wwPDB EM Validation Summary Report (i)

Dec 10, 2022 – 11:44 pm GMT

PDB ID	:	6R81
EMDB ID	:	EMD-4749
Title	:	Multidrug resistance transporter BmrA mutant E504A bound with ATP and Mg solved by Cryo-EM
Authors	:	Wiseman, B.; Chaptal, V.; Zampieri, V.; Magnard, S.; Hogbom, M.; Falson, P.
Deposited on	:	2019-03-30
Resolution	:	3.90 Å(reported)

This is a wwPDB EM Validation Summary Report for a publicly released PDB entry.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/EMValidationReportHelp
with specific help available everywhere you see the (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:

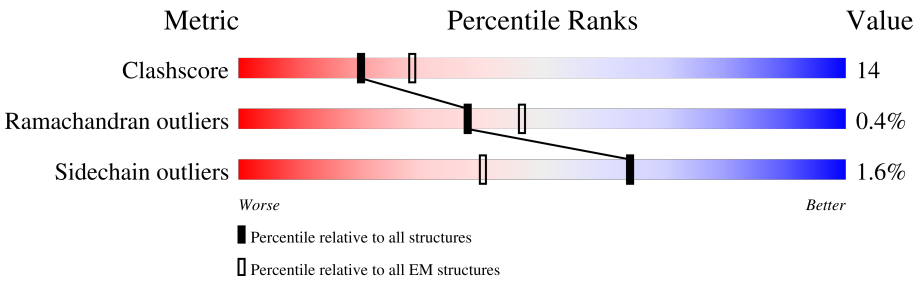
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.4, CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	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 3.90 Å.

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



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

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=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	599	7% 75% 19% • 5%
1	B	599	7% 75% 19% • 5%

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 8861 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.

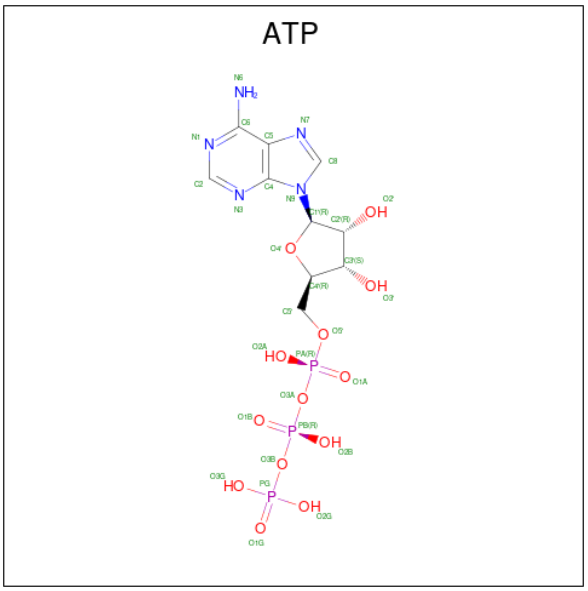
- Molecule 1 is a protein called Lipid A export ATP-binding/permease protein MsbA.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	572	4405	2828	736	824	17	0	0
1	B	569	4392	2820	733	821	18	0	0

