



## wwPDB EM Validation Summary Report ⓘ

Nov 11, 2021 – 04:09 pm GMT

PDB ID : 7PIV  
EMDB ID : EMD-13454  
Title : Active Melanocortin-4 receptor (MC4R)- Gs protein complex bound to agonist  
NDP-alpha-MSH at 2.86 Å resolution.  
Authors : Heyder, N.A.; Schmidt, A.; Kleinau, G.; Hilal, T.; Scheerer, P.  
Deposited on : 2021-08-23  
Resolution : 2.86 Å (reported)  
Based on initial models : 6W25, 3SN6

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.

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

EMDB validation analysis : 0.0.0.dev97  
Mogul : 1.8.4 (270009), CSD as541be (2020)  
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

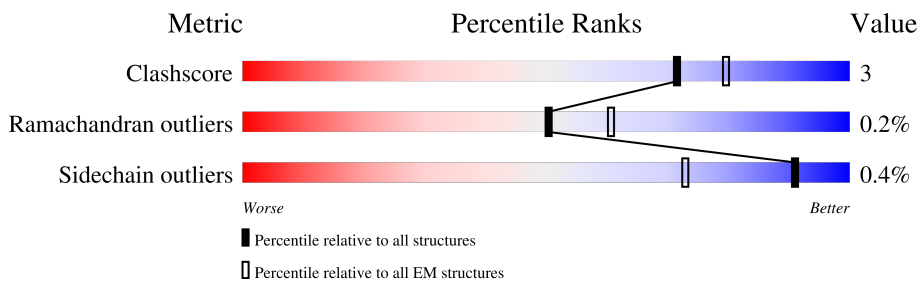
# 1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

*ELECTRON MICROSCOPY*

The reported resolution of this entry is 2.86 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  $\geq 3$ , 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	R	346	
2	P	15	
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 7814 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	260	1996	1328	310	335	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 NDP-alpha-MSH (other names Afamelanotide; Scenesse).

Mol	Chain	Residues	Atoms				AltConf	Trace
			Total	C	N	O		
2	P	13	114	76	20	18	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	183	1570	1005	296	263	6	5	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	338	2613	1622	474	494	23	9	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 VHH fragment - nanobody 35.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
6	N	128	982	612	172	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
			Total	O	
8	R	8	8	8	0
8	P	1	1	1	0

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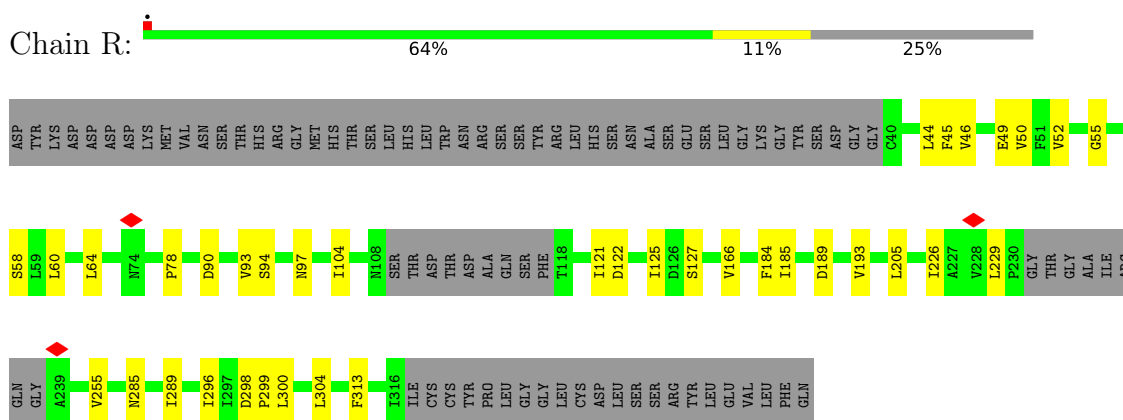
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Mol	Chain	Residues	Atoms	AltConf
8	A	23	Total O 24 24	1
8	B	66	Total O 67 67	1
8	G	5	Total O 5 5	0
8	N	12	Total O 12 12	0

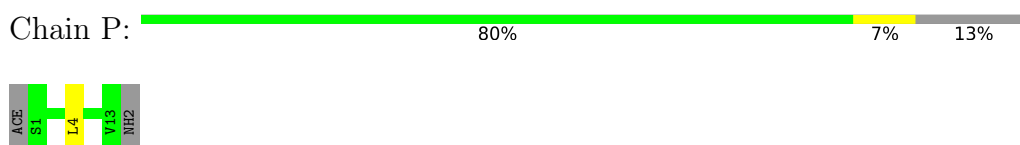
### 3 Residue-property plots [i](#)

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.

