wwPDB EM Validation Summary Report (i)

Dec 10, 2022 – 01:25 pm GMT

PDB ID	:	6QPB
EMDB ID	:	EMD-4612
Title	:	Cryo-EM structure of calcium-free mTMEM16F lipid scramblase in digitonin
Authors	:	Alvadia, C.; Lim, N.K.; Clerico Mosina, V.; Oostergetel, G.T.; Dutzler, R.; Paulino, C.
Deposited on	:	2019-02-13
Resolution	:	3.60 Å(reported)
Based on initial model	:	?

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 (1)) were used in the production of this report:

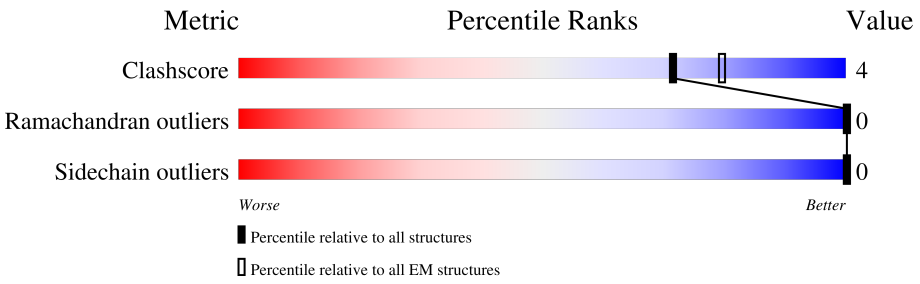
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.4, CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	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 3.60 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	158937	4297
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5% The upper red bar (where present) indicates the fraction of residues that have poor fit to the EM map (all-atom inclusion < 40%). The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	911	10% 72% 8% 20%
1	B	911	10% 72% 8% 20%

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
2	P1O	A	1001	X	-	-	-
2	P1O	B	1001	X	-	-	-

2 Entry composition (i)

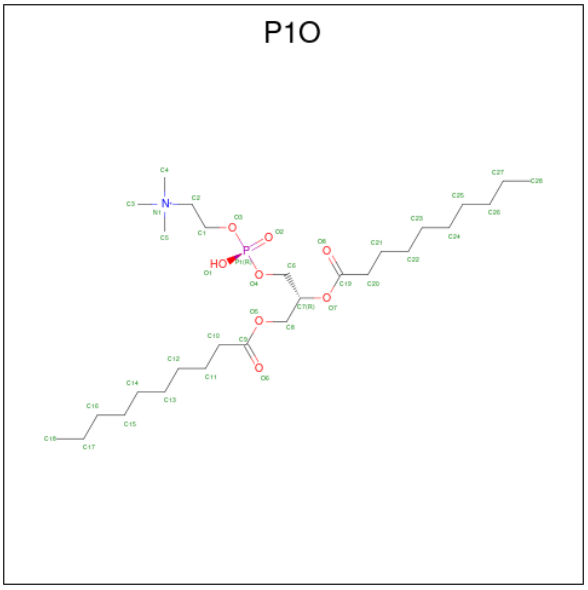
There are 2 unique types of molecules in this entry. The entry contains 12154 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 Anoctamin-6.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	731	6039	3972	968	1059	40	0	0
1	B	731	6039	3972	968	1059	40	0	0

- Molecule 2 is 1,2-DIDECANOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (three-letter code: P1O) (formula: $C_{28}H_{57}NO_8P$).



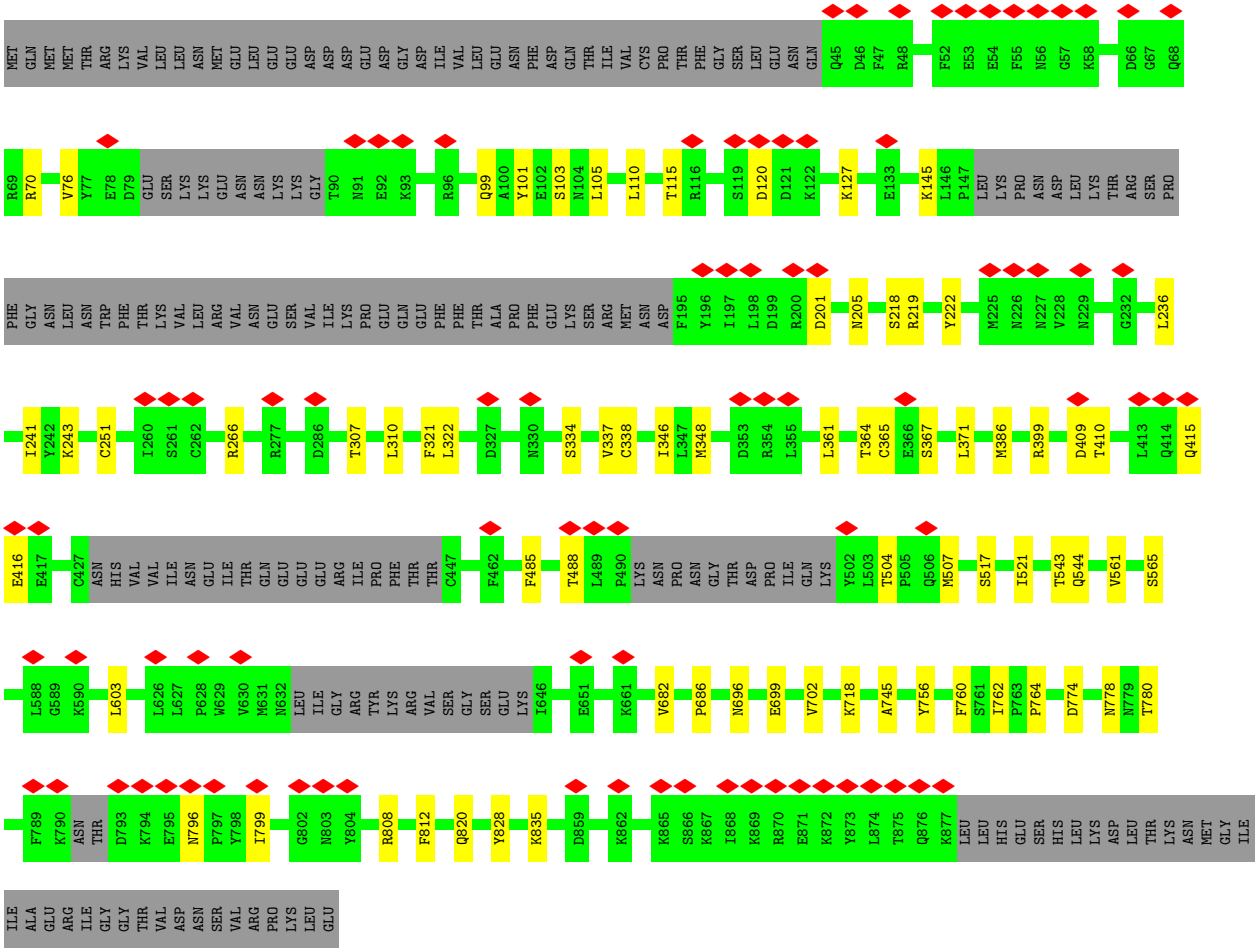
Mol	Chain	Residues	Atoms					AltConf
			Total	C	N	O	P	
2	A	1	38	28	1	8	1	0
2	B	1	38	28	1	8	1	0

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.

