



## wwPDB EM Validation Summary Report ⓘ

Mar 3, 2025 – 06:09 PM EST

PDB ID : 8G94  
EMDB ID : EMD-29861  
Title : Structure of CD69-bound S1PR1 coupled to heterotrimeric Gi  
Authors : Chen, H.; Li, X.  
Deposited on : 2023-02-21  
Resolution : 3.15 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](mailto: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.dev117  
MolProbity : 4.02b-467  
Percentile statistics : 20231227.v01 (using entries in the PDB archive December 27th 2023)  
MapQ : 1.9.13  
Ideal geometry (proteins) : Engh & Huber (2001)  
Ideal geometry (DNA, RNA) : Parkinson et al. (1996)  
Validation Pipeline (wwPDB-VP) : 2.41.4

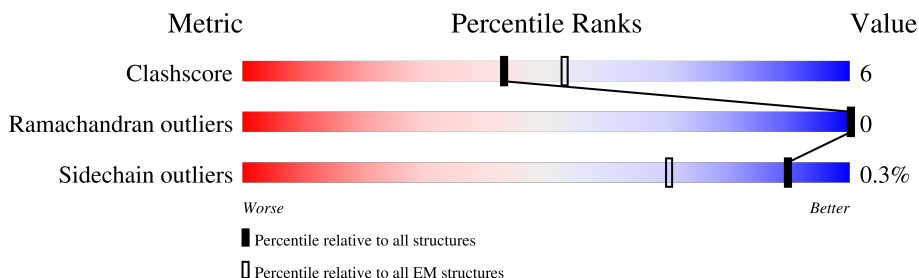
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*ELECTRON MICROSCOPY*

The reported resolution of this entry is 3.15 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	210492	15764
Ramachandran outliers	207382	16835
Sidechain outliers	206894	16415

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  $\geq 3$ , 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	355	
2	B	354	
3	C	345	
4	D	71	
5	E	259	
6	F	211	
6	G	211	

## 2 Entry composition

There are 6 unique types of molecules in this entry. The entry contains 9221 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 Sphingosine 1-phosphate receptor 1.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	288	Total	C	N	O	S	0	0
			2316	1549	377	374	16		

There are 8 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	348	ASP	-	expression tag	UNP P21453
A	349	TYR	-	expression tag	UNP P21453
A	350	LYS	-	expression tag	UNP P21453
A	351	ASP	-	expression tag	UNP P21453
A	352	ASP	-	expression tag	UNP P21453
A	353	ASP	-	expression tag	UNP P21453
A	354	ASP	-	expression tag	UNP P21453
A	355	LYS	-	expression tag	UNP P21453

- Molecule 2 is a protein called Guanine nucleotide-binding protein G(i) subunit alpha-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	221	Total	C	N	O	S	0	0
			1780	1135	296	336	13		

There are 2 discrepancies between the modelled and reference sequences:

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

- Molecule 3 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	C	337	Total	C	N	O	S	0	0
			2592	1599	466	506	21		

There are 6 discrepancies between the modelled and reference sequences:

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

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

Mol	Chain	Residues	Atoms					AltConf	Trace
4	D	58	Total	C	N	O	S	0	0
			444	277	79	85	3		

- Molecule 5 is a protein called scFv16.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	E	231	Total	C	N	O	S	0	0
			1777	1128	294	345	10		

- Molecule 6 is a protein called Early activation antigen CD69.

Mol	Chain	Residues	Atoms					AltConf	Trace
6	F	25	Total	C	N	O	S	0	0
			179	123	26	28	2		
6	G	27	Total	C	N	O		0	0
			133	79	27	27			

There are 28 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
F	1	MET	-	expression tag	UNP Q07108
F	2	ALA	-	expression tag	UNP Q07108
F	200	GLY	-	expression tag	UNP Q07108
F	201	SER	-	expression tag	UNP Q07108
F	202	ALA	-	expression tag	UNP Q07108
F	203	SER	-	expression tag	UNP Q07108
F	204	TRP	-	expression tag	UNP Q07108
F	205	SER	-	expression tag	UNP Q07108
F	206	HIS	-	expression tag	UNP Q07108
F	207	PRO	-	expression tag	UNP Q07108

*Continued on next page...*

*Continued from previous page...*

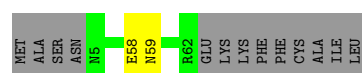
Chain	Residue	Modelled	Actual	Comment	Reference
F	208	GLN	-	expression tag	UNP Q07108
F	209	PHE	-	expression tag	UNP Q07108
F	210	GLU	-	expression tag	UNP Q07108
F	211	LYS	-	expression tag	UNP Q07108
G	1	MET	-	expression tag	UNP Q07108
G	2	ALA	-	expression tag	UNP Q07108
G	200	GLY	-	expression tag	UNP Q07108
G	201	SER	-	expression tag	UNP Q07108
G	202	ALA	-	expression tag	UNP Q07108
G	203	SER	-	expression tag	UNP Q07108
G	204	TRP	-	expression tag	UNP Q07108
G	205	SER	-	expression tag	UNP Q07108
G	206	HIS	-	expression tag	UNP Q07108
G	207	PRO	-	expression tag	UNP Q07108
G	208	GLN	-	expression tag	UNP Q07108
G	209	PHE	-	expression tag	UNP Q07108
G	210	GLU	-	expression tag	UNP Q07108
G	211	LYS	-	expression tag	UNP Q07108





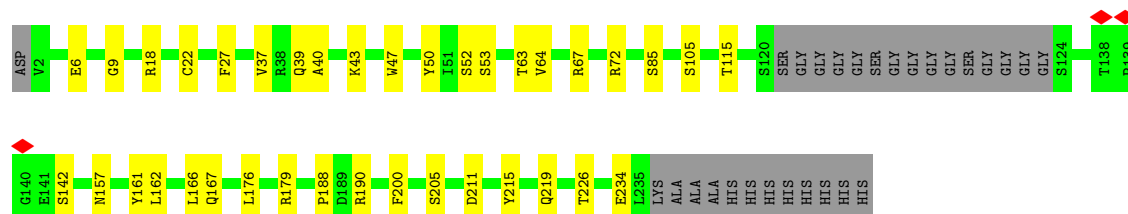
- Molecule 4: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2

Chain D: 79% 18%



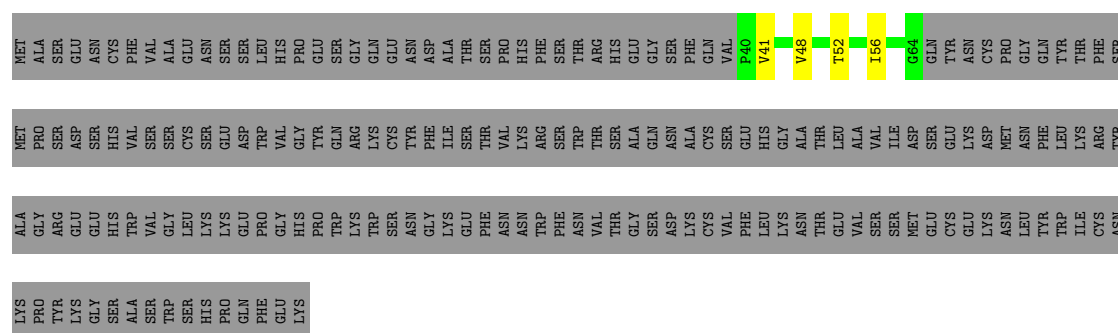
- Molecule 5: scFv16

Chain E: 75% 14% 11%



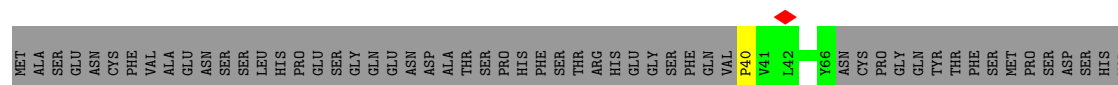
- Molecule 6: Early activation antigen CD69

