDB EM Validation Summary Report (i)

Jun 5, 2023 – 02:21 PM JST

PDB ID	:	8IW9
EMDB ID	:	EMD-35764
Title	:	Cryo-EM structure of the CAD-bound mTAAR9-Gs complex
Authors	:	Sun, J.P.; Li, Q.; Yang, F.; Xu, Y.F.; Guo, L.L.; Lian, S.; Zhang, M.H.; Rong, N.K.
Deposited on	:	2023-03-29
Resolution	:	3.08 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 (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:

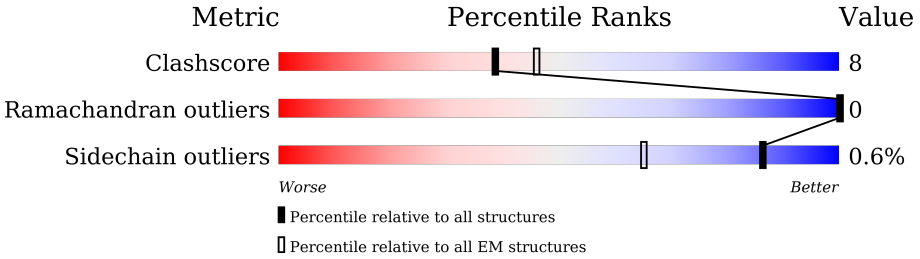
EMDB validation analysis	:	0.0.1.dev50
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.33

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 3.08 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	362	56% 8% 35%
2	B	377	77% 13% 10%
3	C	59	81% 8% 10%
4	R	348	73% 16% 11%
5	S	285	65% 15% • 19%
6	N	128	91% 9%

2 Entry composition (i)

There are 6 unique types of molecules in this entry. The entry contains 9693 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 isoforms short.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	234	1843	1169	337	329	8	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	2488	1547	447	476	18	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
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

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Chain	Residue	Modelled	Actual	Comment	Reference
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	C	53	363	230	66	66	1	0	0

- Molecule 4 is a protein called Trace amine-associated receptor 9.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
4	R	309	2328	1555	364	394	15	0	0

- Molecule 5 is a protein called scFv16.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
5	S	231	1741	1108	287	336	10	0	0

- Molecule 6 is a protein called Nanobody-35.

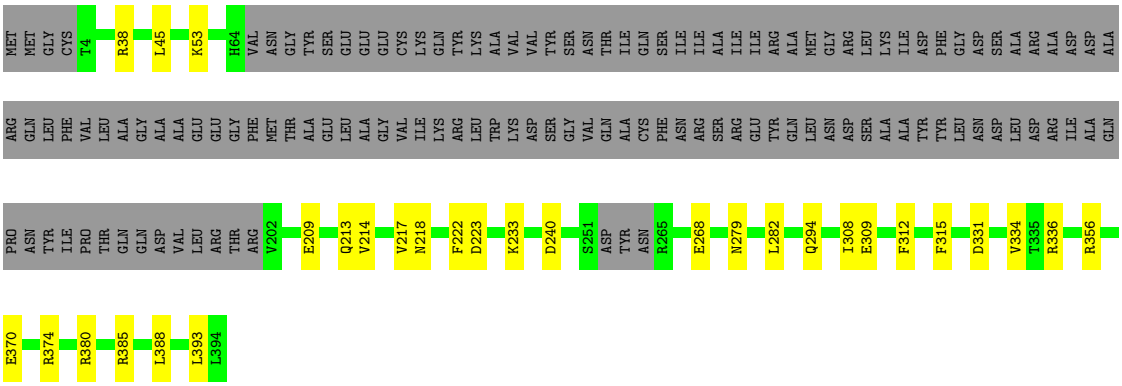
Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
6	N	128	930	583	162	179	6	0	0

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 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.

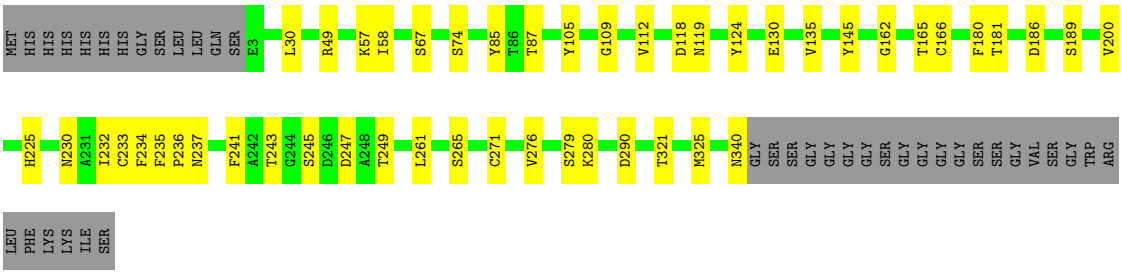
• Molecule 1: Guanine nucleotide-binding protein G(s) subunit alpha isoforms short

Chain A: 56% 8% 35%



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

Chain B: 77% 13% 10%



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

Chain C: 81% 8% 10%

• Molecule 4: Trace amine-associated receptor 9

Chain R: 73% 16% 11%

MET THR SER ASP PHE SER PRO GLU PRO PRO MET GLU LEU CYS TYR GLU ASN VAL ASN GLY SER C22 I23 S26 Y36 L41 A46 N50 L51 I55 A56 I57 K61 Q62 L63 H64 L74 A77 D78 F79 L80 V81 G82 M86 V94 E95 S96 C97 W98

Y99 C105 D112 L120 F121 L138 T139 Y140 P141 S156 C186 L194 N195 Q196 N197 W198 V199 L205 Q227 I231 A235 ASN GLN ALA GLN ALA SER SER GLU S244 A250 R254 F267 V279 A282 Y283 W298 Y301 Y302 A305 M306

N307 Y311 G328 K329 V330 T337 T338 ASN LEU PHE SER GLU GLU ALA GLY ALA GLY

- Molecule 5: scFv16

Chain S: 65% 15% • 19%

MET LEU LEU VAL ASN GLN SER HIS GLN GLY PHE ASN LYS GLU HIS THR SER LYS MET VAL SER ALA ILE VAL LEU TYR VAL LEU LEU ALA ALA ALA ALA HIS SER ALA PHE ALA V2 L11 K19 L20 S21 Q39 L45 E46 W47 V48 A49 Y50 I51 S52 S53 G54 S55