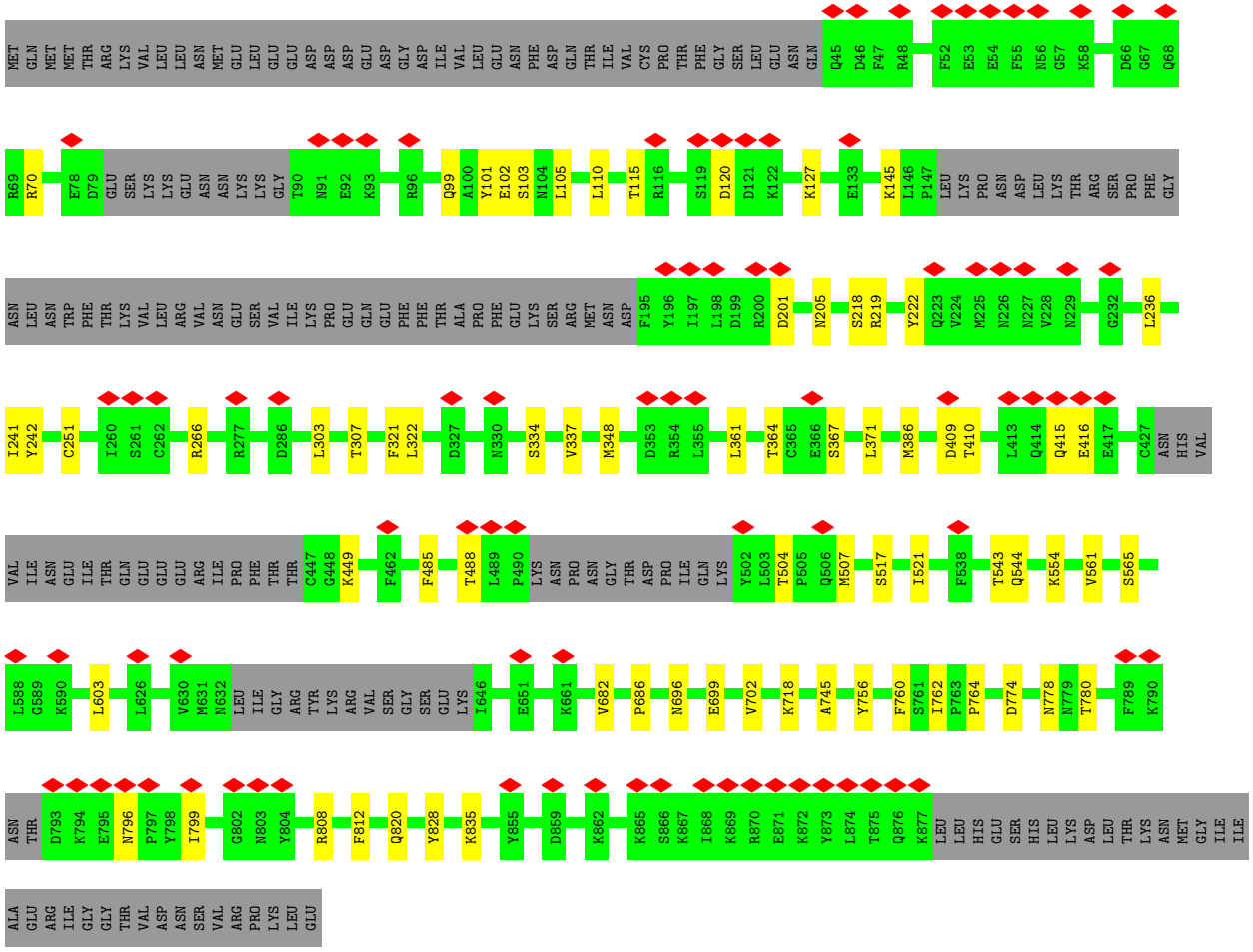
- Molecule 1: Anoctamin-6

Chain A:



- Molecule 1: Anoctamin-6

Chain B:


MET GLN MET MET THR ARG LYS VAL LEU LEU ASN MET GLU LEU GLU GLU ASP ASP ASP GLU ASP GLY ASP ILE VAL LEU GLU ASN PHE ASP GLN THR ILE VAL CYS PRO THR PHE GLY SER LEU GLU ASN GLN Q45 D46 F47 R48 F52 E53 E54 F55 N56 G57 K58 D66 G67 Q68
R69 R70 E78 D79 GLU SER LYS LYS GLU ASN ASN LYS LYS GLY T90 N91 E92 K93 R96 Q99 A100 Y101 E102 S103 N104 L105 L110 T115 R116 S119 D120 D121 K122 K127 E133 K145 L146 P147 LEU LYS PRO ASN ASP LEU LYS THR ARG SER PRO PHE GLY
ASN LEU ASN TRP PHE THR LYS VAL LEU ARG VAL ASN GLU SER VAL ILE LYS PRO GLU GLN GLU PHE PHE THR ALA PRO PHE GLU LYS SER ARG MET ASN ASP F195 Y196 I197 L198 D199 R200 D201 N205 S218 R219 Y222 Q223 V224 M225 N226 N227 V228 N229 G232 L236
I241 Y242 C251 I260 S261 C262 R266 R277 D286 L303 T307 F321 L322 D327 N330 S334 V337 M348 D353 R354 L355 L361 T364 C365 E366 S367 L371 M386 D409 T410 L413 Q414 Q415 E416 E417 C427 ASN HIS VAL
VAL ILE ASN GLU ILE THR GLN GLU GLU GLU ARG ILE PRO PHE THR THR C447 G448 K449 F462 F485 T488 L489 P490 LYS ASN PRO ASN GLY THR ASP PRO ILE GLN LYS Y502 L503 T504 P505 Q506 M507 S517 I521 F538 T543 Q544 K554 V561 S565
L588 G589 K590 L603 L626 V630 M631 N632 LEU ILE GLY ARG TYR LYS ARG VAL SER GLY SER GLU LYS I646 E651 K661 V682 P686 N696 E699 V702 K718 A745 Y756 F760 S761 I762 P763 P764 D774 N778 N779 T780 F789 K790
ASN THR D793 K794 E795 N796 P797 Y798 I799 G802 N803 Y804 R808 F812 Q820 Y828 K835 Y855 D859 K862 K865 S866 K867 I868 K869 R870 E871 K872 Y873 L874 T875 Q876 K877 LEU LEU HIS GLU SER HIS LEU LYS ASP LEU THR LYS ASN MET GLY ILE ILE
ALA GLU ARG ILE GLY GLY THR VAL ASP ASN SER VAL ARG PRO LYS LEU GLU

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C2	Depositor
Number of particles used	194284	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	FEI TALOS ARCTICA	Depositor
Voltage (kV)	200	Depositor
Electron dose ($e^-/Å^2$)	52	Depositor
Minimum defocus (nm)	300	Depositor
Maximum defocus (nm)	3000	Depositor
Magnification	49407	Depositor
Image detector	GATAN K2 SUMMIT (4k x 4k)	Depositor
Maximum map value	0.403	Depositor
Minimum map value	-0.194	Depositor
Average map value	0.002	Depositor
Map value standard deviation	0.014	Depositor
Recommended contour level	0.07	Depositor
Map size (Å)	222.64, 222.64, 222.64	wwPDB
Map dimensions	220, 220, 220	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.012, 1.012, 1.012	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: P1O

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	0.31	0/6207	0.49	0/8419
1	B	0.31	0/6207	0.49	0/8419
All	All	0.31	0/12414	0.49	0/16838

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts (i)

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in the chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, whereas Symm-Clashes lists symmetry-related clashes.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	6039	0	5997	44	0
1	B	6039	0	5997	43	0
2	A	38	0	56	0	0
2	B	38	0	56	0	0
All	All	12154	0	12106	85	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 85 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:762:ILE:HG22	1:A:764:PRO:HD2	1.75	0.68
1:B:762:ILE:HG22	1:B:764:PRO:HD2	1.75	0.68
1:A:364:THR:O	1:A:367:SER:OG	2.08	0.66
1:B:364:THR:O	1:B:367:SER:OG	2.09	0.65
1:A:561:VAL:O	1:A:565:SER:HB3	1.99	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	A	717/911 (79%)	699 (98%)	18 (2%)	0	100	100
1	B	717/911 (79%)	699 (98%)	18 (2%)	0	100	100
All	All	1434/1822 (79%)	1398 (98%)	36 (2%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2 Protein sidechains (i)

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

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

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	659/827 (80%)	659 (100%)	0	100	100
1	B	659/827 (80%)	659 (100%)	0	100	100
All	All	1318/1654 (80%)	1318 (100%)	0	100	100

There are no protein residues with a non-rotameric sidechain to report.