Chain F: 10% 88%



- Molecule 6: Early activation antigen CD69

Chain G: 12% 87%



SER	VAL	SER	SER
TRP	GLY	TRP	TRP
SER	LEU	SER	SER
HIS	LYS	HIS	SER
PRO	LYS	PRO	GLU
GLN	GLU	GLN	ASP
PHE	PRO	PHE	TRP
GLU	GLY	GLU	VAL
PHE	HIS	PHE	GLY
LYS	PRO	LYS	TYR
	TRP		GLN
	LYS		ARG
	TRP		LYS
	SER		CYS
	ASN		TYR
	GLY		PHE
	LYS		ILE
	PHE		SER
	GLU		THR
	ASN		VAL
	ASN		LYS
	TRP		ARG
	PHE		SER
	ASN		TRP
	VAL		THR
	THR		SER
	GLY		ALA
	SER		GLN
	ASP		ASN
	LYS		ALA
	CYS		CYS
	VAL		SER
	PHE		GLU
	LEU		HIS
	LYS		GLY
	ASN		ALA
	THR		THR
	GLU		LEU
	VAL		VAL
	SER		VAL
	SER		ILE
	MET		ASP
	GLU		SER
	CYS		GLU
	GLU		LYS
	LYS		ASP
	ASN		MET
	LEU		ASN
	TYR		PHE
	TRP		LYS
	ILE		LEV
	CYS		ARG
	ASN		THR
	LYS		ALA
	PRO		GLY
	TYR		ARG
	LYS		GLU
	GLY		GLU
	SER		THR
	ALA		FED



## 4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	293516	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 ( $e^-/\text{\AA}^2$ )	60	Depositor
Minimum defocus (nm)	800	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	Not provided	
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor
Maximum map value	0.084	Depositor
Minimum map value	-0.047	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.003	Depositor
Recommended contour level	0.01	Depositor
Map size ( $\text{\AA}$ )	239.04, 239.04, 239.04	wwPDB
Map dimensions	288, 288, 288	wwPDB
Map angles ( $^\circ$ )	90.0, 90.0, 90.0	wwPDB
Pixel spacing ( $\text{\AA}$ )	0.83, 0.83, 0.83	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	0.28	0/2366	0.50	0/3210
2	B	0.32	0/1809	0.45	0/2426
3	C	0.40	0/2639	0.53	0/3577
4	D	0.27	0/450	0.42	0/608
5	E	0.41	0/1821	0.53	0/2469
6	F	0.26	0/180	0.49	0/246
6	G	0.29	0/132	0.55	1/182 (0.5%)
All	All	0.35	0/9397	0.50	1/12718 (0.0%)

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
6	G	40	PRO	N-CA-CB	5.84	110.31	103.30

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	2316	0	2483	38	0
2	B	1780	0	1782	21	0
3	C	2592	0	2499	38	0
4	D	444	0	454	2	0
5	E	1777	0	1714	25	0

*Continued on next page...*

*Continued from previous page...*

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
6	F	179	0	210	4	0
6	G	133	0	62	0	0
All	All	9221	0	9204	118	0

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 6.

The worst 5 of 118 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)
5:E:39:GLN:HE22	5:E:167:GLN:HE22	1.33	0.76
1:A:78:ARG:HB3	1:A:81:TYR:HB2	1.68	0.74
2:B:8:GLU:OE2	5:E:161:TYR:HD1	1.71	0.73
3:C:271:CYS:HB2	3:C:290:ASP:HB2	1.70	0.73
2:B:39:LEU:HD13	2:B:253:ILE:HD13	1.69	0.72

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	282/355 (79%)	269 (95%)	13 (5%)	0	100	100
2	B	215/354 (61%)	209 (97%)	6 (3%)	0	100	100
3	C	335/345 (97%)	318 (95%)	17 (5%)	0	100	100
4	D	56/71 (79%)	55 (98%)	1 (2%)	0	100	100
5	E	227/259 (88%)	216 (95%)	11 (5%)	0	100	100
6	F	23/211 (11%)	23 (100%)	0	0	100	100
6	G	25/211 (12%)	24 (96%)	1 (4%)	0	100	100
All	All	1163/1806 (64%)	1114 (96%)	49 (4%)	0	100	100

There are no Ramachandran outliers to report.

### 5.3.2 Protein sidechains ⓘ

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all PDB entries followed by that with respect to all EM entries.

The Analysed column shows the number of residues for which the sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	259/317 (82%)	259 (100%)	0	100	100
2	B	197/306 (64%)	195 (99%)	2 (1%)	73	85
3	C	280/285 (98%)	279 (100%)	1 (0%)	89	94
4	D	47/58 (81%)	47 (100%)	0	100	100
5	E	196/209 (94%)	196 (100%)	0	100	100
6	F	21/187 (11%)	21 (100%)	0	100	100
All	All	1000/1362 (73%)	997 (100%)	3 (0%)	90	95

All (3) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	B	205	ARG
2	B	243	MET
3	C	214	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 17 such sidechains are listed below:

Mol	Chain	Res	Type
4	D	59	ASN
6	F	47	ASN
2	B	333	GLN
3	C	16	ASN
3	C	156	GLN

### 5.3.3 RNA ⓘ

There are no RNA molecules in this entry.

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

There are no non-standard protein/DNA/RNA residues in this entry.

## 5.5 Carbohydrates [i](#)

There are no oligosaccharides in this entry.

## 5.6 Ligand geometry [i](#)

There are no ligands in this entry.

## 5.7 Other polymers [i](#)

There are no such residues in this entry.

## 5.8 Polymer linkage issues [i](#)

There are no chain breaks in this entry.

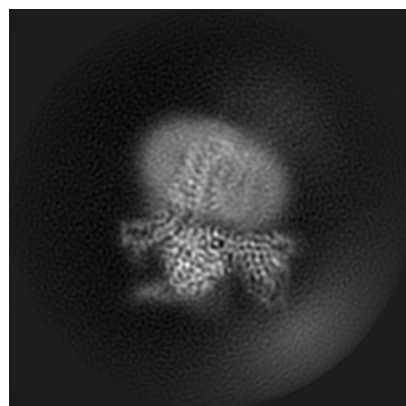
## 6 Map visualisation [i](#)

This section contains visualisations of the EMDB entry EMD-29861. These allow visual inspection of the internal detail of the map and identification of artifacts.

