wwPDB EM Validation Summary Report (i)

Nov 22, 2022 – 10:55 PM JST

PDB ID	:	7F4H
EMDB ID	:	EMD-31452
Title	:	Cryo-EM structure of afamelanotide-bound melanocortin-1 receptor in complex with Gs protein, Nb35 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	:	2.70 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:

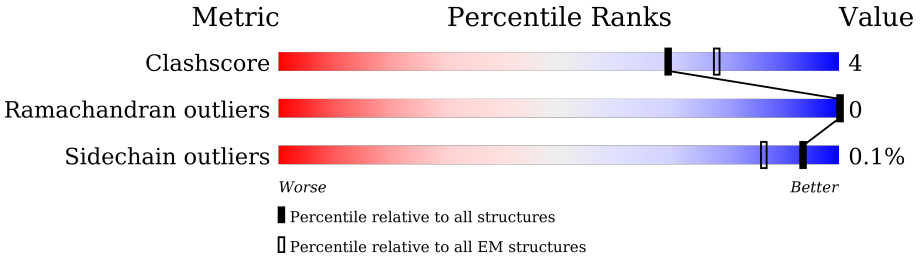
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (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 2.70 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	G	71	75% • 23%
2	A	361	57% 7% 35%
3	N	160	68% 12% 21%
4	B	371	81% 11% • 8%
5	R	621	40% • 57%
6	S	285	73% 8% 19%
7	F	13	69% 23% 8%

2 Entry composition (i)

There are 8 unique types of molecules in this entry. The entry contains 9849 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	G	55	427	268	75	81	3	0	0

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

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
2	A	234	1942	1227	349	358	8	0	0

- Molecule 3 is a protein called Nanobody 35.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
3	N	127	963	600	169	188	6	0	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	341	2590	1601	468	500	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
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

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Chain	Residue	Modelled	Actual	Comment	Reference
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 5 is a protein called Melanocortin-1 receptor.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
5	R	267	2054	1361	335	341	17	0	0

- Molecule 6 is a protein called scFv16.

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

- Molecule 7 is a protein called Afamelanotide.

Mol	Chain	Residues	Atoms				AltConf	Trace
			Total	C	N	O		
7	F	12	101	67	19	15	0	0

- Molecule 8 is CALCIUM ION (three-letter code: CA) (formula: Ca) (labeled as "Ligand of Interest" by depositor).

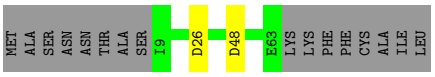
Mol	Chain	Residues	Atoms		AltConf
			Total	Ca	
8	R	1	1	1	0

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.

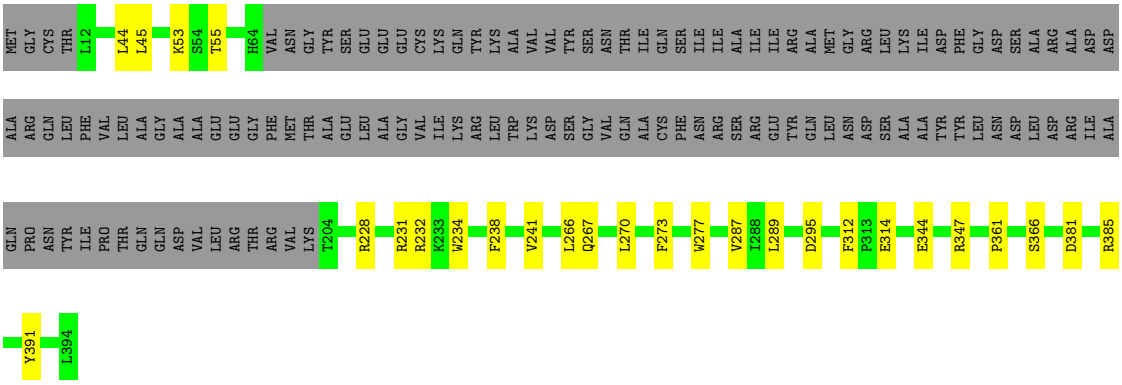
• Molecule 1: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2

Chain G: 75% • 23%



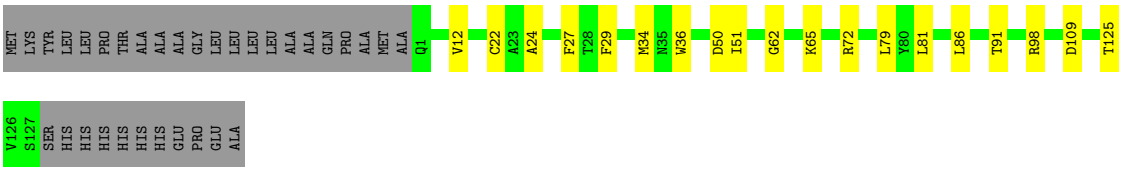
• Molecule 2: Guanine nucleotide-binding protein G(i) subunit alpha-1

