wwPDB EM Validation Summary Report (i)

Dec 10, 2022 – 01:25 pm GMT

PDB ID	:	6QP6
EMDB ID	:	EMD-4611
Title	:	Cryo-EM structure of calcium-bound 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	:	3.20 Å(reported)
Based on initial model	:	5YOB

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:

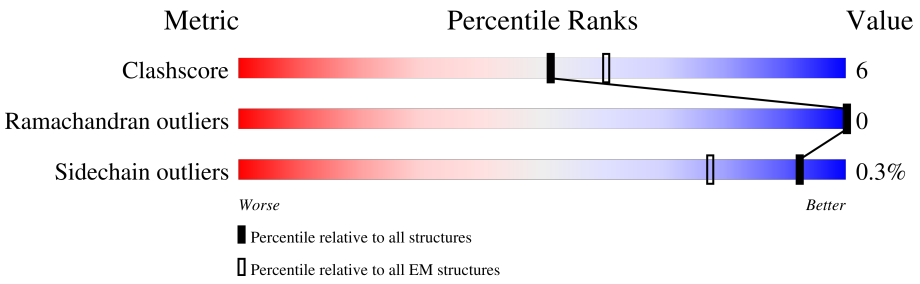
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.4, CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	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 3.20 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% 69% 12% 19%
1	B	911	10% 70% 11% 19%

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
3	P1O	A	1003	X	-	-	-
3	P1O	B	1003	X	-	-	-

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 12330 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.

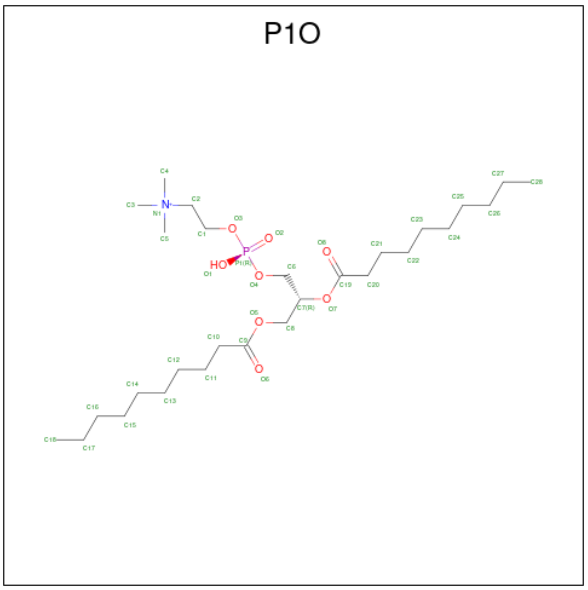
- Molecule 1 is a protein called Anoctamin-6.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	741	6124	4022	988	1074	40	0	0
1	B	741	6124	4022	988	1074	40	0	0

- Molecule 2 is CALCIUM ION (three-letter code: CA) (formula: Ca) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms		AltConf
			Total	Ca	
2	A	3	3	3	0
2	B	3	3	3	0

- Molecule 3 is 1,2-DIDECANOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (three-letter code: P1O) (formula: $C_{28}H_{57}NO_8P$) (labeled as "Ligand of Interest" by depositor).



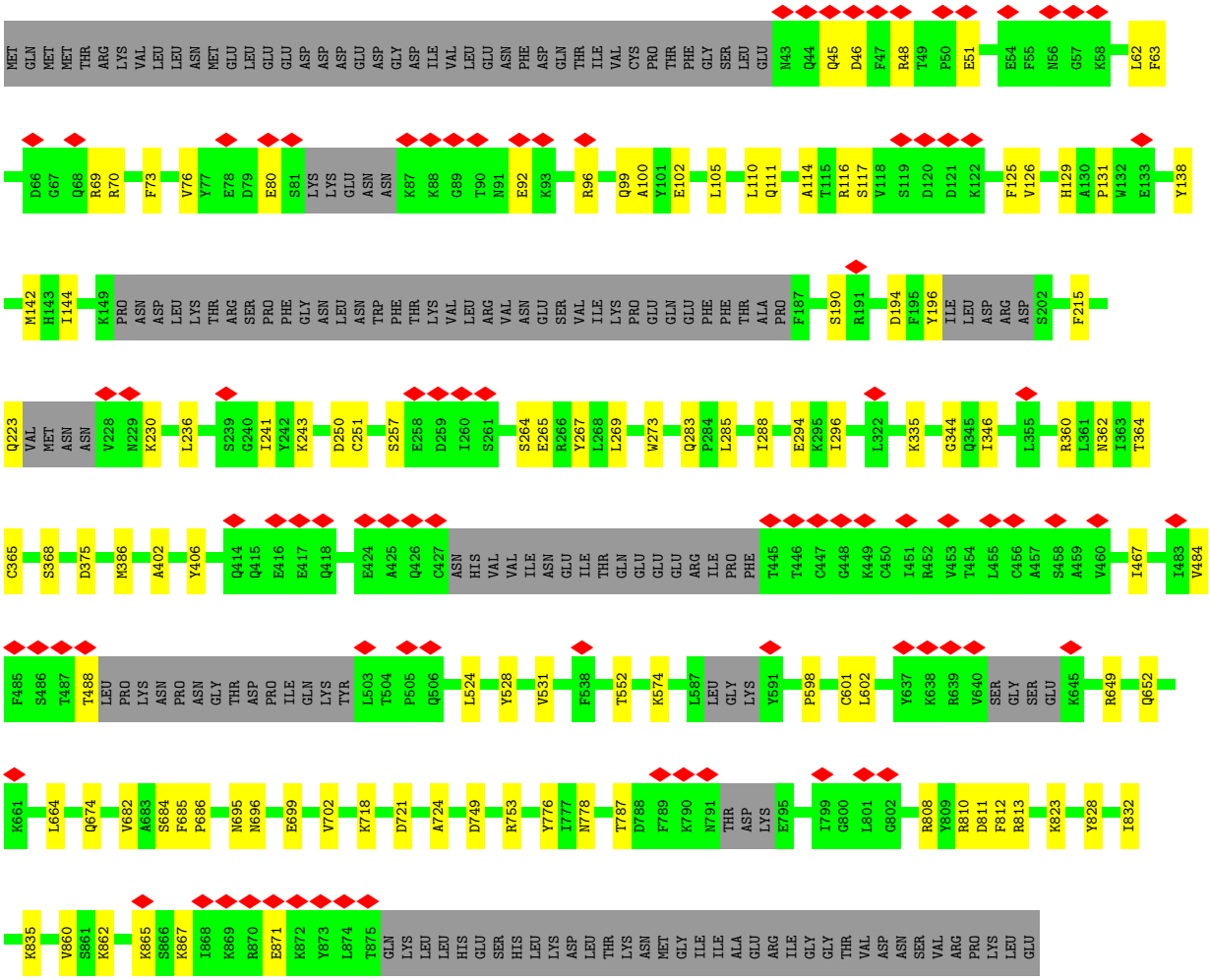
Mol	Chain	Residues	Atoms					AltConf
			Total	C	N	O	P	
3	A	1	38	28	1	8	1	0
			Total	C	N	O	P	
3	B	1	38	28	1	8	1	0

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.