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 10 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	623	GLN
1	B	696	ASN
1	B	796	ASN
1	A	696	ASN
1	A	796	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)

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

5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

5.6 Ligand geometry (i)

2 ligands 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	$\#\|Z\| > 2$
2	P1O	B	1001	-	37,37,37	1.55	8 (21%)	43,45,45	0.96	2 (4%)
2	P1O	A	1001	-	37,37,37	1.54	8 (21%)	43,45,45	0.96	2 (4%)

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
2	P1O	B	1001	-	1/1/5/5	18/41/41/41	-
2	P1O	A	1001	-	1/1/5/5	18/41/41/41	-

The worst 5 of 16 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	B	1001	P1O	P1-O3	4.66	1.78	1.59
2	A	1001	P1O	P1-O3	4.64	1.78	1.59
2	A	1001	P1O	C8-C7	2.80	1.59	1.50
2	B	1001	P1O	C8-C7	2.80	1.59	1.50
2	A	1001	P1O	P1-O4	2.60	1.69	1.59

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	A	1001	P1O	O7-C19-C20	3.13	118.24	111.50
2	B	1001	P1O	O7-C19-C20	3.10	118.19	111.50
2	B	1001	P1O	O5-C9-C10	2.22	118.88	111.91
2	A	1001	P1O	O5-C9-C10	2.22	118.86	111.91

All (2) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
2	A	1001	P1O	C7
2	B	1001	P1O	C7

5 of 36 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	1001	P1O	O3-C1-C2-N1
2	A	1001	P1O	O4-C6-C7-O7
2	B	1001	P1O	O3-C1-C2-N1
2	B	1001	P1O	O4-C6-C7-O7
2	A	1001	P1O	C1-O3-P1-O4

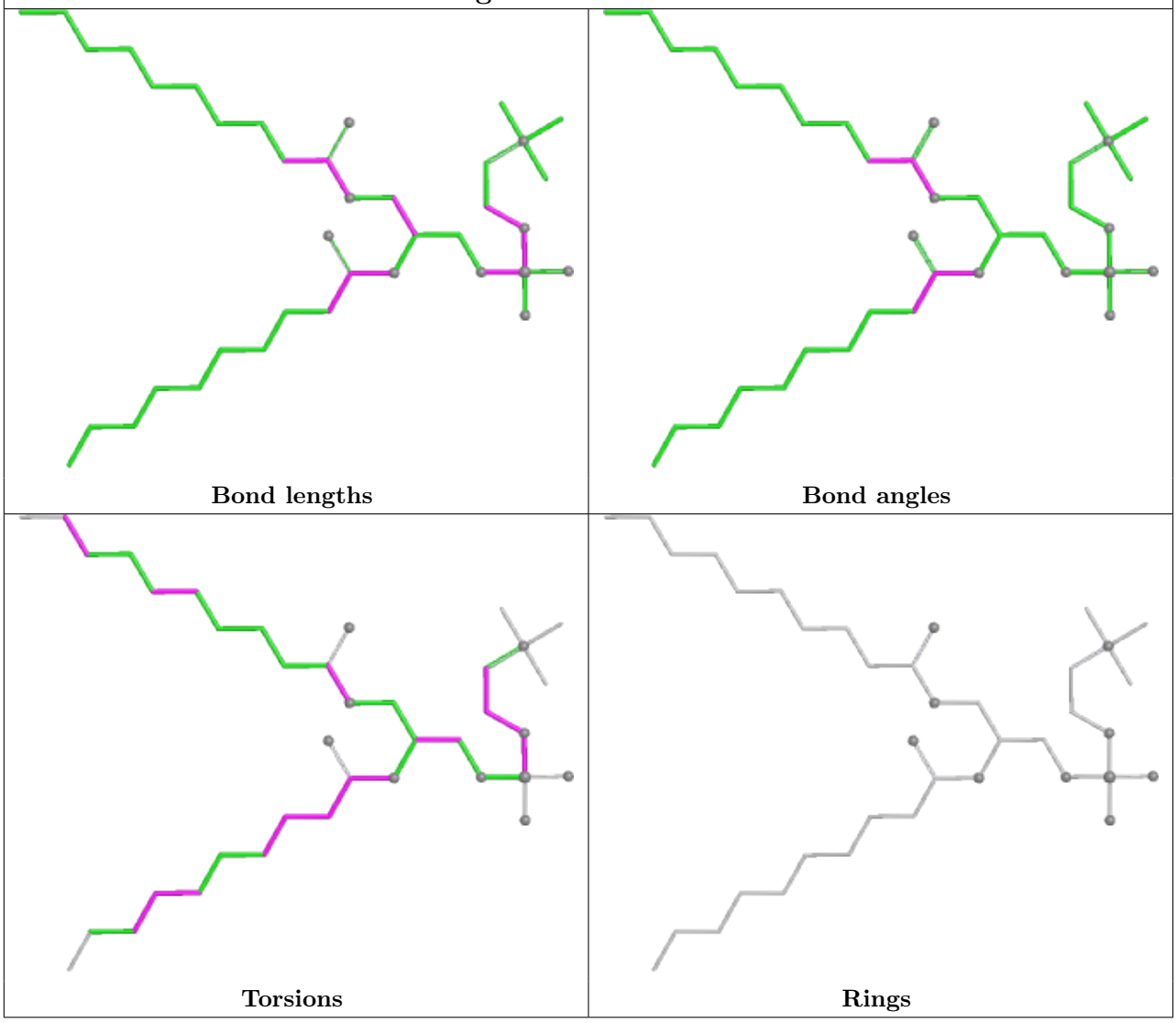
There are no ring outliers.

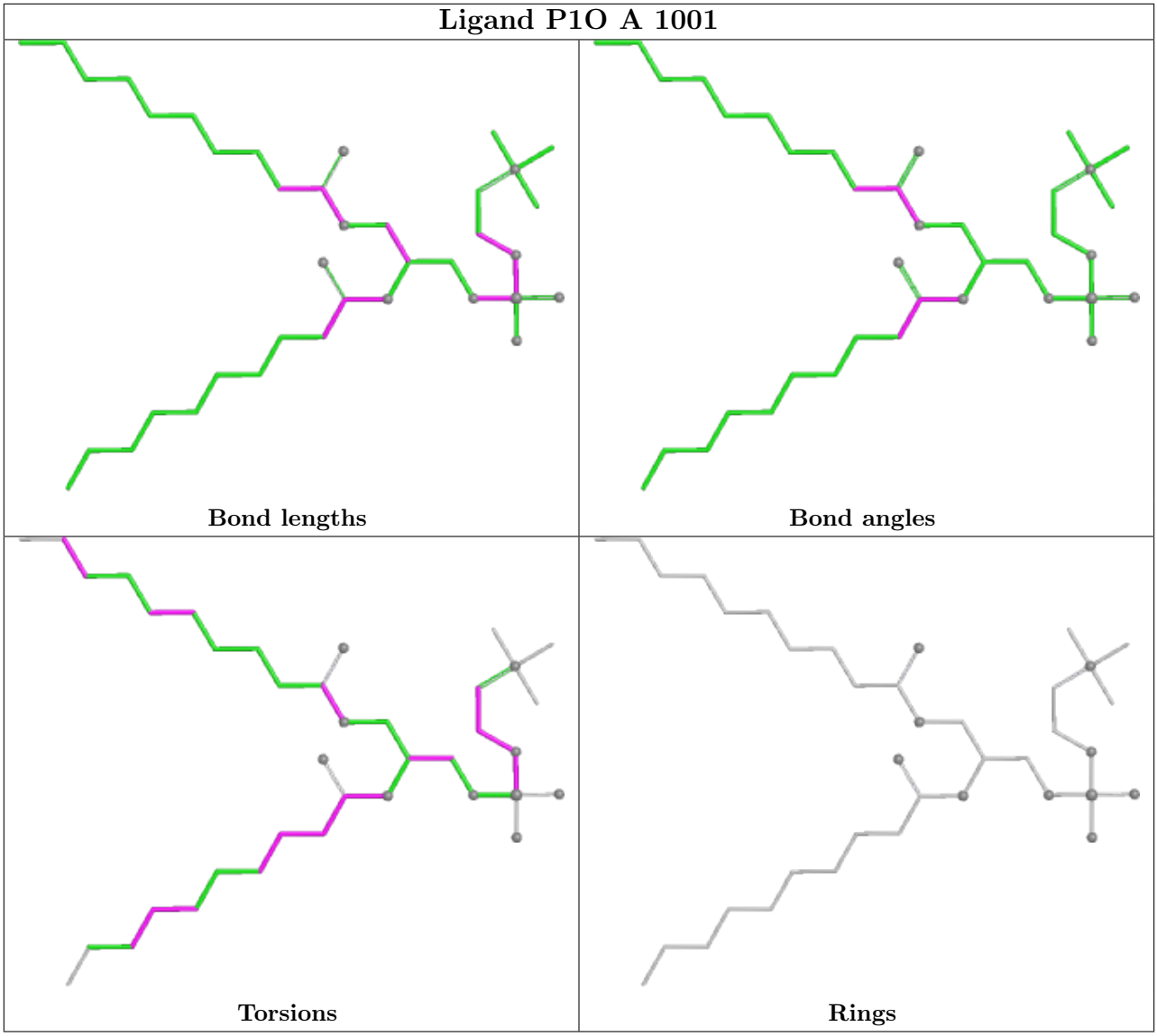
No monomer is involved in short contacts.