Chain A: 57% 7% 35%



• Molecule 3: Nanobody 35

Chain N: 68% 12% 21%



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

Chain B: 81% 11% • 8%

MET GLY SER LEU LEU GLN SER E3 C25 L30 T34 R42 I43 Q44 M45 R48 K57 L69 L79 W82 V90 H91 A92 I93 P94 L95 D186 L198 L210 W211 C218 I232 T243 C250 Q259 M262 T263 Y264 T274 S275

V276 S281 L285 L286 A287 G288 C294 L300 L308 A309 G310 V327 A328 T329 W332 K337 I338 W339 N340 S343 GLY GLY GLY SER GLY GLY GLY GLY SER SER GLY VAL SER GLY TRP ARG LEU PHE LYS LYS ILE SER

- Molecule 5: Melanocortin-1 receptor

Chain R: 40% • 57%

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

ALA ALA ALA LEU ASP ALA GLN LYS ALA THR PRO PRO LYS LEU GLU ASP LYS SER PRO ASP SER PRO GLU MET LYS ASP PHE ARG HIS GLY PHE ASP ILE LEU VAL GLY GLN ILE ASP ASP ALA LEU LYS LEU ALA ASN GLU GLY LYS VAL LYS GLU ALA GLN ALA ALA ALA GLU GLN LEU

LYS THR THR ARG ASN ALA TYR ILE GLN LYS TYR LEU ALA VAL GLN GLY SER GLN ARG ARG LEU LEU GLY SER LEU ASN SER THR PRO THR ALA ILE PRO GLN LEU GLY LEU ALA ALA ASN GLN THR GLY ALA ARG CYS L36 E37 V38 F45 I63 C78 N91 T95

D117 R142 I180 R213 Q216 K226 ARG GLN ARG PRO VAL HIS GLN GLY PHE GLY L237 C253 L263 C267 L282 N290 V312 LEU THR CYS SER TRP GLY SER SER GLY GLY GLY GLY GLY SER GLY GLY GLY SER SER GLY VAL PHE THR LEU GLU ASP

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

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

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

- Molecule 6: scFv16

Chain S: 73% 8% 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 HIS SER ALA PHE ALA V2 A24 F27 A28 F29 A40 K43 R67 L86 D90 T91 Y102 L117

T118 V119 S120 ALA GLY GLY GLY GLY SER GLY GLY GLY GLY SER GLY GLY GLY GLY SER ALA D137 M140 T144 V147 L174 Y175 W176 L188 V199 R202 E222 T238 L247

- Molecule 7: Afamelanotide

Chain F: 69% 23% 8%

SER Y2 S3 L4 E5 H6 F7 V13

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	460989	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 ($e^-/Å^2$)	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	0.112	Depositor
Minimum map value	-0.050	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.004	Depositor
Recommended contour level	0.01	Depositor
Map size (Å)	208.99998, 208.99998, 208.99998	wwPDB
Map dimensions	200, 200, 200	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	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: CA, DPN, 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	G	0.46	0/433	0.70	0/584
2	A	0.52	0/1981	0.69	0/2667
3	N	0.50	0/983	0.70	0/1332
4	B	0.50	0/2637	0.71	0/3577
5	R	0.50	0/2095	0.64	0/2856
6	S	0.54	0/1815	0.67	0/2461
7	F	0.48	0/83	0.66	0/108
All	All	0.51	0/10027	0.68	0/13585

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
3	N	0	1
4	B	0	1
All	All	0	2

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
4	B	340	ASN	Mainchain
3	N	72	ARG	Sidechain

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	G	427	0	437	2	0
2	A	1942	0	1916	17	0
3	N	963	0	929	11	0
4	B	2590	0	2489	25	0
5	R	2054	0	2161	12	0
6	S	1771	0	1707	12	0
7	F	101	0	92	4	0
8	R	1	0	0	0	0
All	All	9849	0	9731	73	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 4.

