



wwPDB EM Validation Summary Report ⓘ

Nov 22, 2022 – 10:48 PM JST

PDB ID : 7F4F
EMDB ID : EMD-31449
Title : Cryo-EM structure of afamelanotide-bound melanocortin-1 receptor in complex with Gs protein and scFv16
Authors : Ma, S.; Chen, Y.; Dai, A.; Yin, W.; Guo, J.; Yang, D.; Zhou, F.; Jiang, Y.; Wang, M.-W.; Xu, H.E.
Deposited on : 2021-06-18
Resolution : 2.90 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.dev43
Mogul : 1.8.5 (274361), CSD as541be (2020)
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) : 2.31.3

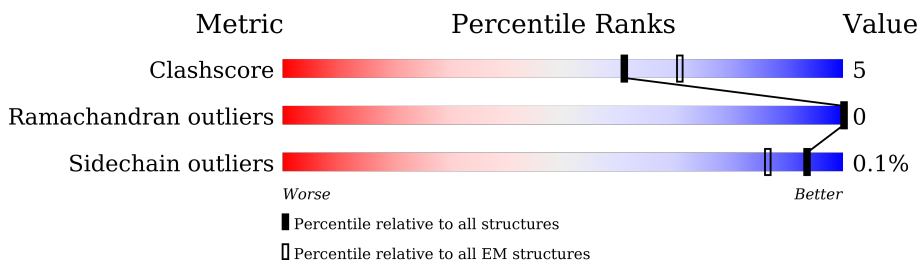
1 Overall quality at a glance i

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 2.90 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	S	285	
2	R	621	
3	A	361	
4	G	71	
5	B	371	
6	F	13	

2 Entry composition [i](#)

There are 7 unique types of molecules in this entry. The entry contains 8881 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 scFv16.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	S	230	1771	1125	293	343	10	0	0

- Molecule 2 is a protein called Melanocortin-1 receptor.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
2	R	269	2079	1376	340	346	17	0	0

- Molecule 3 is a protein called Guanine nucleotide-binding protein G(i) subunit alpha-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
3	A	232	1916	1209	344	355	8	0	0

- 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
			Total	C	N	O	S		
4	G	55	423	266	75	79	3	0	0

- Molecule 5 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		
5	B	341	2583	1598	466	498	21	0	0

There are 32 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	-4	MET	-	initiating methionine	UNP P62873

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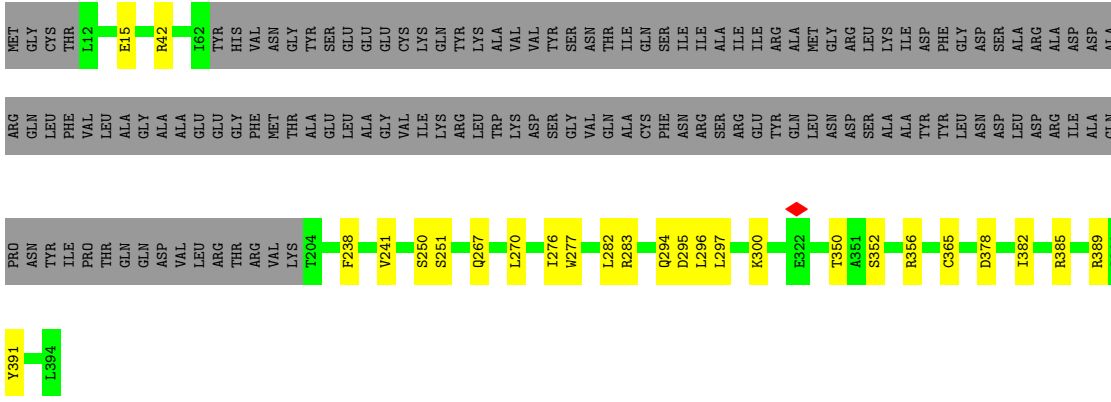
Chain	Residue	Modelled	Actual	Comment	Reference
B	-3	GLY	-	expression tag	UNP P62873
B	-2	SER	-	expression tag	UNP P62873
B	-1	LEU	-	expression tag	UNP P62873
B	0	LEU	-	expression tag	UNP P62873
B	1	GLN	-	expression tag	UNP P62873
B	341	GLY	-	expression tag	UNP P62873
B	342	SER	-	expression tag	UNP P62873
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
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 6 is a protein called Afamelanotide.