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In

addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.

Ligand P1O B 1001





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.

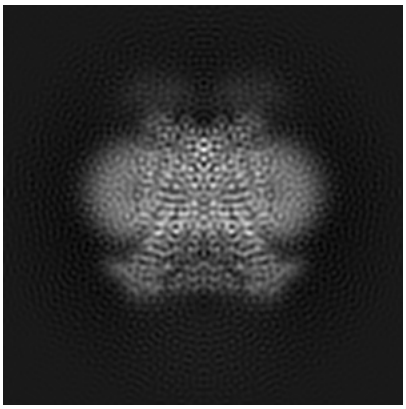
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-4612. These allow visual inspection of the internal detail of the map and identification of artifacts.

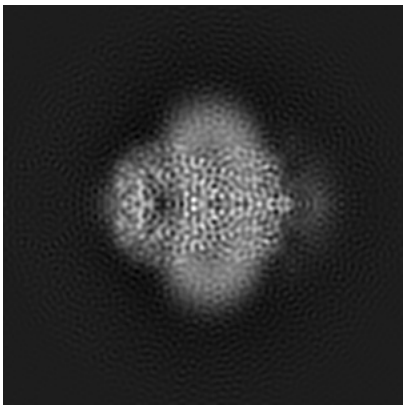
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections (i)

6.1.1 Primary map



X



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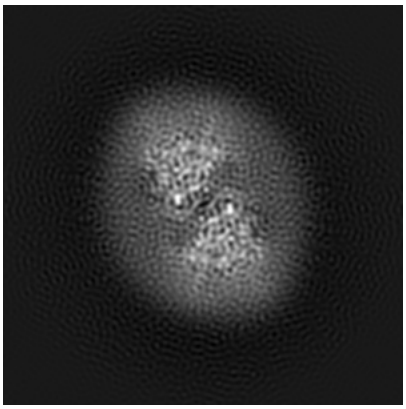
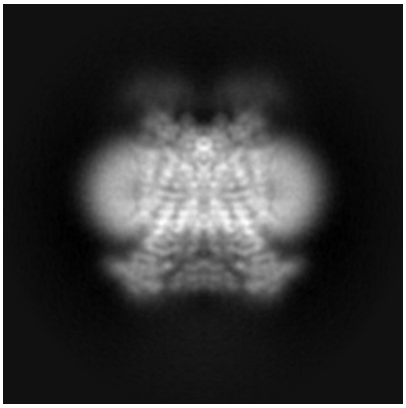


Z

6.1.2 Raw map



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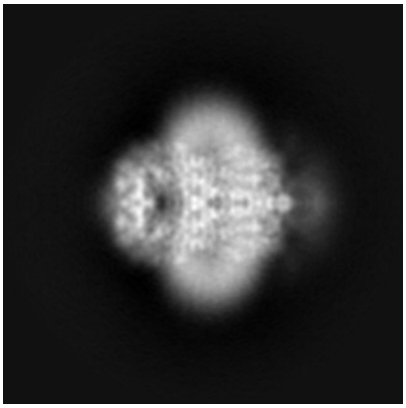
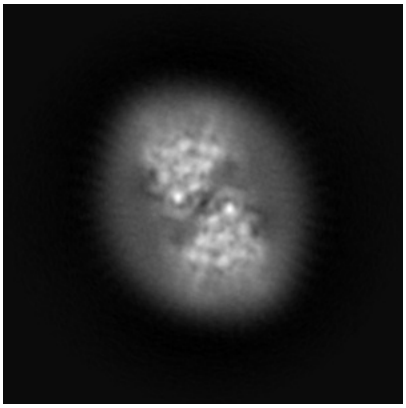


Y

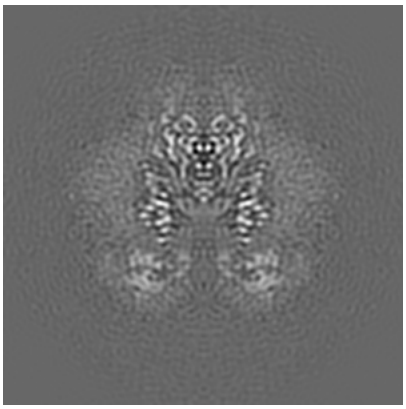


Z

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

6.2 Central slices (i)

6.2.1 Primary map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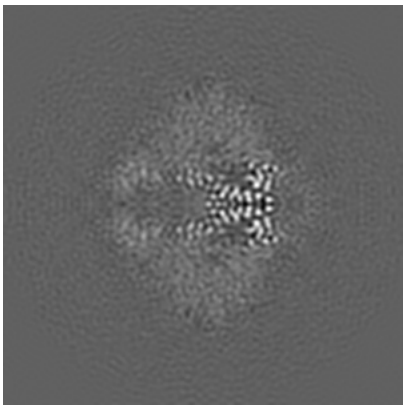
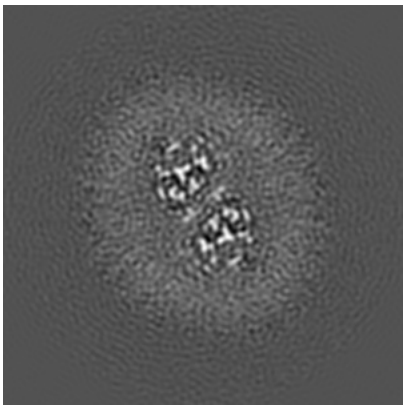
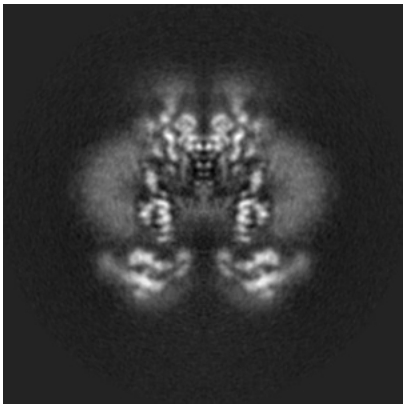
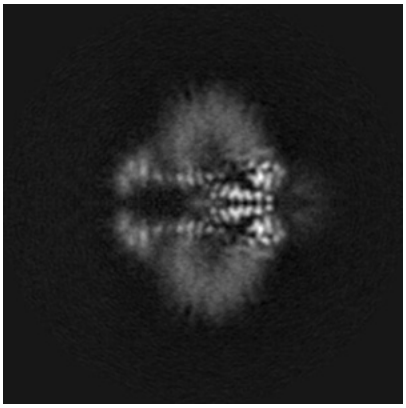
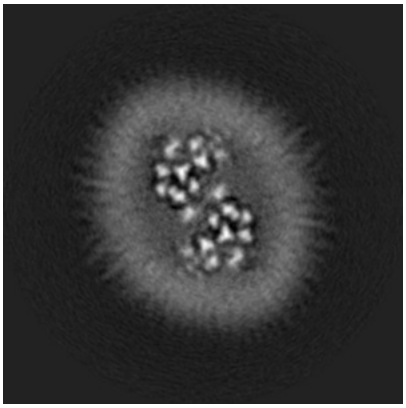