Y60 A61 D62 T63 V64 K65 G66 R67 I70 S71 R72 K76 P80 L81 Q82 M83 L86 R87 S120 ALA GLY GLY GLY GLY SER GLY GLY GLY GLY SER GLY GLY GLY GLY S135 I138 Q142 A143 T144 S145 S146 V155 C159 R160 R180 Q183 L188 I189

P200 D201 R202 G207 F212 L219 E220 E234 G242 E246 LEU

- Molecule 6: Nanobody-35

Chain N: 91% 9%

Q1 Q39 L45 K65 T91 C99 C107 F108 D109 S112 R118 T125 S128

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	494227	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	NONE	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	1.875	Depositor
Minimum defocus (nm)	1000	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	0.092	Depositor
Minimum map value	-0.035	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.003	Depositor
Recommended contour level	0.01	Depositor
Map size (Å)	216.00002, 216.00002, 216.00002	wwPDB
Map dimensions	200, 200, 200	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.08, 1.08, 1.08	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	0.26	0/1879	0.50	0/2539
2	B	0.26	0/2534	0.51	0/3451
3	C	0.28	0/369	0.46	0/507
4	R	0.28	0/2398	0.47	0/3285
5	S	0.28	0/1785	0.53	0/2425
6	N	0.27	0/950	0.52	0/1293
All	All	0.27	0/9915	0.50	0/13500

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	A	1843	0	1772	29	0
2	B	2488	0	2343	38	0
3	C	363	0	335	3	0
4	R	2328	0	2210	48	0
5	S	1741	0	1656	30	0
6	N	930	0	855	7	0
All	All	9693	0	9171	148	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 8.

The worst 5 of 148 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:214:VAL:O	1:A:217:VAL:HG12	1.80	0.82
1:A:380:ARG:HA	4:R:138:LEU:HD21	1.62	0.81
4:R:41:LEU:O	4:R:41:LEU:HD23	1.83	0.79
5:S:142:GLN:HG3	5:S:242:GLY:H	1.48	0.78
2:B:165:THR:HG22	2:B:181:THR:HG22	1.65	0.78

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	228/362 (63%)	222 (97%)	6 (3%)	0	100	100
2	B	336/377 (89%)	325 (97%)	11 (3%)	0	100	100
3	C	51/59 (86%)	47 (92%)	4 (8%)	0	100	100
4	R	305/348 (88%)	288 (94%)	17 (6%)	0	100	100
5	S	227/285 (80%)	212 (93%)	15 (7%)	0	100	100
6	N	126/128 (98%)	123 (98%)	3 (2%)	0	100	100
All	All	1273/1559 (82%)	1217 (96%)	56 (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	187/316 (59%)	187 (100%)	0	100	100
2	B	252/308 (82%)	252 (100%)	0	100	100
3	C	31/48 (65%)	31 (100%)	0	100	100
4	R	227/296 (77%)	223 (98%)	4 (2%)	59	80
5	S	187/228 (82%)	185 (99%)	2 (1%)	73	88
6	N	92/106 (87%)	92 (100%)	0	100	100
All	All	976/1302 (75%)	970 (99%)	6 (1%)	86	93

5 of 6 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
4	R	302	TYR
5	S	80	PHE
5	S	81	LEU
4	R	99	TYR
4	R	98	TRP

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

Mol	Chain	Res	Type
1	A	213	GLN
2	B	119	ASN
4	R	62	GLN

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.

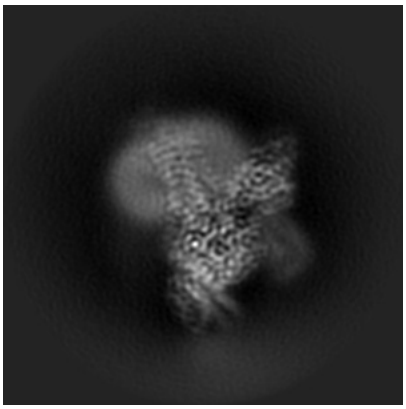
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-35764. These allow visual inspection of the internal detail of the map and identification of artifacts.

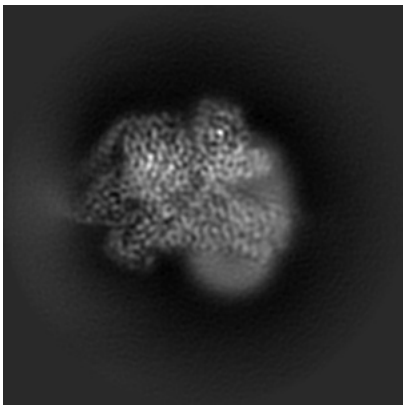
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections (i)

6.1.1 Primary map



X



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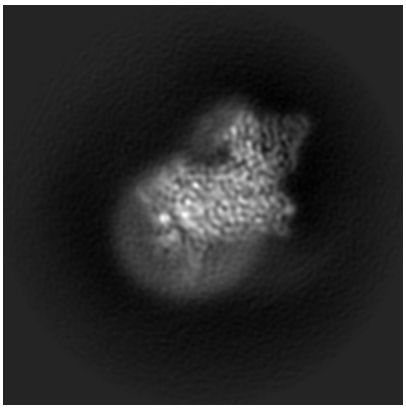
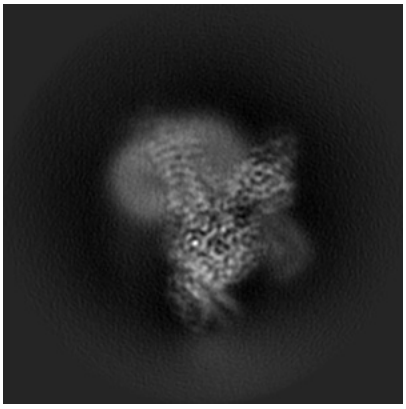