The worst 5 of 73 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)
4:B:25:CYS:SG	4:B:259:GLN:NE2	2.62	0.73
4:B:329:THR:HG1	4:B:339:TRP:HE1	1.35	0.72
2:A:314:GLU:OE2	3:N:65:LYS:NZ	2.26	0.66
2:A:231:ARG:HG3	2:A:234:TRP:CZ2	2.32	0.65
4:B:250:CYS:HB3	4:B:264:TYR:HB2	1.79	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	G	53/71 (75%)	52 (98%)	1 (2%)	0	100	100
2	A	230/361 (64%)	223 (97%)	7 (3%)	0	100	100
3	N	125/160 (78%)	117 (94%)	8 (6%)	0	100	100
4	B	339/371 (91%)	319 (94%)	20 (6%)	0	100	100
5	R	263/621 (42%)	257 (98%)	6 (2%)	0	100	100
6	S	226/285 (79%)	217 (96%)	9 (4%)	0	100	100
7	F	8/13 (62%)	8 (100%)	0	0	100	100
All	All	1244/1882 (66%)	1193 (96%)	51 (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	G	45/58 (78%)	45 (100%)	0	100	100
2	A	211/315 (67%)	211 (100%)	0	100	100
3	N	104/129 (81%)	104 (100%)	0	100	100
4	B	275/302 (91%)	274 (100%)	1 (0%)	91	97
5	R	225/518 (43%)	225 (100%)	0	100	100
6	S	195/228 (86%)	195 (100%)	0	100	100
7	F	8/10 (80%)	8 (100%)	0	100	100
All	All	1063/1560 (68%)	1062 (100%)	1 (0%)	93	98

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

Mol	Chain	Res	Type
4	B	48	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
4	B	259	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)

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	$\#\|Z\| > 2$
7	NLE	F	4	7	6,7,8	0.53	0	2,7,9	0.53	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
7	NLE	F	4	7	-	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
7	F	4	NLE	CA-CB-CG-CD
7	F	4	NLE	N-CA-CB-CG

There are no ring outliers.

1 monomer is involved in 1 short contact:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
7	F	4	NLE	1	0

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.

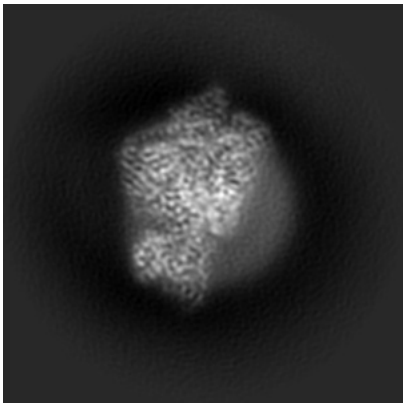
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-31452. These allow visual inspection of the internal detail of the map and identification of artifacts.

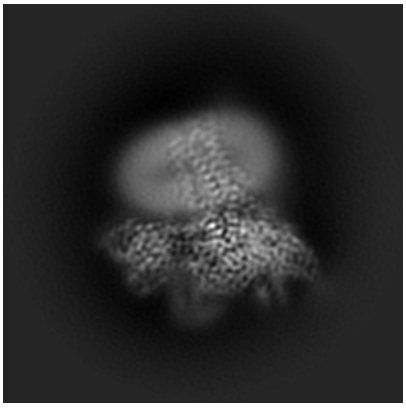
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections (i)

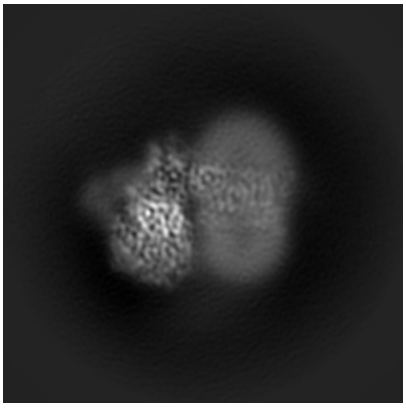
6.1.1 Primary map



X



Y

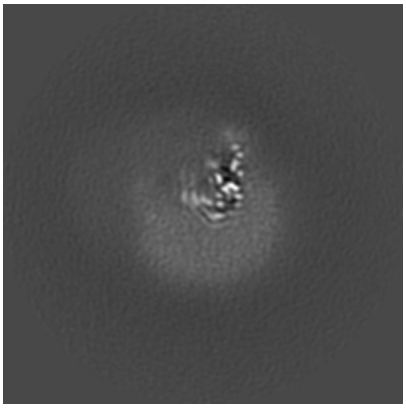


Z

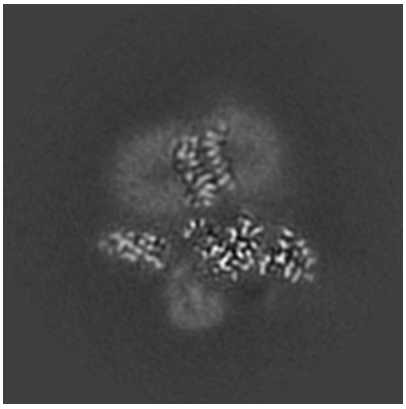
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