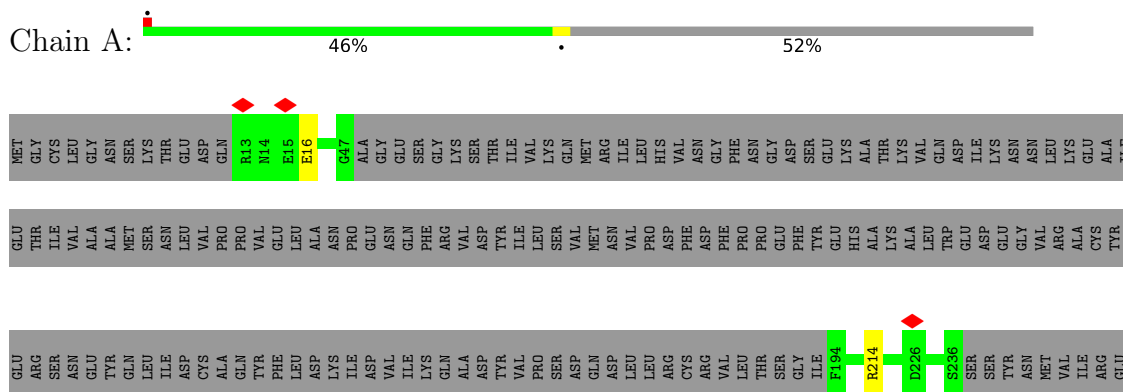
- Molecule 1: Melanocortin receptor 4

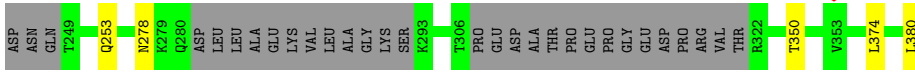


- Molecule 2: NDP-alpha-MSH (other names Afamelanotide; Scenesse)

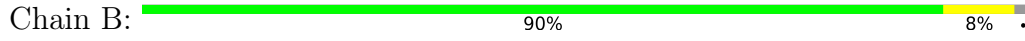


- Molecule 3: Isoform Gnas-2 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short

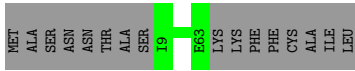
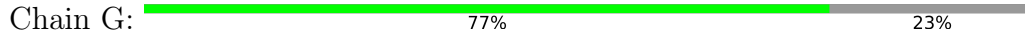




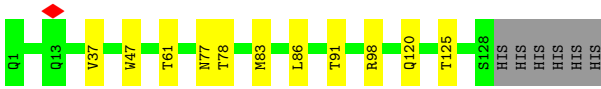
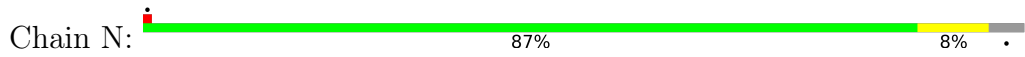
- Molecule 4: Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1



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



- Molecule 6: Camelid antibody VHH fragment - nanobody 35



## 4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	221682	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	0.938	Depositor
Minimum map value	-0.288	Depositor
Average map value	0.001	Depositor
Map value standard deviation	0.010	Depositor
Recommended contour level	0.05	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: DPN, CA, NLE

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	0.69	0/2046	0.71	0/2788
2	P	0.63	0/97	0.66	0/127
3	A	0.63	0/1614	0.68	0/2161
4	B	0.66	0/2687	0.72	0/3641
5	G	0.65	0/430	0.70	0/581
6	N	0.66	0/1008	0.72	0/1365
All	All	0.66	0/7882	0.70	0/10663

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	1996	0	2029	27	0
2	P	114	0	107	4	0
3	A	1570	0	1568	4	0
4	B	2613	0	2535	18	0
5	G	421	0	429	0	0
6	N	982	0	946	7	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	24	0	0	0	0
8	B	67	0	0	0	0
8	G	5	0	0	0	0
8	N	12	0	0	0	0
8	P	1	0	0	0	0
8	R	8	0	0	0	0
All	All	7814	0	7614	53	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.

The worst 5 of 53 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:104:ILE:HG22	2:P:4:NLE:HE3	1.51	0.93
1:R:104:ILE:CG2	2:P:4:NLE:HE3	2.13	0.79
1:R:58:SER:HB3	1:R:296:ILE:HG22	1.70	0.74
4:B:226:GLU:O	6:N:98:ARG:NH1	2.23	0.71
1:R:55:GLY:HA2	1:R:94:SER:OG	1.98	0.63

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	257/346 (74%)	245 (95%)	12 (5%)	0	100	100
2	P	9/15 (60%)	8 (89%)	1 (11%)	0	100	100
3	A	178/380 (47%)	166 (93%)	12 (7%)	0	100	100
4	B	345/345 (100%)	323 (94%)	20 (6%)	2 (1%)	25	53
5	G	54/71 (76%)	52 (96%)	2 (4%)	0	100	100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
6	N	128/134 (96%)	121 (94%)	7 (6%)	0	100	100
All	All	971/1291 (75%)	915 (94%)	54 (6%)	2 (0%)	50	75

