wwPDB EM Validation Summary Report (i)

Dec 10, 2022 – 01:30 pm GMT

PDB ID	:	6QPC
EMDB ID	:	EMD-4613
Title	:	Cryo-EM structure of calcium-bound mTMEM16F lipid scramblase in nanodisc
Authors	:	Alvadia, C.; Lim, N.K.; Clerico Mosina, V.; Oostergetel, G.T.; Dutzler, R.; Paulino, C.
Deposited on	:	2019-02-13
Resolution	:	3.50 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:

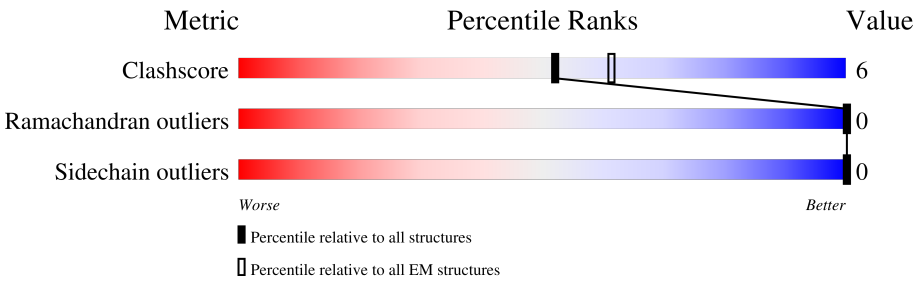
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.4, CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	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 3.50 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	54% 10% 37%
1	B	911	53% 10% 37%

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 9140 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.

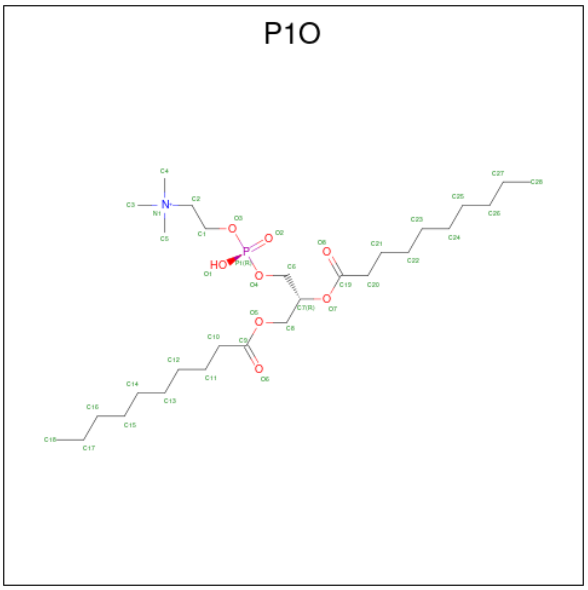
- Molecule 1 is a protein called Anoctamin-6.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	577	4530	2992	726	781	31	0	0
1	B	577	4530	2992	726	781	31	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	2	2	2	0
2	B	2	2	2	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).



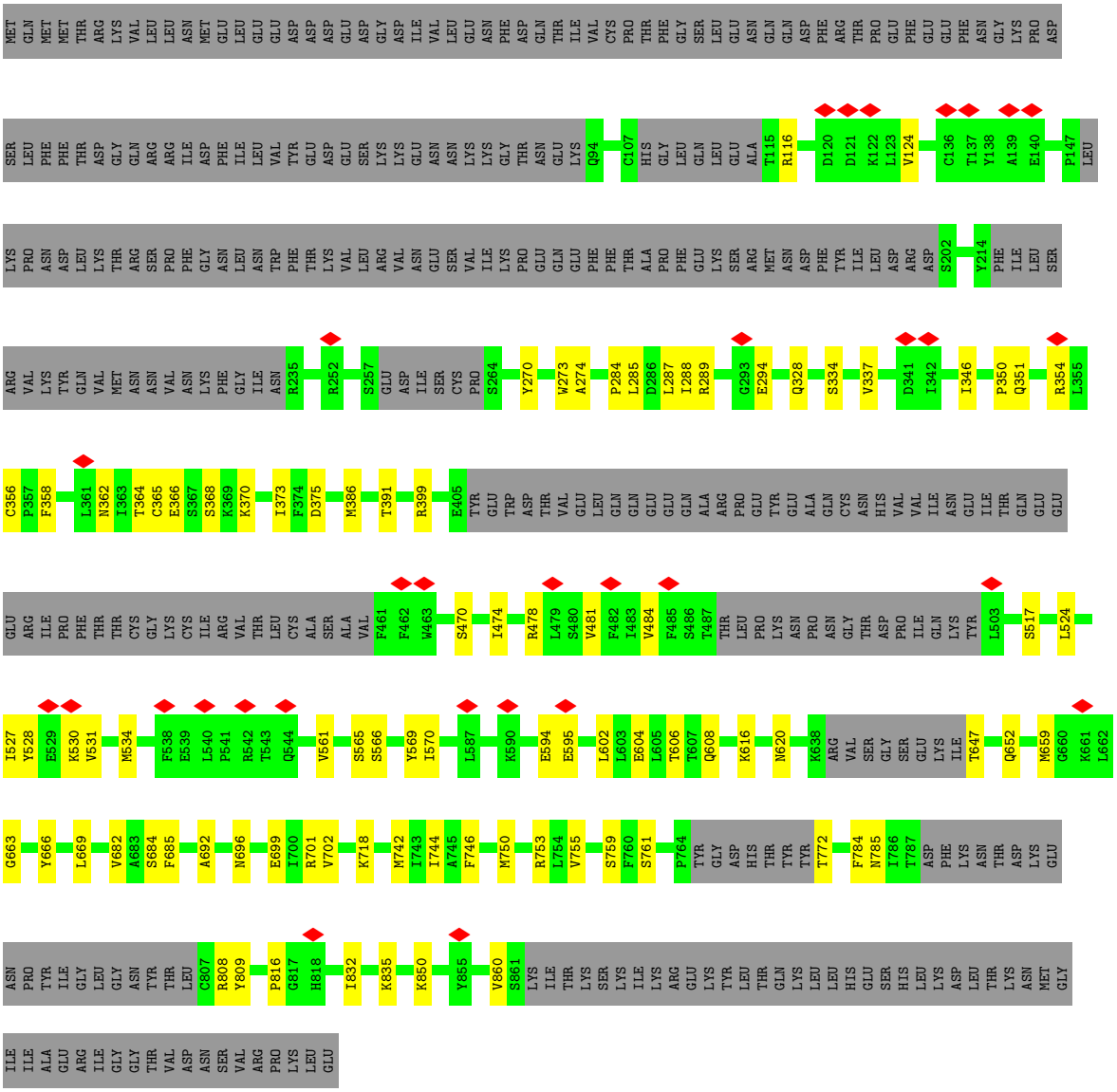
Mol	Chain	Residues	Atoms					AltConf
			Total	C	N	O	P	
3	A	1	38	28	1	8	1	0
			Total	C	N	O	P	
3	B	1	38	28	1	8	1	0