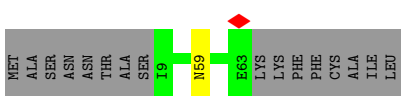
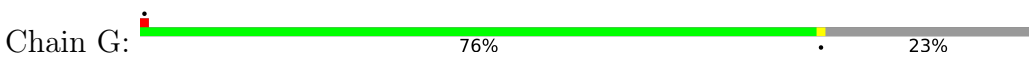
Mol	Chain	Residues	Atoms				AltConf	Trace
			Total	C	N	O		
6	F	12	108	73	19	16	0	0

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

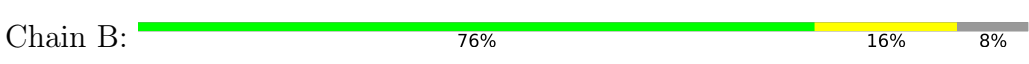
Mol	Chain	Residues	Atoms		AltConf
7	R	1	Total 1	Ca 1	0



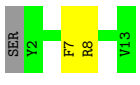
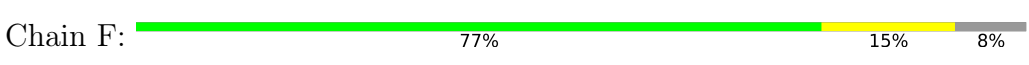
• Molecule 4: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2



• Molecule 5: Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1



• Molecule 6: Afamelanotide



4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	312962	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 ($e^-/\text{\AA}^2$)	80	Depositor
Minimum defocus (nm)	Not provided	
Maximum defocus (nm)	Not provided	
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	0.111	Depositor
Minimum map value	-0.047	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.004	Depositor
Recommended contour level	0.01	Depositor
Map size (\AA)	208.99998, 208.99998, 208.99998	wwPDB
Map dimensions	200, 200, 200	wwPDB
Map angles ($^\circ$)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (\AA)	1.045, 1.045, 1.045	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, NLE, CA

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	S	0.50	0/1815	0.69	0/2461
2	R	0.47	0/2120	0.66	2/2889 (0.1%)
3	A	0.62	0/1953	0.78	4/2630 (0.2%)
4	G	0.51	0/429	0.66	0/579
5	B	0.46	0/2630	0.72	0/3568
6	F	0.62	0/91	0.71	0/119
All	All	0.51	0/9038	0.71	6/12246 (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
1	S	0	2
5	B	0	1
All	All	0	3

There are no bond length outliers.

The worst 5 of 6 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
2	R	109	ARG	NE-CZ-NH1	7.94	124.27	120.30
3	A	283	ARG	NE-CZ-NH1	7.28	123.94	120.30
3	A	356	ARG	NE-CZ-NH1	6.39	123.49	120.30
3	A	389	ARG	NE-CZ-NH1	6.19	123.40	120.30
3	A	385	ARG	NE-CZ-NH1	5.32	122.96	120.30

There are no chirality outliers.

All (3) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
5	B	340	ASN	Mainchain
1	S	191	ARG	Sidechain
1	S	236	PRO	Peptide

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	S	1771	0	1709	13	0
2	R	2079	0	2194	19	0
3	A	1916	0	1889	14	0
4	G	423	0	433	2	0
5	B	2583	0	2479	39	0
6	F	108	0	99	5	0
7	R	1	0	0	0	0
All	All	8881	0	8803	82	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 5.

The worst 5 of 82 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)
3:A:295:ASP:OD1	3:A:296:LEU:N	2.23	0.72
2:R:118:ASN:OD1	6:F:8:ARG:NH2	2.27	0.68
2:R:125:CYS:HB2	6:F:7:DPN:HE1	1.76	0.66
3:A:378:ASP:O	3:A:382:ILE:HG13	1.96	0.66
2:R:133:CYS:HG	2:R:195:PHE:HZ	1.45	0.65

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	S	226/285 (79%)	219 (97%)	7 (3%)	0	100	100
2	R	265/621 (43%)	256 (97%)	9 (3%)	0	100	100
3	A	228/361 (63%)	219 (96%)	9 (4%)	0	100	100
4	G	53/71 (75%)	53 (100%)	0	0	100	100
5	B	339/371 (91%)	321 (95%)	18 (5%)	0	100	100
6	F	8/13 (62%)	8 (100%)	0	0	100	100
All	All	1119/1722 (65%)	1076 (96%)	43 (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	S	195/228 (86%)	195 (100%)	0	100	100
2	R	229/518 (44%)	229 (100%)	0	100	100
3	A	208/315 (66%)	207 (100%)	1 (0%)	88	96
4	G	44/58 (76%)	44 (100%)	0	100	100
5	B	273/302 (90%)	273 (100%)	0	100	100
6	F	9/10 (90%)	9 (100%)	0	100	100
All	All	958/1431 (67%)	957 (100%)	1 (0%)	93	98

All (1) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
3	A	42	ARG

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

sidechains are listed below:

Mol	Chain	Res	Type
3	A	390	GLN
5	B	237	ASN
5	B	340	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](#)

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

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. 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 $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	# Z > 2	Counts	RMSZ	# Z > 2
6	NLE	F	4	6	6,7,8	0.42	0	2,7,9	0.41	0

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
6	NLE	F	4	6	-	2/5/6/8	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (2) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
6	F	4	NLE	CA-CB-CG-CD

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Mol	Chain	Res	Type	Atoms
6	F	4	NLE	CE-CD-CG-CB

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.