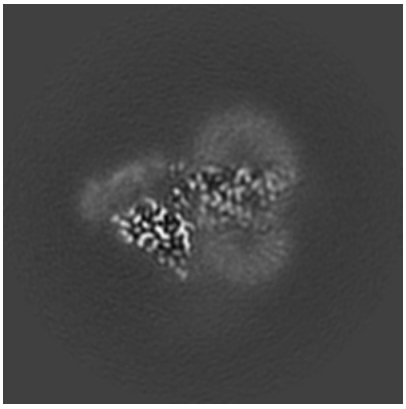
6.2.1 Primary map



X Index: 100



Y Index: 100

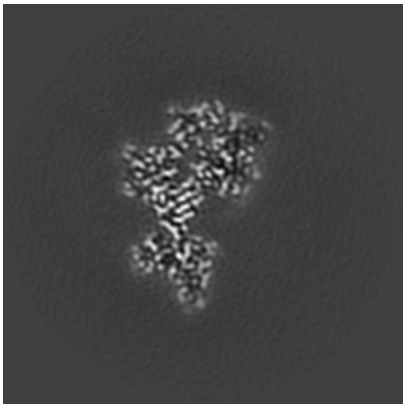


Z Index: 100

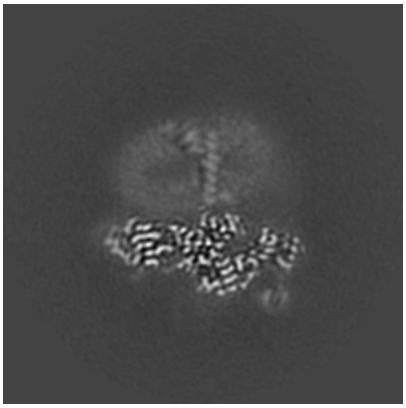
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

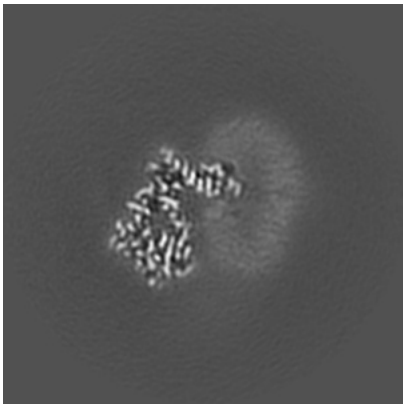
6.3.1 Primary map



X Index: 80



Y Index: 89



Z Index: 115

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.

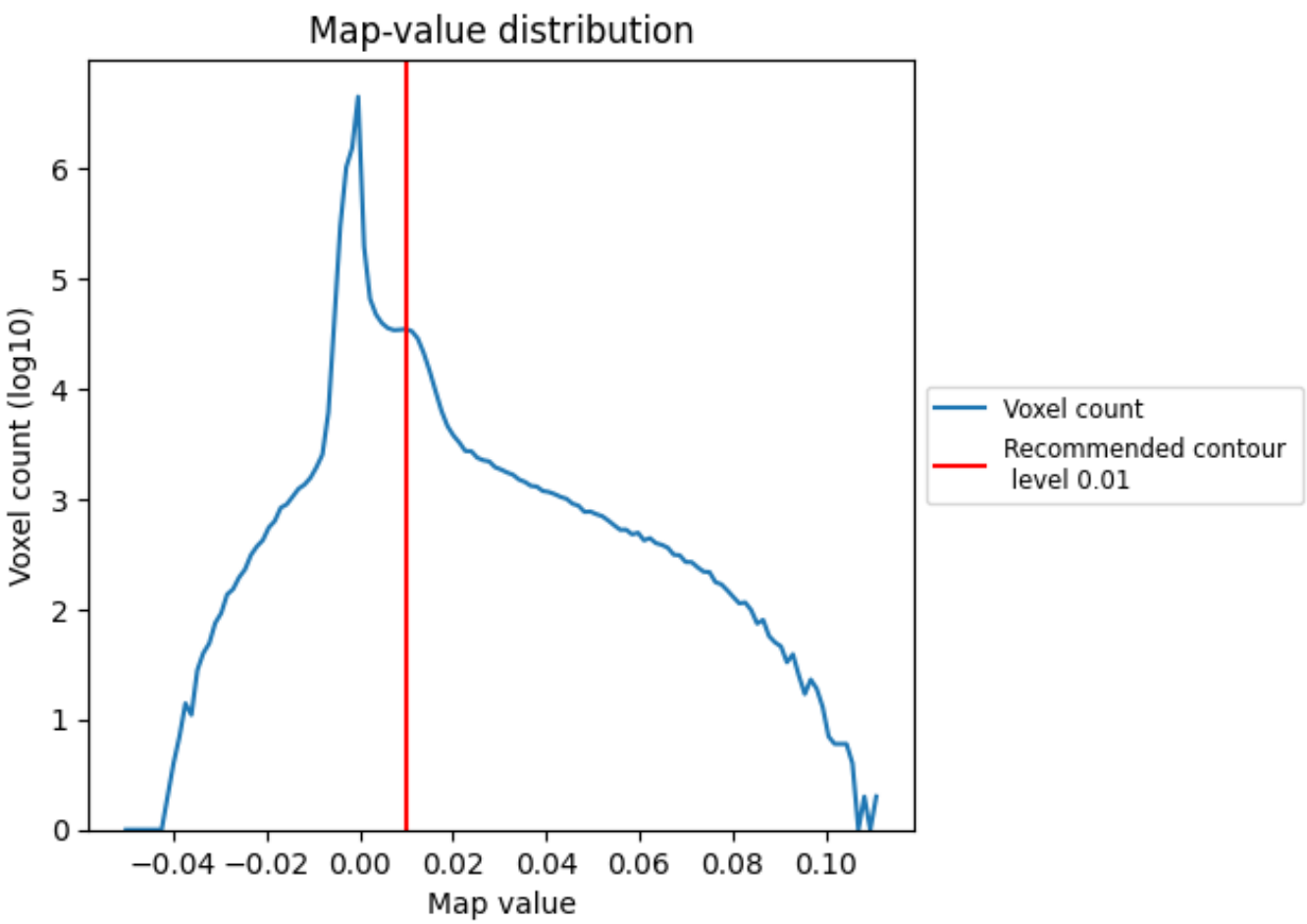
6.5 Mask visualisation (i)