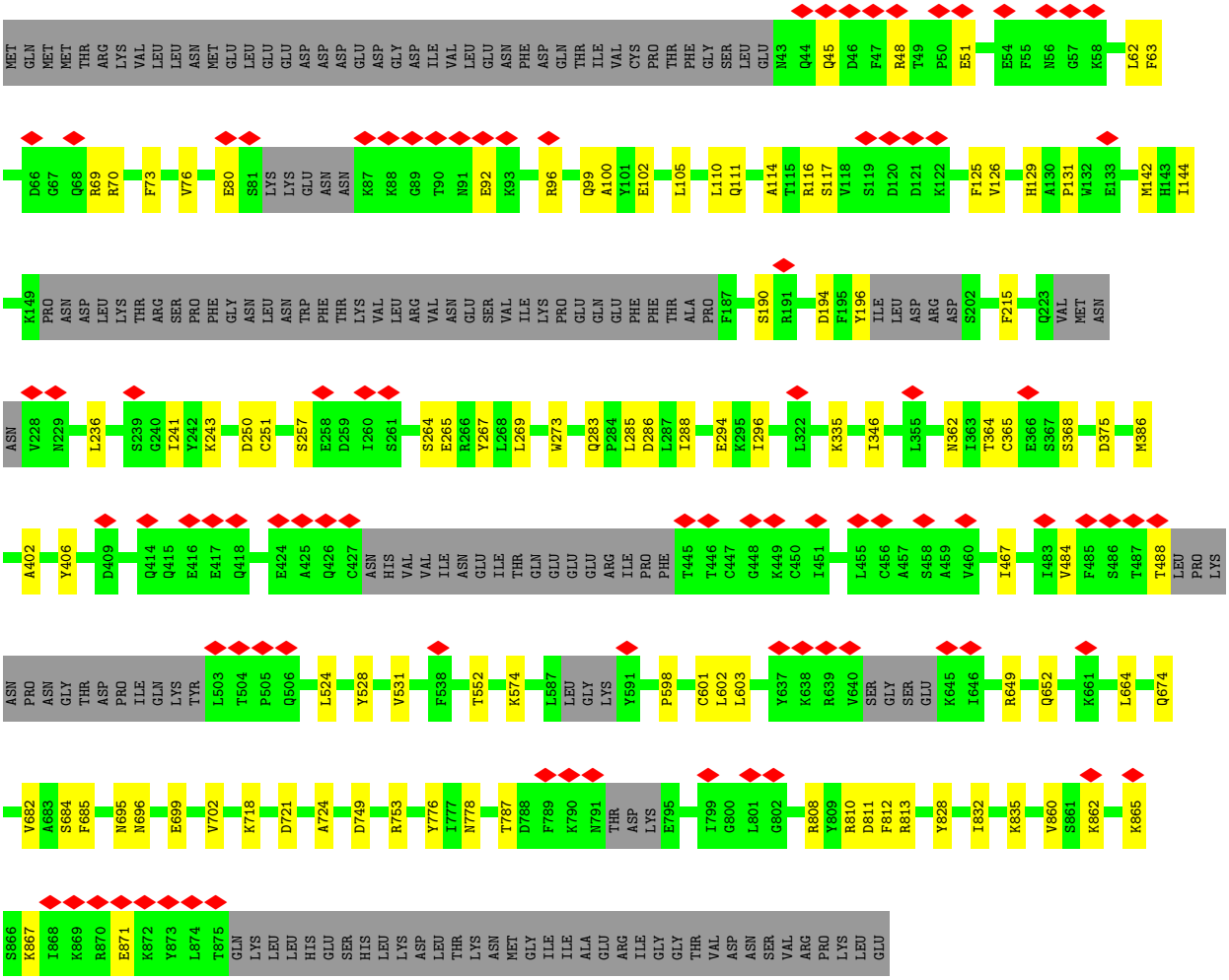
- Molecule 1: Anoctamin-6

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 N43 Q44 Q45 D46 F47 R48 T49 P50 E51 E54 F55 N56 G57 K58 L62 F63
D66 G67 Q68 R69 R70 F73 V76 E80 S81 LYS LYS GLU ASN ASN K87 K88 G89 T90 N91 E92 K93 R96 Q99 A100 Y101 E102 L105 L110 Q111 A114 T115 R116 S117 V118 S119 D120 D121 K122 F125 V126 H129 A130 P131 W132 E133 M142 H143 I144
K149 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 F187 S190 R191 D194 F195 Y196 ILE LEU ASP ARG ASP S202 F215 Q223 VAL MET ASN
ASN V228 N229 L236 S239 G240 I241 Y242 K243 D250 C251 S257 E258 D259 I260 S261 S264 E265 R266 Y267 L268 L269 W273 Q283 P284 L285 D286 L287 I288 E294 K295 I296 L322 K335 I346 L355 N362 I363 T364 C365 E366 S367 S368 D375 M386
A402 Y406 D409 Q414 Q415 E416 E417 Q418 E424 A425 Q426 C427 ASN HIS VAL VAL ILE ASN GLU ILE THR GLN GLU GLU GLU ARG ILE PRO PHE T445 T446 C447 G448 K449 C450 I451 L455 C456 A457 S458 A459 V460 I467 I483 V484 F485 S486 T487 T488 LEU PRO LYS
ASN PRO ASN GLY THR ASP PRO ILE GLN LYS TYR L503 T504 P505 Q506 L524 Y528 V531 F538 T552 K574 L587 LEU GLY LYS Y591 P598 C601 L602 L603 Y637 K638 R639 V640 SER GLY SER GLU K645 T646 R649 Q652 K661 L664 Q674
V682 A683 S684 F685 N695 N696 E699 V702 K718 D721 A724 D749 R753 Y776 I777 N778 T787 D788 F789 K790 N791 THR ASP LYS E795 I799 G800 L801 G802 R808 Y809 R810 D811 F812 R813 Y828 I832 K835 V860 S861 K862 K865
S866 K867 I868 K869 R870 E871 K872 Y873 L874 T875 GLN LYS 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	219302	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	0.352	Depositor
Minimum map value	-0.282	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.011	Depositor
Recommended contour level	0.04	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, 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	A	0.48	0/6289	0.58	0/8518
1	B	0.48	0/6289	0.58	0/8518
All	All	0.48	0/12578	0.58	0/17036

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	6124	0	6085	75	0
1	B	6124	0	6085	67	0
2	A	3	0	0	0	0
2	B	3	0	0	0	0
3	A	38	0	56	0	0
3	B	38	0	56	0	0
All	All	12330	0	12282	142	0

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

The worst 5 of 142 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:B:467:ILE:HD13	1:B:524:LEU:CD2	1.48	1.43
1:A:467:ILE:HD13	1:A:524:LEU:CD2	1.48	1.40
1:A:467:ILE:CD1	1:A:524:LEU:HD23	1.83	1.08
1:B:467:ILE:CD1	1:B:524:LEU:HD23	1.83	1.08
1:A:467:ILE:HD13	1:A:524:LEU:HD23	0.99	0.98

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	721/911 (79%)	701 (97%)	20 (3%)	0	100	100
1	B	721/911 (79%)	701 (97%)	20 (3%)	0	100	100
All	All	1442/1822 (79%)	1402 (97%)	40 (3%)	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	668/827 (81%)	666 (100%)	2 (0%)	92	96
1	B	668/827 (81%)	666 (100%)	2 (0%)	92	96

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
All	All	1336/1654 (81%)	1332 (100%)	4 (0%)	92 96

All (4) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	73	PHE
1	A	196	TYR
1	B	73	PHE
1	B	196	TYR

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 16 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	730	GLN
1	B	632	ASN
1	B	99	GLN
1	B	549	ASN
1	A	814	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)

Of 8 ligands modelled in this entry, 6 are monoatomic - leaving 2 for Mogul analysis.