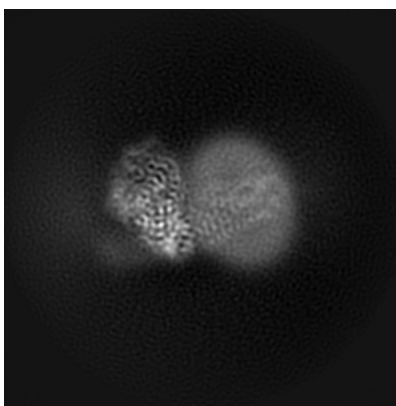
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

### 6.1 Orthogonal projections [i](#)

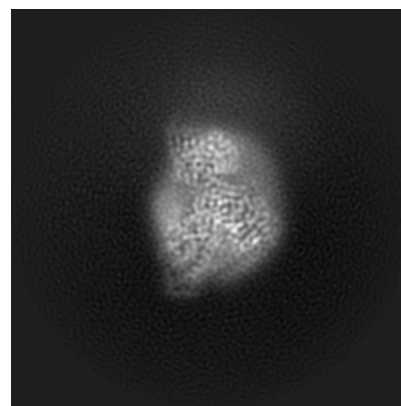
#### 6.1.1 Primary map



X

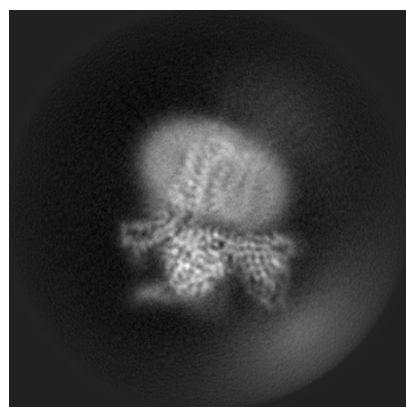


Y

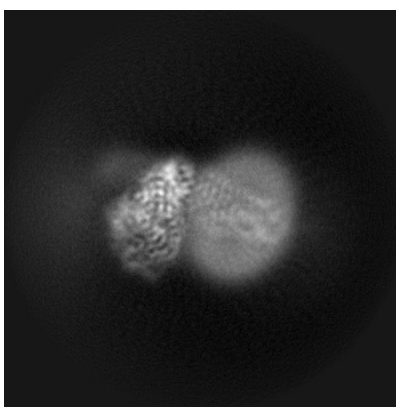


Z

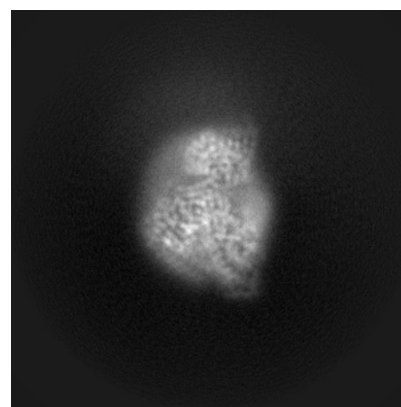
#### 6.1.2 Raw map



X



Y

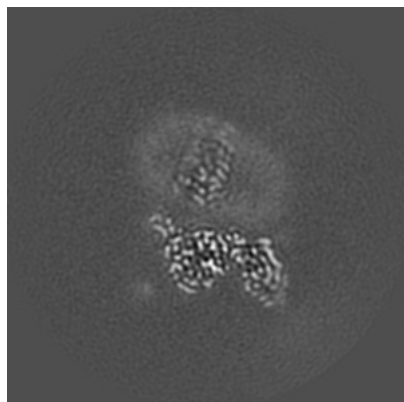


Z

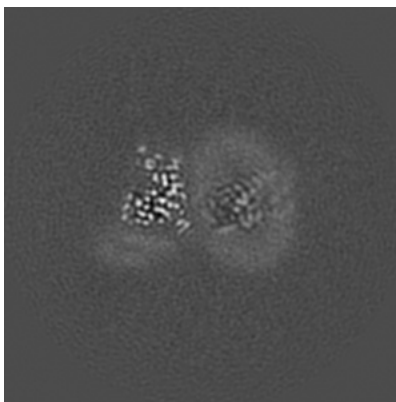
The images above show the map projected in three orthogonal directions.

## 6.2 Central slices [i](#)

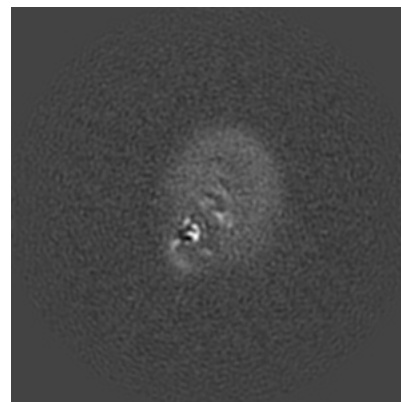
### 6.2.1 Primary map



X Index: 144

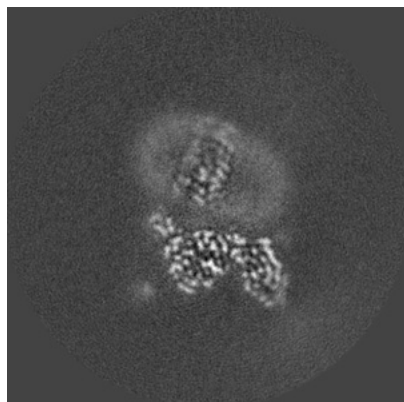


Y Index: 144

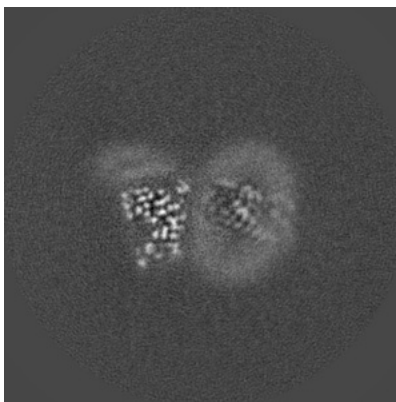


Z Index: 144

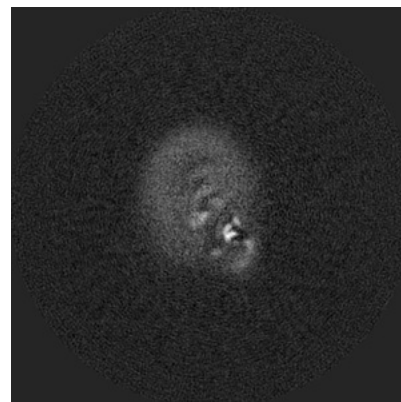
### 6.2.2 Raw map



X Index: 144



Y Index: 144

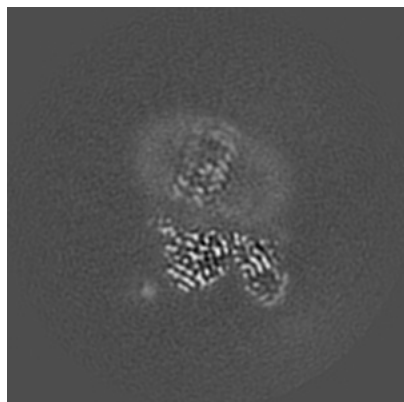


Z Index: 144

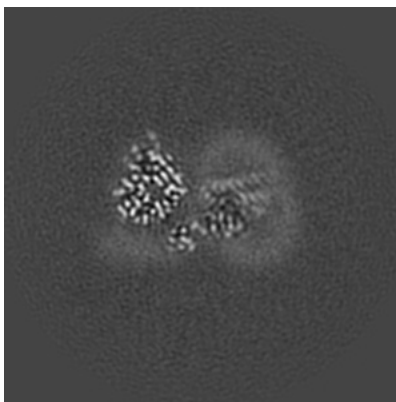
The images above show central slices of the map in three orthogonal directions.