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

7 Map analysis (i)

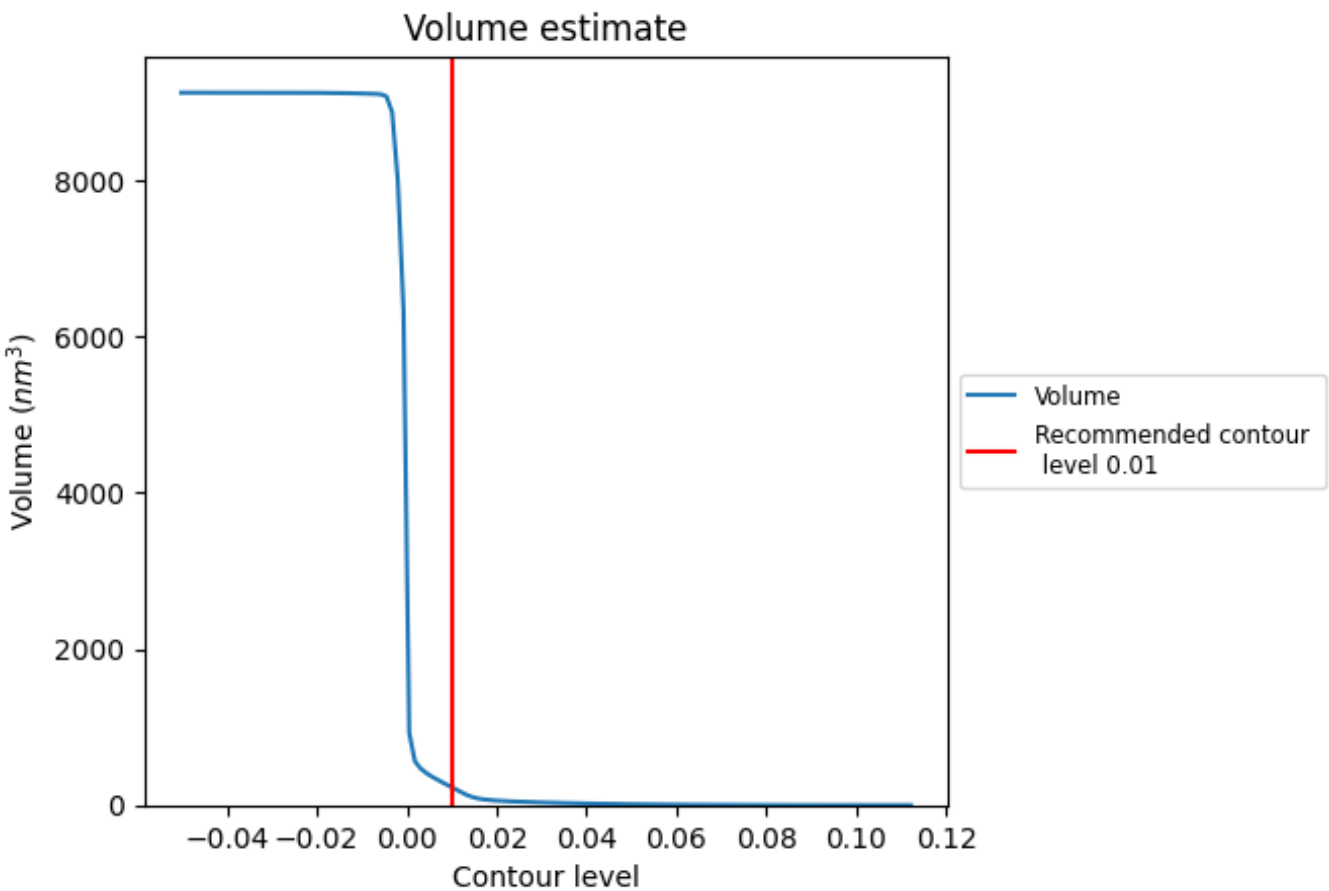
This section contains the results of statistical analysis of the map.