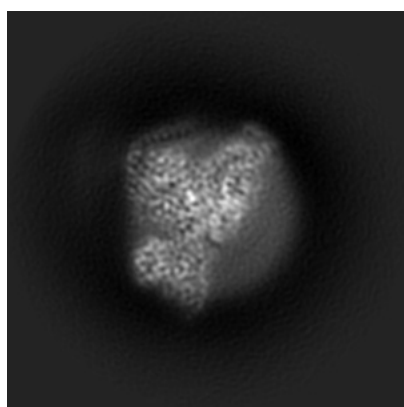
6 Map visualisation [i](#)

This section contains visualisations of the EMDB entry EMD-31449. These allow visual inspection of the internal detail of the map and identification of artifacts.

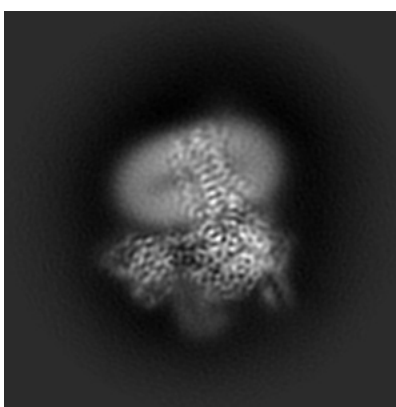
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections [i](#)

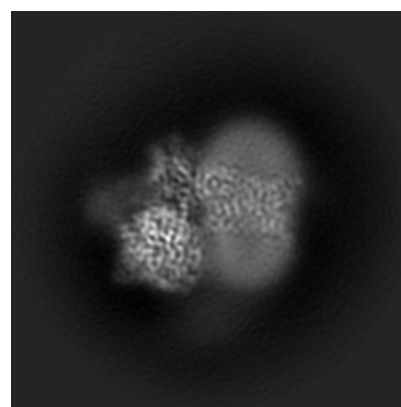
6.1.1 Primary map



X



Y

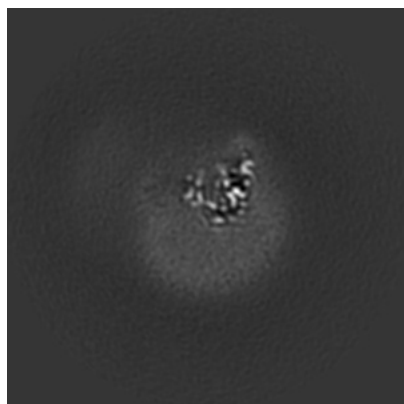


Z

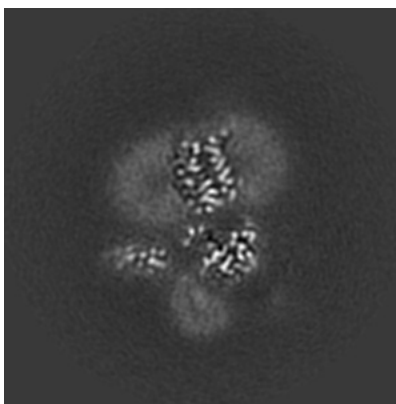
The images above show the map projected in three orthogonal directions.

6.2 Central slices [i](#)

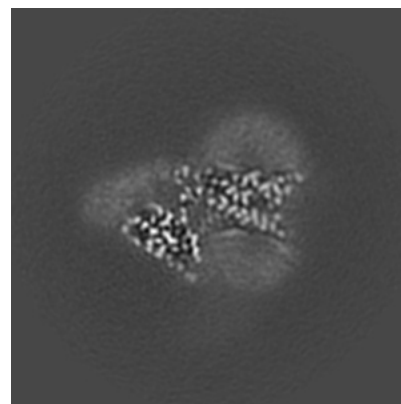
6.2.1 Primary map



X Index: 100



Y Index: 100

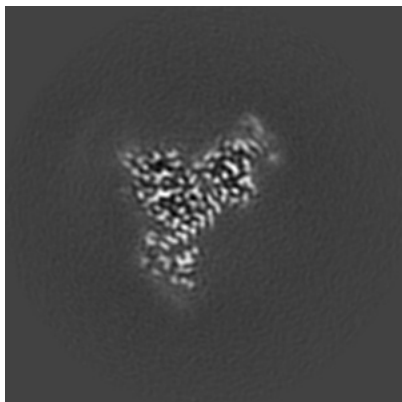


Z Index: 100

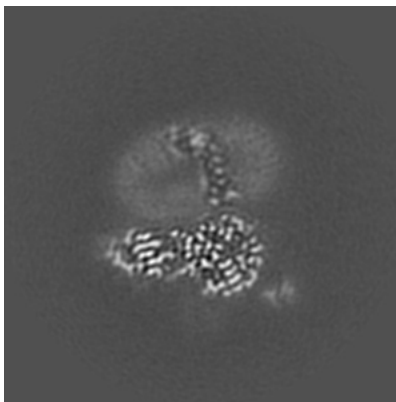
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices [i](#)