## 6.3 Largest variance slices [i](#)

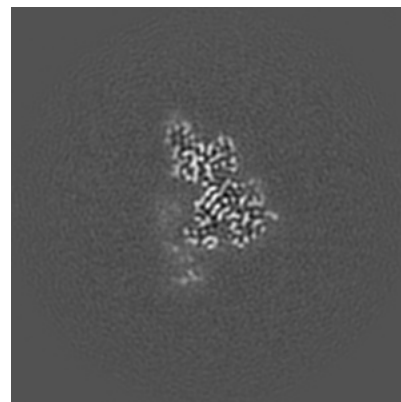
### 6.3.1 Primary map



X Index: 146

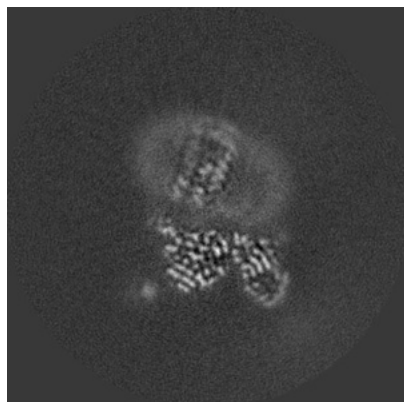


Y Index: 132

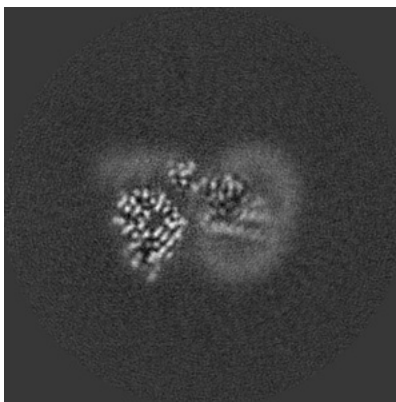


Z Index: 113

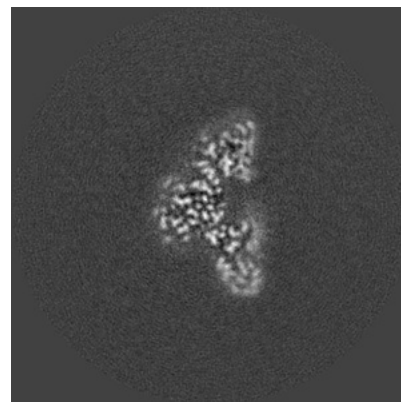
### 6.3.2 Raw map



X Index: 142



Y Index: 132



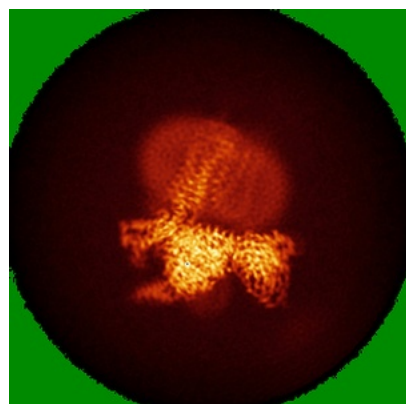
Z Index: 119

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

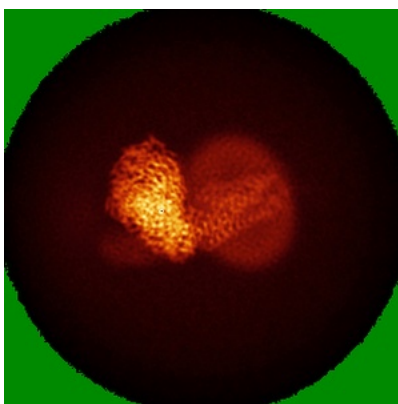


## 6.4 Orthogonal standard-deviation projections (False-color) [i](#)

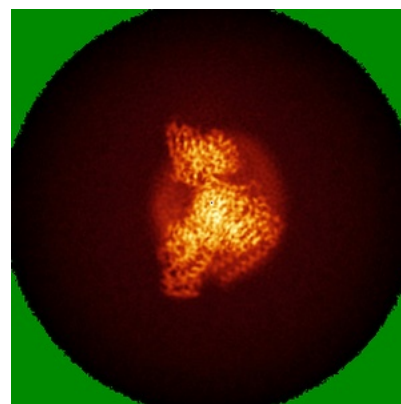
### 6.4.1 Primary map



X

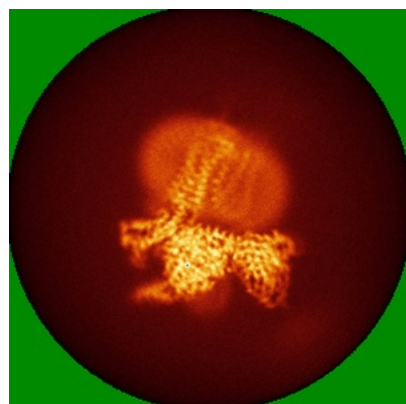


Y

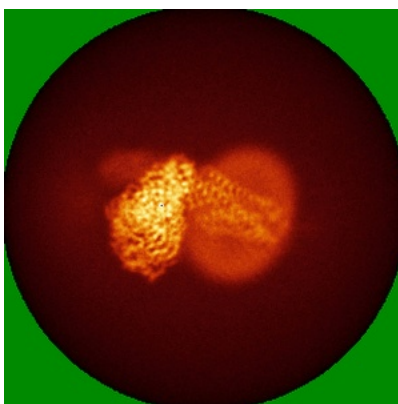


Z

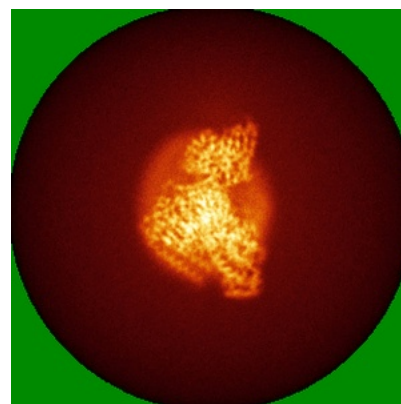
### 6.4.2 Raw map



X



Y

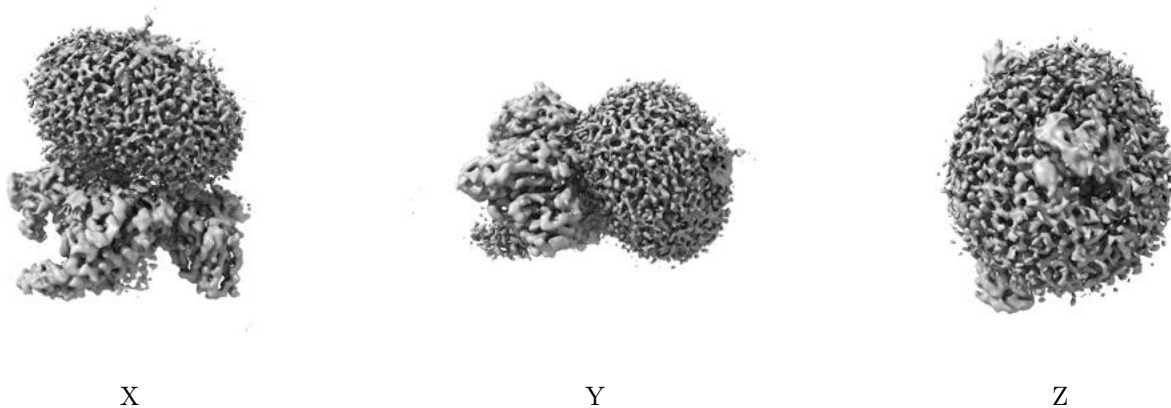


Z

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.