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

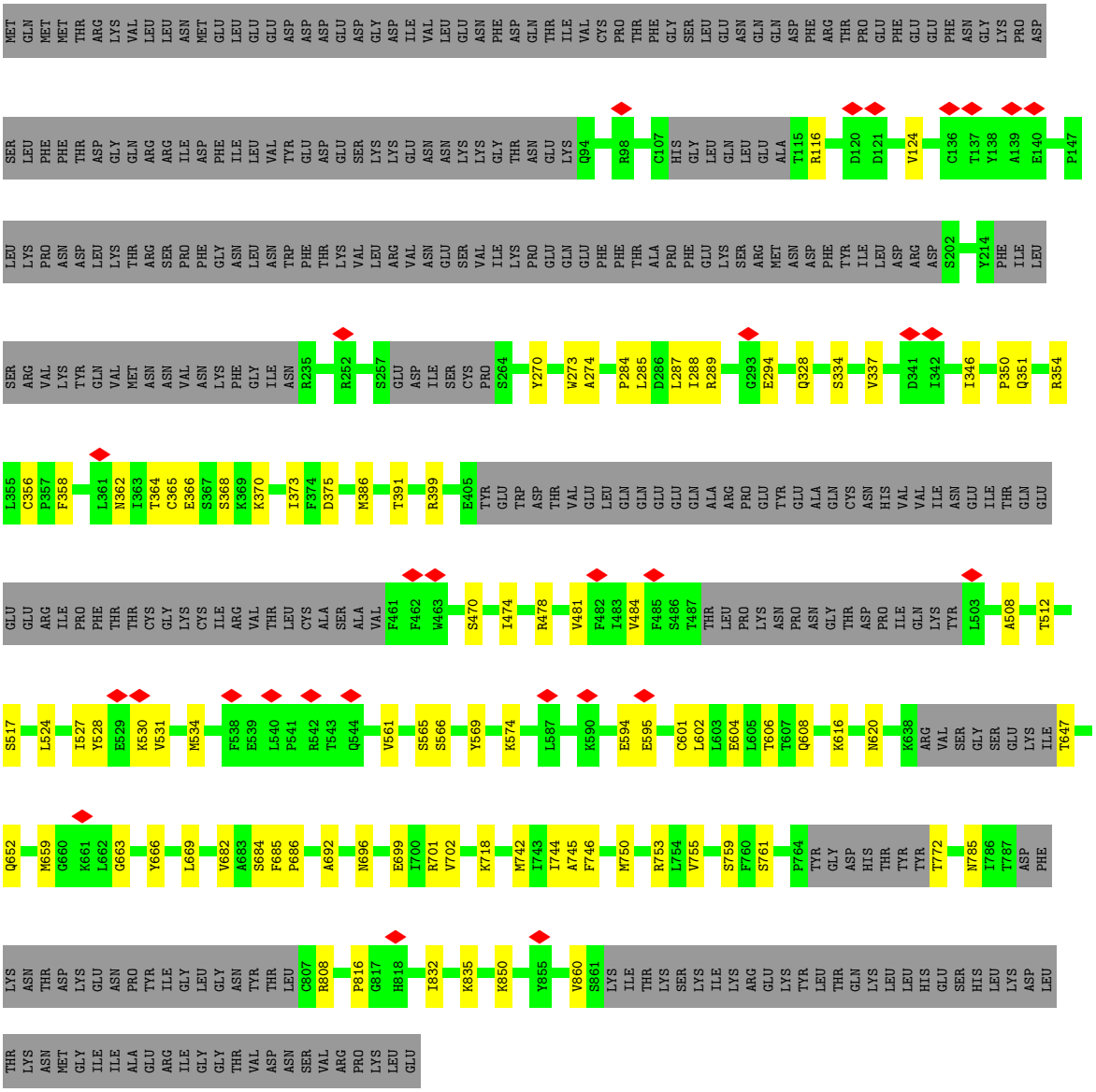
Chain A: 54% 10% 37%



- Molecule 1: Anoctamin-6

Chain B:

53%
10%
37%


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 GLN ASP PHE ARG THR PRO GLU PHE GLU GLU PHE ASN GLY LYS PRO ASP
SER LEU PHE PHE THR ASP GLY GLN ARG ARG ILE ASP PHE ILE LEU VAL TYR GLU ASP GLU SER LYS LYS GLU ASN ASN LYS LYS GLY THR ASN GLU LYS Q94 R98 C107 HIS GLY LEU GLN LEU GLU ALA T115 R116 D120 D121 V124 C136 T137 Y138 A139 E140 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 PHE TYR ILE LEU ASP ARG ASP S202 Y214 PHE ILE LEU
SER ARG VAL LYS TYR GLN VAL MET ASN ASN VAL ASN LYS PHE GLY ILE ASN R235 R252 S257 GLU ASP ILE SER CYS PRO S264 Y270 W273 A274 P284 L285 D286 L287 I288 R289 G293 E294 Q328 S334 V337 D341 I342 I346 P350 Q351 R354
L355 C356 P357 F358 L361 N362 I363 T364 C365 E366 S367 S368 K369 K370 I373 F374 D375 M386 T391 R399 E405 TYR GLU TRP ASP THR VAL GLU LEU GLN GLN GLU GLU GLN ALA ARG PRO GLU TYR GLU ALA GLN CYS ASN HIS VAL VAL ILE ASN GLU ILE THR GLN GLU
GLU GLU ARG ILE PRO PHE THR THR CYS GLY LYS CYS ILE ARG VAL THR LEU CYS ALA SER ALA VAL F461 F462 W463 S470 I474 R478 V481 F482 I483 V484 F485 S486 T487 THR LEU PRO LYS ASN PRO ASN GLY THR ASP PRO ILE GLN LYS TYR L503 A508 T512
S517 L524 I527 Y528 E529 K530 V531 M534 F538 E539 L540 P541 R542 T543 Q544 V561 S565 S566 Y569 K574 L587 K590 E594 E595 C601 L602 L603 E604 L605 T606 T607 Q608 K616 N620 K638 ARG VAL SER GLY SER GLU LYS ILE T647
Q652 M659 G660 K661 L662 G663 Y666 L669 V682 A683 S684 F685 P686 A692 N696 E699 I700 R701 V702 K718 M742 I743 I744 A745 F746 M750 R753 L754 V755 S759 F760 S761 P764 TYR GLY ASP HIS THR TYR TYR T772 N785 I786 T787 ASP PHE
LYS ASN THR ASP LYS GLU ASN PRO TYR ILE GLY LEU GLY ASN TYR THR LEU C807 R808 P816 G817 H818 I832 K835 K850 Y855 V860 S861 LYS ILE THR LYS SER LYS ILE LYS ARG GLU LYS TYR LEU THR 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, Not provided	
Number of particles used	186487	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	0.283	Depositor
Minimum map value	-0.169	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.010	Depositor
Recommended contour level	0.05	Depositor
Map size (Å)	259.072, 259.072, 259.072	wwPDB
Map dimensions	256, 256, 256	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: CA, 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	0.42	0/4649	0.55	0/6322
1	B	0.42	0/4649	0.55	0/6322
All	All	0.42	0/9298	0.55	0/12644

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	A	0	2
1	B	0	2
All	All	0	4

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (4) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	354	ARG	Peptide
1	A	356	CYS	Peptide
1	B	354	ARG	Peptide
1	B	356	CYS	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	A	4530	0	4327	50	0
1	B	4530	0	4327	52	0
2	A	2	0	0	0	0
2	B	2	0	0	0	0
3	A	38	0	54	0	0
3	B	38	0	54	0	0
All	All	9140	0	8762	102	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 102 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:616:LYS:O	1:B:620:ASN:HB2	1.87	0.75
1:A:616:LYS:O	1:A:620:ASN:HB2	1.87	0.75
1:B:481:VAL:HG21	1:B:517:SER:HB3	1.76	0.68
1:A:481:VAL:HG21	1:A:517:SER:HB3	1.76	0.66
1:A:375:ASP:O	1:A:753:ARG:NH1	2.32	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	555/911 (61%)	541 (98%)	14 (2%)	0	100 100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	B	555/911 (61%)	541 (98%)	14 (2%)	0	100	100
All	All	1110/1822 (61%)	1082 (98%)	28 (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	454/827 (55%)	454 (100%)	0	100	100
1	B	454/827 (55%)	454 (100%)	0	100	100
All	All	908/1654 (55%)	908 (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	608	GLN
1	B	695	ASN
1	B	730	GLN
1	A	695	ASN
1	A	730	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)

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 6 ligands modelled in this entry, 4 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	B	1003	1	37,37,37	1.45	6 (16%)	43,45,45	0.91	3 (6%)
3	P1O	A	1003	1	37,37,37	1.45	6 (16%)	43,45,45	0.91	3 (6%)

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	B	1003	1	1/1/5/5	19/41/41/41	-
3	P1O	A	1003	1	1/1/5/5	19/41/41/41	-

The worst 5 of 12 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	1003	P1O	P1-O3	4.49	1.77	1.59
3	B	1003	P1O	P1-O3	4.49	1.77	1.59
3	A	1003	P1O	C20-C19	2.77	1.58	1.50
3	B	1003	P1O	C20-C19	2.75	1.58	1.50
3	B	1003	P1O	C2-C1	2.57	1.59	1.51