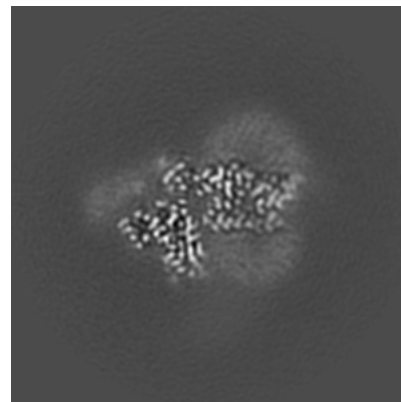
6.3.1 Primary map



X Index: 85



Y Index: 88

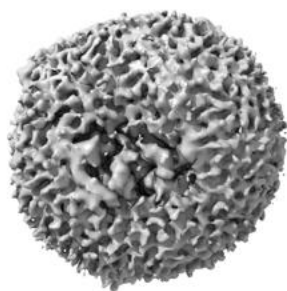


Z Index: 104

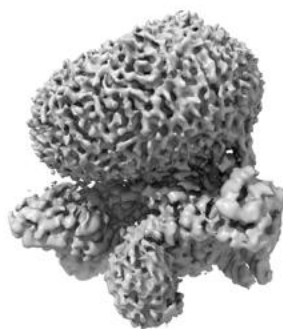
The images above show the largest variance slices of the map in three orthogonal directions.