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	$\#\|Z\| > 2$
3	P1O	A	1003	-	37,37,37	1.51	7 (18%)	43,45,45	1.00	2 (4%)
3	P1O	B	1003	-	37,37,37	1.51	7 (18%)	43,45,45	1.00	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
3	P1O	A	1003	-	1/1/5/5	20/41/41/41	-
3	P1O	B	1003	-	1/1/5/5	20/41/41/41	-

The worst 5 of 14 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	B	1003	P1O	P1-O3	4.64	1.78	1.59
3	A	1003	P1O	P1-O3	4.63	1.78	1.59
3	B	1003	P1O	C20-C19	2.77	1.58	1.50
3	A	1003	P1O	C20-C19	2.74	1.58	1.50
3	A	1003	P1O	O7-C19	2.49	1.41	1.34

All (4) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	B	1003	P1O	O7-C19-C20	3.21	118.42	111.50
3	A	1003	P1O	O7-C19-C20	3.20	118.40	111.50
3	B	1003	P1O	O5-C9-C10	2.42	119.49	111.91
3	A	1003	P1O	O5-C9-C10	2.39	119.40	111.91

All (2) chirality outliers are listed below:

Mol	Chain	Res	Type	Atom
3	A	1003	P1O	C7
3	B	1003	P1O	C7

5 of 40 torsion outliers are listed below:

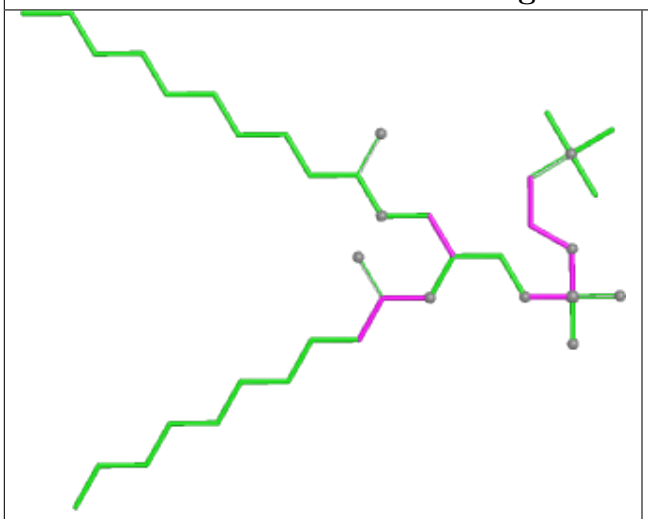
Mol	Chain	Res	Type	Atoms
3	A	1003	P1O	C1-O3-P1-O4
3	B	1003	P1O	C1-O3-P1-O4
3	A	1003	P1O	C24-C25-C26-C27
3	B	1003	P1O	C24-C25-C26-C27
3	A	1003	P1O	C9-C10-C11-C12

There are no ring outliers.

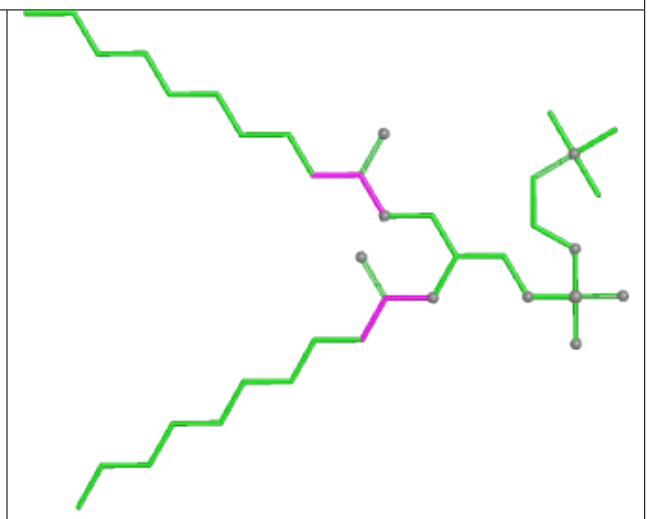
No monomer is involved in short contacts.

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.