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

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	A	1003	P1O	O7-C19-C20	3.06	118.09	111.50

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Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	B	1003	P1O	O7-C19-C20	3.05	118.08	111.50
3	A	1003	P1O	O1-P1-O2	2.17	122.98	112.24
3	B	1003	P1O	O1-P1-O2	2.17	122.97	112.24
3	B	1003	P1O	O5-C9-C10	2.12	118.57	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 38 torsion outliers are listed below:

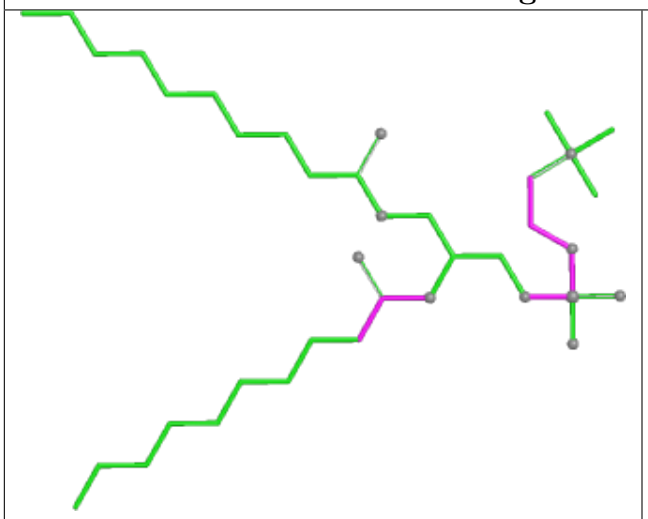
Mol	Chain	Res	Type	Atoms
3	A	1003	P1O	C1-O3-P1-O1
3	A	1003	P1O	O3-C1-C2-N1
3	B	1003	P1O	C1-O3-P1-O1
3	B	1003	P1O	O3-C1-C2-N1
3	A	1003	P1O	C24-C25-C26-C27

There are no ring outliers.

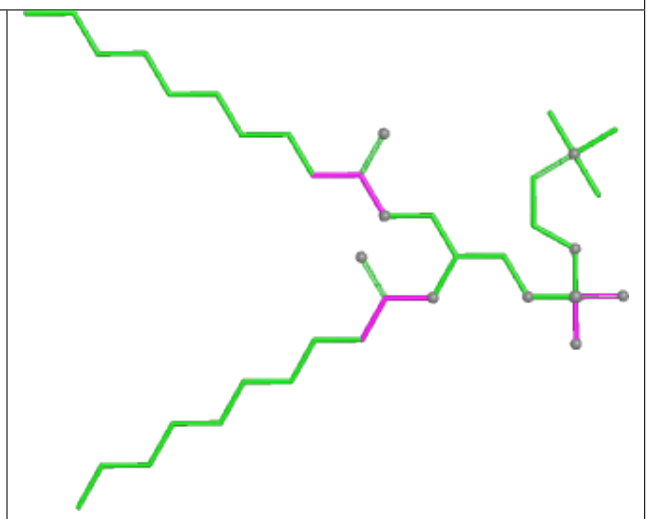
No monomer is involved in short contacts.

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.