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

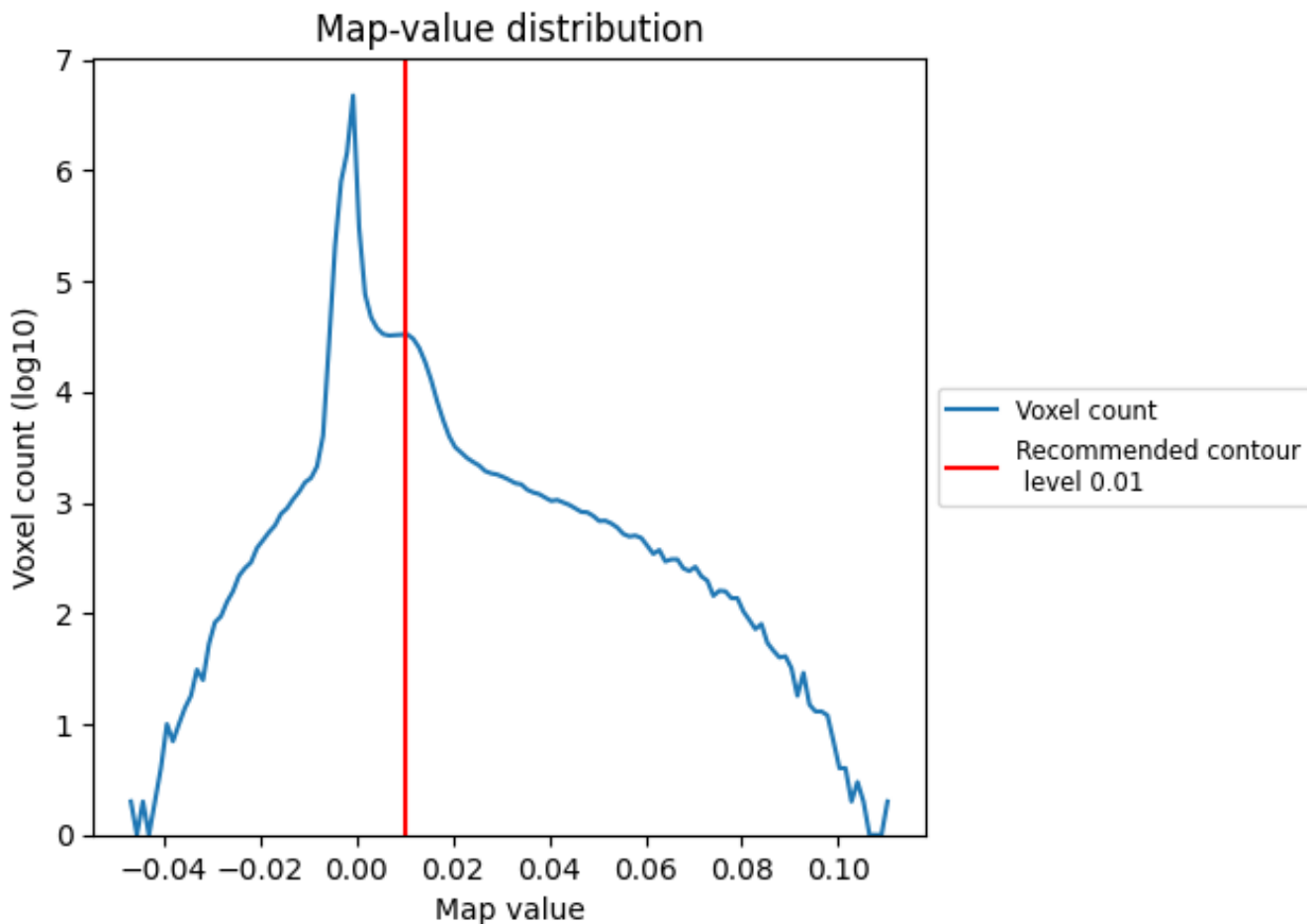
6.5 Mask visualisation

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

7 Map analysis [i](#)

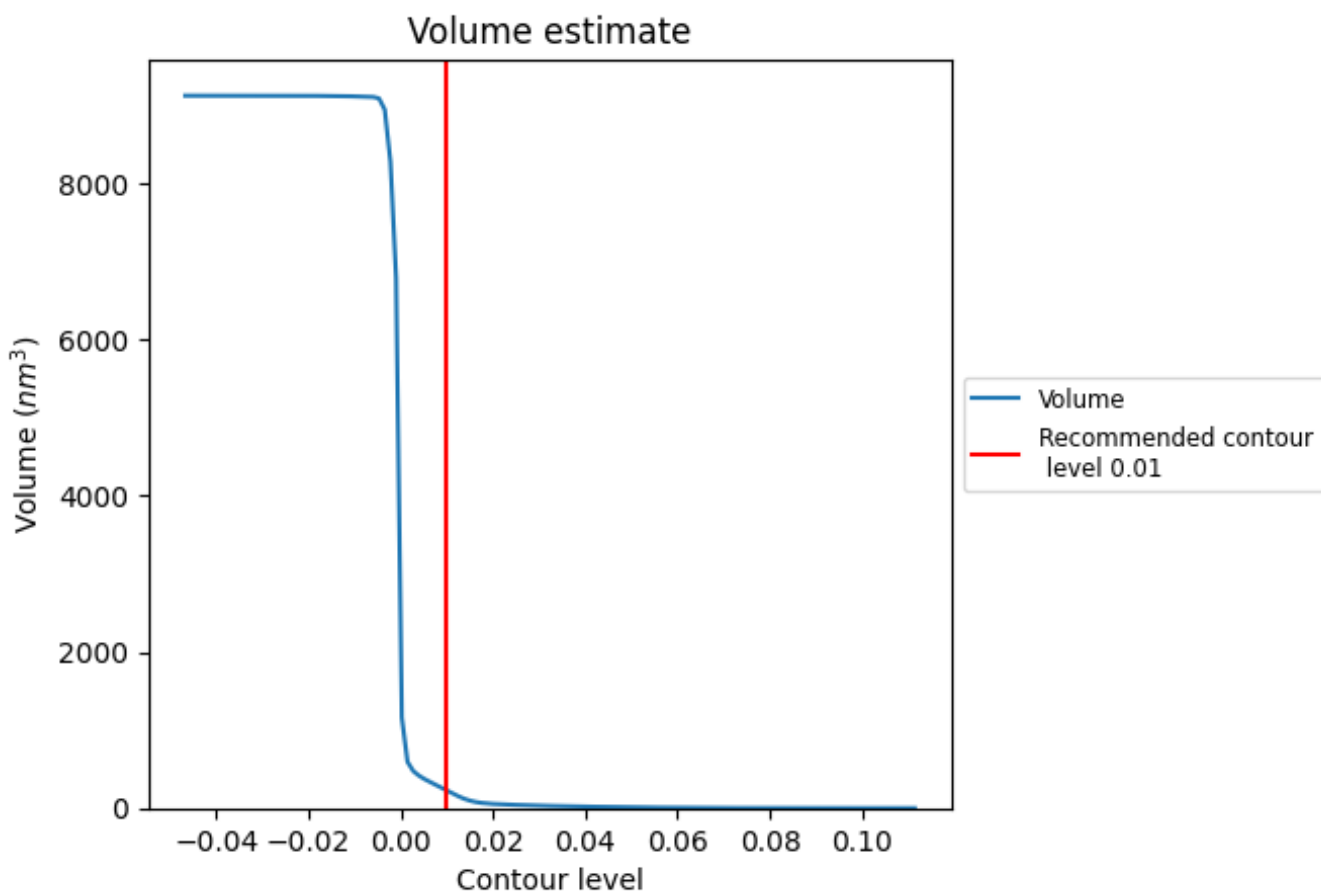
This section contains the results of statistical analysis of the map.