Y Index: 110


Z Index: 110

6.2.2 Raw map


X Index: 110

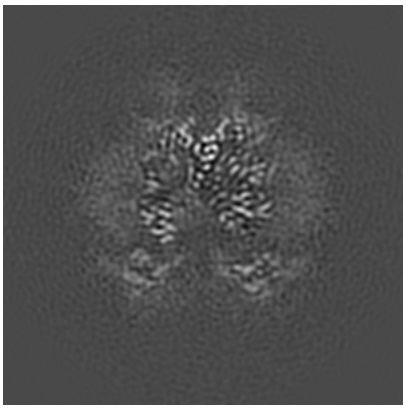

Y Index: 110


Z Index: 110

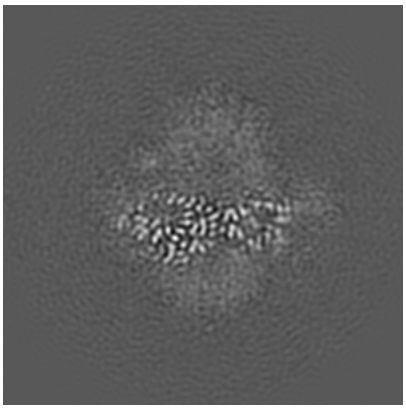
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

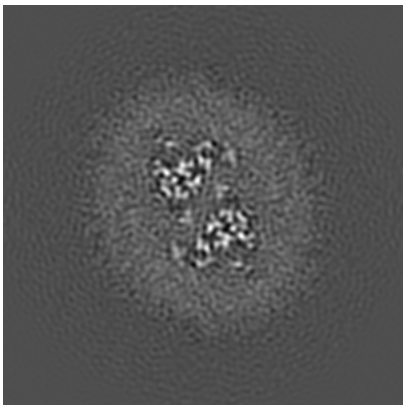
6.3.1 Primary map



X Index: 108

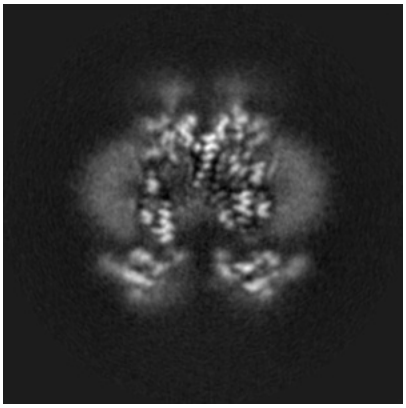


Y Index: 129

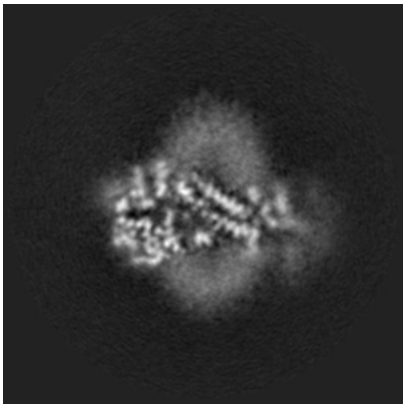


Z Index: 105

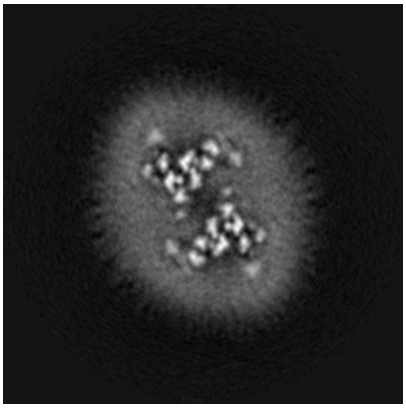
6.3.2 Raw map



X Index: 108



Y Index: 139



Z Index: 100

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

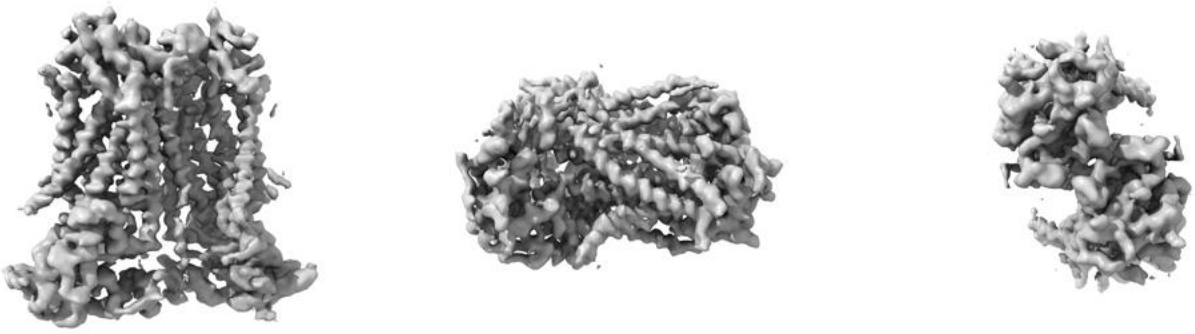
6.4 Orthogonal surface views (i)

6.4.1 Primary map

X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.07. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.4.2 Raw map



X Y Z

These images show the 3D surface of the raw map. The raw map's contour level was selected so that its surface encloses the same volume as the primary map does at its recommended contour level.

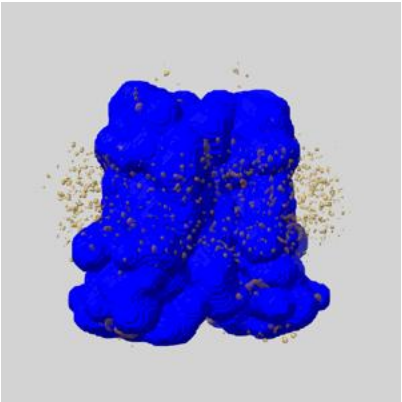
6.5 Mask visualisation (i)

This section shows the 3D surface view of the primary map at 50% transparency overlaid with the specified mask at 0% transparency

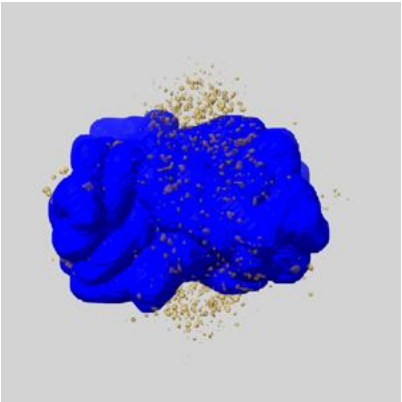
A mask typically either:

- Encompasses the whole structure
- Separates out a domain, a functional unit, a monomer or an area of interest from a larger structure

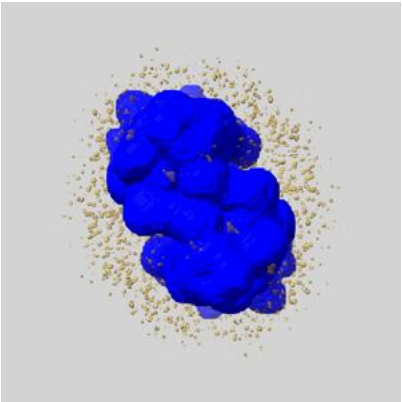
6.5.1 emd_4612_msk_1.map (i)