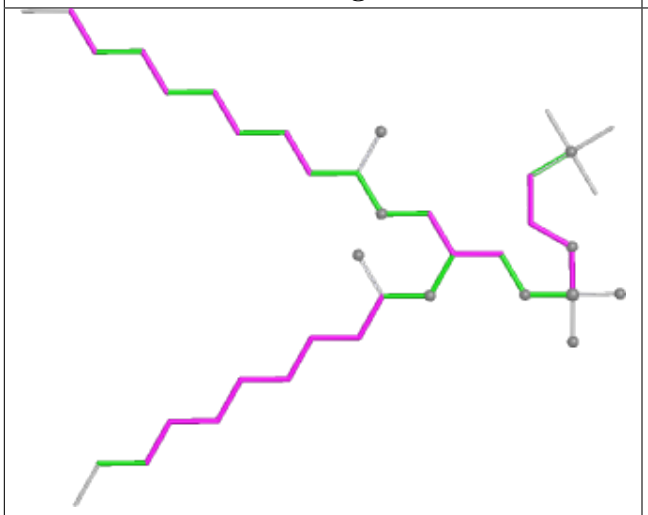
Ligand P1O A 1003



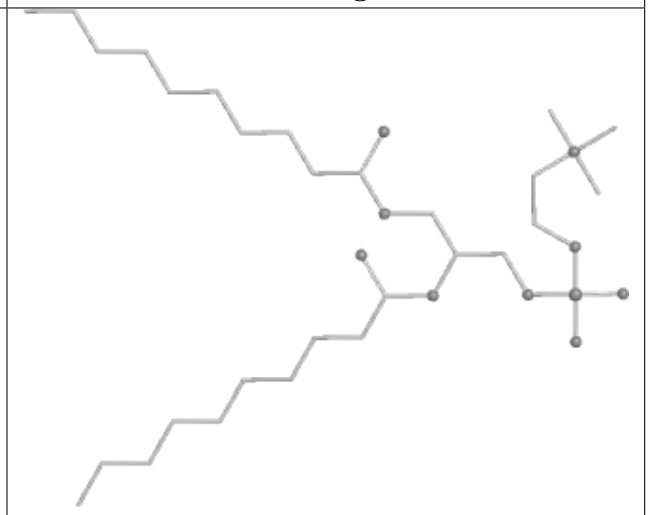
Bond lengths



Bond angles

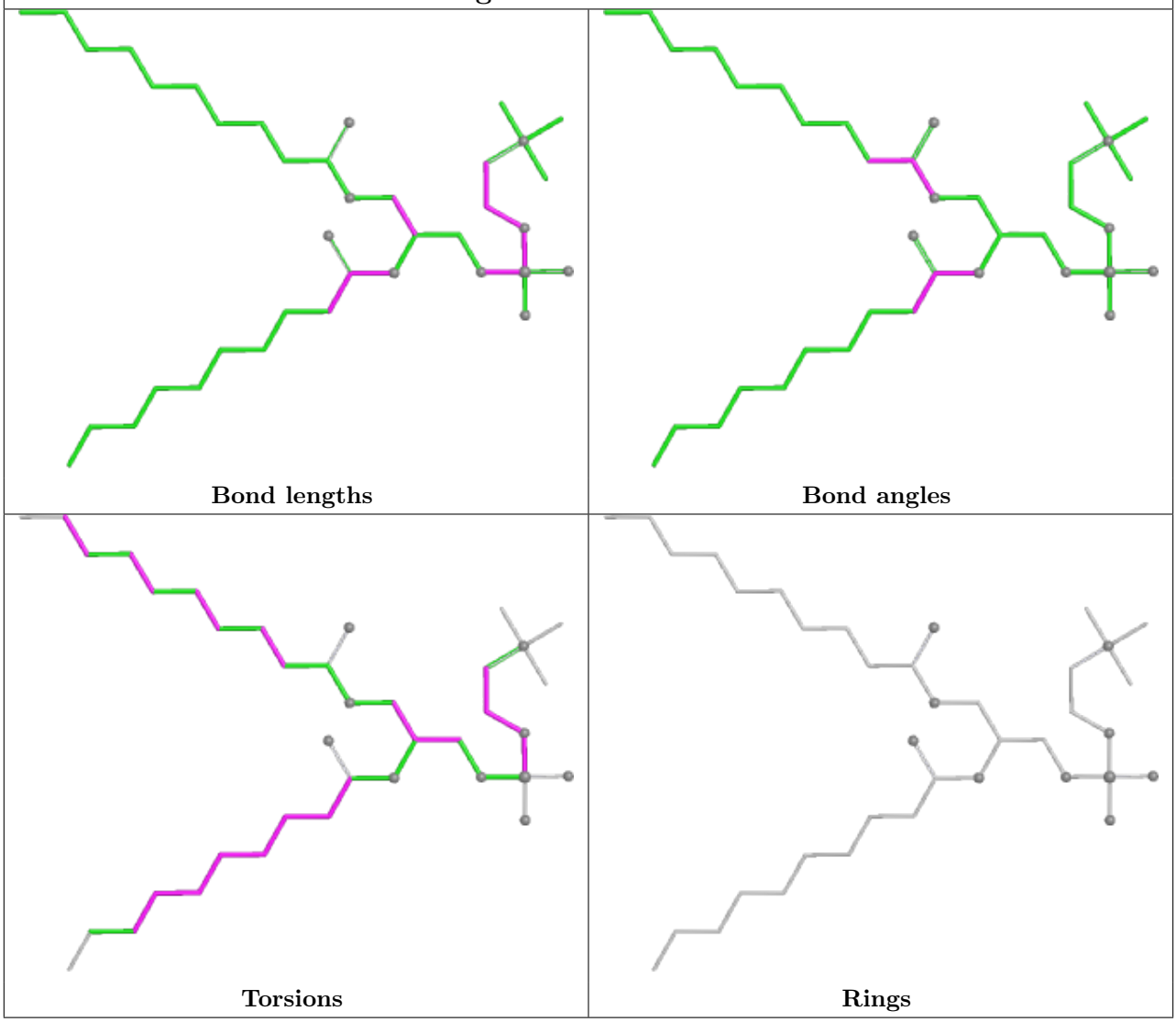


Torsions



Rings

Ligand P1O B 1003



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.

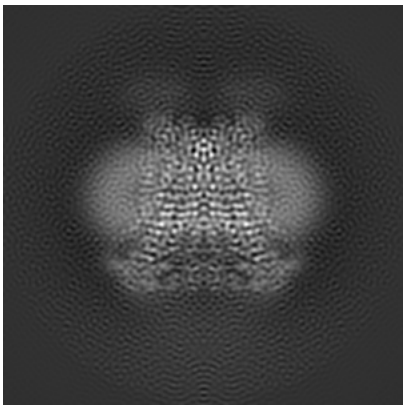
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-4611. These allow visual inspection of the internal detail of the map and identification of artifacts.

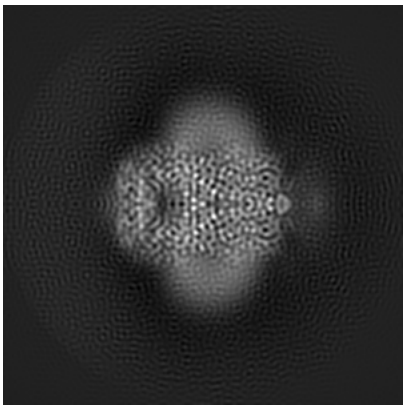
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections (i)

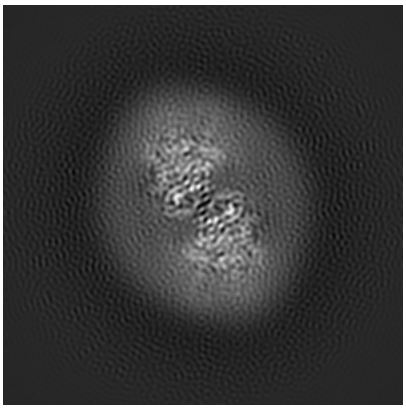
6.1.1 Primary map



X

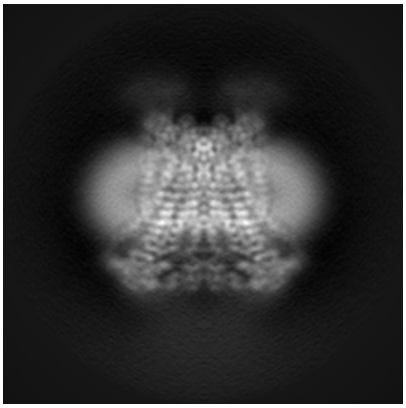


Y

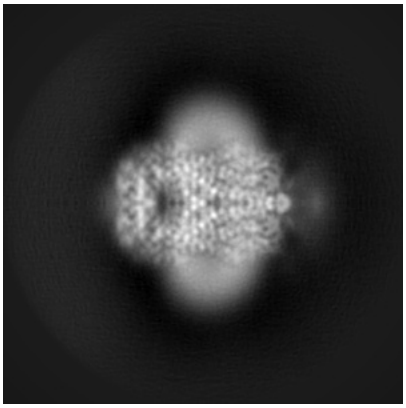


Z

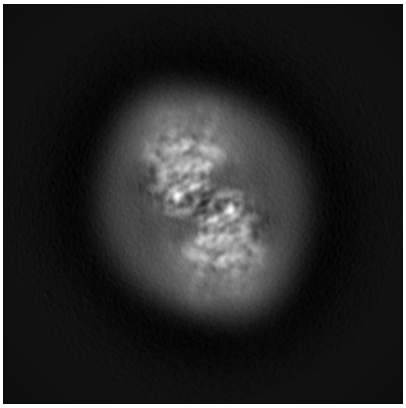
6.1.2 Raw map



X



Y

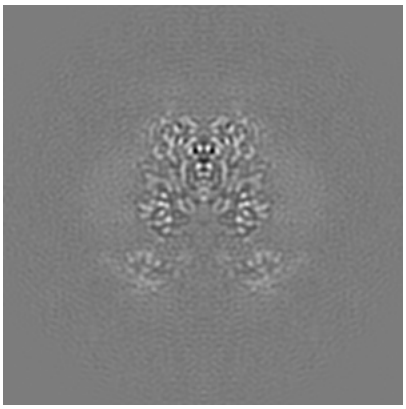


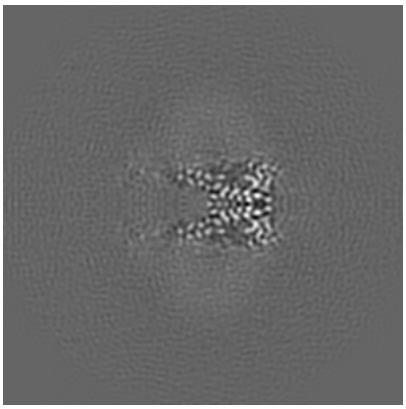
Z

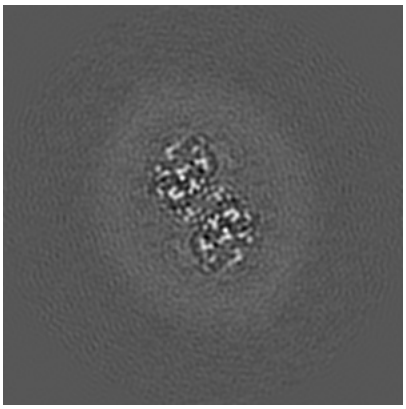
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