There are 22 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-9	MET	-	initiating methionine	UNP A0A164TVX9
A	-8	SER	-	expression tag	UNP A0A164TVX9
A	-7	SER	-	expression tag	UNP A0A164TVX9
A	-6	SER	-	expression tag	UNP A0A164TVX9
A	-5	HIS	-	expression tag	UNP A0A164TVX9
A	-4	HIS	-	expression tag	UNP A0A164TVX9
A	-3	HIS	-	expression tag	UNP A0A164TVX9
A	-2	HIS	-	expression tag	UNP A0A164TVX9
A	-1	HIS	-	expression tag	UNP A0A164TVX9
A	0	HIS	-	expression tag	UNP A0A164TVX9
A	504	ALA	GLU	engineered mutation	UNP A0A164TVX9
B	-9	MET	-	initiating methionine	UNP A0A164TVX9
B	-8	SER	-	expression tag	UNP A0A164TVX9
B	-7	SER	-	expression tag	UNP A0A164TVX9
B	-6	SER	-	expression tag	UNP A0A164TVX9
B	-5	HIS	-	expression tag	UNP A0A164TVX9
B	-4	HIS	-	expression tag	UNP A0A164TVX9
B	-3	HIS	-	expression tag	UNP A0A164TVX9
B	-2	HIS	-	expression tag	UNP A0A164TVX9
B	-1	HIS	-	expression tag	UNP A0A164TVX9
B	0	HIS	-	expression tag	UNP A0A164TVX9
B	504	ALA	GLU	engineered mutation	UNP A0A164TVX9

- Molecule 2 is ADENOSINE-5'-TRIPHOSPHATE (three-letter code: ATP) (formula: $C_{10}H_{16}N_5O_{13}P_3$).



Mol	Chain	Residues	Atoms					AltConf
			Total	C	N	O	P	
2	A	1	62	20	10	26	6	0
			Total	C	N	O	P	
2	A	1	62	20	10	26	6	0

- Molecule 3 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

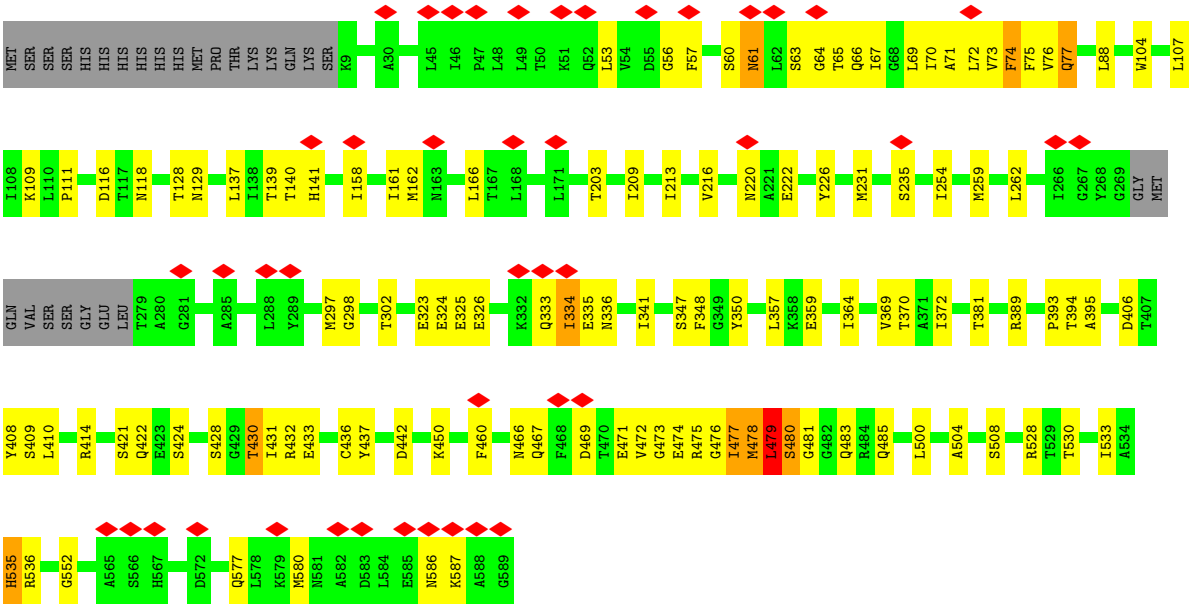
Mol	Chain	Residues	Atoms		AltConf
			Total	Mg	
3	A	1	1	1	0
			Total	Mg	
3	B	1	1	1	0

3 Residue-property plots (i)

These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and atom inclusion in map density. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. A red diamond above a residue indicates a poor fit to the EM map for this residue (all-atom inclusion < 40%). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