Z

6.1.2 Raw map



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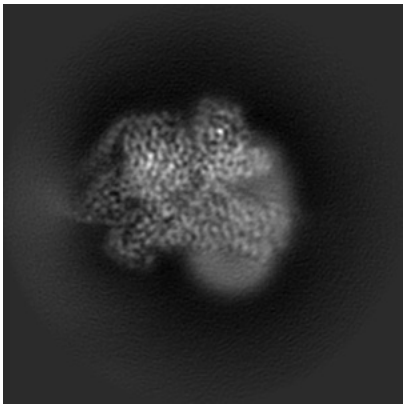

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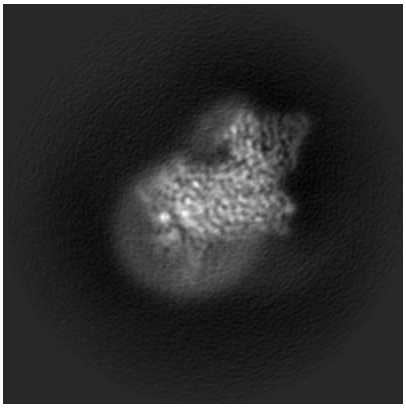
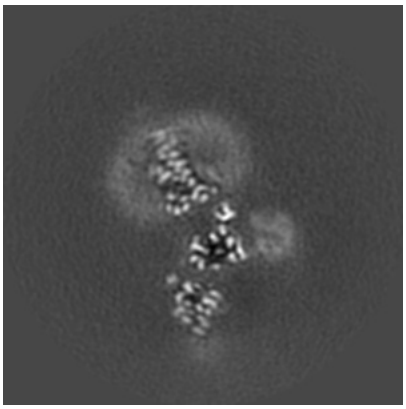


Z

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

6.2 Central slices (i)

6.2.1 Primary map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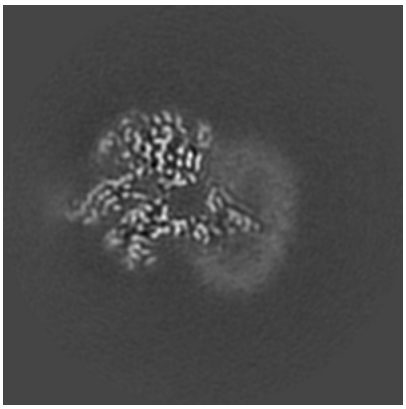
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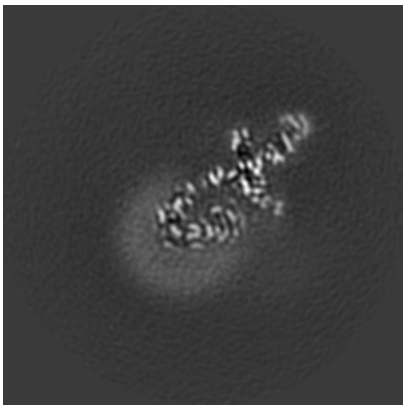
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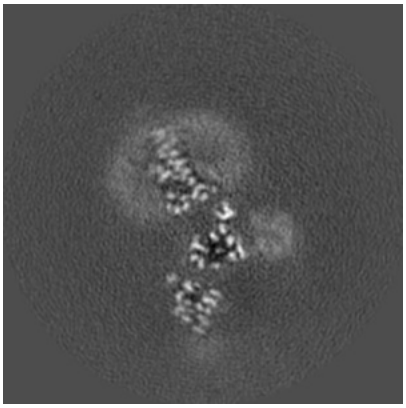
6.2.2 Raw map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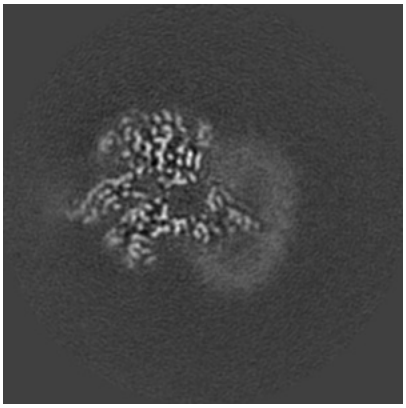
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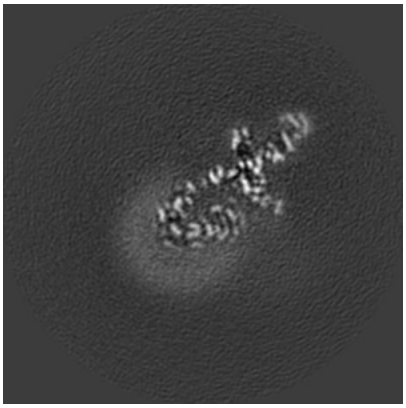
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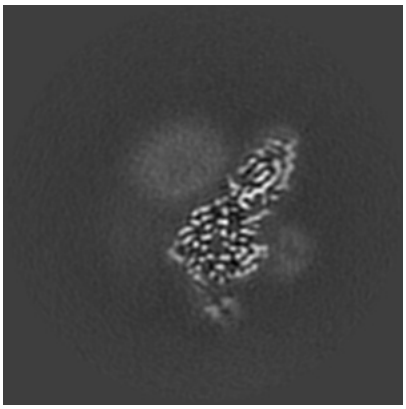
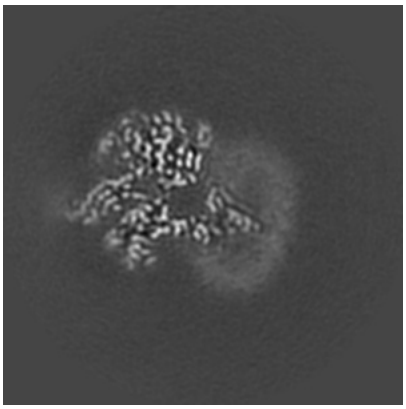
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

6.3.1 Primary map


X Index: 123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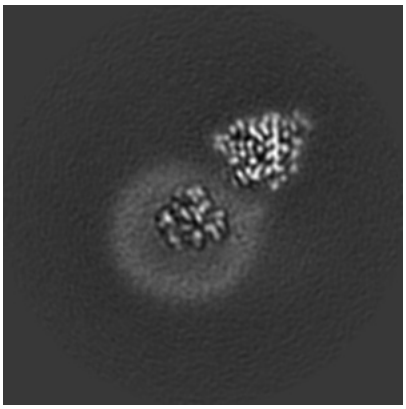
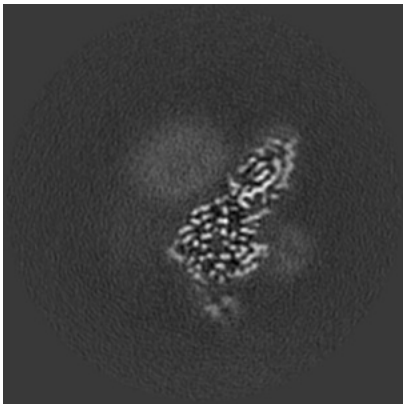