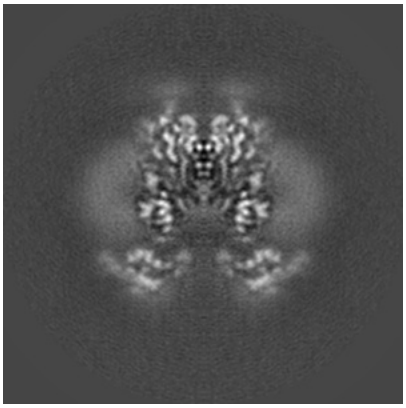
6.2.1 Primary map

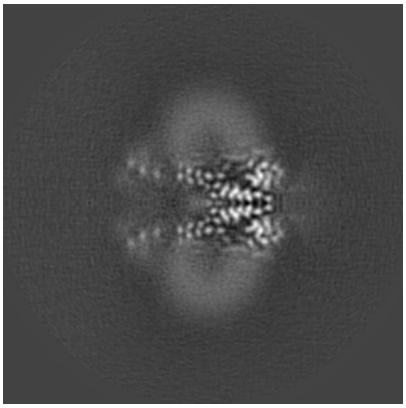

X Index: 110

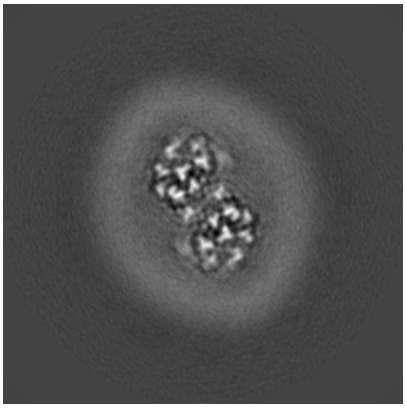

Y Index: 110


Z Index: 110

6.2.2 Raw map


X Index: 110

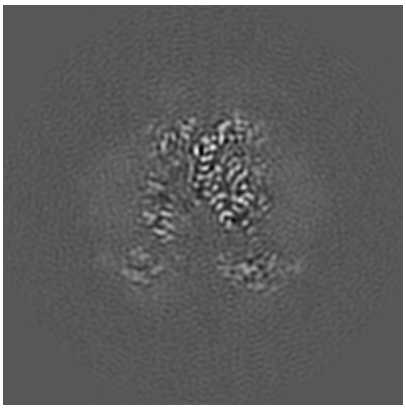

Y Index: 110

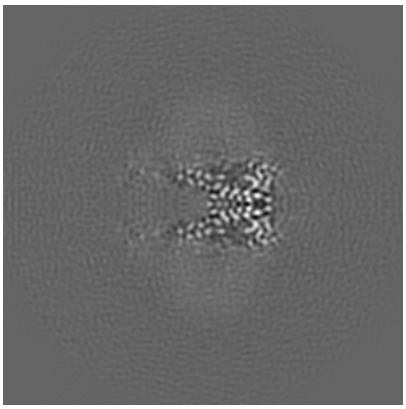

Z Index: 110

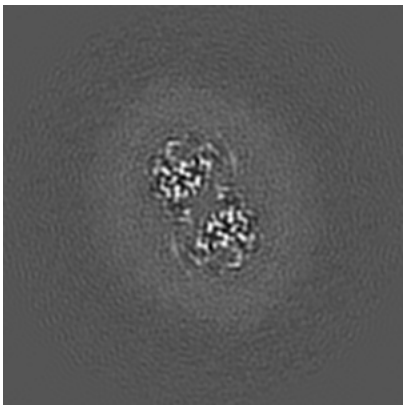
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

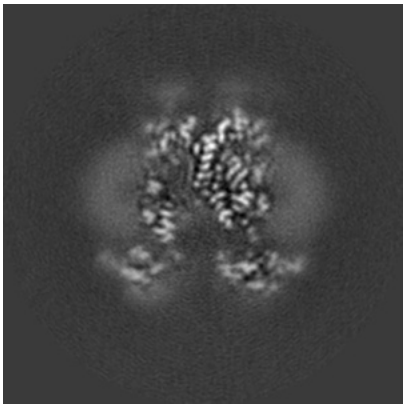
6.3.1 Primary map

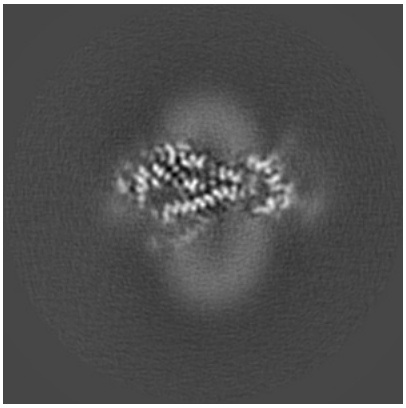

X Index: 107

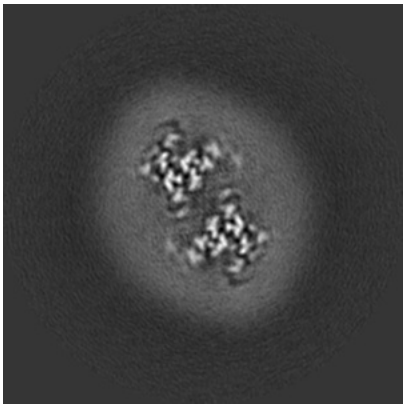

Y Index: 110


Z Index: 105

6.3.2 Raw map


X Index: 107


Y Index: 89


Z Index: 100

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

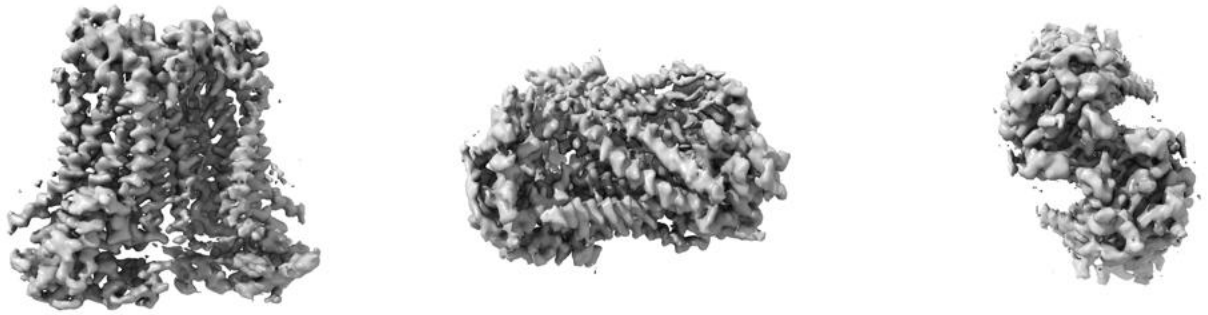
6.4 Orthogonal surface views (i)

6.4.1 Primary map

X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.04. 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_4611_msk_1.map (i)