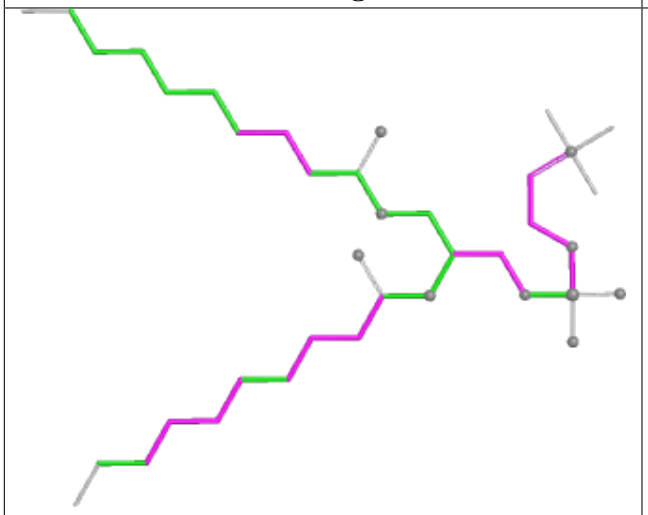
Ligand P1O B 1003



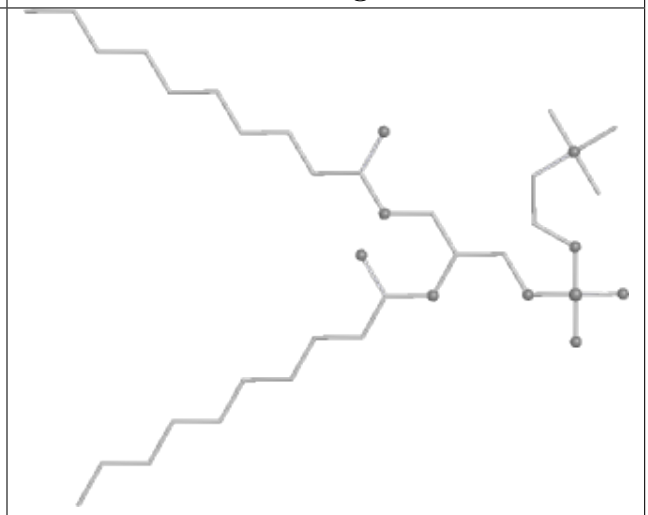
Bond lengths



Bond angles

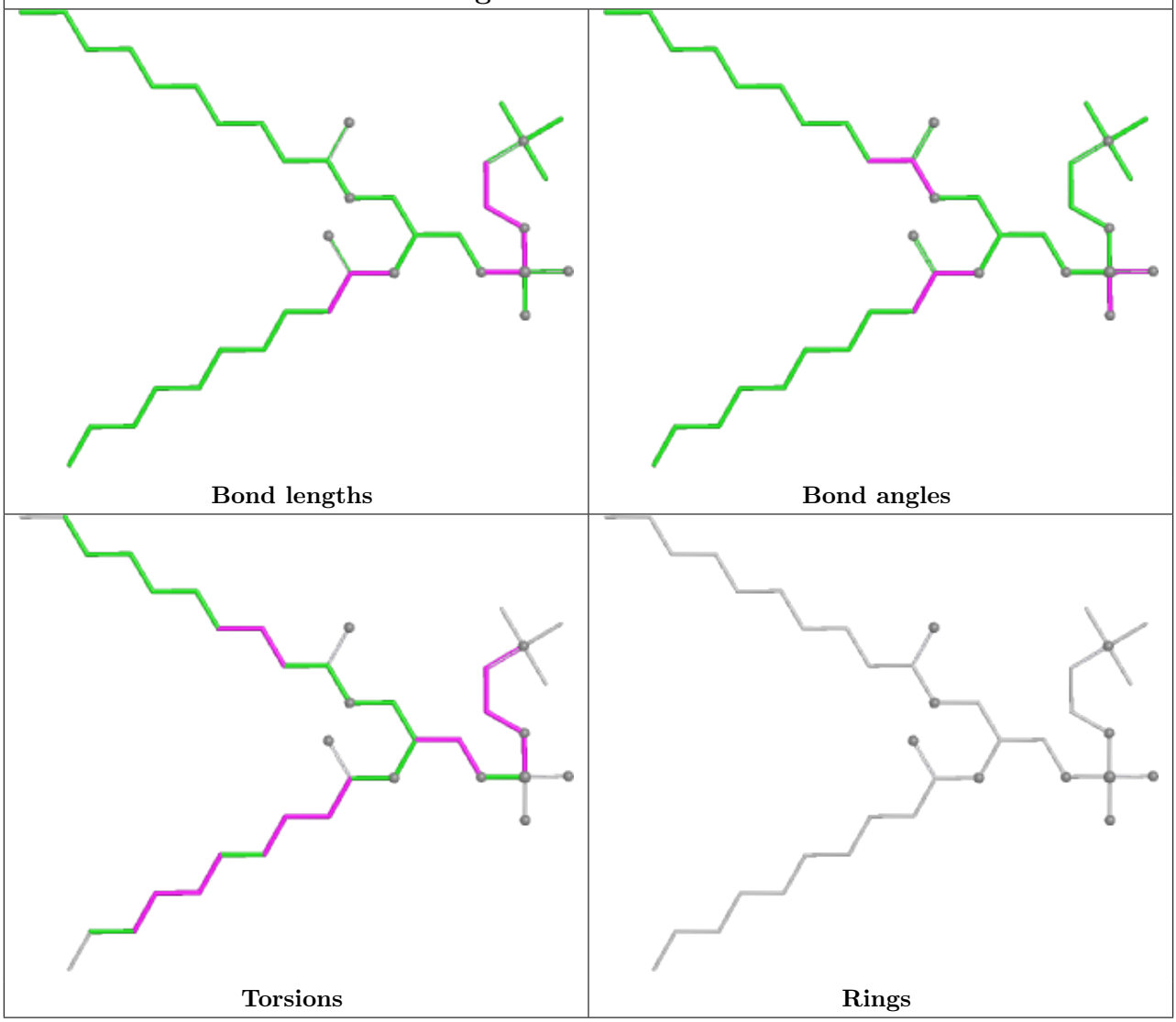


Torsions



Rings

Ligand P1O A 1003



5.7 Other polymers (i)

There are no such residues in this entry.

5.8 Polymer linkage issues (i)

The following chains have linkage breaks:

Mol	Chain	Number of breaks
1	A	1
1	B	1

All chain breaks are listed below:

Model	Chain	Residue-1	Atom-1	Residue-2	Atom-2	Distance (Å)
1	A	815:PRO	C	816:PRO	N	3.10
1	B	815:PRO	C	816:PRO	N	3.10

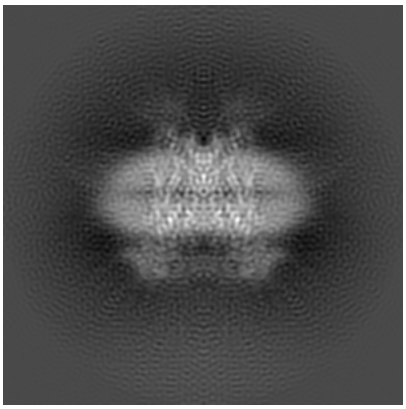
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-4613. These allow visual inspection of the internal detail of the map and identification of artifacts.

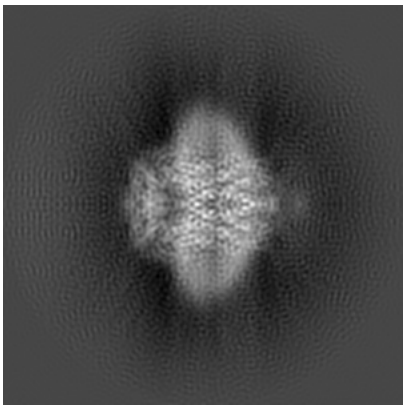
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections (i)

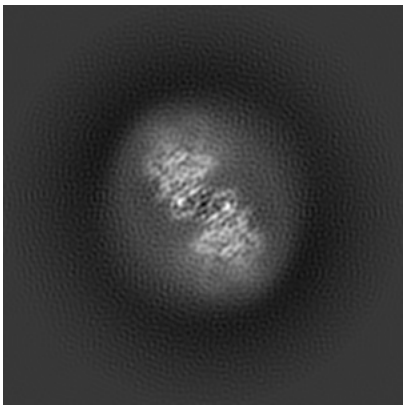
6.1.1 Primary map



X

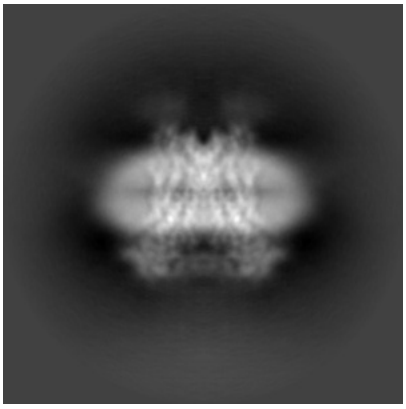


Y

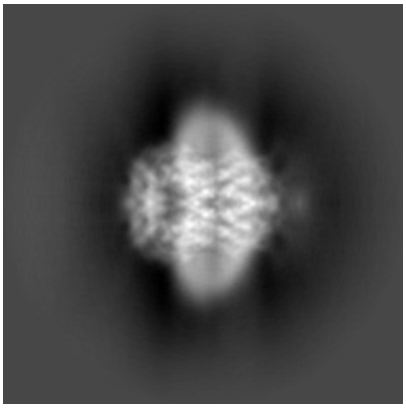


Z

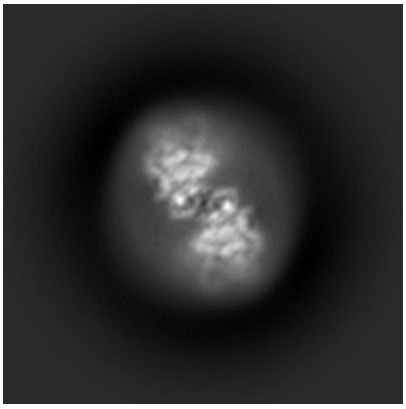
6.1.2 Raw map



X



Y

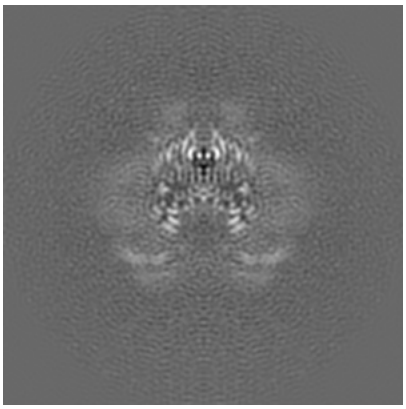


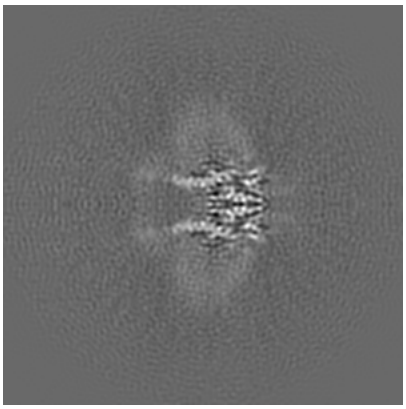
Z

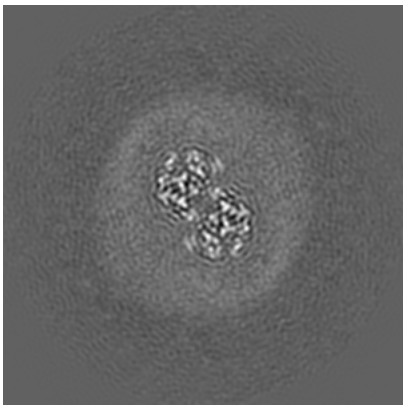
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