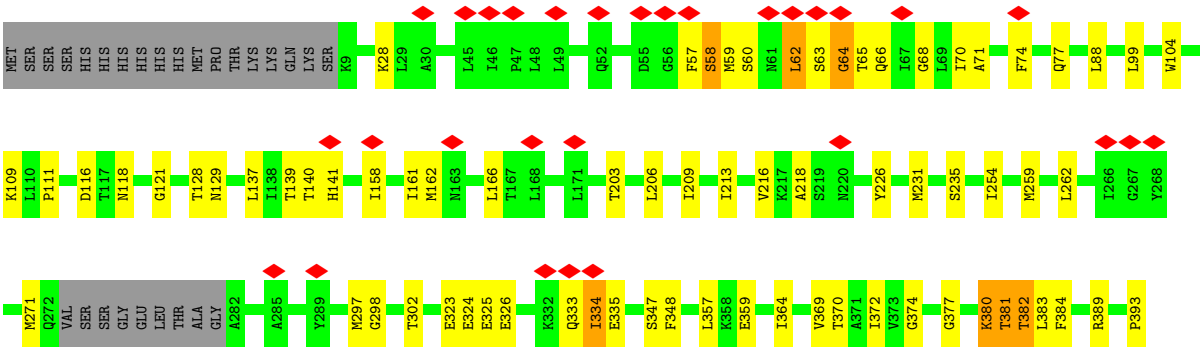
• Molecule 1: Lipid A export ATP-binding/permease protein MsbA

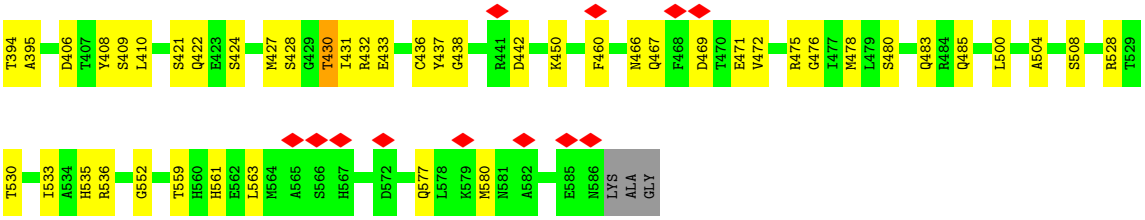
Chain A:



• Molecule 1: Lipid A export ATP-binding/permease protein MsbA

Chain B:




T394
A395
D406
T407
Y408
S409
L410
S421
Q422
E423
S424
M427
S428
G429
T430
I431
R432
E433
C436
Y437
G438
R441
D442
K450
F460
N466
Q467
F468
D469
T470
E471
V472
R475
G476
I477
M478
L479
S480
Q483
R484
Q485
L500
A504
S508
R528
T529
T530
T533
A534
H535
R536
G552
T559
H560
H561
E562
L563
N564
A565
S566
H567
D572
Q577
L578
K579
N580
N581
A582
E585
N586
LYS
ALA
GLY

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C2	Depositor
Number of particles used	128372	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	40	Depositor
Minimum defocus (nm)	Not provided	
Maximum defocus (nm)	Not provided	
Magnification	Not provided	
Image detector	GATAN K2 SUMMIT (4k x 4k)	Depositor
Maximum map value	1.462	Depositor
Minimum map value	-0.765	Depositor
Average map value	0.010	Depositor
Map value standard deviation	0.063	Depositor
Recommended contour level	0.33	Depositor
Map size (Å)	211.99998, 211.99998, 211.99998	wwPDB
Map dimensions	200, 200, 200	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.06, 1.06, 1.06	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: ATP, MG

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	0.55	2/4471 (0.0%)	0.74	6/6049 (0.1%)
1	B	0.52	2/4458 (0.0%)	0.70	6/6031 (0.1%)
All	All	0.53	4/8929 (0.0%)	0.72	12/12080 (0.1%)