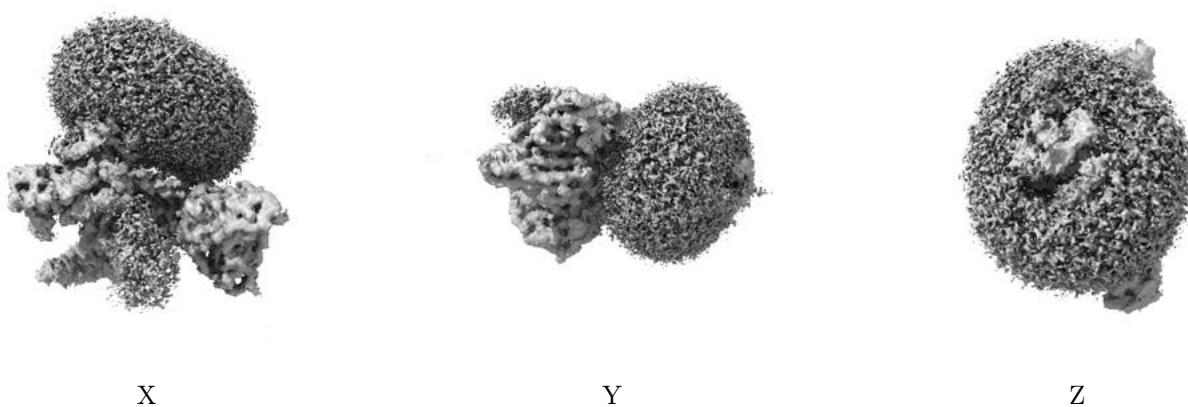
## 6.5 Orthogonal surface views [i](#)

### 6.5.1 Primary map



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



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.

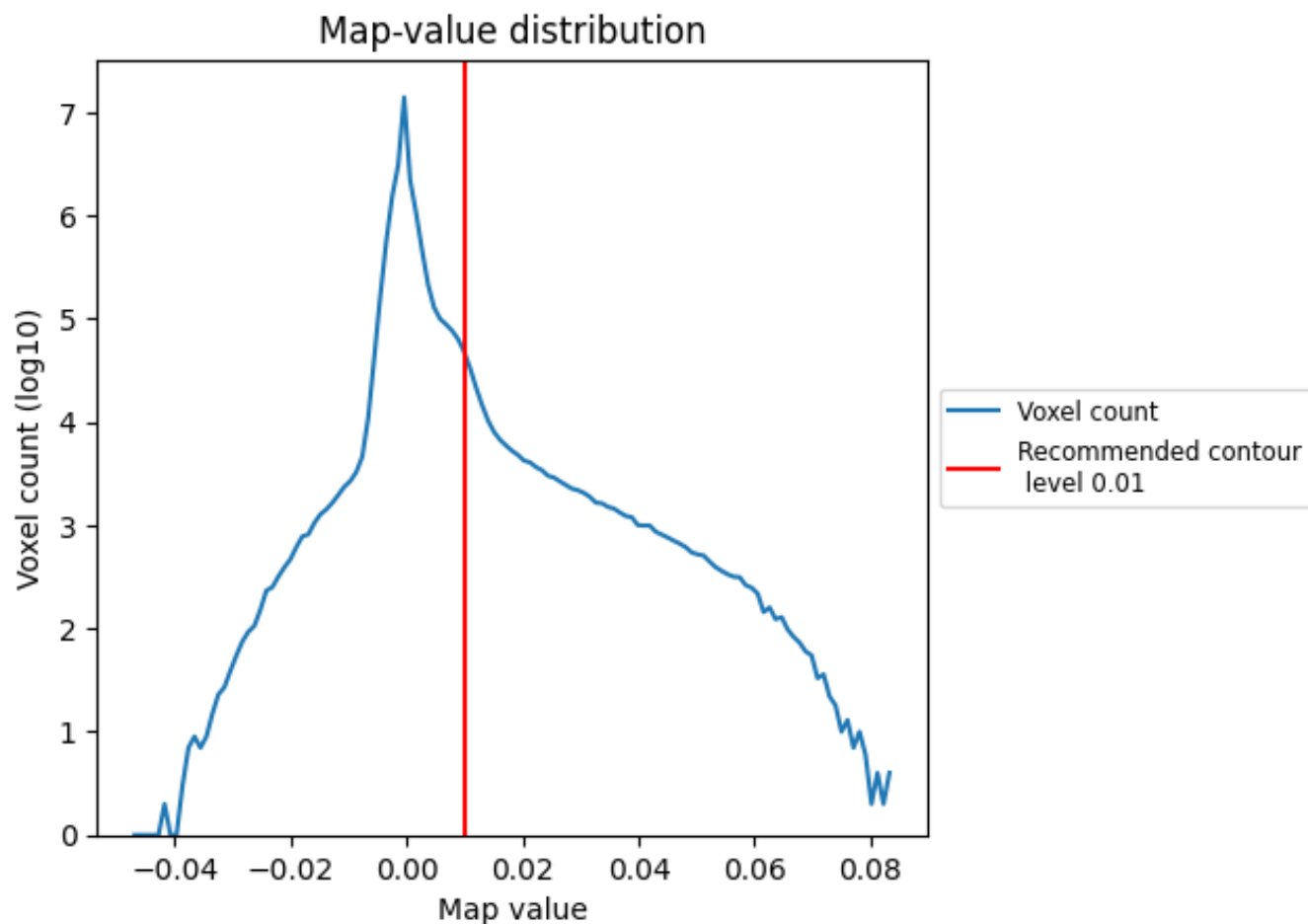
## 6.6 Mask visualisation [i](#)