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.

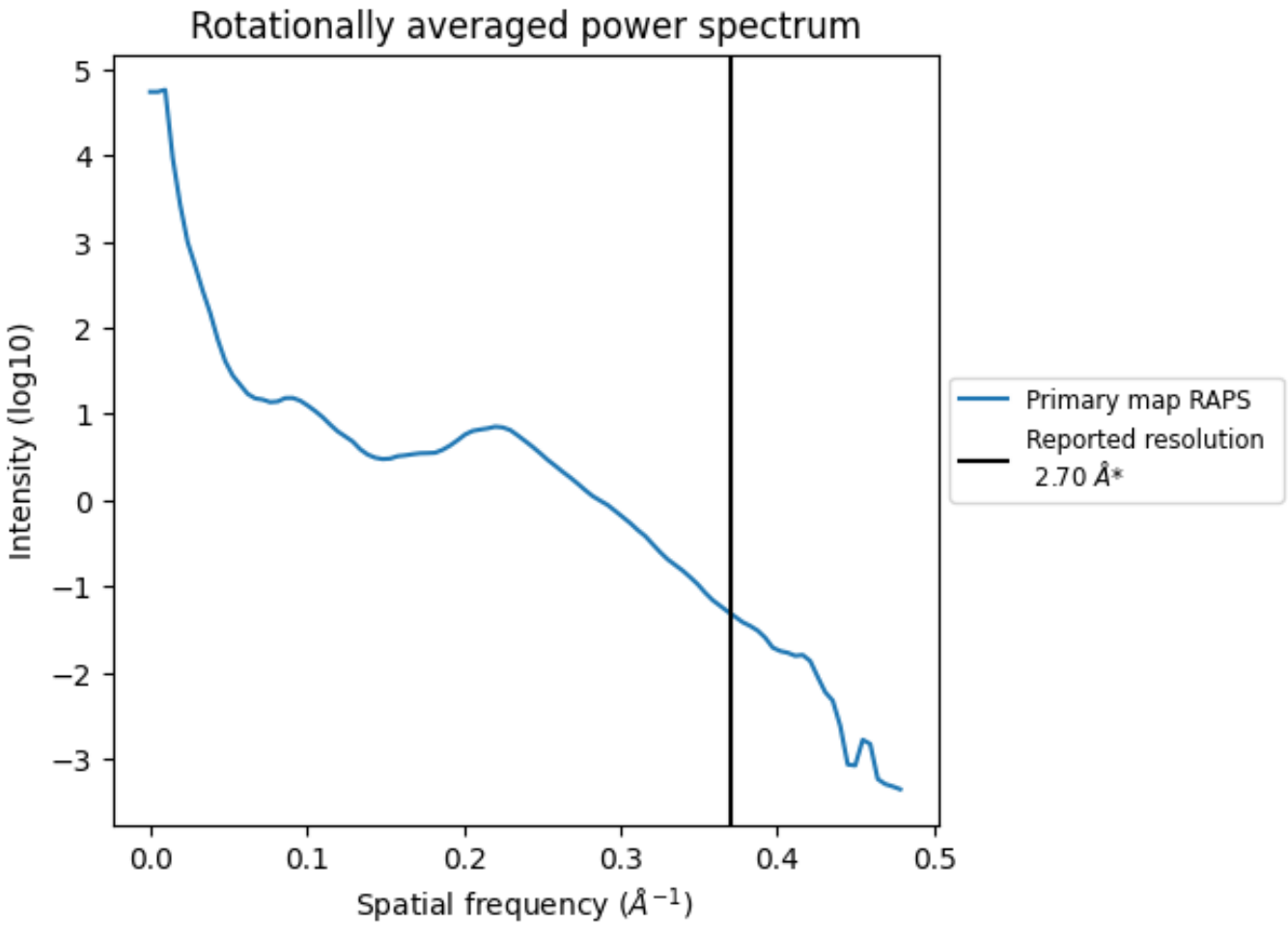
7.2 Volume estimate (i)