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
4	B	128	THR
4	B	334	SER

### 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	218/309 (71%)	217 (100%)	1 (0%)	88	96
2	P	10/10 (100%)	10 (100%)	0	100	100
3	A	164/341 (48%)	163 (99%)	1 (1%)	86	95
4	B	277/285 (97%)	276 (100%)	1 (0%)	91	96
5	G	43/58 (74%)	43 (100%)	0	100	100
6	N	106/112 (95%)	106 (100%)	0	100	100
All	All	818/1115 (73%)	815 (100%)	3 (0%)	91	96

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

Mol	Chain	Res	Type
1	R	97	ASN
3	A	16	GLU
4	B	340	ASN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

### 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 [i](#)

There are no chain breaks in this entry.

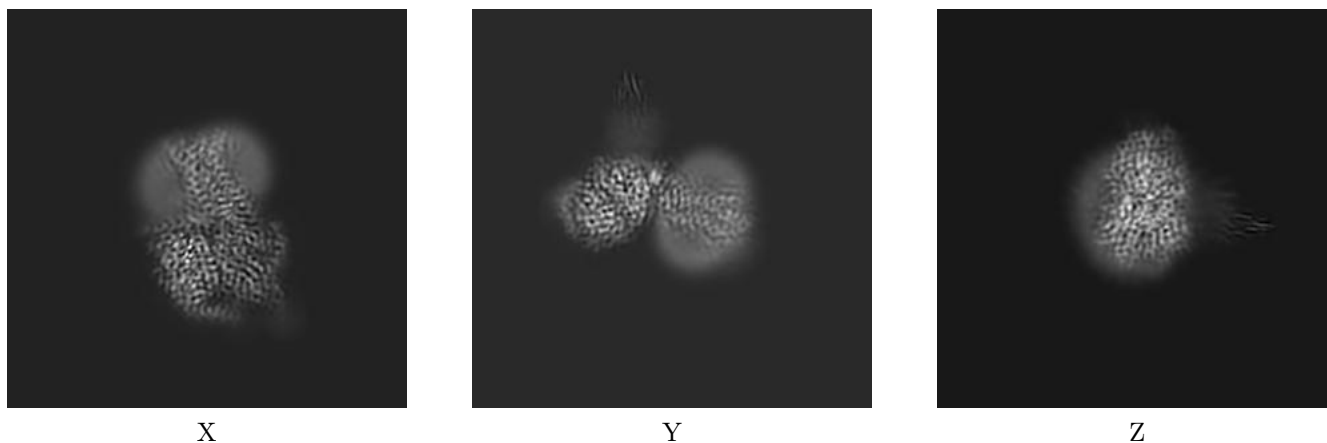
## 6 Map visualisation [i](#)

This section contains visualisations of the EMDB entry EMD-13454. 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](#)