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	6
1	B	0	7
All	All	0	13

All (4) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	476	GLY	CA-C	-6.06	1.42	1.51
1	B	380	LYS	C-O	-5.73	1.12	1.23
1	A	476	GLY	C-O	-5.53	1.14	1.23
1	B	381	THR	C-O	-5.45	1.12	1.23

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

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	480	SER	CB-CA-C	-11.00	89.20	110.10
1	B	381	THR	OG1-CB-CG2	-7.38	93.04	110.00
1	A	74	PHE	C-N-CA	-7.21	103.68	121.70
1	B	325	GLU	CA-CB-CG	7.06	128.94	113.40
1	A	325	GLU	CA-CB-CG	6.96	128.72	113.40

There are no chirality outliers.

5 of 13 planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	118	ASN	Peptide
1	A	323	GLU	Peptide
1	A	324	GLU	Peptide
1	A	335	GLU	Peptide
1	A	428	SER	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	4405	0	4577	147	0
1	B	4392	0	4560	133	0
2	A	62	0	24	13	0
3	A	1	0	0	0	0
3	B	1	0	0	0	0
All	All	8861	0	9161	247	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 14.

The worst 5 of 247 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:475:ARG:NH1	1:B:475:ARG:HD2	1.37	1.38
1:B:57:PHE:HE1	1:B:64:GLY:CA	1.38	1.36
1:B:374:GLY:N	1:B:380:LYS:HE2	1.42	1.32
1:A:475:ARG:CZ	1:B:475:ARG:HD2	1.61	1.30
1:B:374:GLY:H	1:B:380:LYS:CE	1.43	1.30

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	568/599 (95%)	500 (88%)	66 (12%)	2 (0%)	34	71
1	B	565/599 (94%)	498 (88%)	64 (11%)	3 (0%)	29	67
All	All	1133/1198 (95%)	998 (88%)	130 (12%)	5 (0%)	38	71

All (5) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	467	GLN
1	B	467	GLN
1	A	431	ILE
1	B	382	THR
1	B	431	ILE

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	481/506 (95%)	472 (98%)	9 (2%)	57	75
1	B	481/506 (95%)	475 (99%)	6 (1%)	71	83
All	All	962/1012 (95%)	947 (98%)	15 (2%)	64	79

5 of 15 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	528	ARG

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Mol	Chain	Res	Type
1	B	437	TYR
1	A	535	HIS
1	B	528	ARG
1	B	158	ILE

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	196	GLN
1	B	459	ASN
1	B	518	GLN
1	A	207	ASN
1	A	459	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 4 ligands modelled in this entry, 2 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	$\#\|Z\| > 2$
2	ATP	A	602	-	26,33,33	0.88	0	31,52,52	1.82	5 (16%)
2	ATP	A	601	-	26,33,33	0.99	1 (3%)	31,52,52	1.68	5 (16%)

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	ATP	A	602	-	-	5/18/38/38	0/3/3/3
2	ATP	A	601	-	-	2/18/38/38	0/3/3/3

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	601	ATP	C2'-C1'	-2.42	1.50	1.53

The worst 5 of 10 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	A	602	ATP	PB-O3B-PG	-4.66	116.84	132.83
2	A	601	ATP	PA-O3A-PB	-4.50	117.39	132.83
2	A	602	ATP	N3-C2-N1	-3.89	122.60	128.68
2	A	601	ATP	N3-C2-N1	-3.78	122.77	128.68
2	A	602	ATP	C3'-C2'-C1'	3.69	106.54	100.98

There are no chirality outliers.