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.

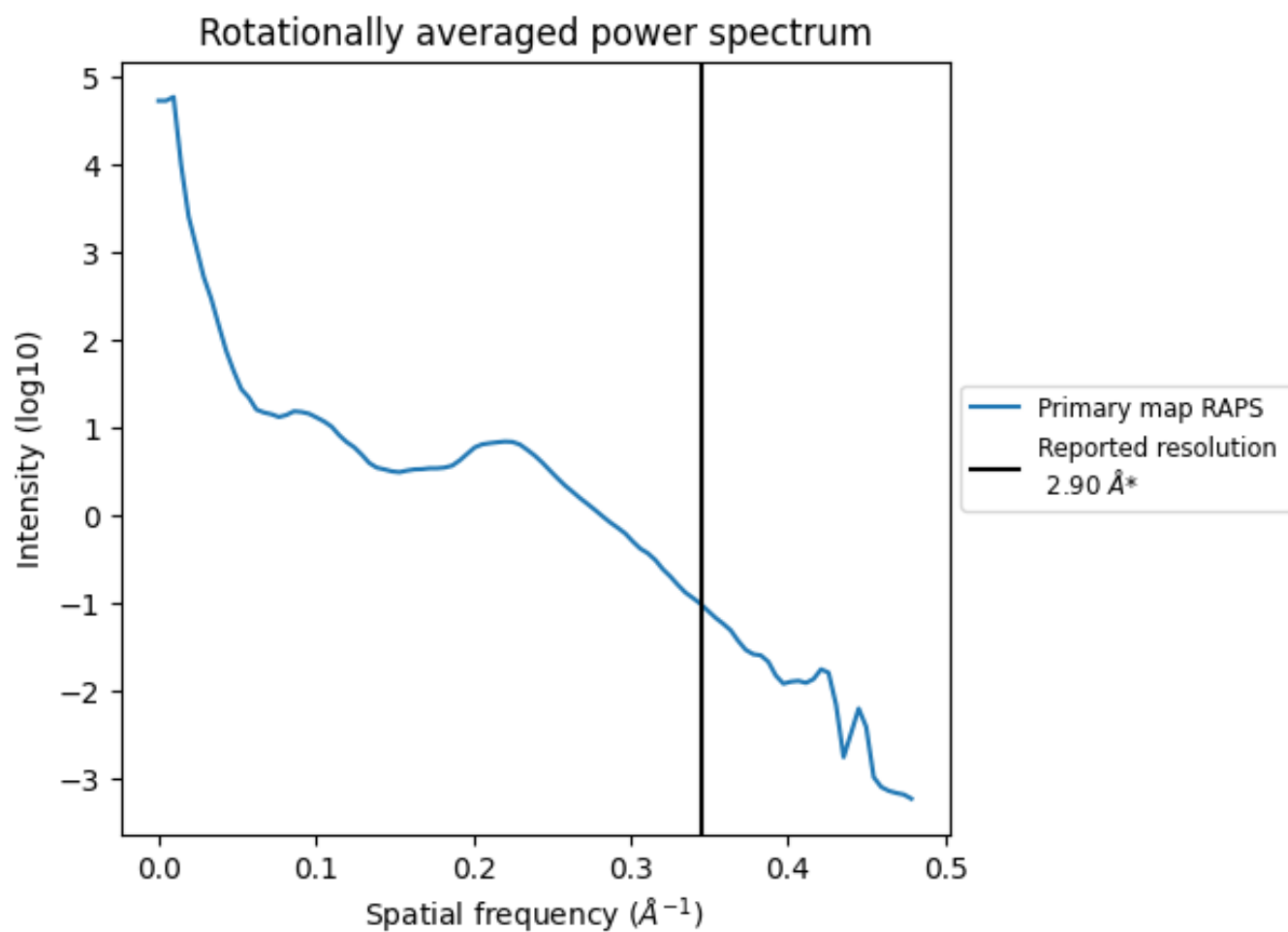
7.2 Volume estimate [i](#)



The volume at the recommended contour level is 227 nm^3 ; this corresponds to an approximate mass of 205 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 0.345 Å⁻¹

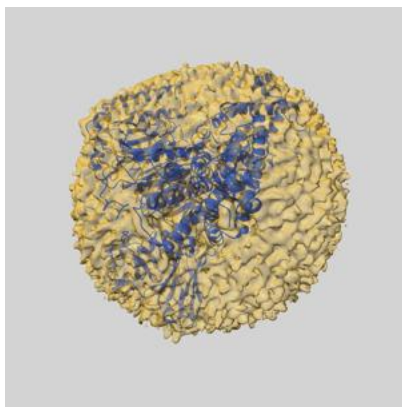
8 Fourier-Shell correlation

This section was not generated. No FSC curve or half-maps provided.