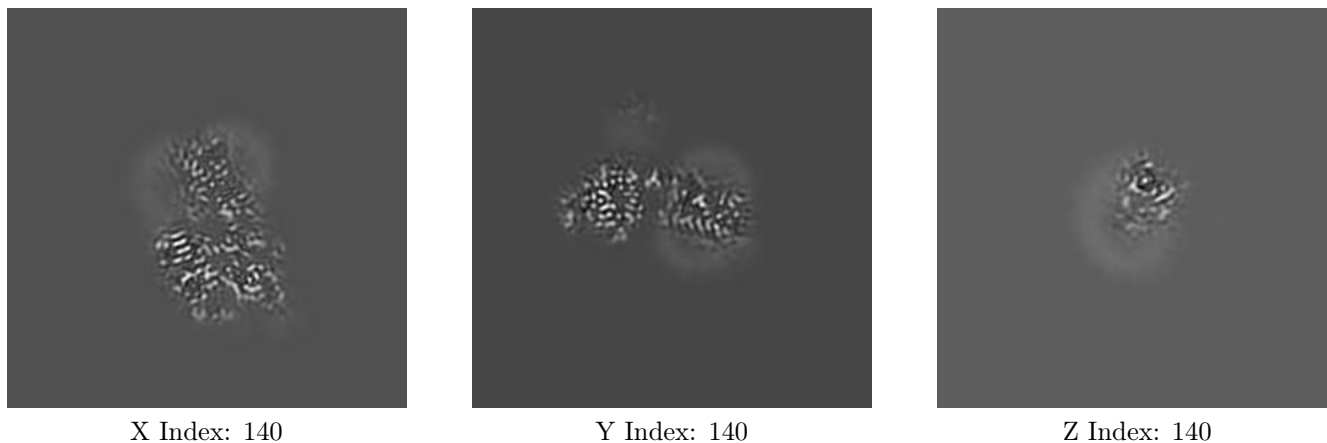
#### 6.1.1 Primary map



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

### 6.2 Central slices [i](#)

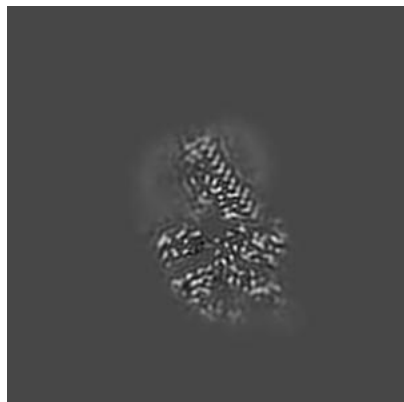
#### 6.2.1 Primary map



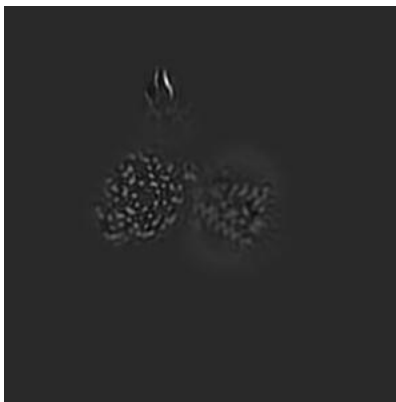
The images above show central slices of the map in three orthogonal directions.

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

### 6.3.1 Primary map



X Index: 146



Y Index: 128

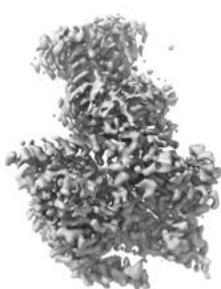


Z Index: 113

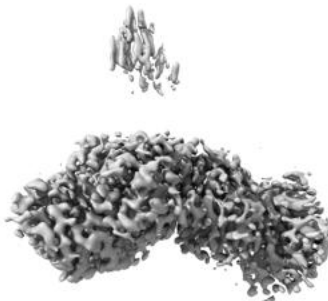
The images above show the largest variance slices of the map in three orthogonal directions.