X



Y

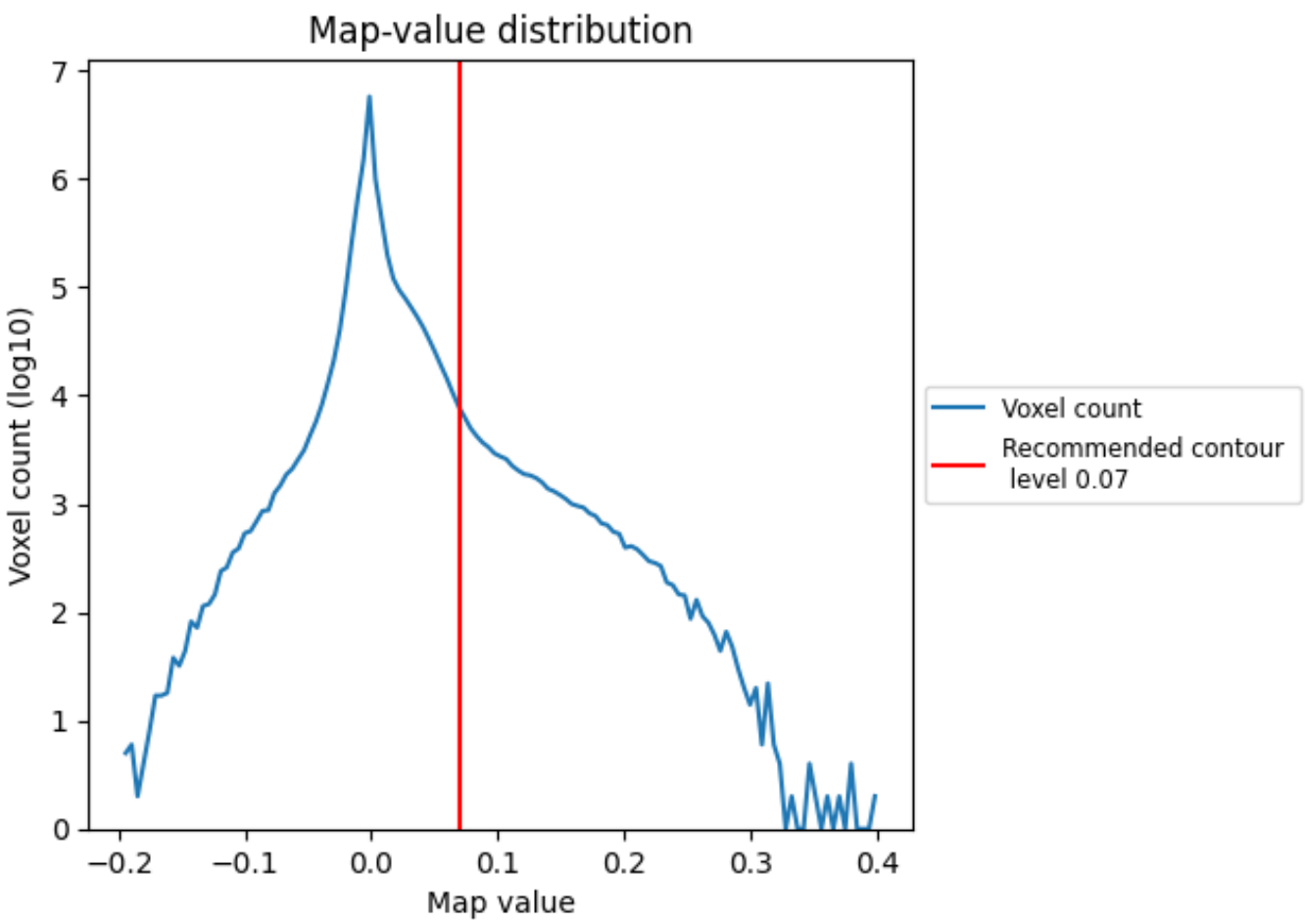


Z

7 Map analysis (i)

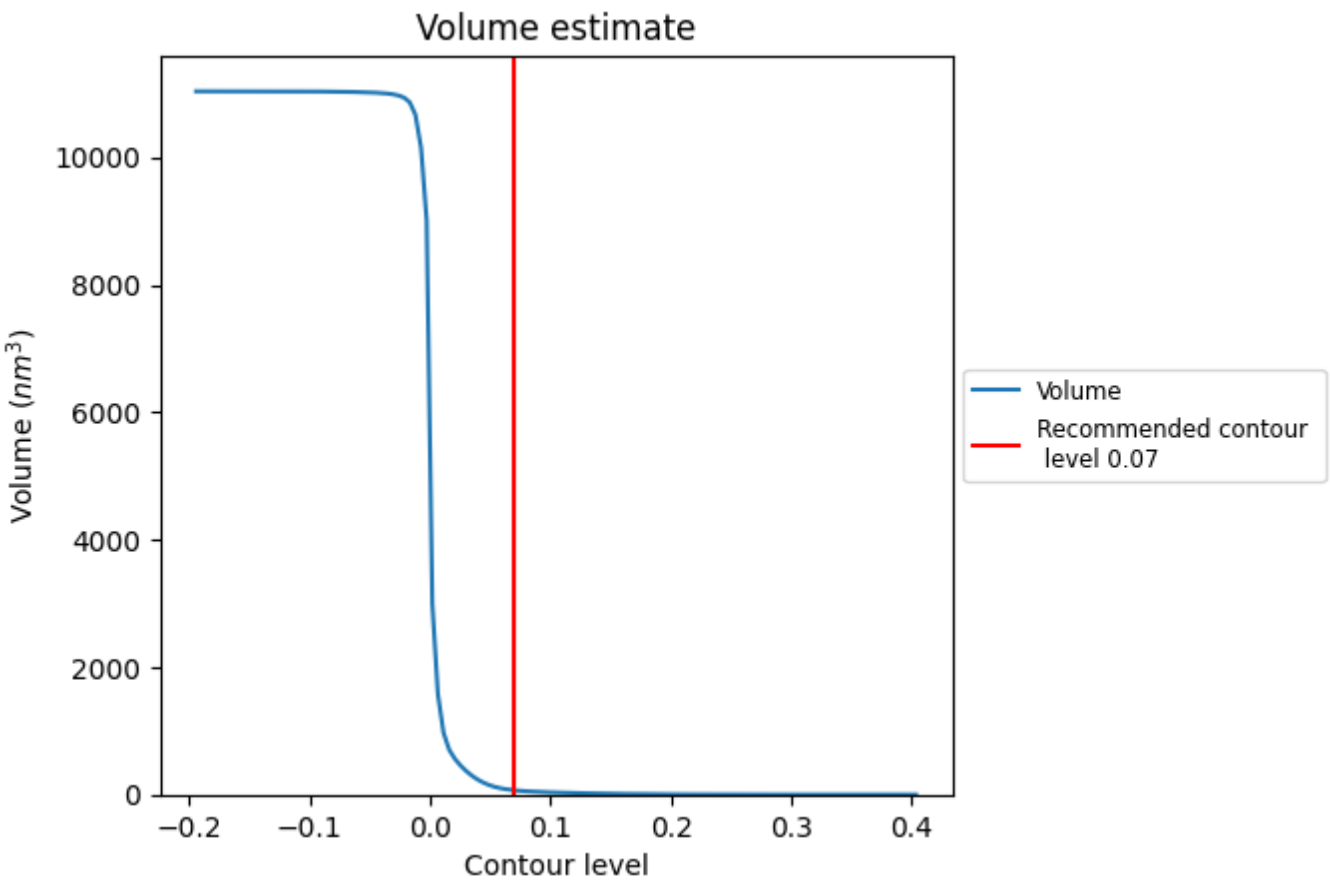
This section contains the results of statistical analysis of the map.

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.