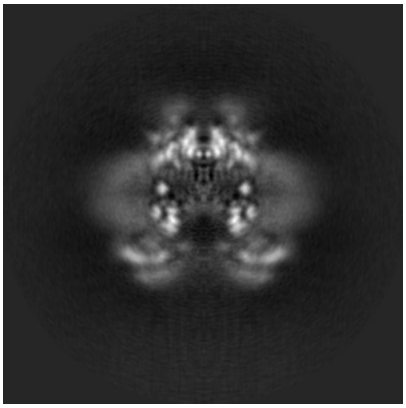
6.2.1 Primary map

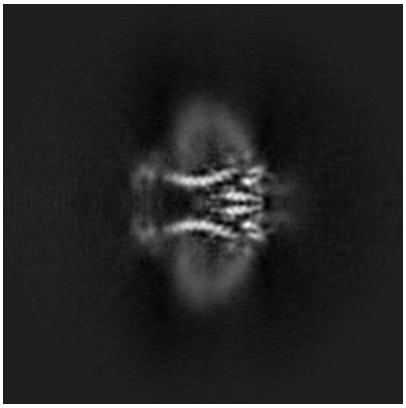

X Index: 128

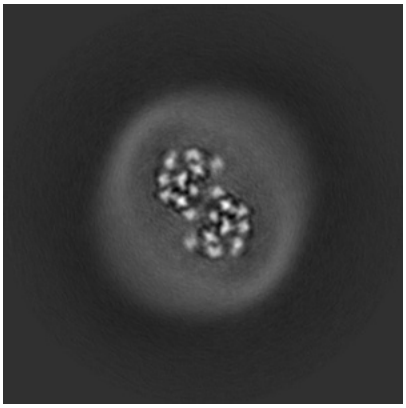

Y Index: 128


Z Index: 128

6.2.2 Raw map


X Index: 128

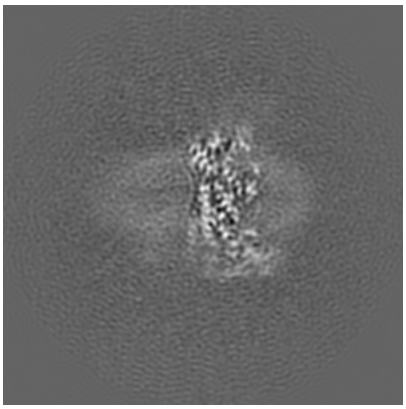

Y Index: 128

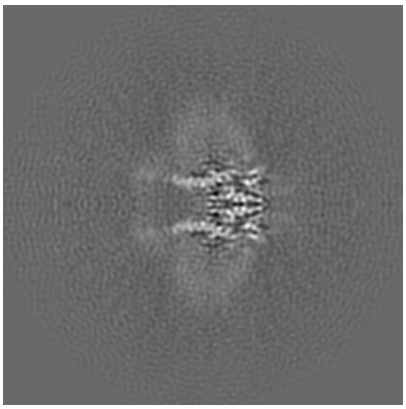

Z Index: 128

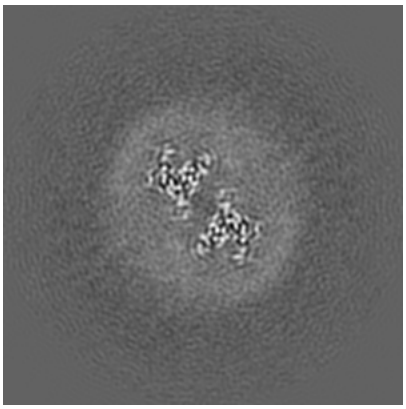
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

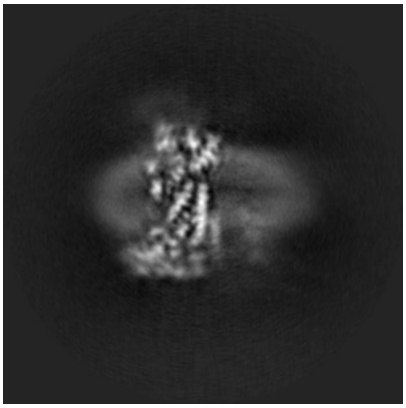
6.3.1 Primary map

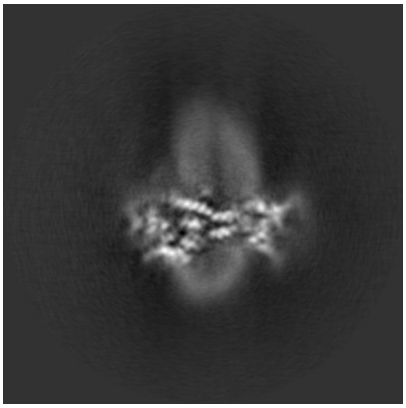

X Index: 113

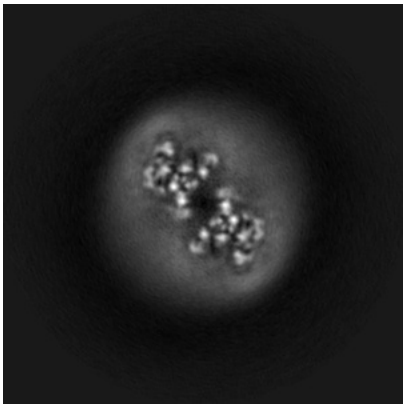

Y Index: 128


Z Index: 119

6.3.2 Raw map


X Index: 143


Y Index: 148


Z Index: 117

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

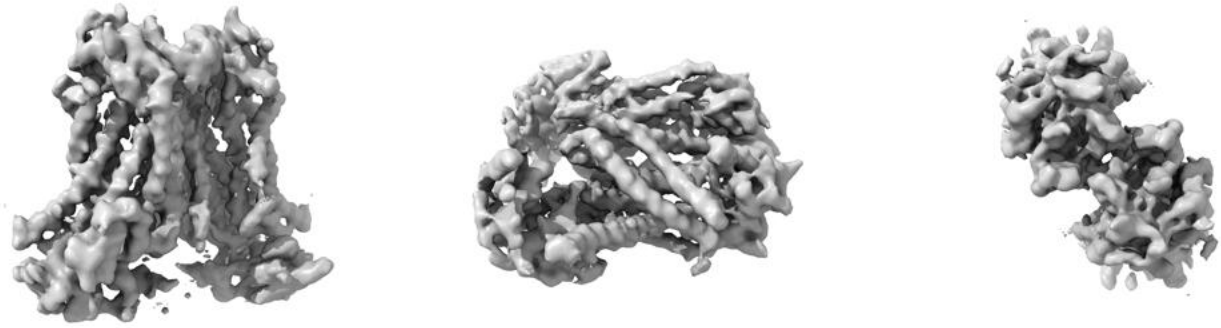
6.4 Orthogonal surface views (i)

6.4.1 Primary map

X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.05. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.4.2 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.

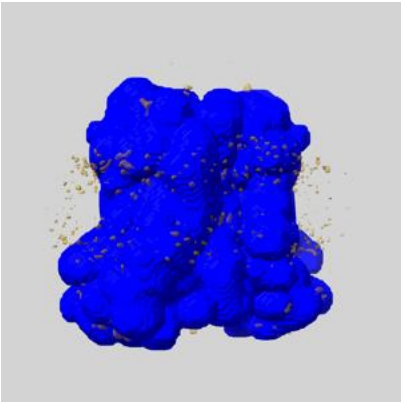
6.5 Mask visualisation (i)

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

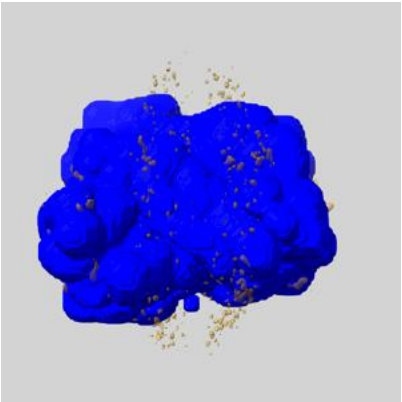
A mask typically either:

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

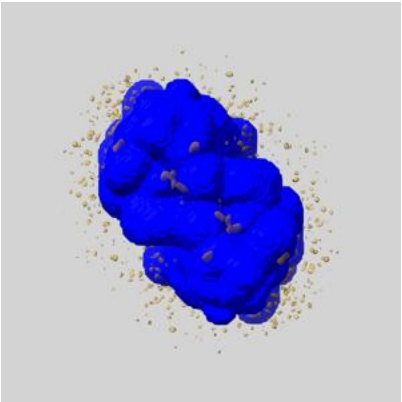
6.5.1 emd_4613_msk_1.map (i)