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

## 7 Map analysis [i](#)

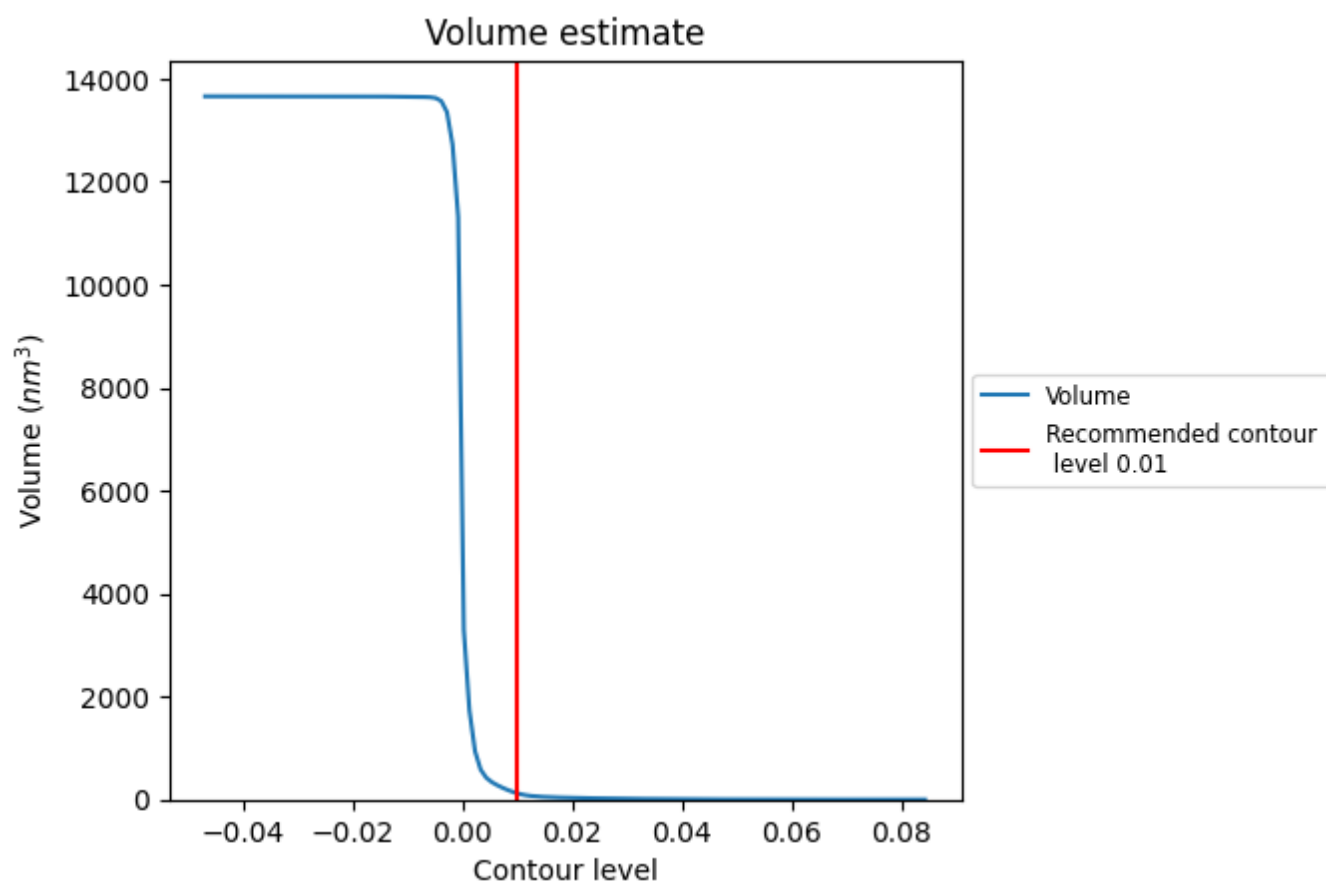
This section contains the results of statistical analysis of the map.

### 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.

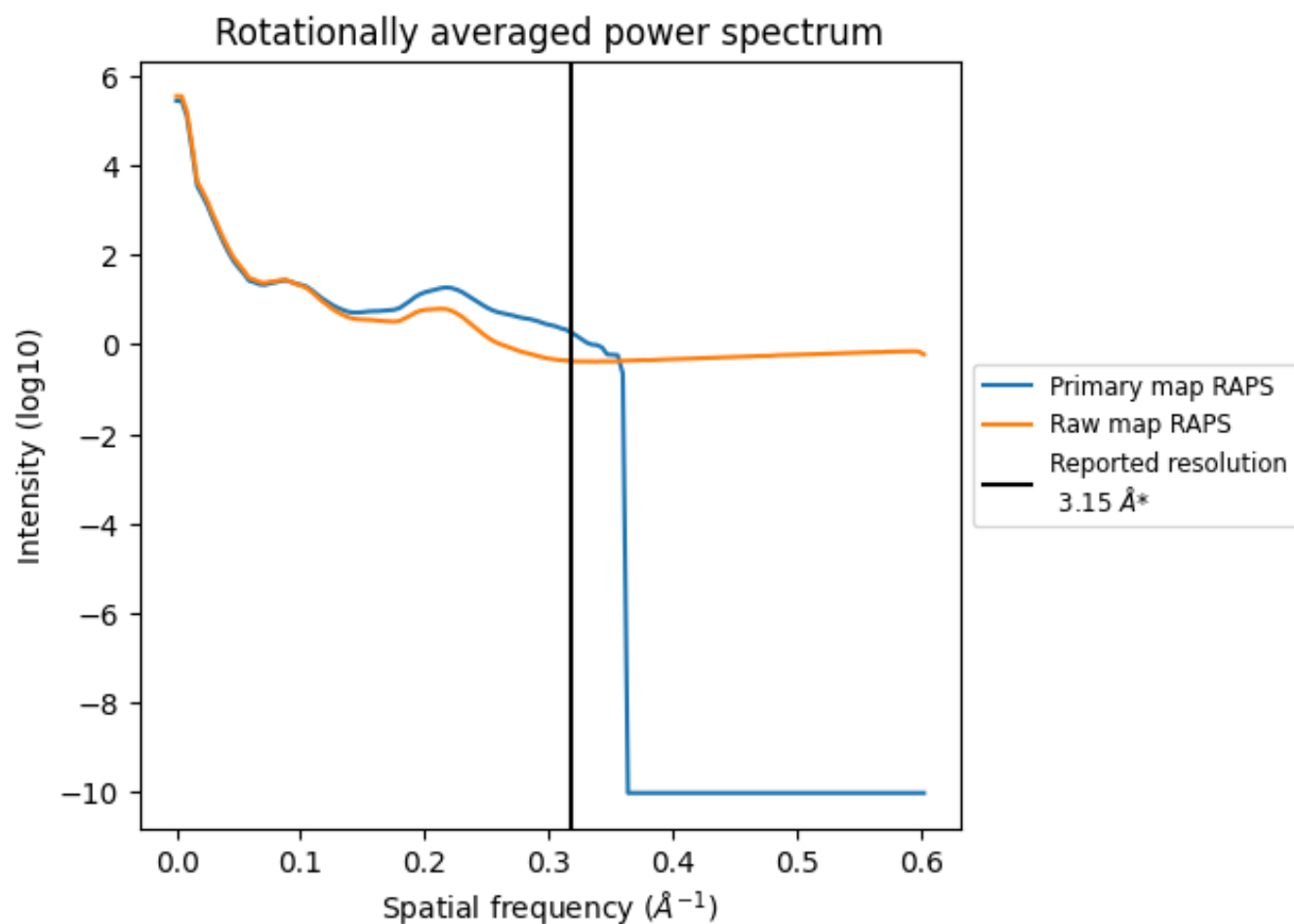
## 7.2 Volume estimate [i](#)



The volume at the recommended contour level is 123 nm<sup>3</sup>; this corresponds to an approximate mass of 111 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 ⓘ