X

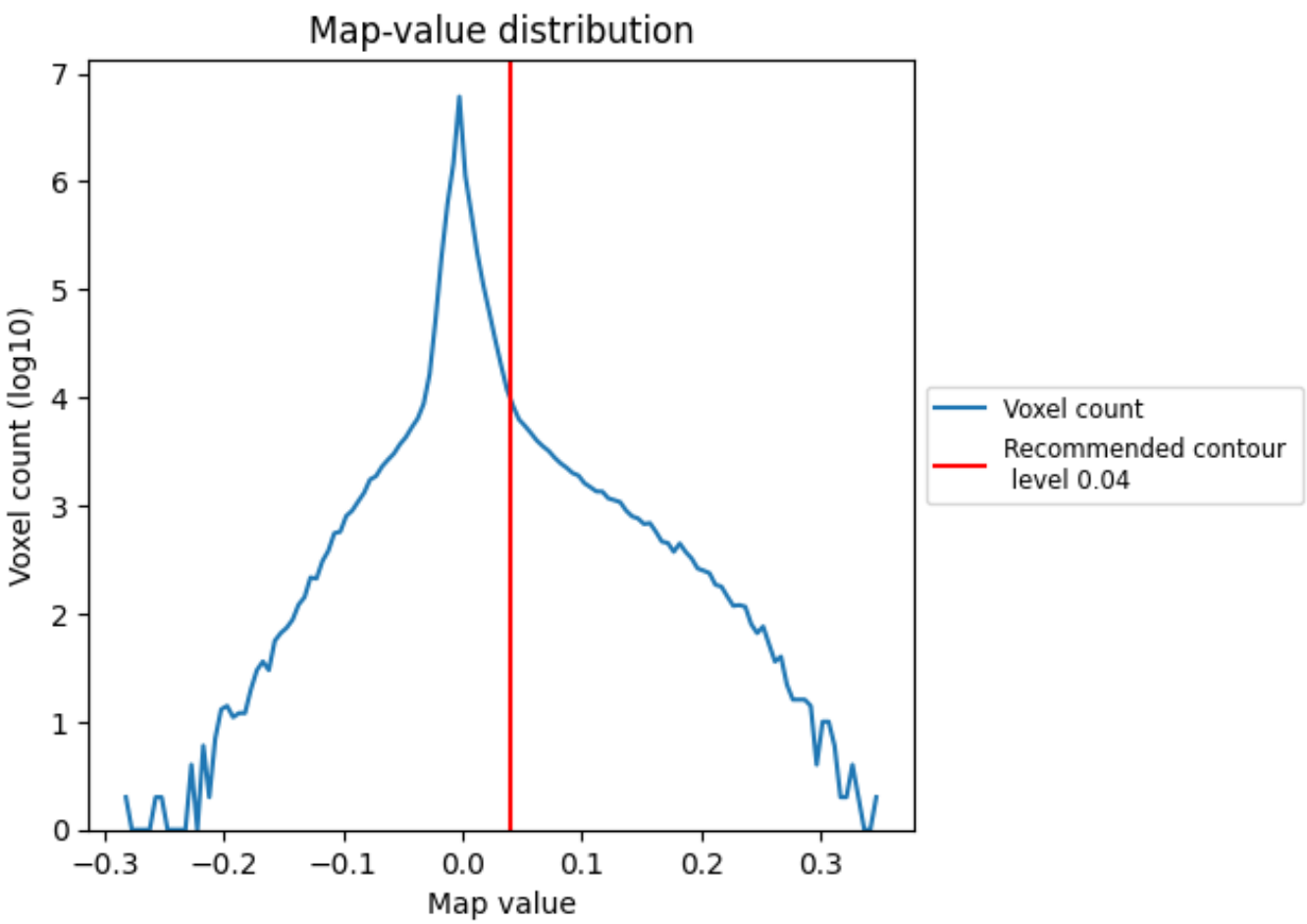
Y

Z

7 Map analysis (i)

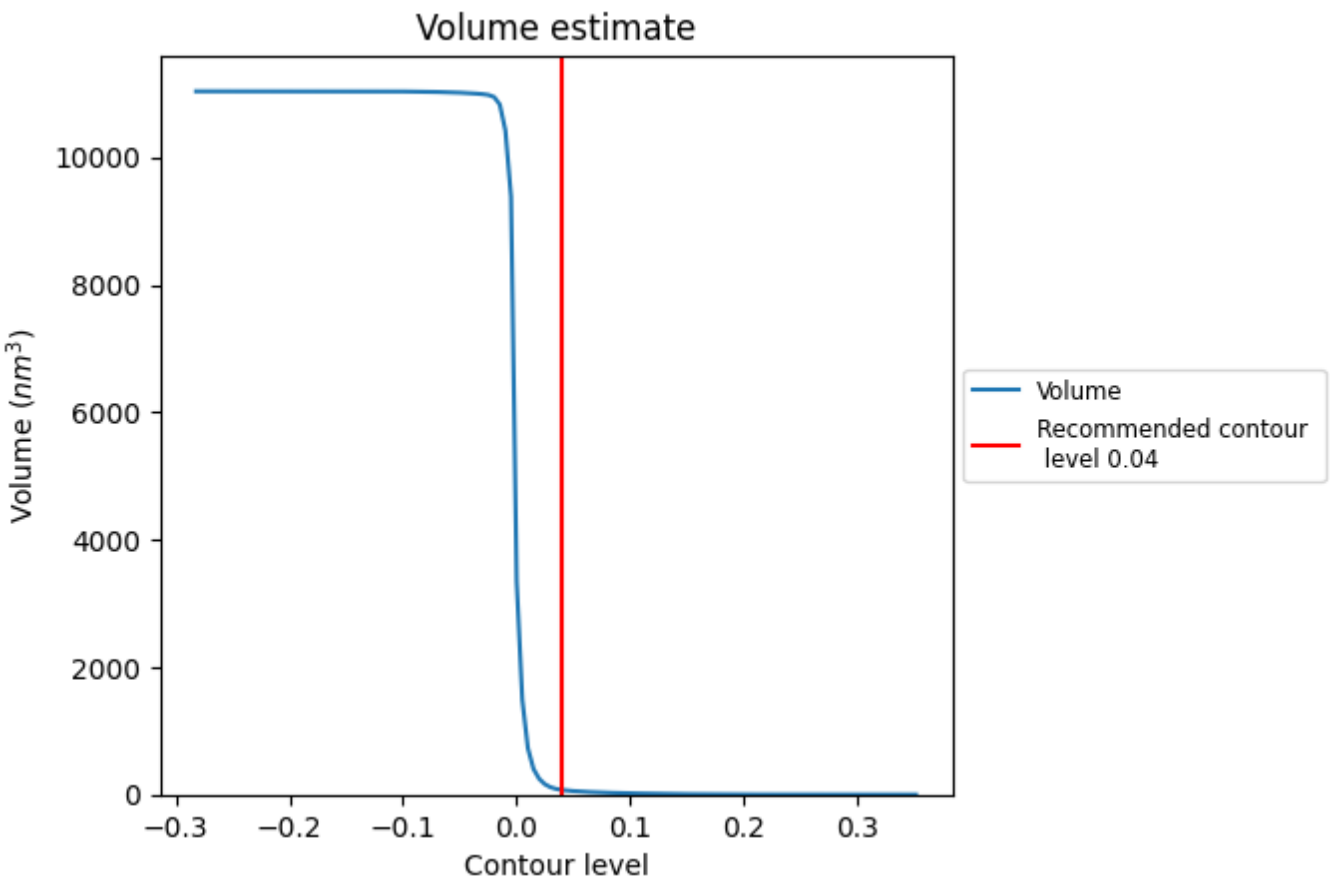
This section contains the results of statistical analysis of the map.

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.