X



Y

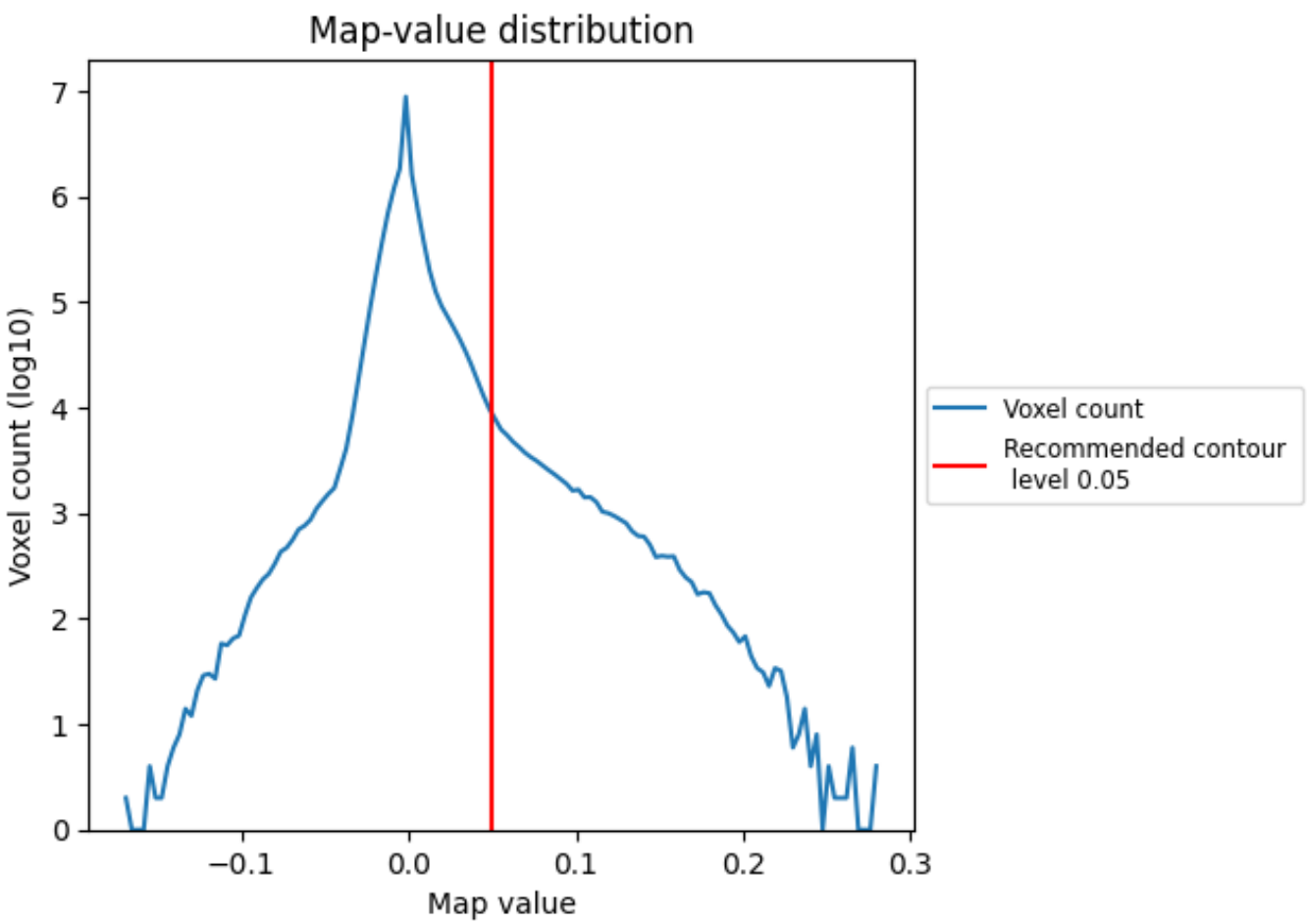


Z

7 Map analysis (i)

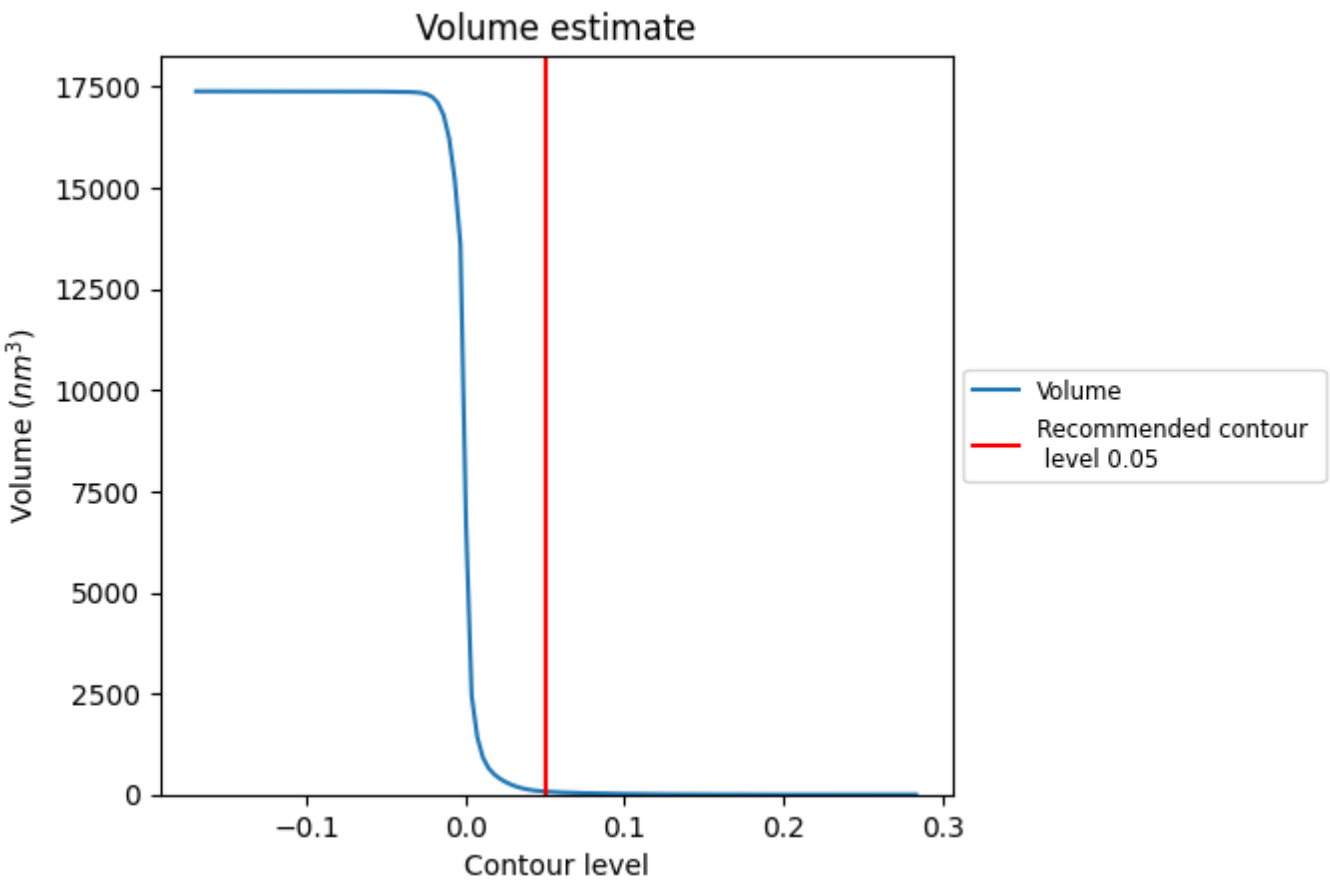
This section contains the results of statistical analysis of the map.

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.