Z Index: 108

6.3.2 Raw map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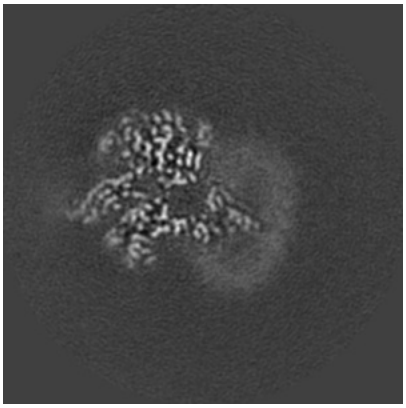
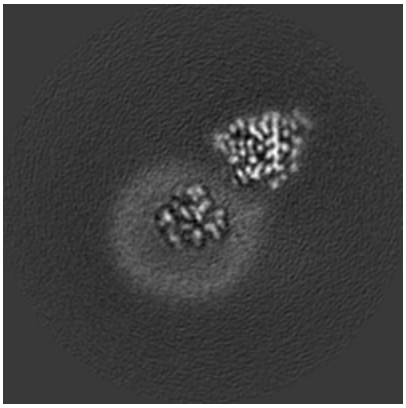
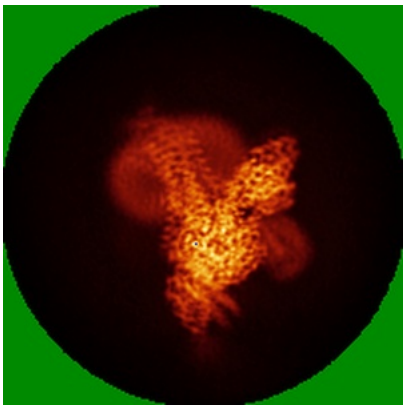

Y Index: 100

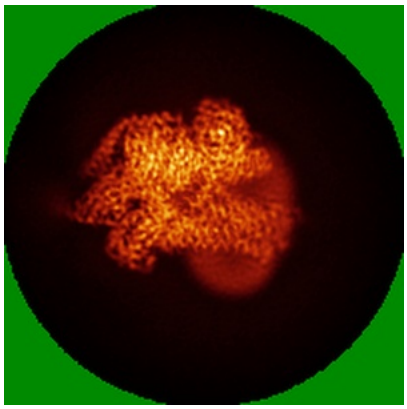

Z Index: 108

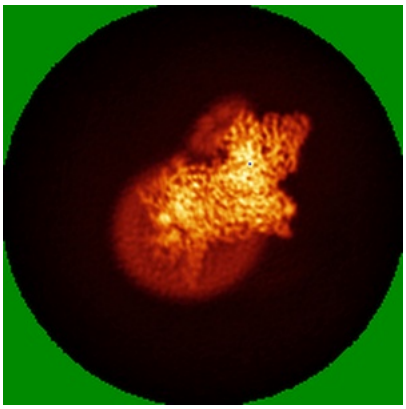
The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal standard-deviation projections (False-color) (i)

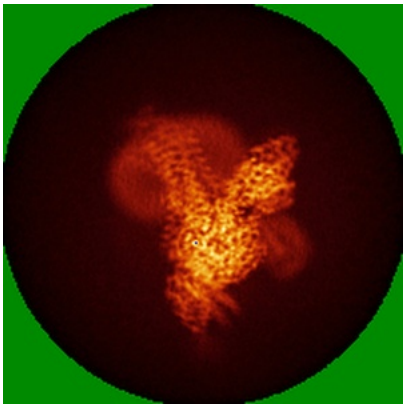
6.4.1 Primary map

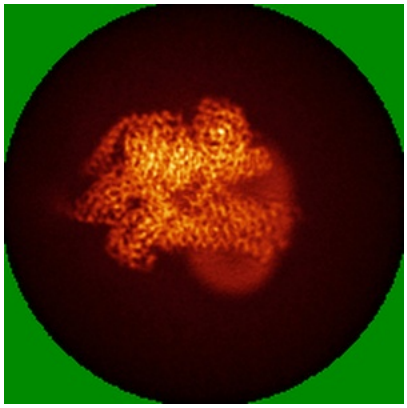

X

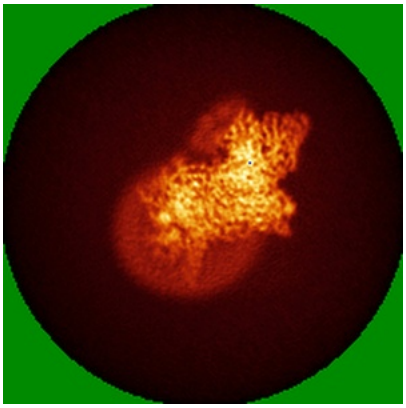

Y


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

X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.01. 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

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.