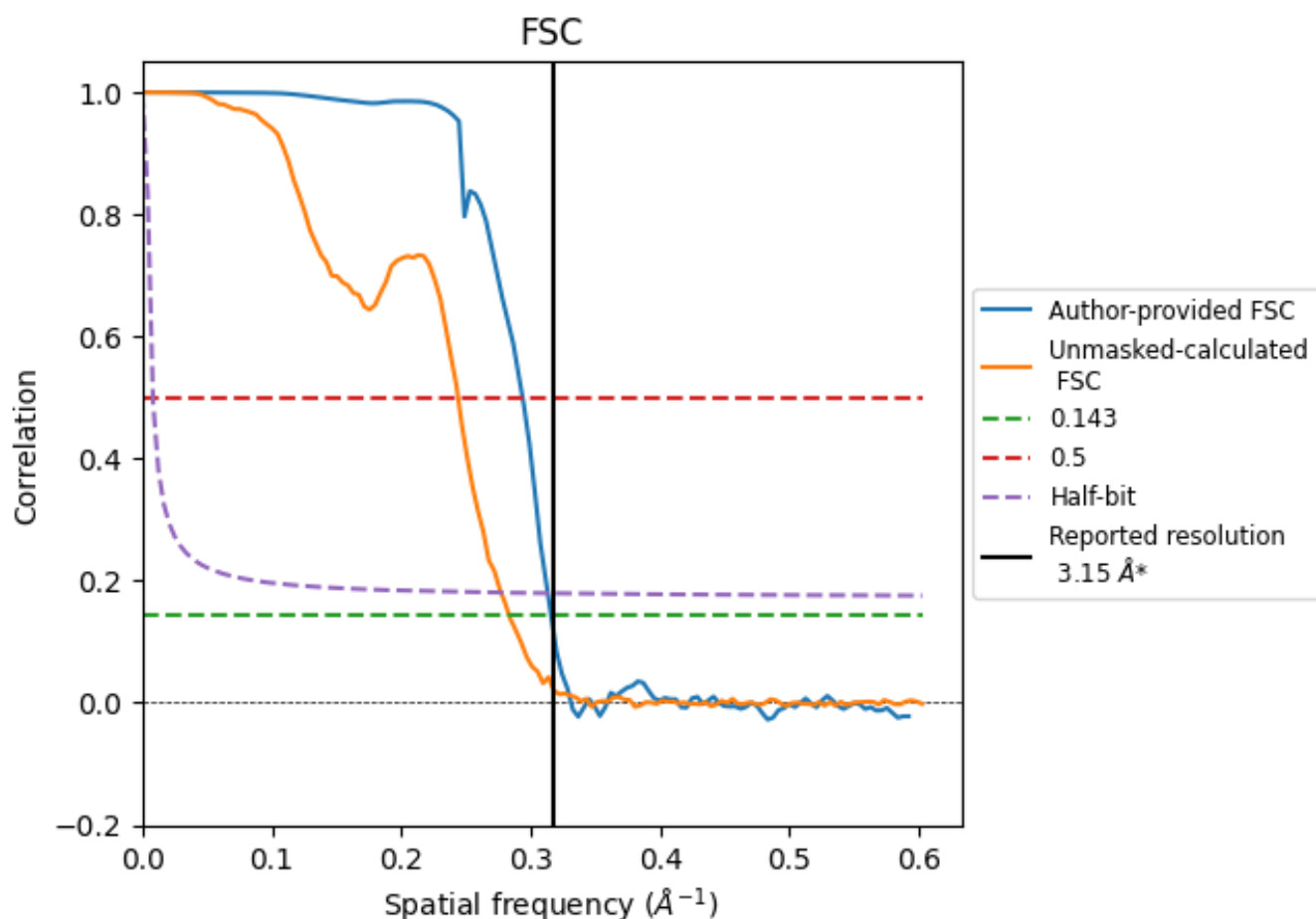


\*Reported resolution corresponds to spatial frequency of 0.317 Å<sup>-1</sup>

## 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  $0.317 \text{ \AA}^{-1}$

## 8.2 Resolution estimates [i](#)

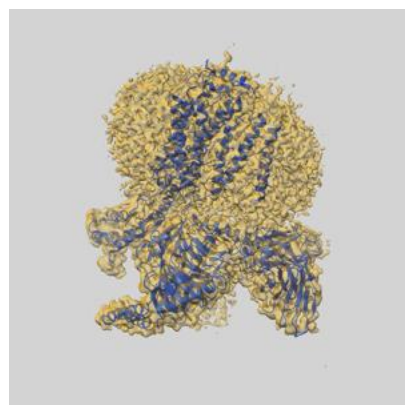
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.15	-	-
Author-provided FSC curve	3.17	3.40	3.19
Unmasked-calculated*	3.53	4.10	3.61

\*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 3.53 differs from the reported value 3.15 by more than 10 %

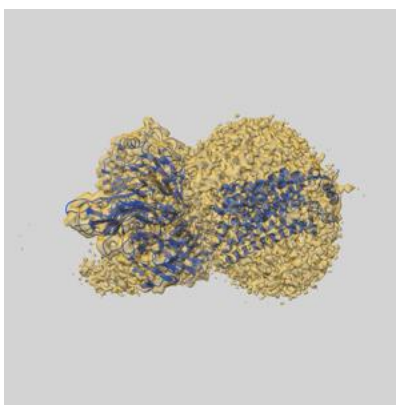
## 9 Map-model fit [i](#)

This section contains information regarding the fit between EMDB map EMD-29861 and PDB model 8G94. Per-residue inclusion information can be found in section [3](#) on page [6](#).

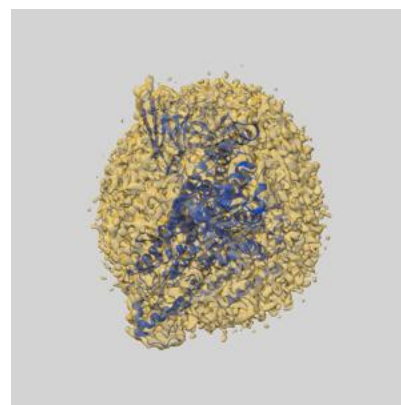
### 9.1 Map-model overlay [i](#)



X



Y

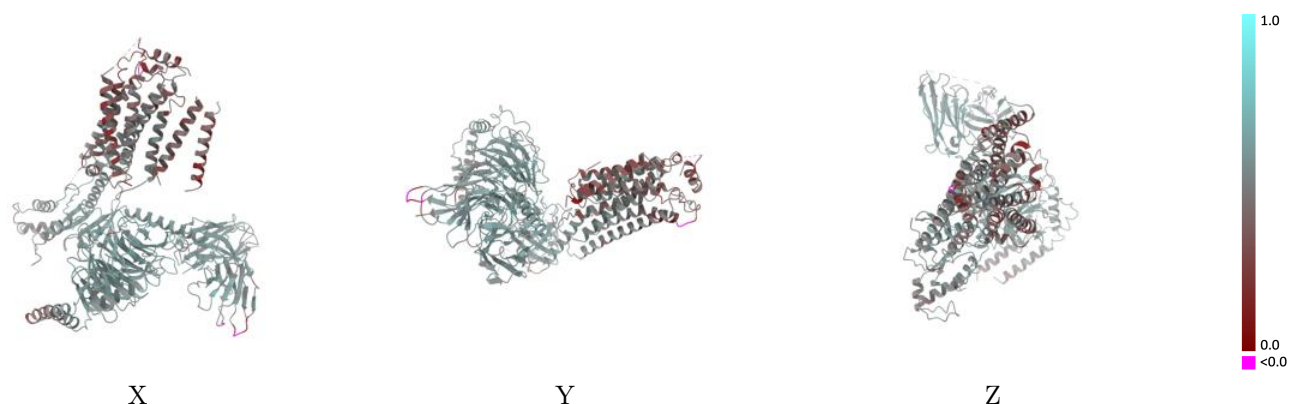


Z

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.