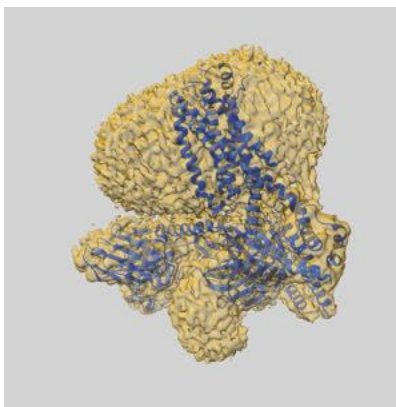
9 Map-model fit [i](#)

This section contains information regarding the fit between EMDB map EMD-31449 and PDB model 7F4F. Per-residue inclusion information can be found in section 3 on page 6.

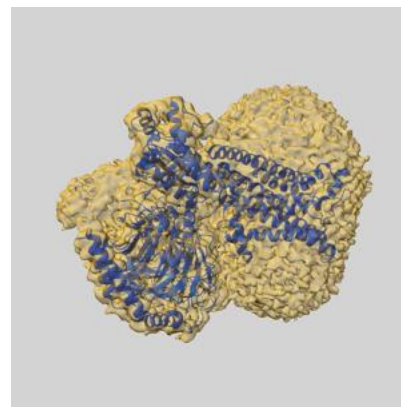
9.1 Map-model overlay [i](#)



X



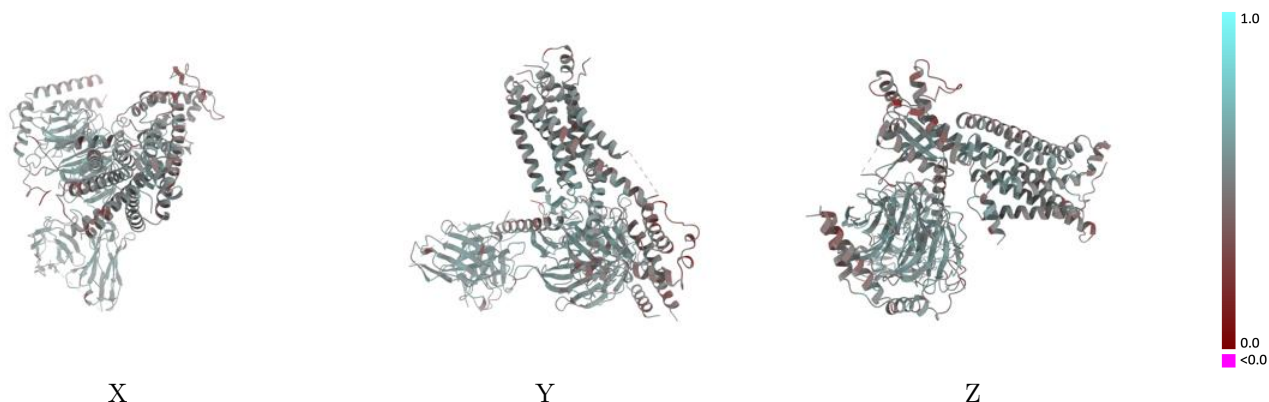
Y



Z

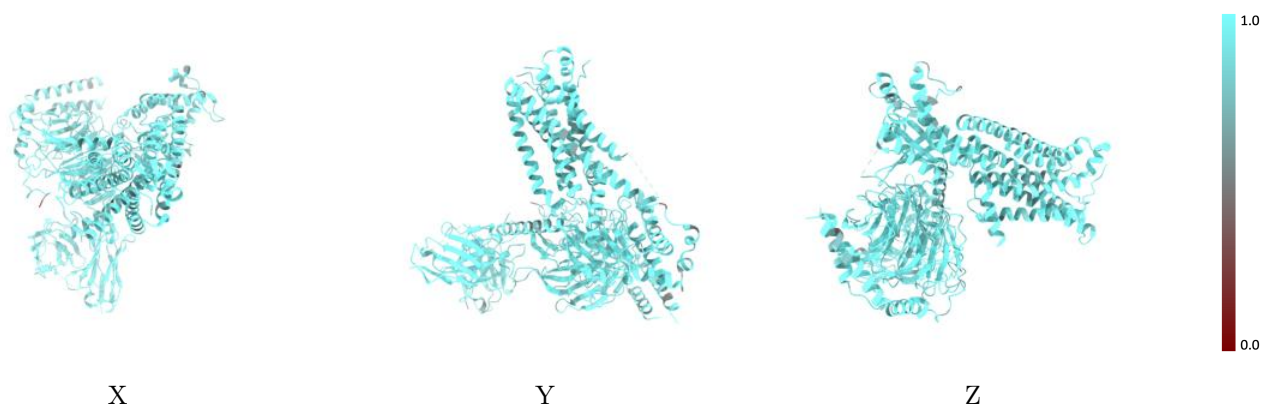
The images above show the 3D surface view of the map at the recommended contour level 0.01 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