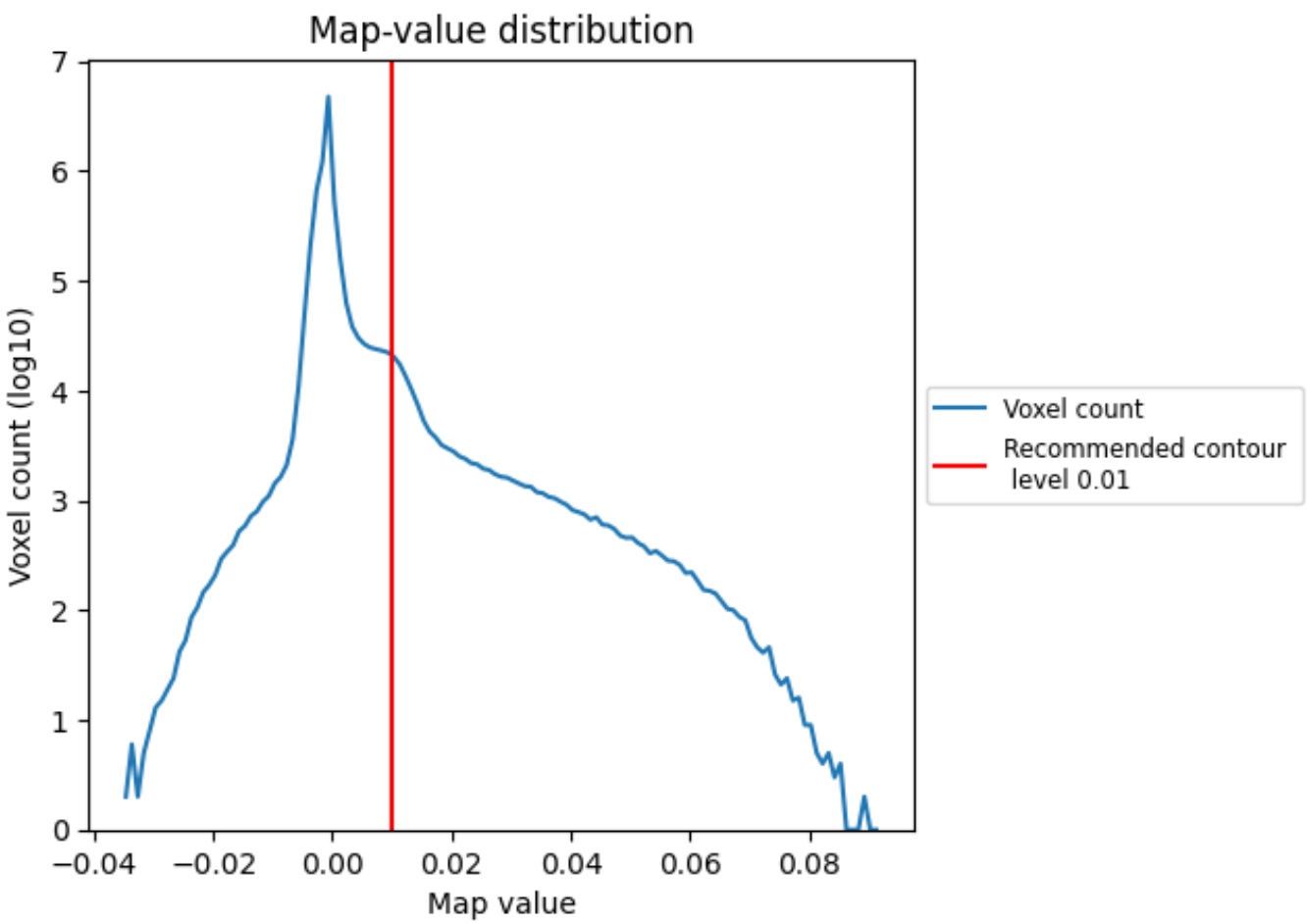
6.6 Mask visualisation (i)

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

7 Map analysis (i)

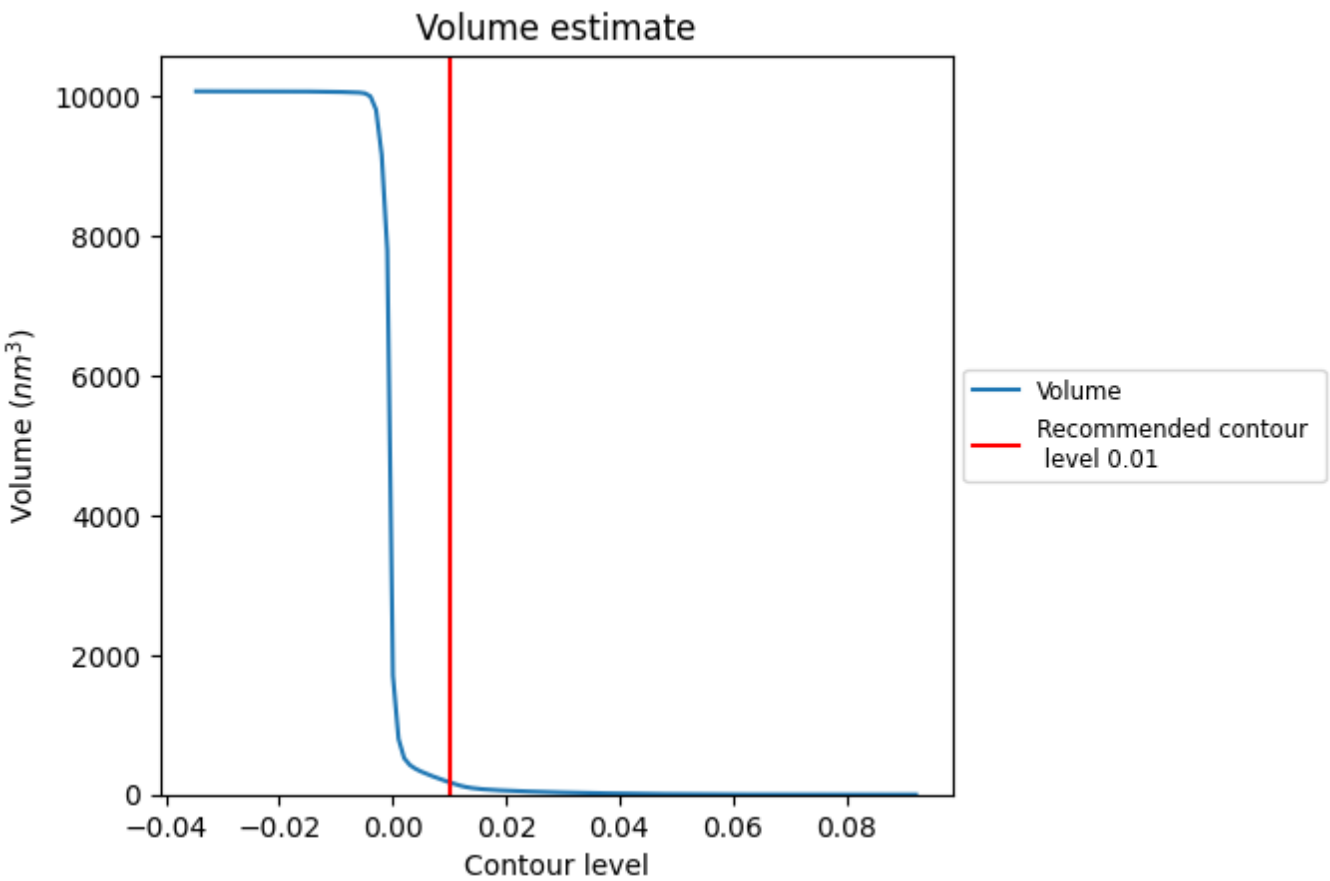
This section contains the results of statistical analysis of the map.