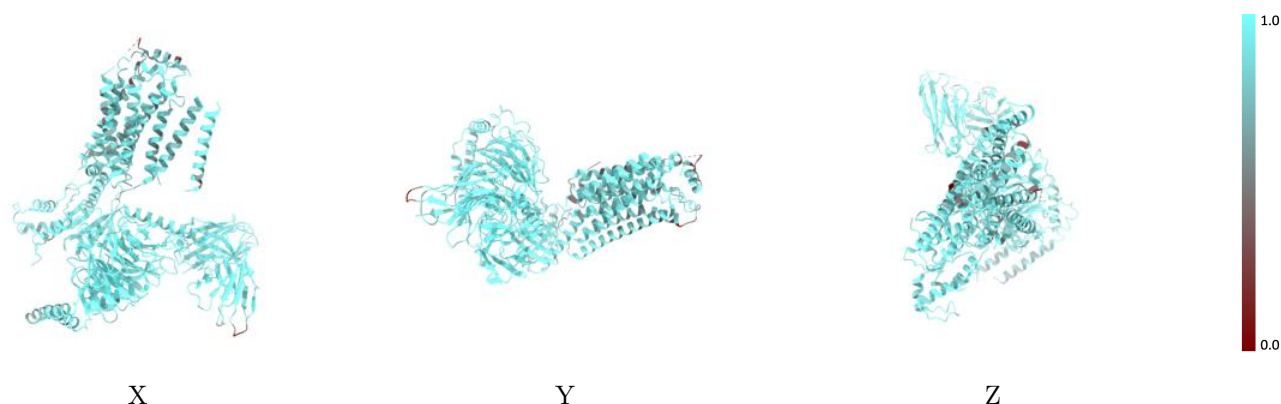


## 9.2 Q-score mapped to coordinate model [i](#)



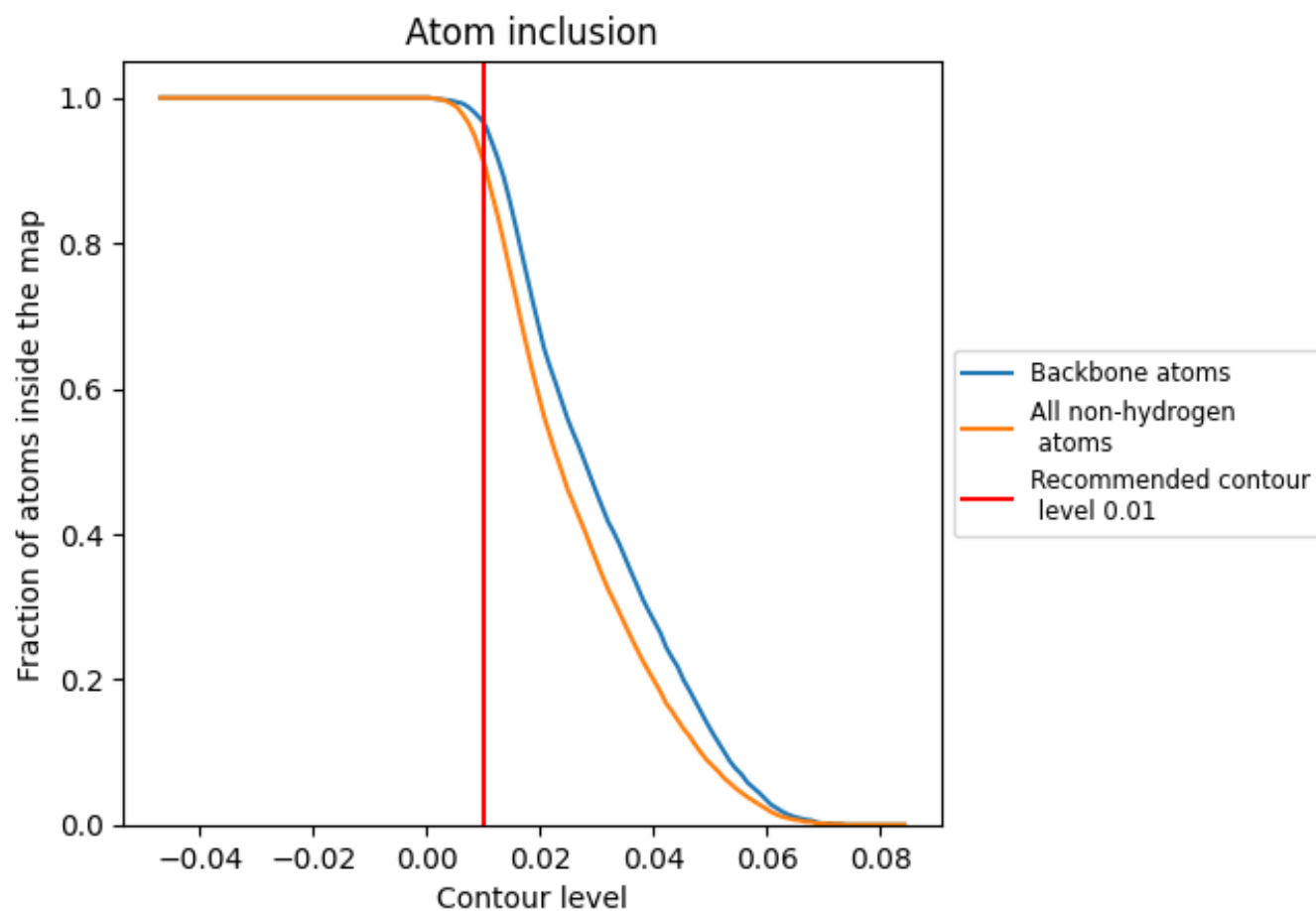
The images above show the model with each residue coloured according to its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions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

## 9.4 Atom inclusion [i](#)



At the recommended contour level, 97% of all backbone atoms, 91% of all non-hydrogen atoms, are inside the map.

9.5 Map-model fit summary ⓘ

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	<div><div></div></div> 0.9140	<div><div></div></div> 0.5070
A	<div><div></div></div> 0.8330	<div><div></div></div> 0.4100
B	<div><div></div></div> 0.9450	<div><div></div></div> 0.5330
C	<div><div></div></div> 0.9590	<div><div></div></div> 0.5690
D	<div><div></div></div> 0.8860	<div><div></div></div> 0.4960
E	<div><div></div></div> 0.9410	<div><div></div></div> 0.5410
F	<div><div></div></div> 0.8320	<div><div></div></div> 0.3910
G	<div><div></div></div> 0.8870	<div><div></div></div> 0.3490

1.0

0.0

<0.0