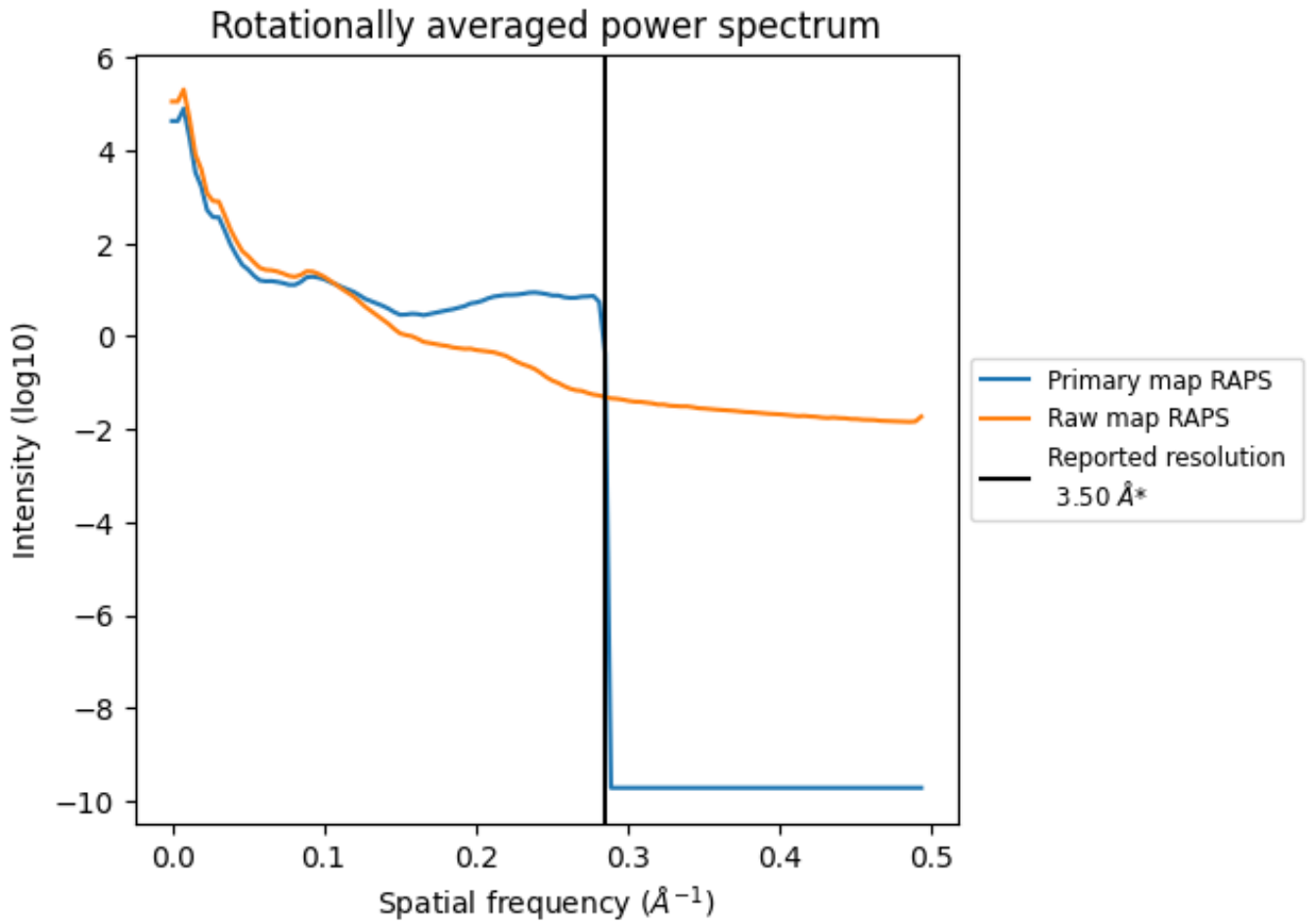
7.2 Volume estimate (i)



The volume at the recommended contour level is 75 nm^3; this corresponds to an approximate mass of 68 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)

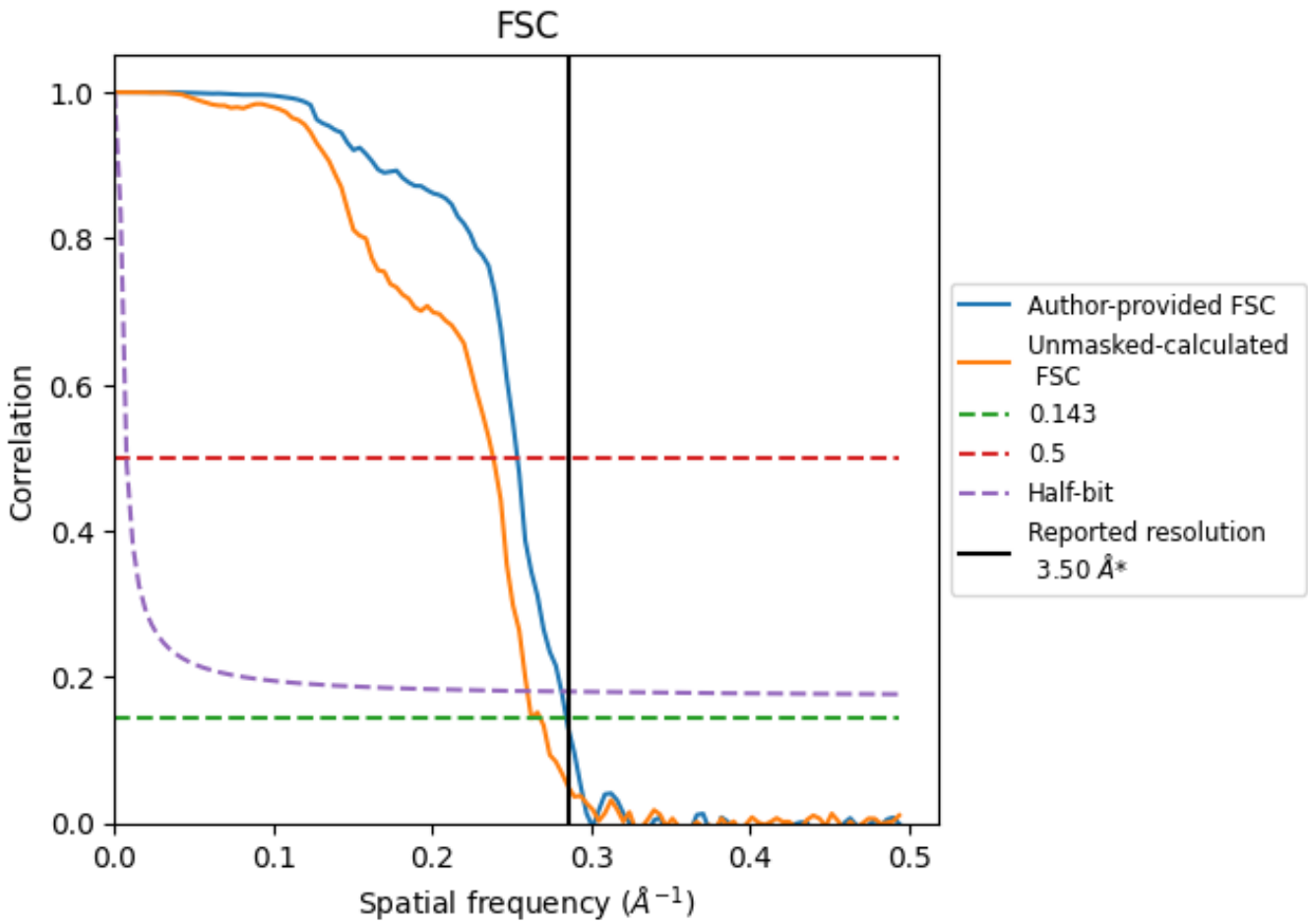


*Reported resolution corresponds to spatial frequency of 0.286 Å^{-1}

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 0.286 Å^{-1}

8.2 Resolution estimates (i)

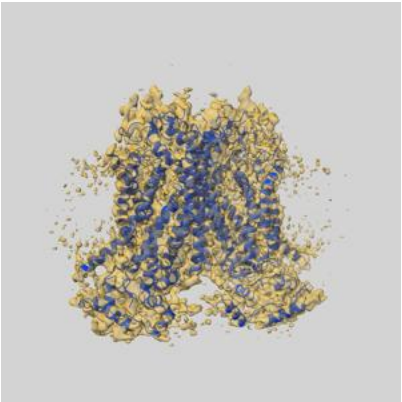
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.50	-	-
Author-provided FSC curve	3.51	3.94	3.55
Unmasked-calculated*	3.73	4.19	3.85

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

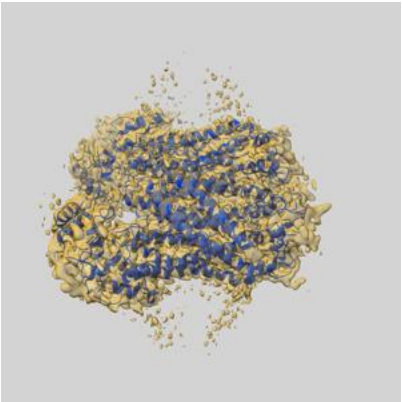
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-4613 and PDB model 6QPC. Per-residue inclusion information can be found in section 3 on page 5.