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.

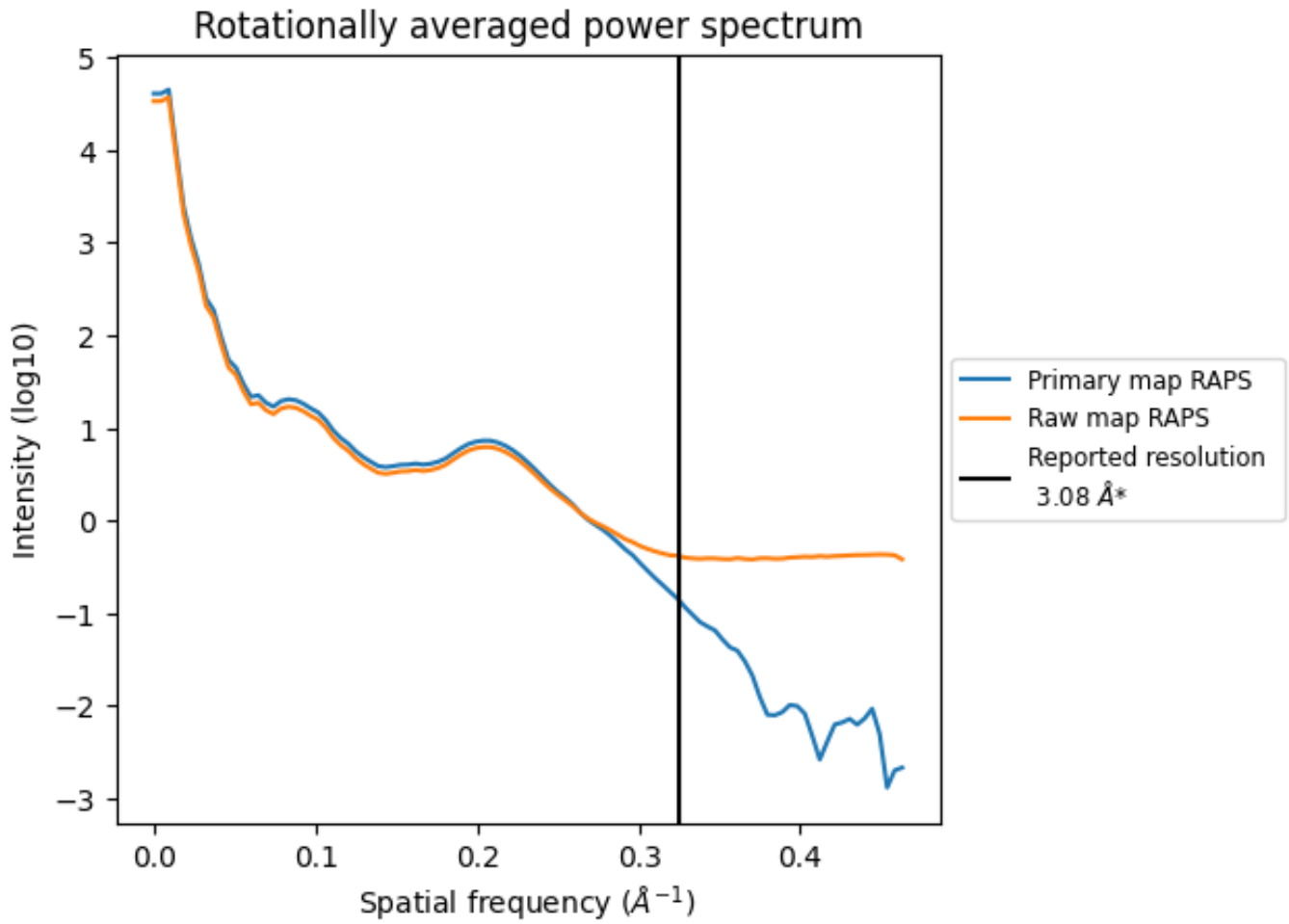
7.2 Volume estimate (i)



The volume at the recommended contour level is 175 nm^3; this corresponds to an approximate mass of 158 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)