The volume at the recommended contour level is 229 nm^3; this corresponds to an approximate mass of 207 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 0.370 Å^{-1}

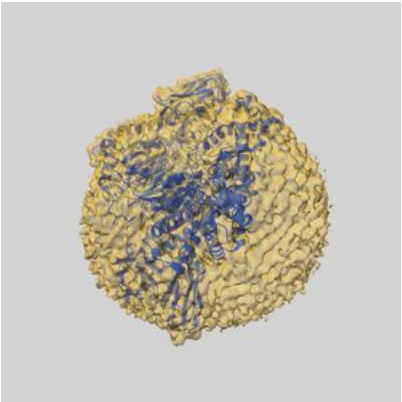
8 Fourier-Shell correlation (i)

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

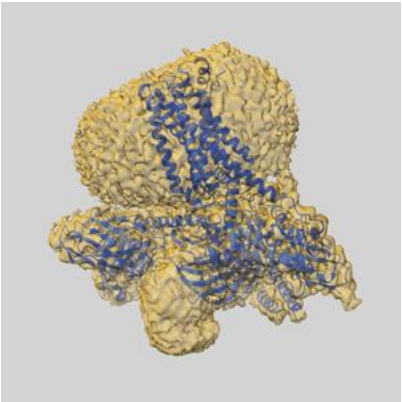
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-31452 and PDB model 7F4H. Per-residue inclusion information can be found in section 3 on page 6.

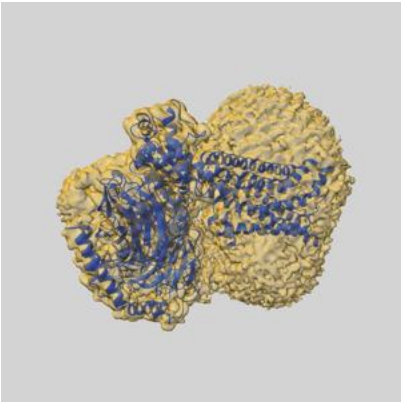
9.1 Map-model overlay (i)



X



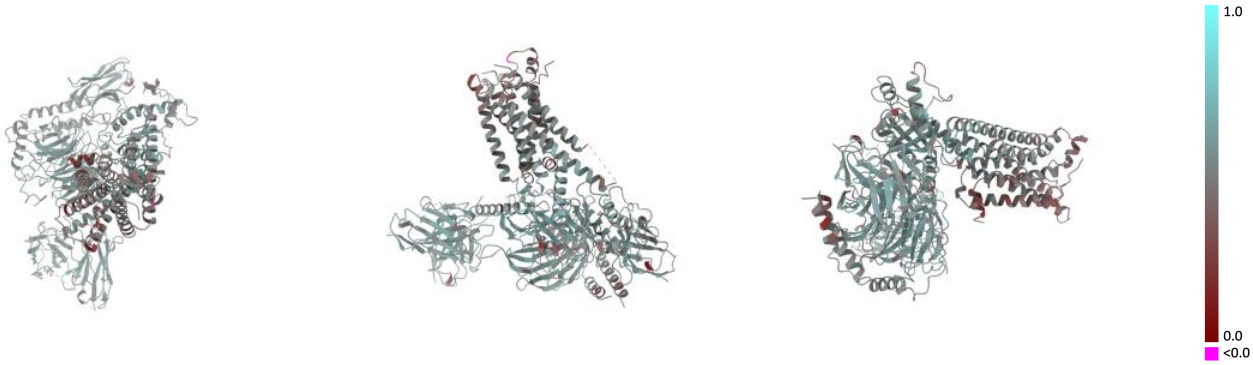
Y



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.

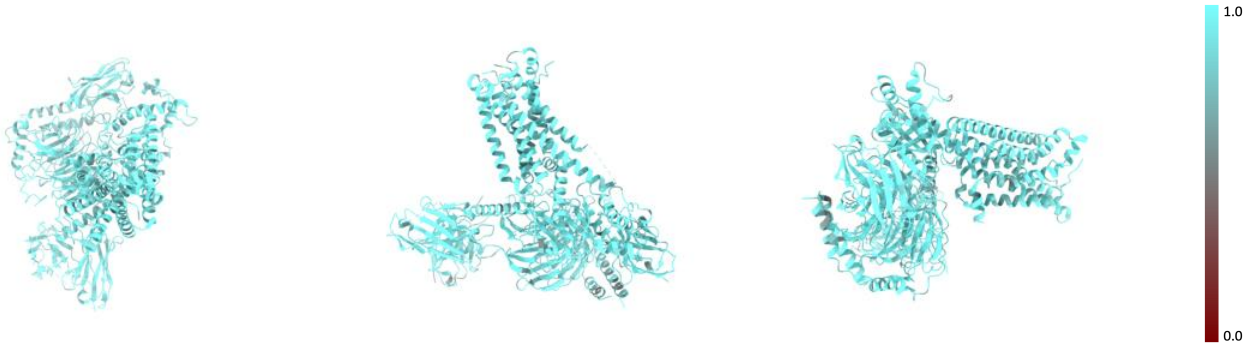
9.2 Q-score mapped to coordinate model (i)



X Y Z

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.