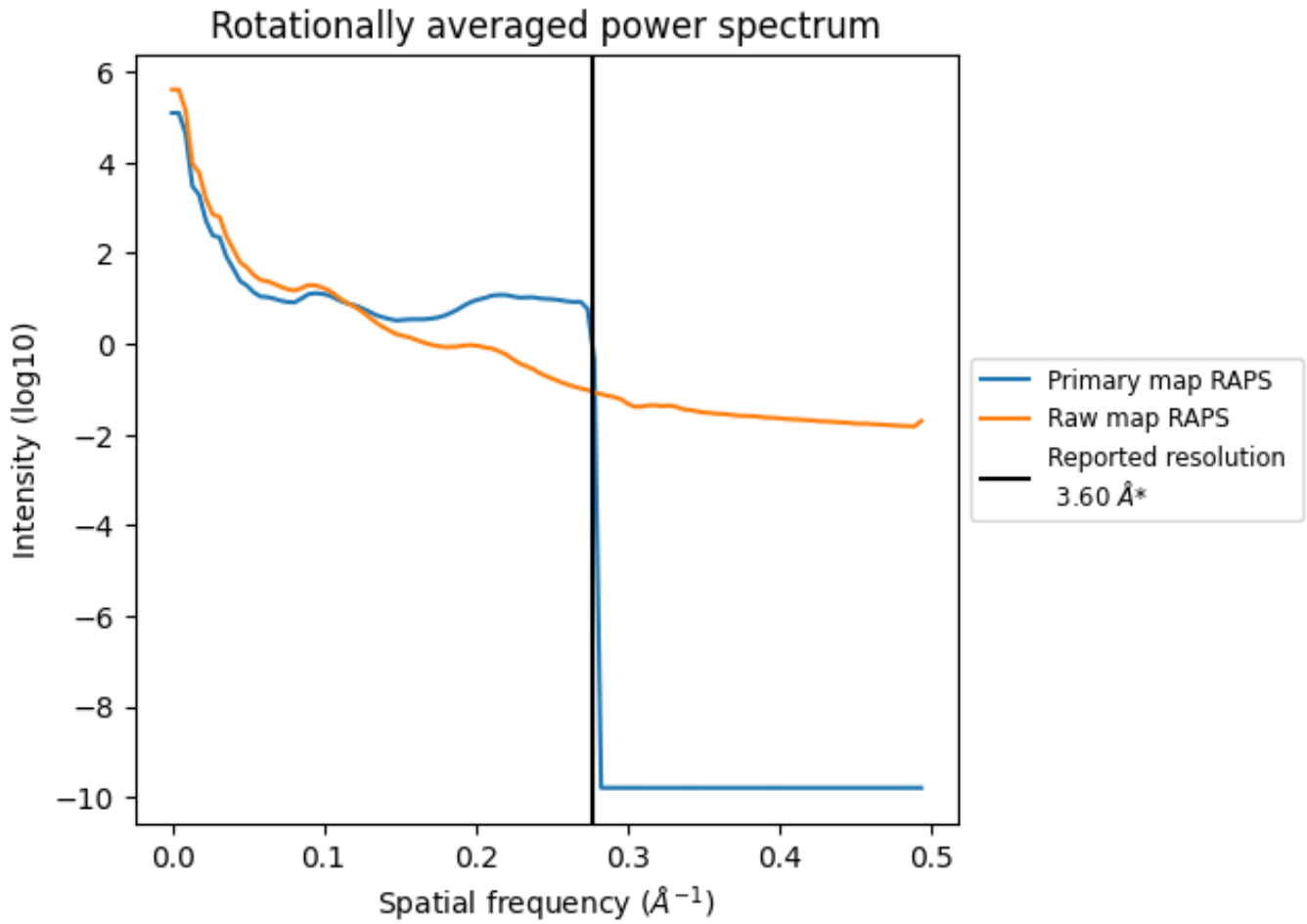
7.2 Volume estimate (i)



The volume at the recommended contour level is 67 nm^3; this corresponds to an approximate mass of 60 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)

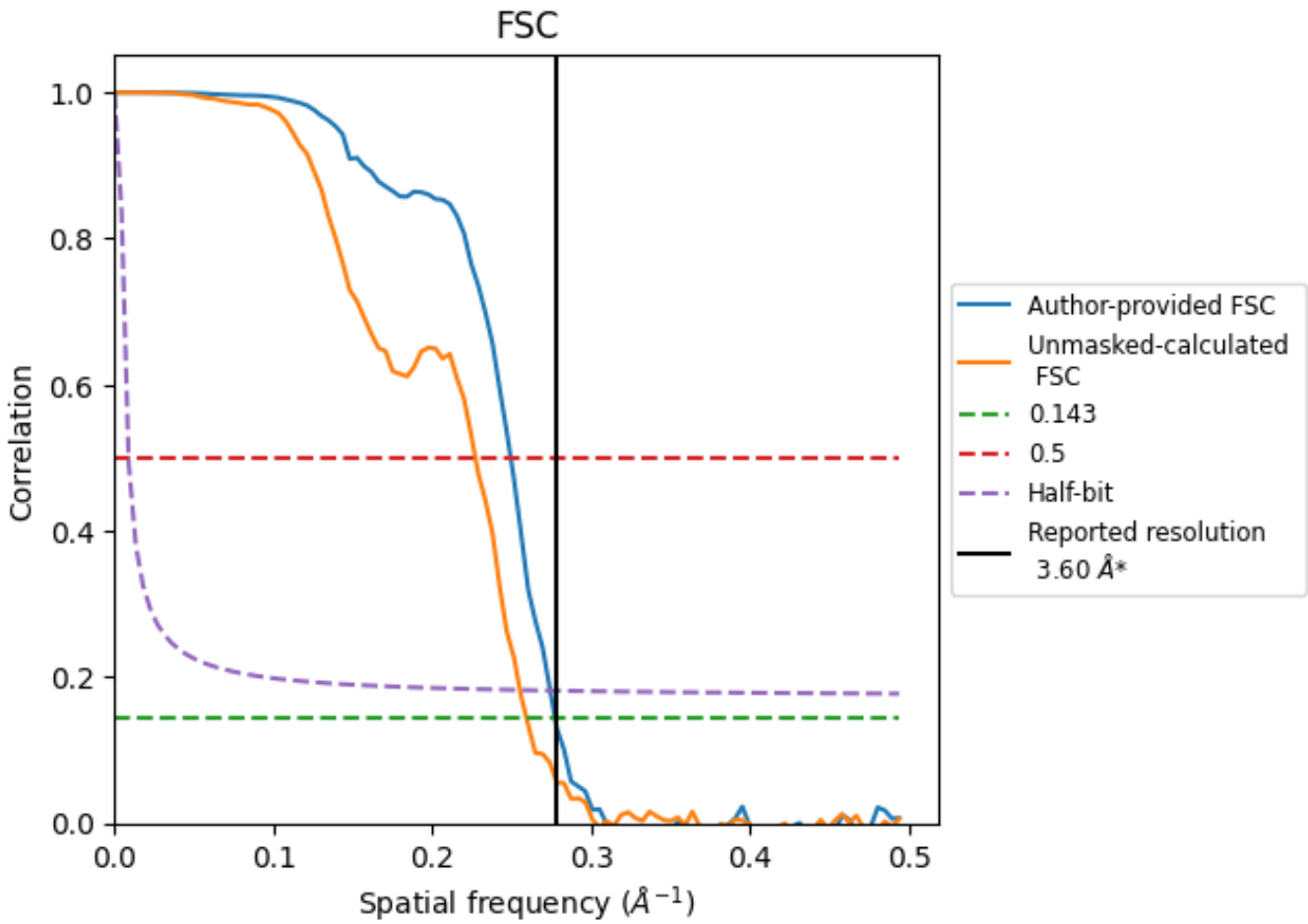


*Reported resolution corresponds to spatial frequency of 0.278 Å^{-1}

8 Fourier-Shell correlation (i)

Fourier-Shell Correlation (FSC) is the most commonly used method to estimate the resolution of single-particle and subtomogram-averaged maps. The shape of the curve depends on the imposed symmetry, mask and whether or not the two 3D reconstructions used were processed from a common reference. The reported resolution is shown as a black line. A curve is displayed for the half-bit criterion in addition to lines showing the 0.143 gold standard cut-off and 0.5 cut-off.

8.1 FSC (i)



*Reported resolution corresponds to spatial frequency of 0.278 Å^{-1}

8.2 Resolution estimates (i)

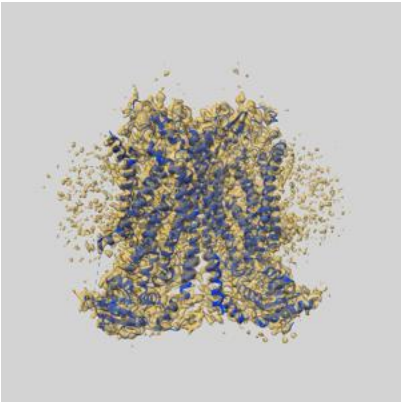
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.60	-	-
Author-provided FSC curve	3.60	4.01	3.64
Unmasked-calculated*	3.85	4.40	3.92

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

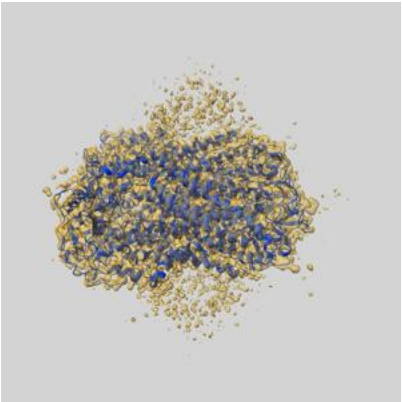
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-4612 and PDB model 6QPB. Per-residue inclusion information can be found in section 3 on page 4.