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



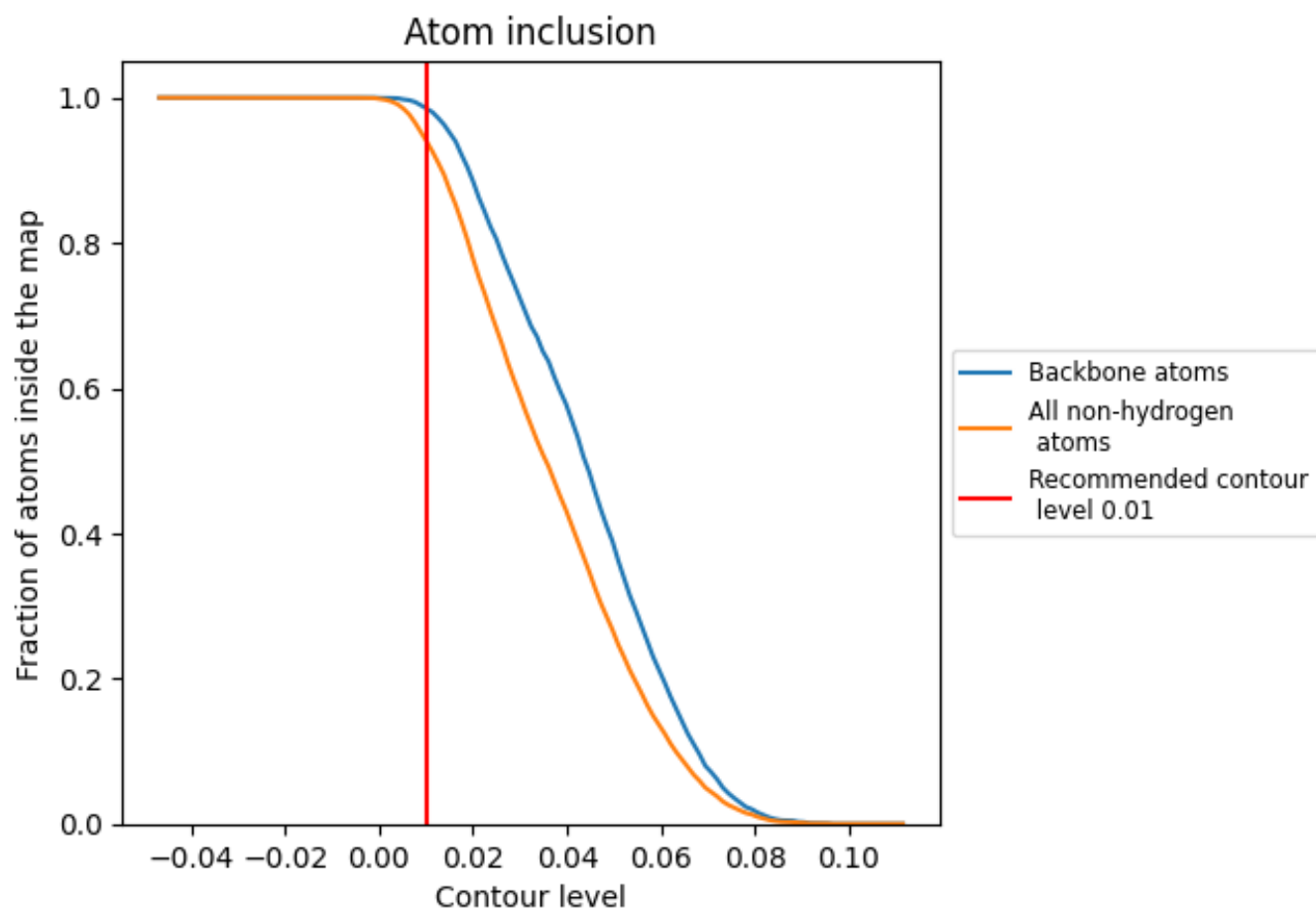
The images above show the model with each residue coloured according to its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions 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, 99% of all backbone atoms, 94% 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	 0.9413	 0.5090
A	 0.9206	 0.4720
B	 0.9592	 0.5400
F	 0.9135	 0.5350
G	 0.8846	 0.4530
R	 0.9390	 0.4950
S	 0.9555	 0.5300