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

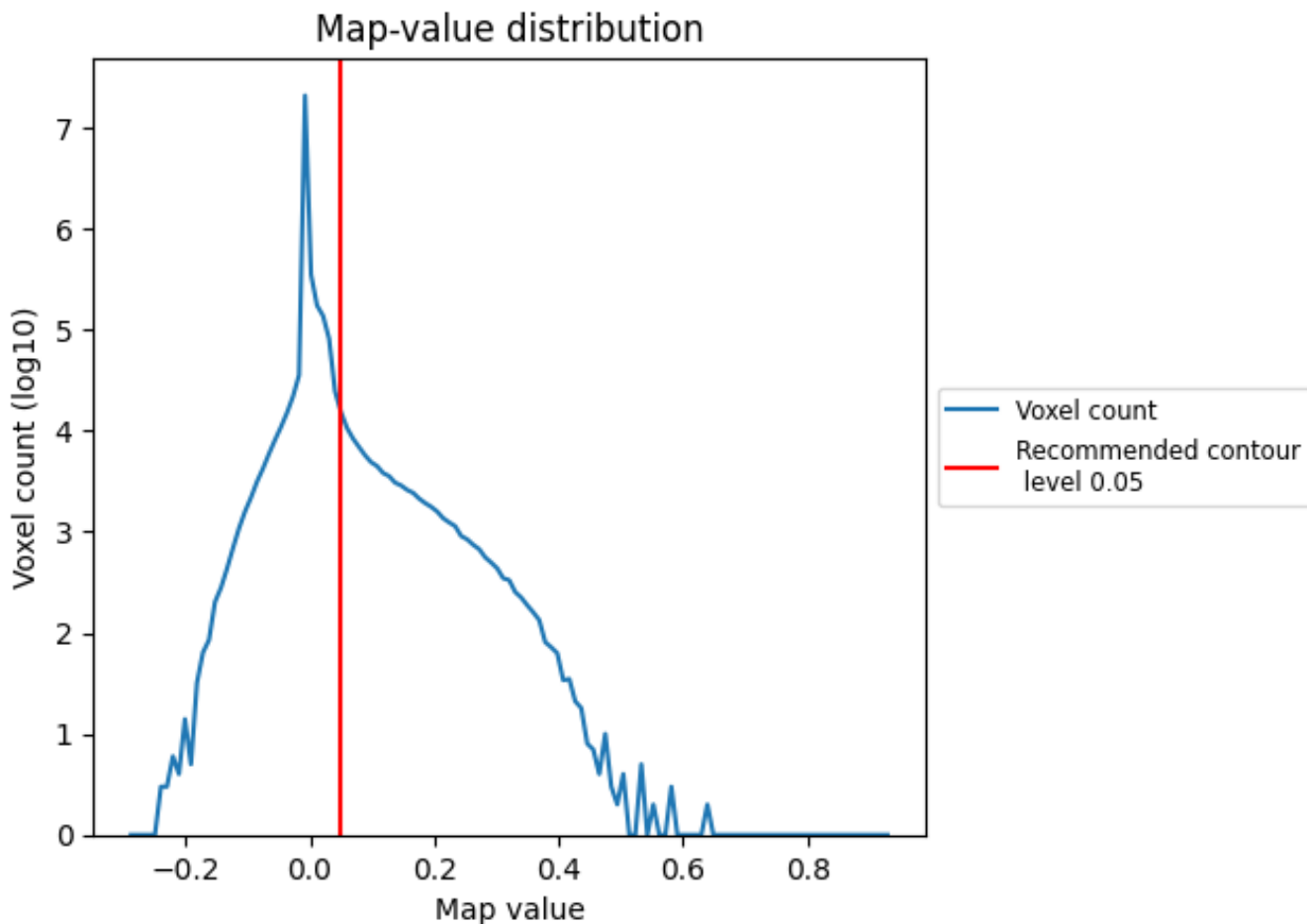
## 6.5 Mask visualisation

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.

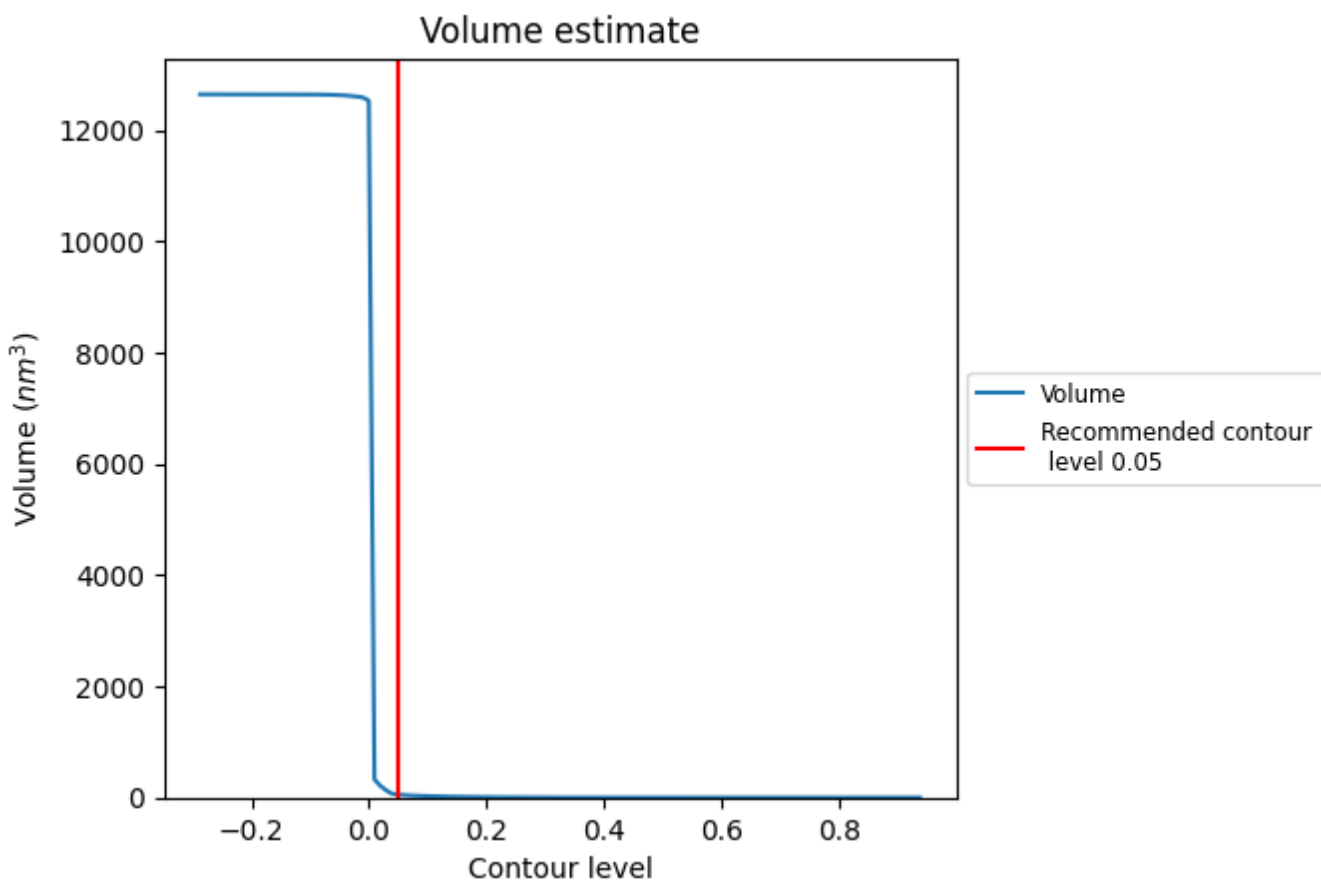
### 7.1 Map-value distribution [i](#)



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.