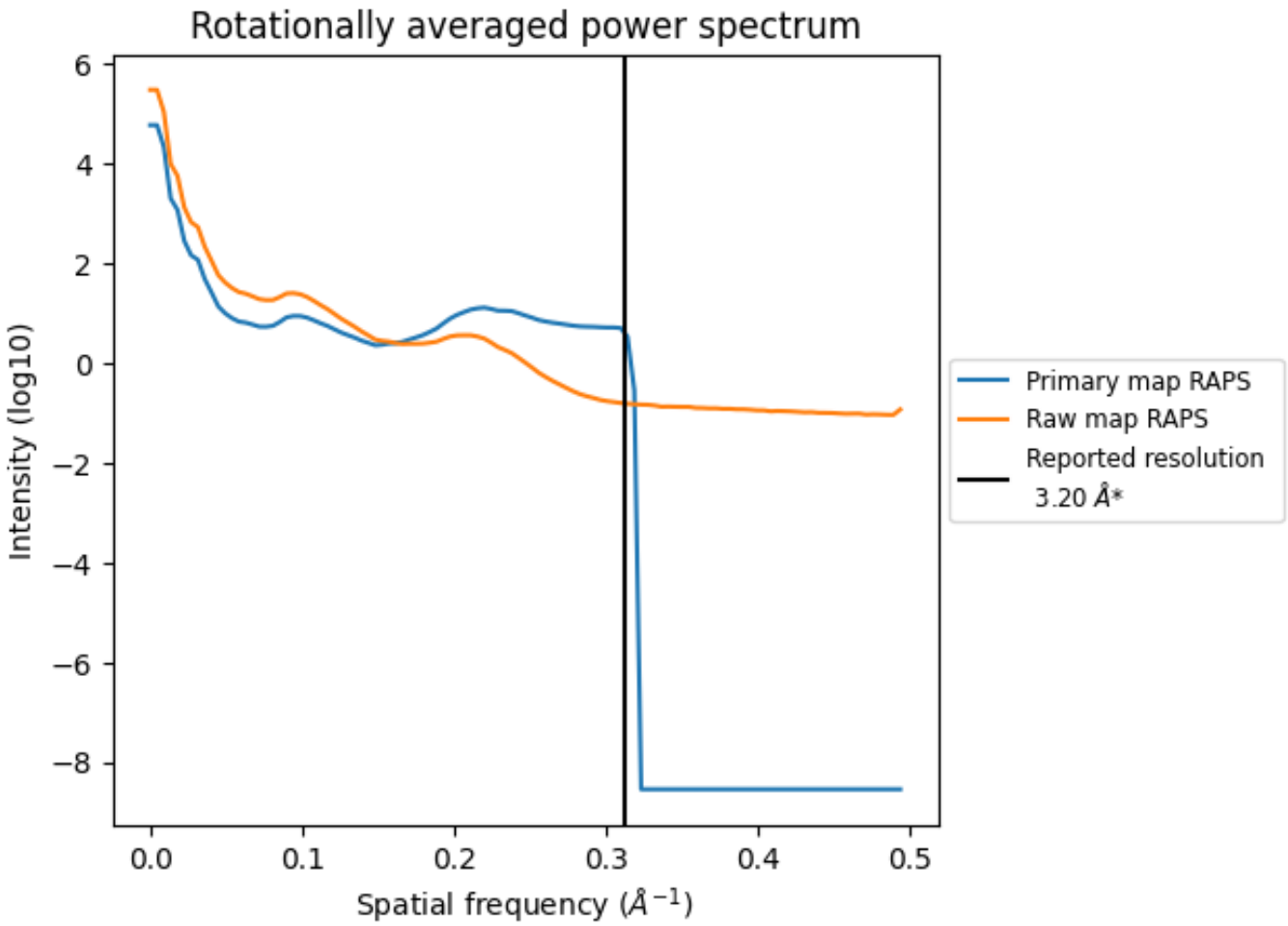
7.2 Volume estimate (i)



The volume at the recommended contour level is 73 nm^3; this corresponds to an approximate mass of 66 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)

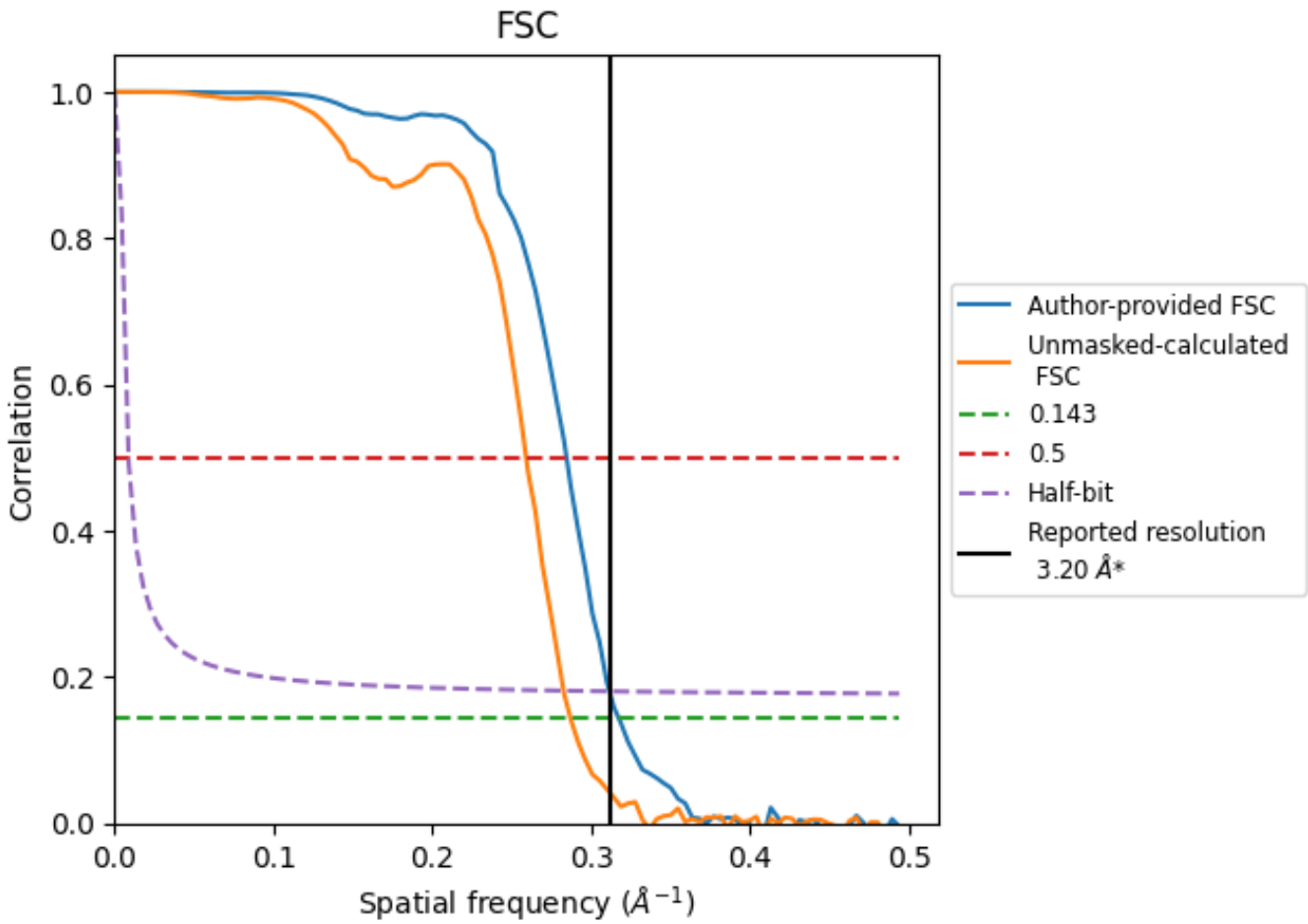


*Reported resolution corresponds to spatial frequency of 0.312 Å^{-1}

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 0.312 Å^{-1}

8.2 Resolution estimates (i)

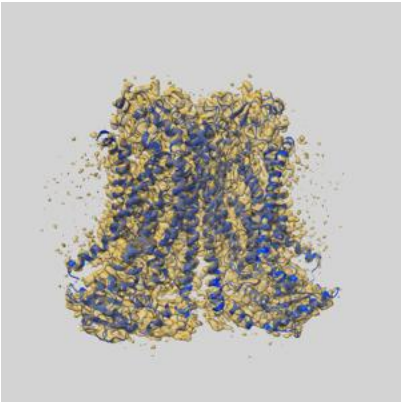
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.20	-	-
Author-provided FSC curve	3.15	3.51	3.21
Unmasked-calculated*	3.48	3.86	3.54

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

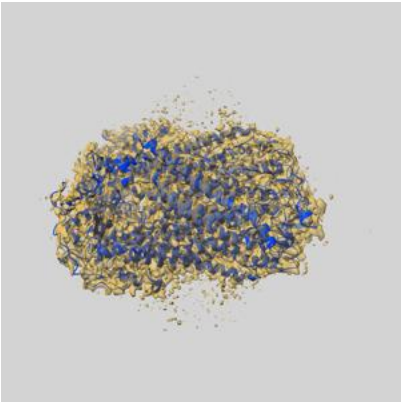
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-4611 and PDB model 6QP6. Per-residue inclusion information can be found in section 3 on page 5.