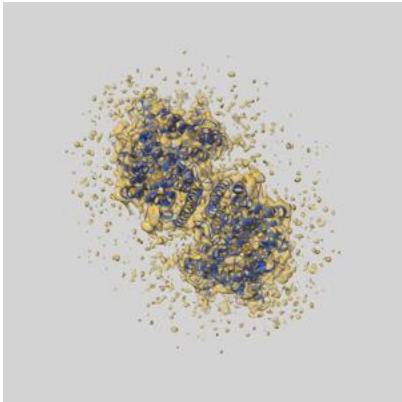
9.1 Map-model overlay (i)



X



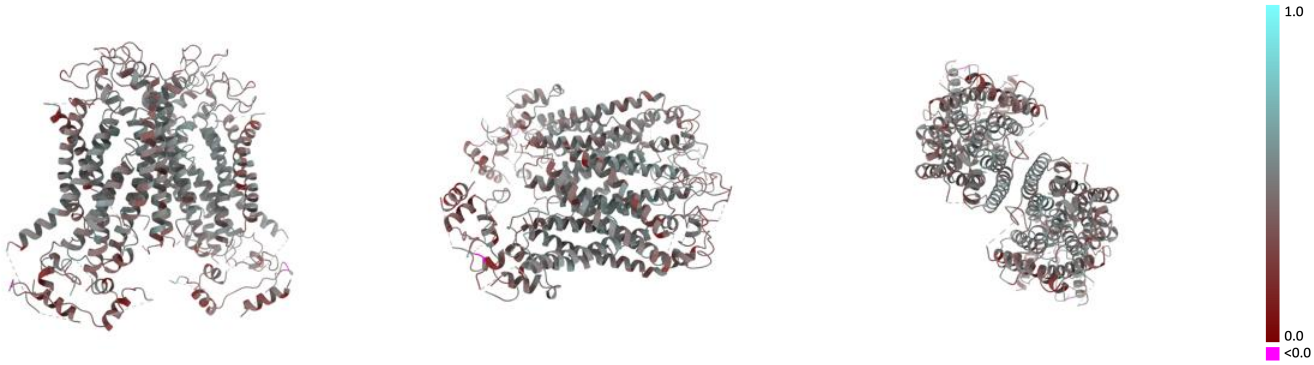
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.05 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

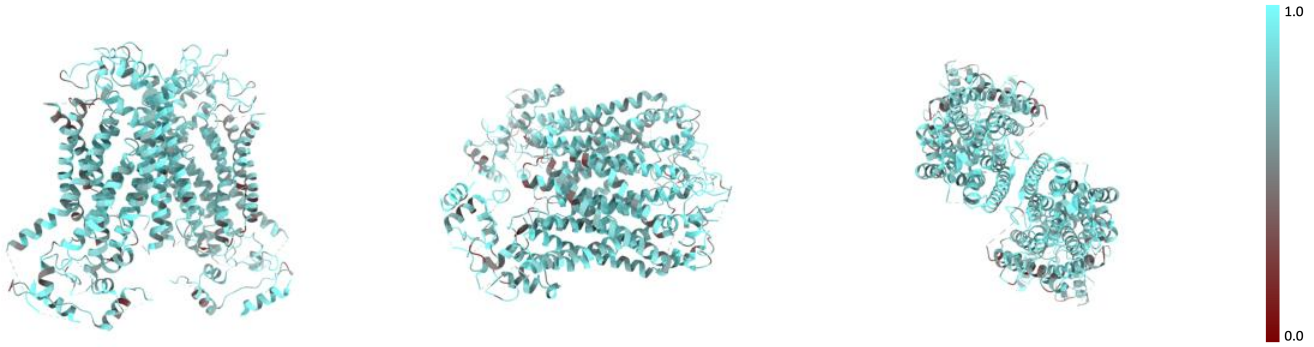
9.2 Q-score mapped to coordinate model (i)



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.

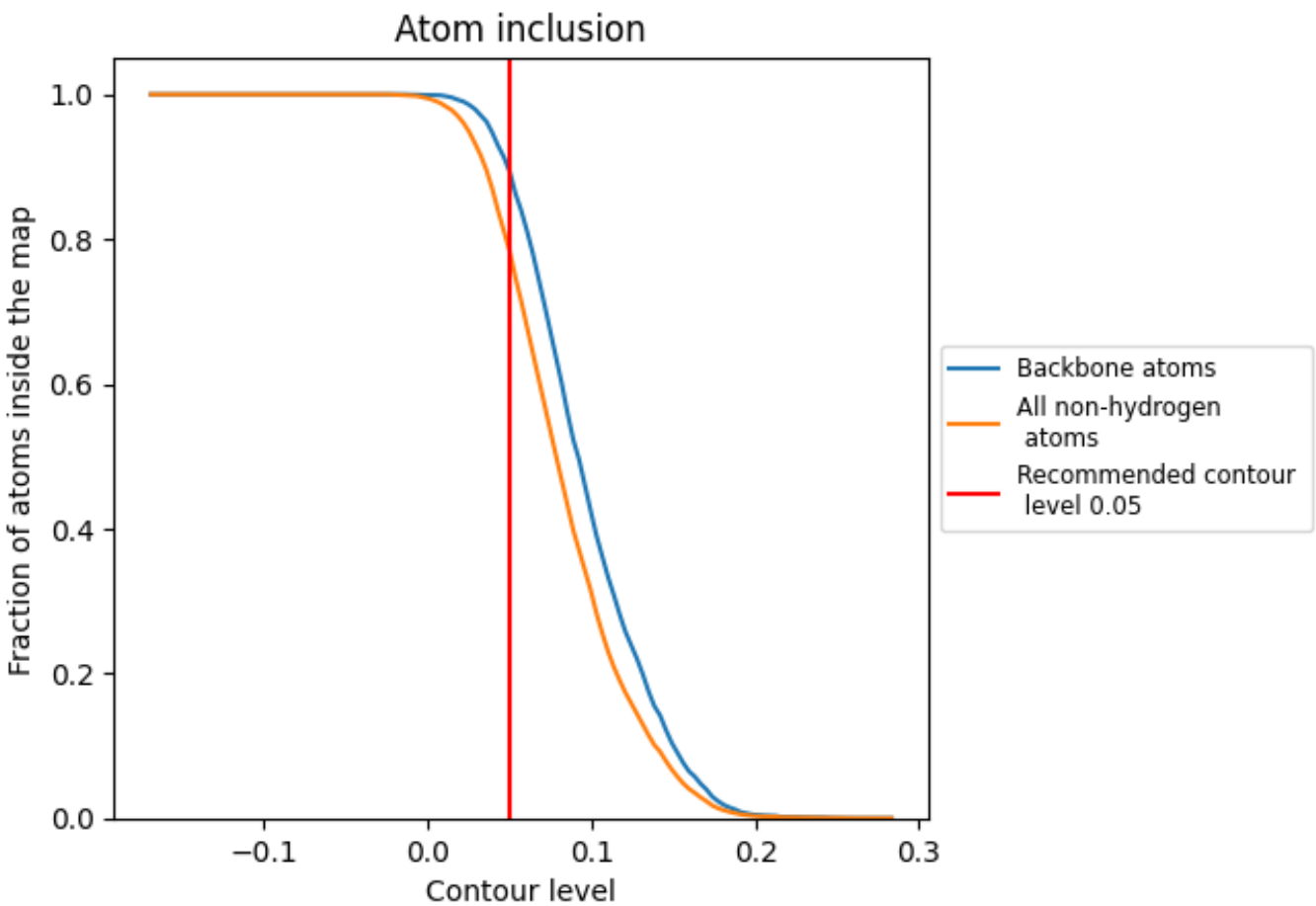
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.05).

9.4 Atom inclusion (i)



At the recommended contour level, 89% of all backbone atoms, 78% 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 (0.05) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.7824	0.4400
A	0.7826	0.4390
B	0.7821	0.4400