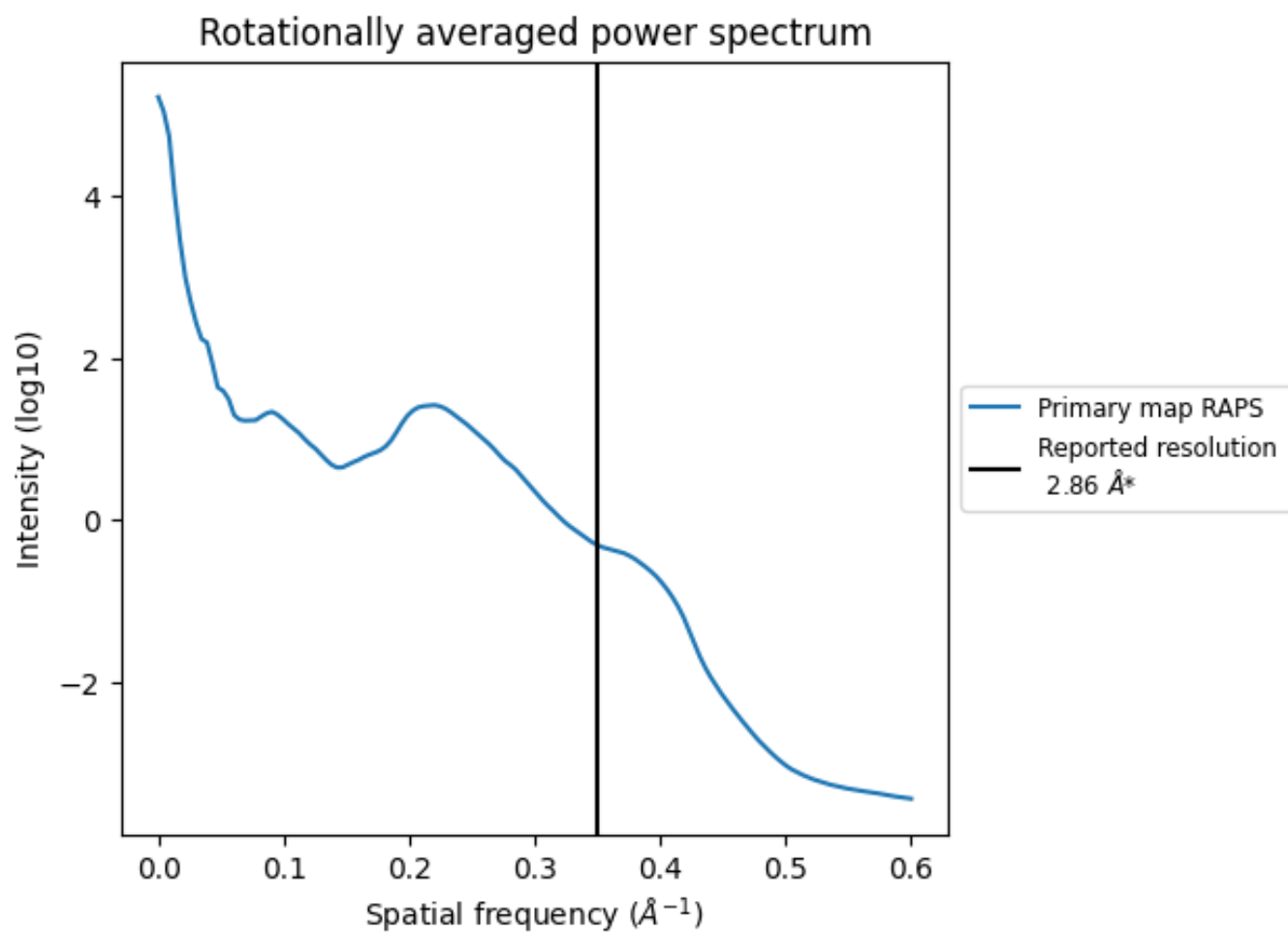
## 7.2 Volume estimate [i](#)



The volume at the recommended contour level is 54 nm<sup>3</sup>; this corresponds to an approximate mass of 49 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](#)