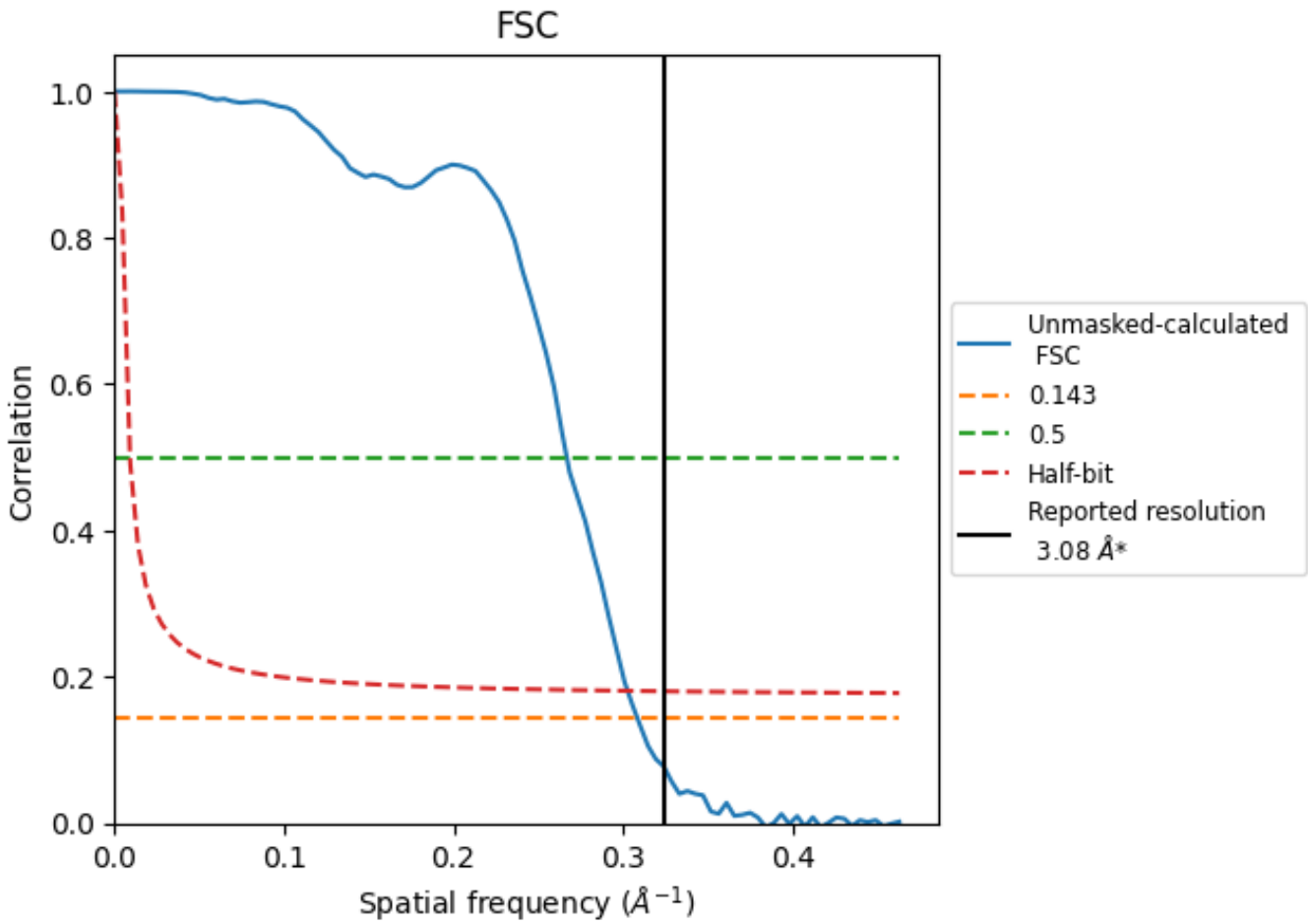


*Reported resolution corresponds to spatial frequency of 0.325 Å^{-1}

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 0.325 Å^{-1}

8.2 Resolution estimates (i)

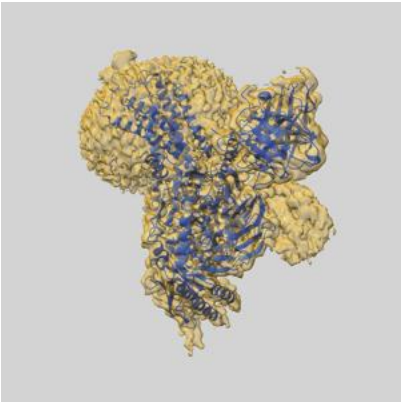
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.08	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	3.24	3.75	3.30

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

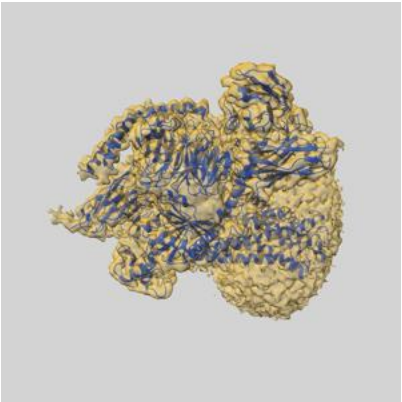
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-35764 and PDB model 8IW9. Per-residue inclusion information can be found in section 3 on page 6.

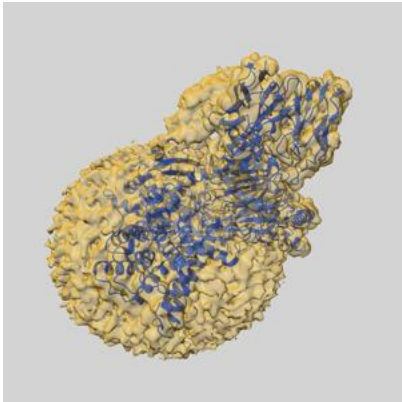
9.1 Map-model overlay (i)