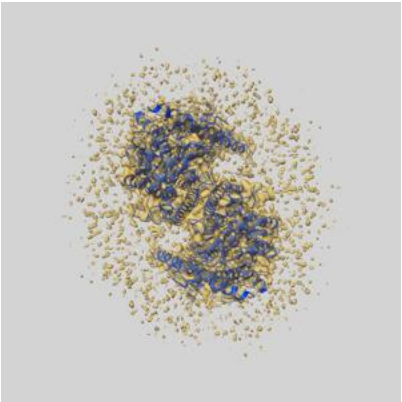
9.1 Map-model overlay (i)



X



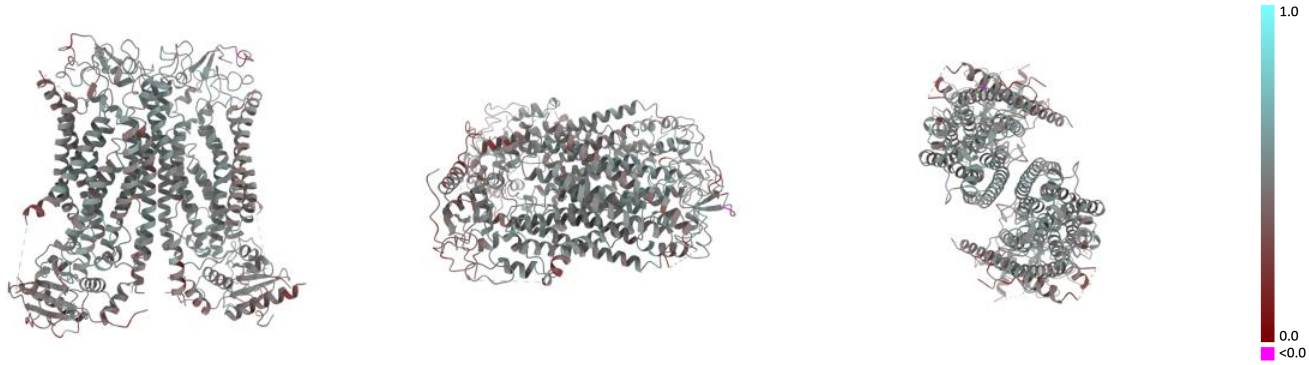
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.07 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.

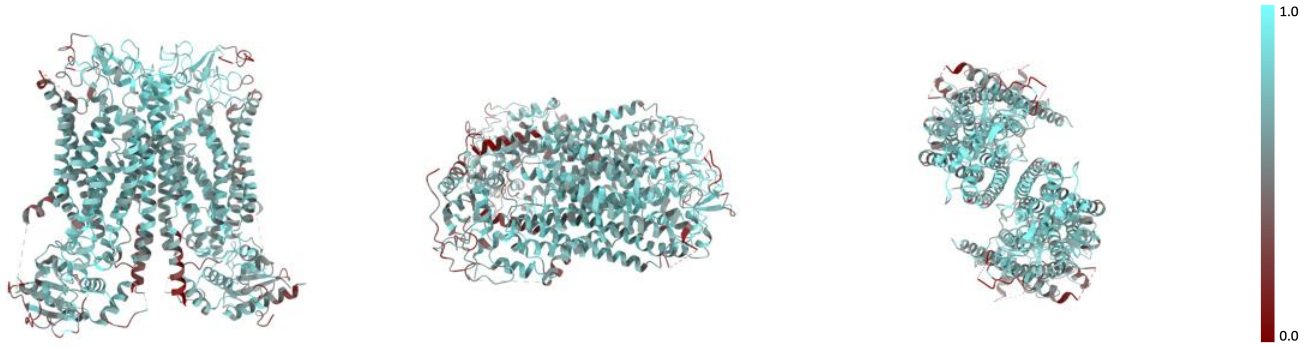
9.2 Q-score mapped to coordinate model (i)



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.

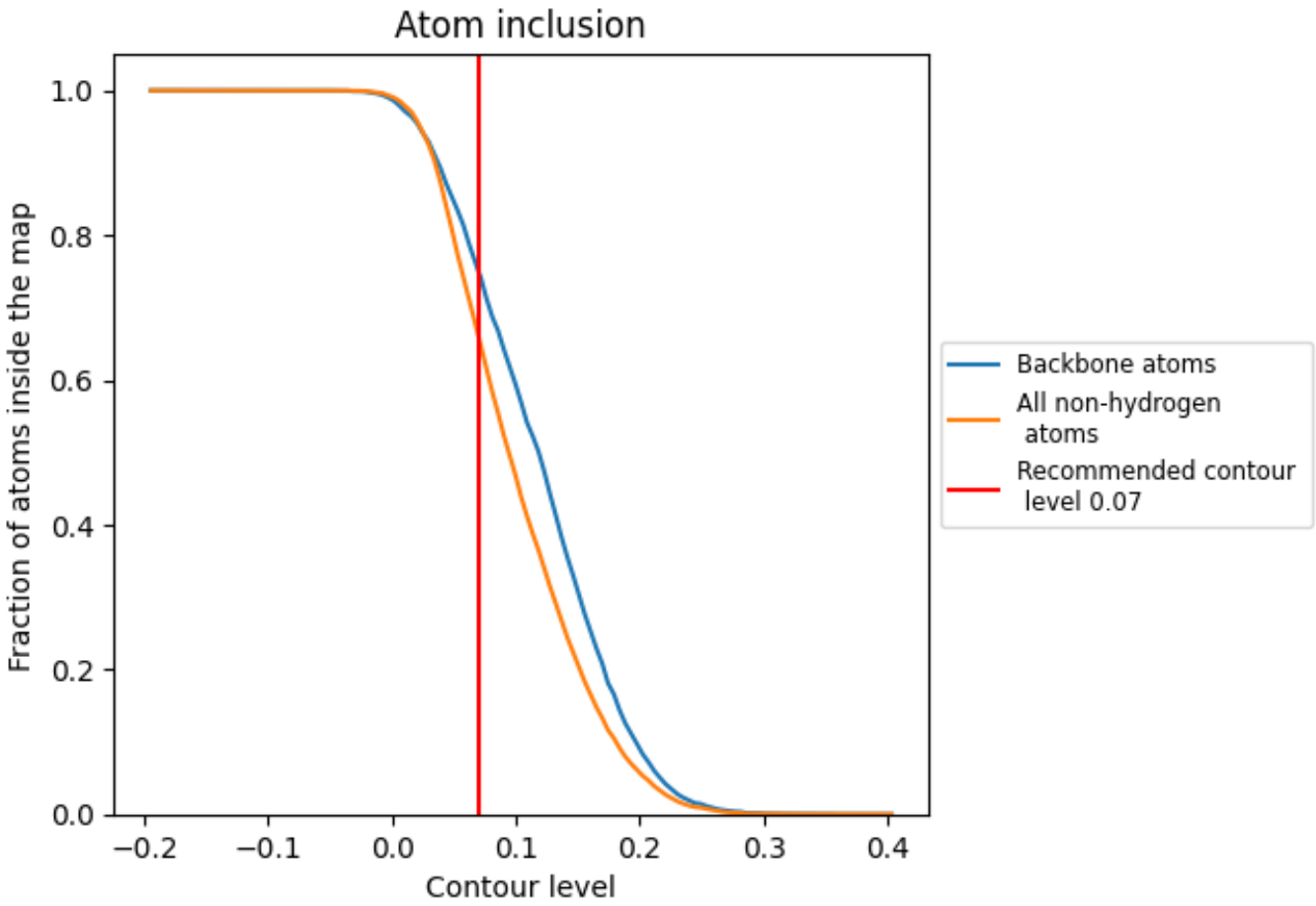
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.07).

9.4 Atom inclusion (i)



At the recommended contour level, 75% of all backbone atoms, 66% 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 (0.07) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.6596	0.4740
A	0.6606	0.4740
B	0.6587	0.4740