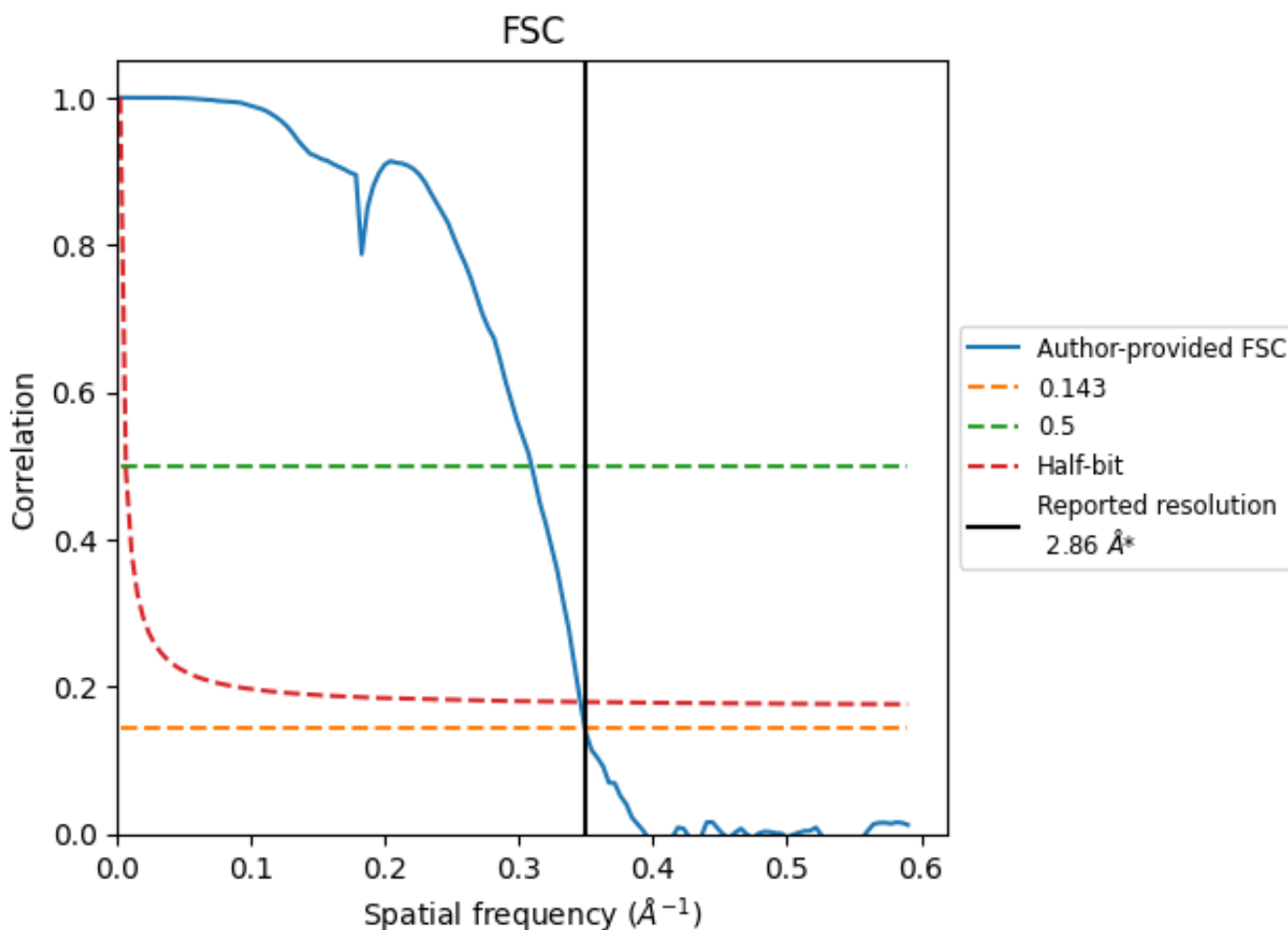


\*Reported resolution corresponds to spatial frequency of  $0.350 \text{ \AA}^{-1}$

## 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 0.350 Å<sup>-1</sup>

## 8.2 Resolution estimates [i](#)

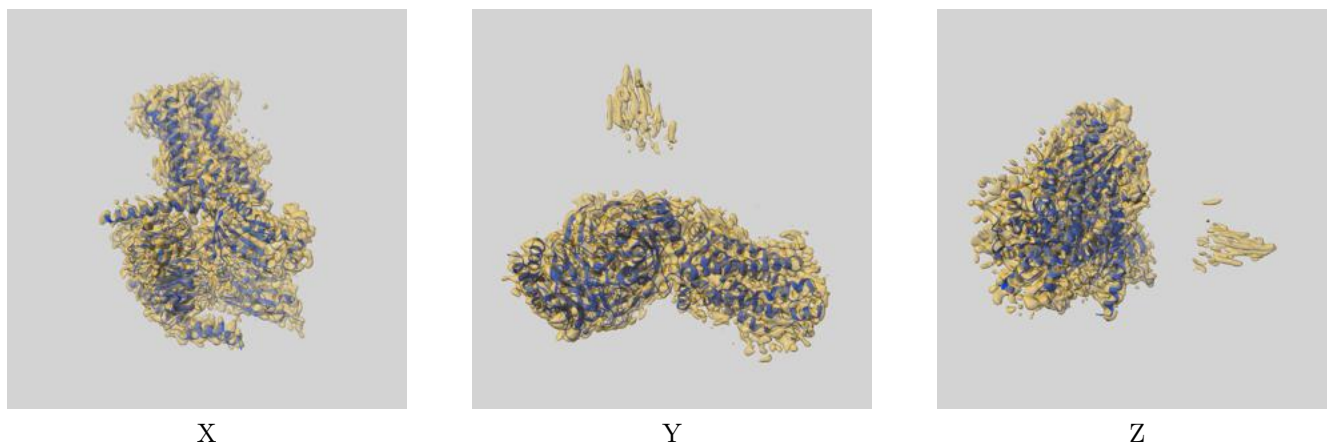
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	2.86	-	-
Author-provided FSC curve	2.86	3.24	2.89
Unmasked-calculated*	-	-	-