X



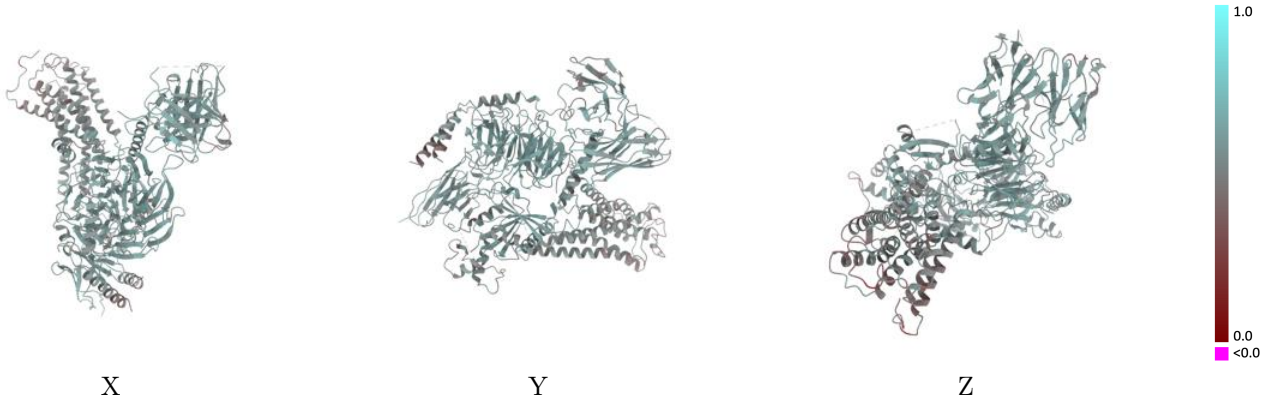
Y



Z

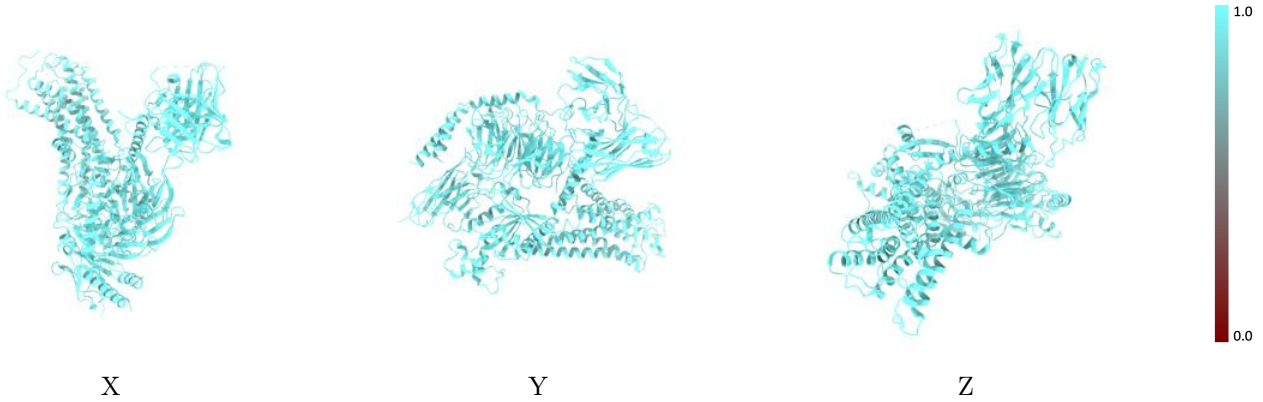
The images above show the 3D surface view of the map at the recommended contour level 0.01 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

9.2 Q-score mapped to coordinate model (i)



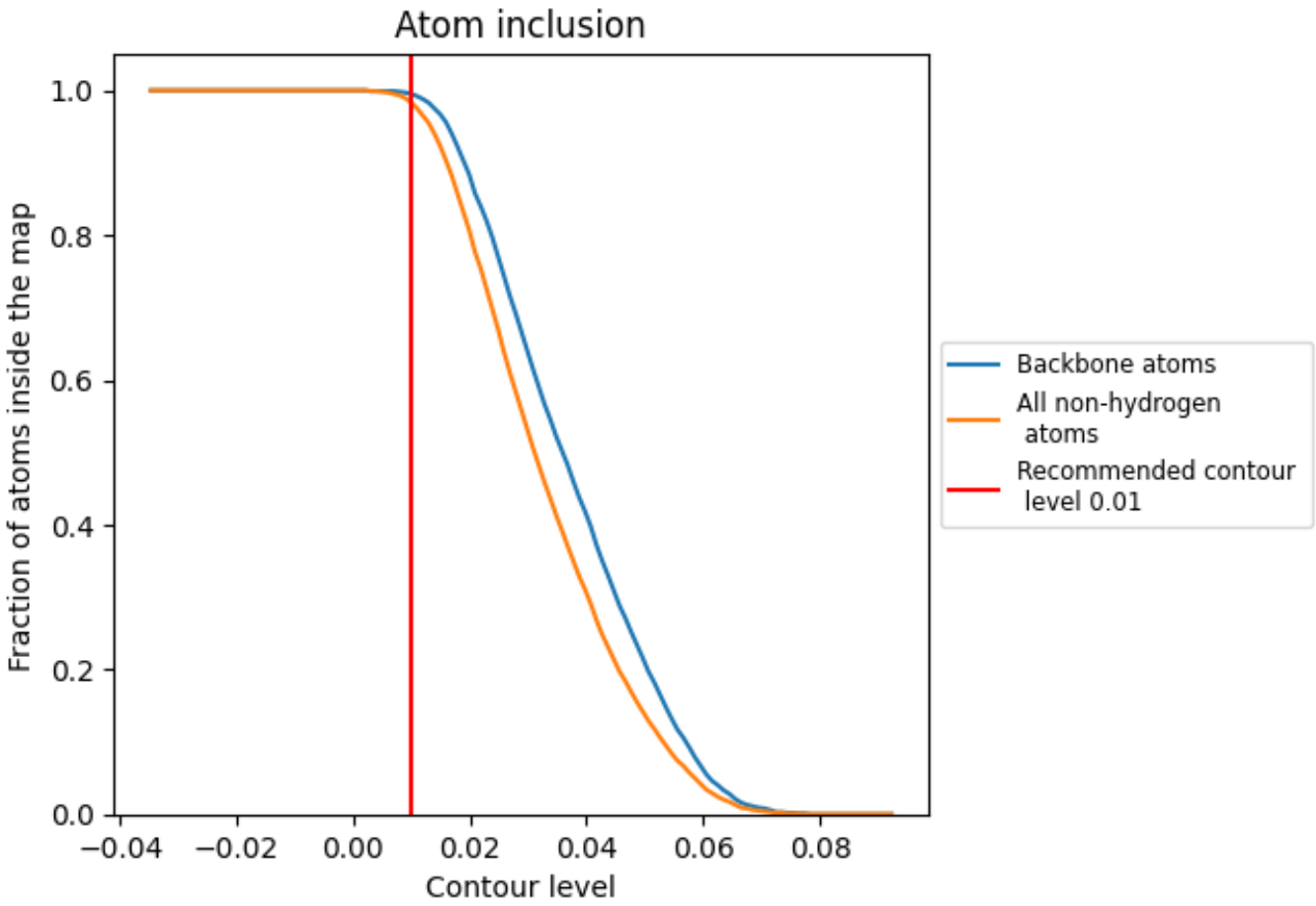
The images above show the model with each residue coloured according its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions 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 (0.01).

9.4 Atom inclusion (i)



At the recommended contour level, 100% of all backbone atoms, 98% 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 (0.01) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.9830	0.5460
A	0.9850	0.5550
B	0.9910	0.5790
C	0.9750	0.5270
N	0.9850	0.5590
R	0.9720	0.4930
S	0.9870	0.5590