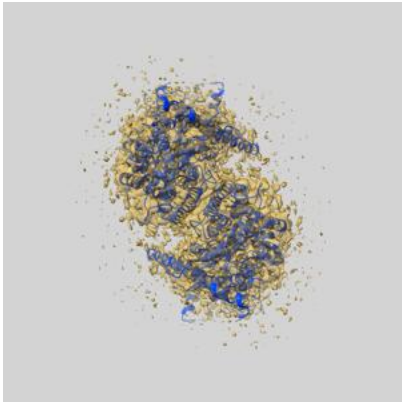
9.1 Map-model overlay (i)



X



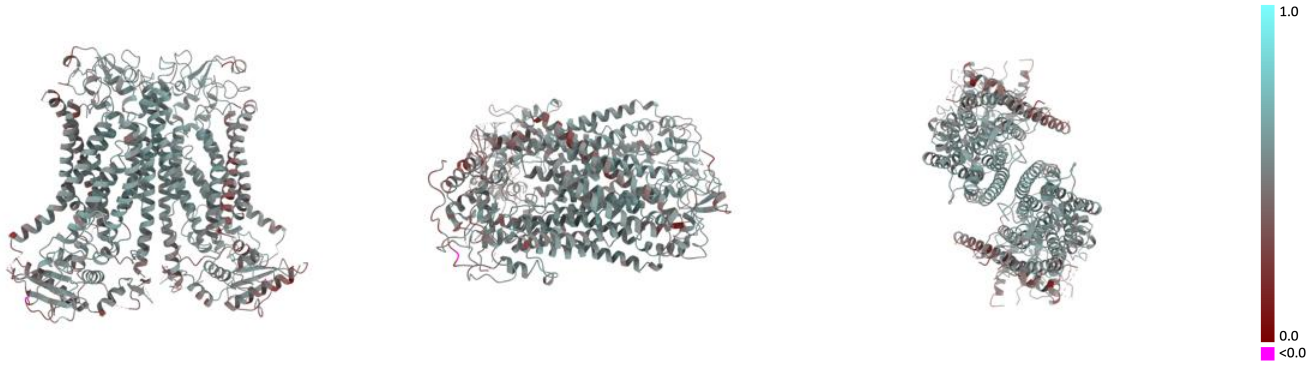
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.04 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.

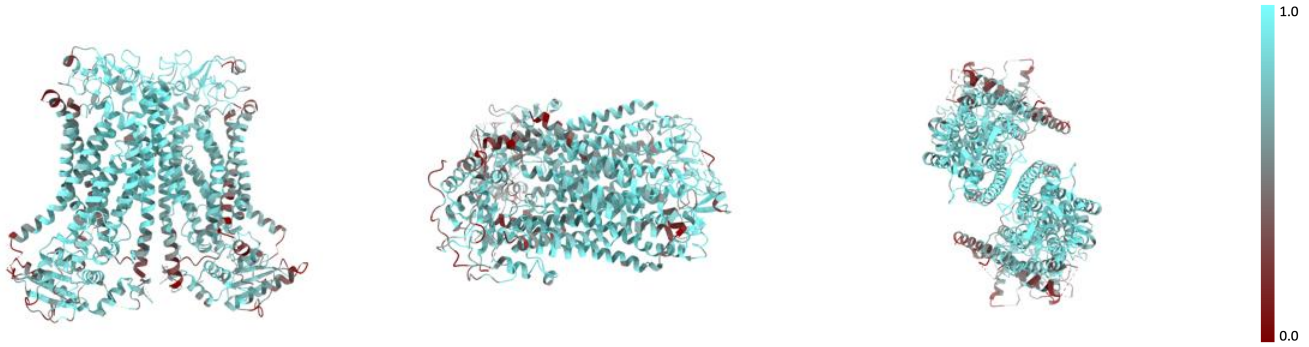
9.2 Q-score mapped to coordinate model (i)



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.

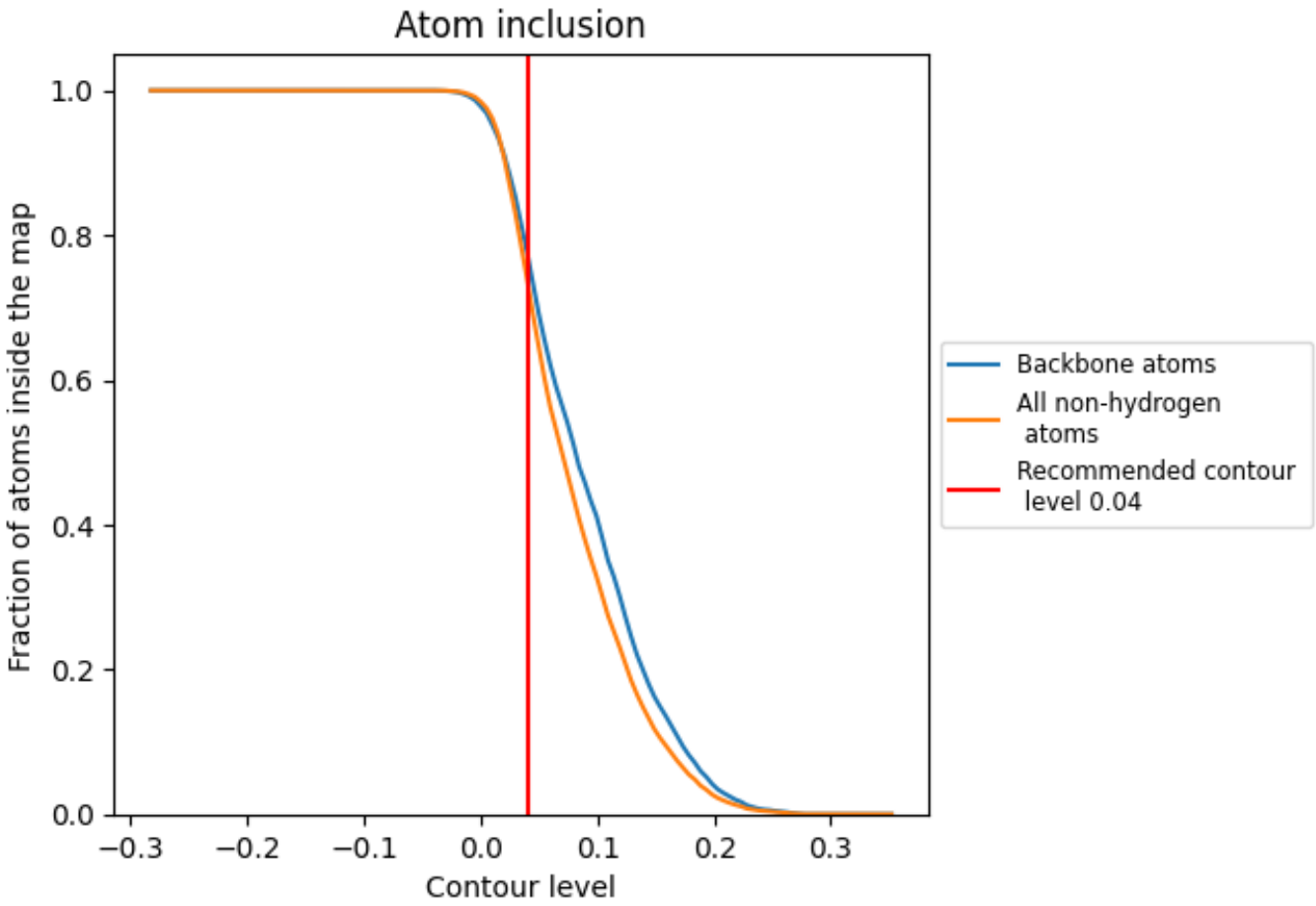
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.04).

9.4 Atom inclusion (i)



At the recommended contour level, 78% of all backbone atoms, 74% 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 (0.04) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.7377	0.5050
A	0.7381	0.5050
B	0.7373	0.5050