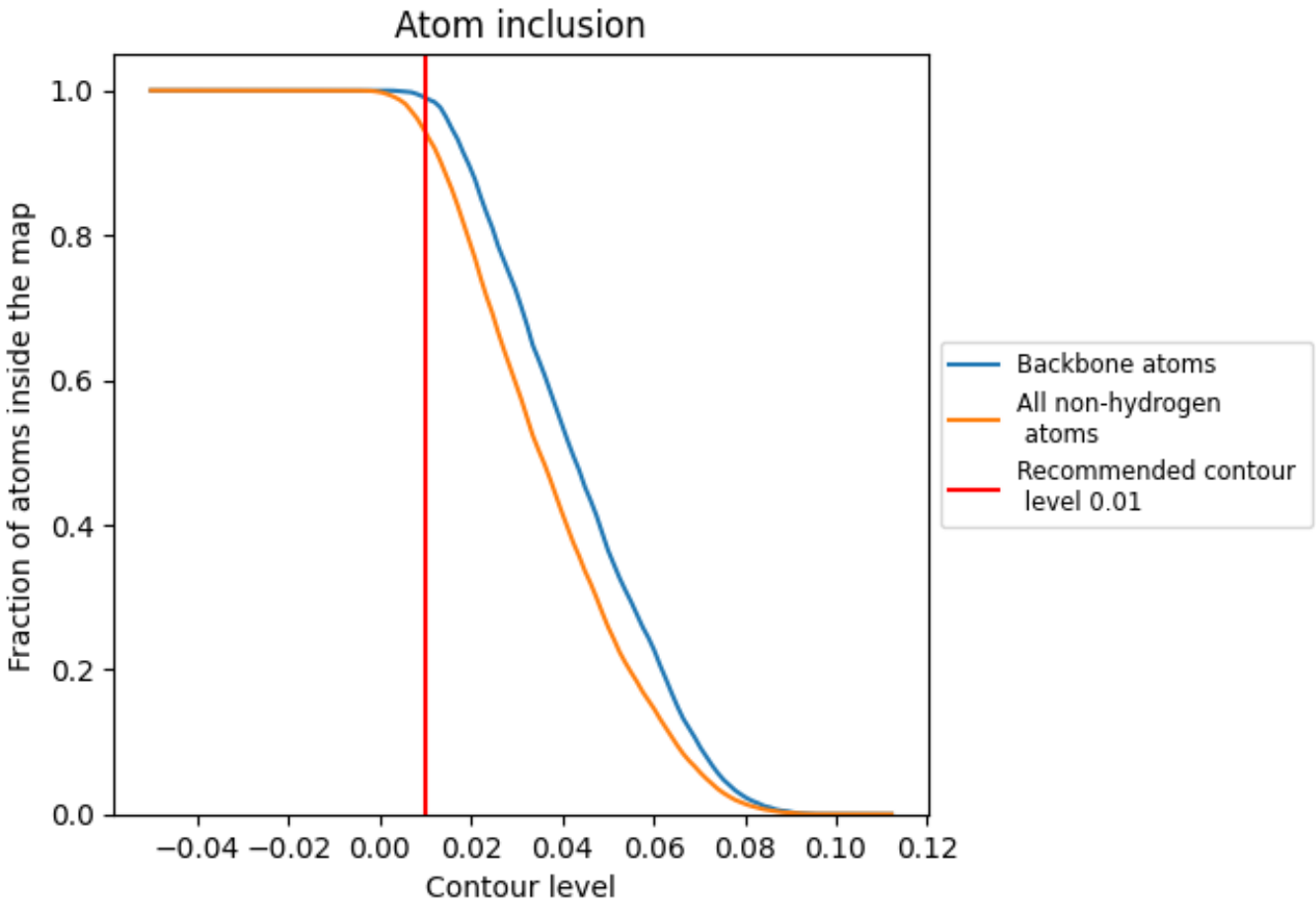
9.3 Atom inclusion mapped to coordinate model (i)



X Y Z

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	0.9422	0.5200
A	0.9417	0.5300
B	0.9490	0.5490
F	0.8980	0.4460
G	0.8952	0.4910
N	0.9426	0.5400
R	0.9343	0.4530
S	0.9555	0.5460