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

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

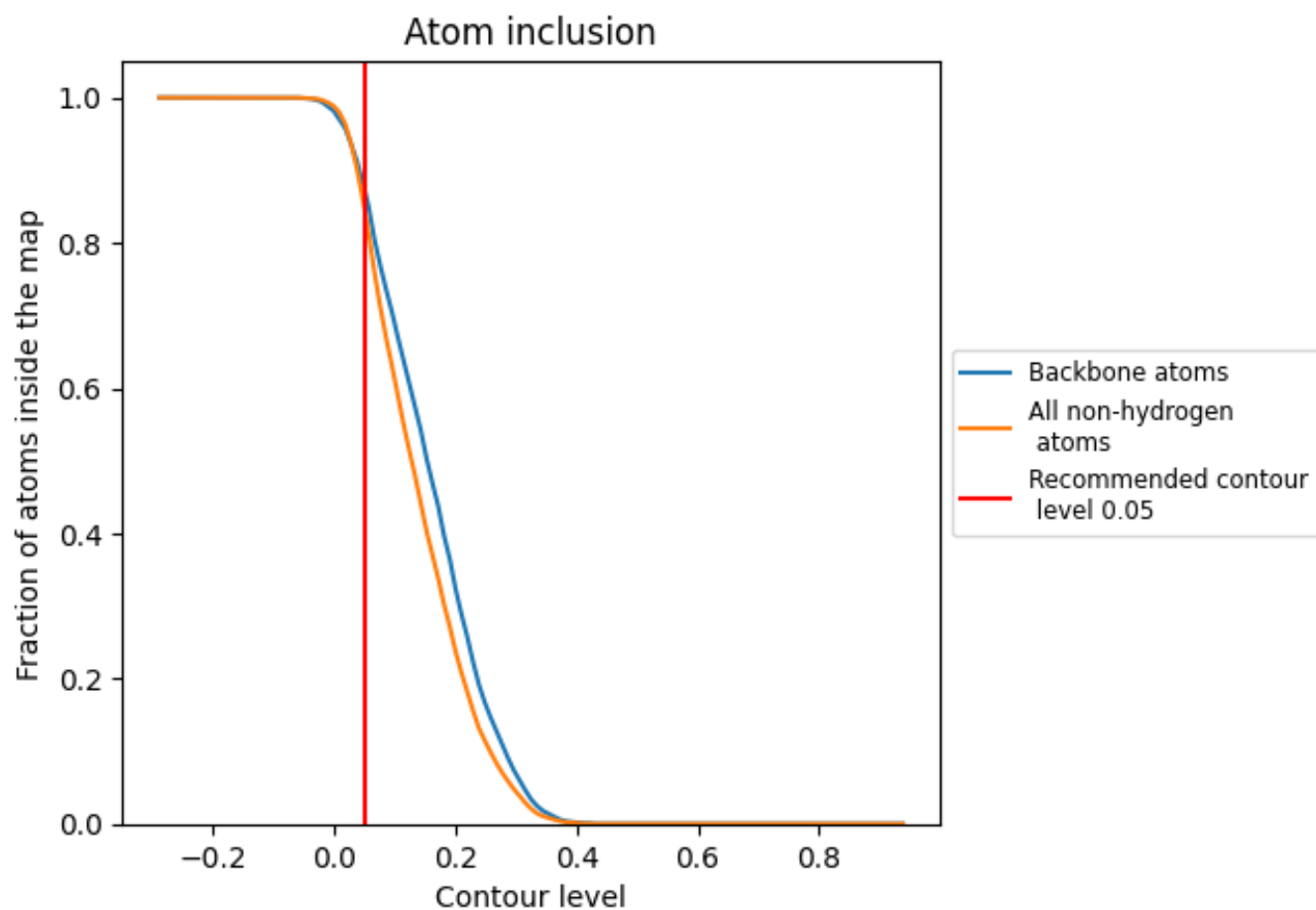
This section contains information regarding the fit between EMDB map EMD-13454 and PDB model 7PIV. Per-residue inclusion information can be found in section 3 on page 6.

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



The images above show the 3D surface view of the map at the recommended contour level 0.05 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, 87% of all backbone atoms, 85% of all non-hydrogen atoms, are inside the map.