5 of 7 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	601	ATP	C5'-O5'-PA-O3A
2	A	602	ATP	O4'-C4'-C5'-O5'
2	A	602	ATP	C3'-C4'-C5'-O5'
2	A	602	ATP	PB-O3A-PA-O1A
2	A	601	ATP	C5'-O5'-PA-O1A

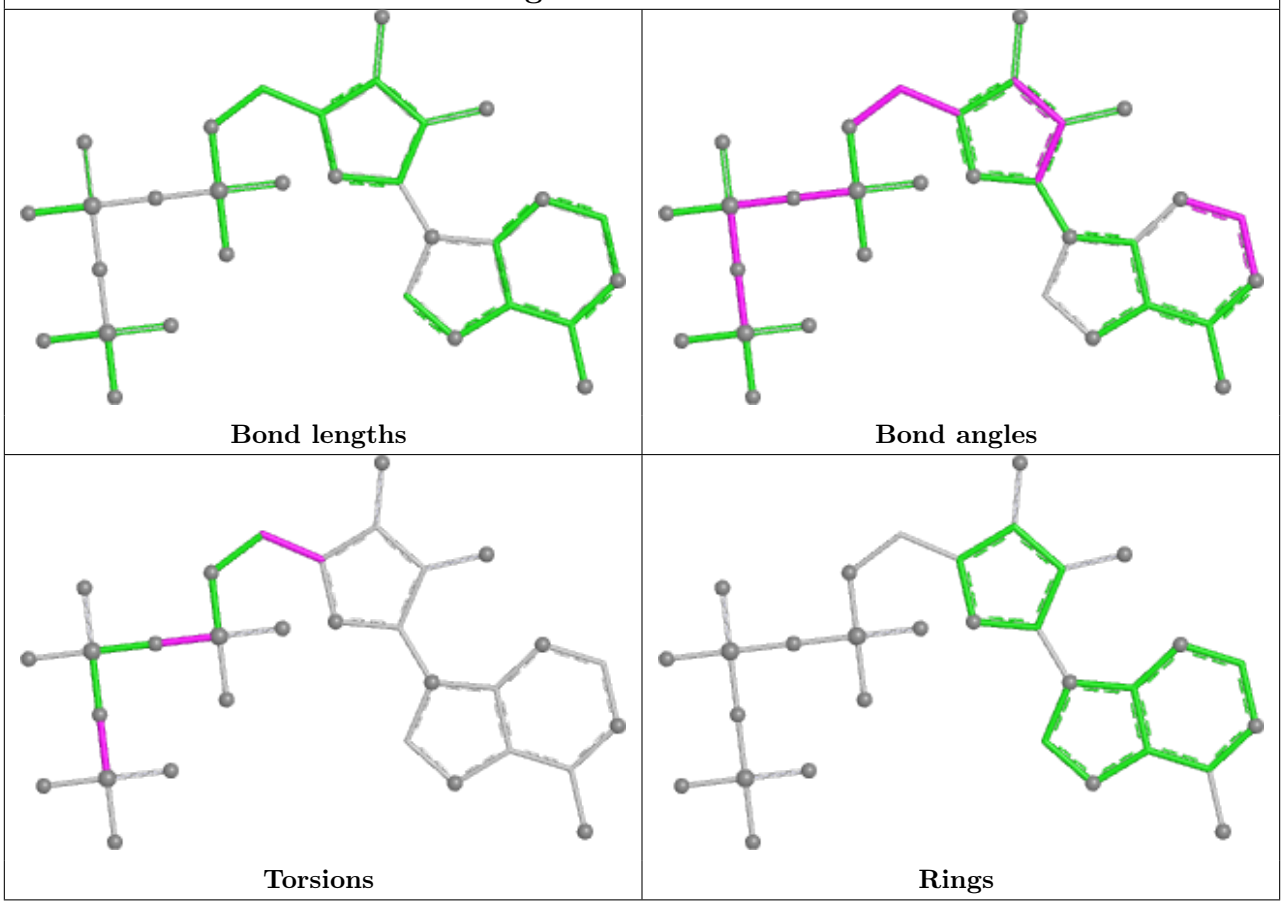
There are no ring outliers.

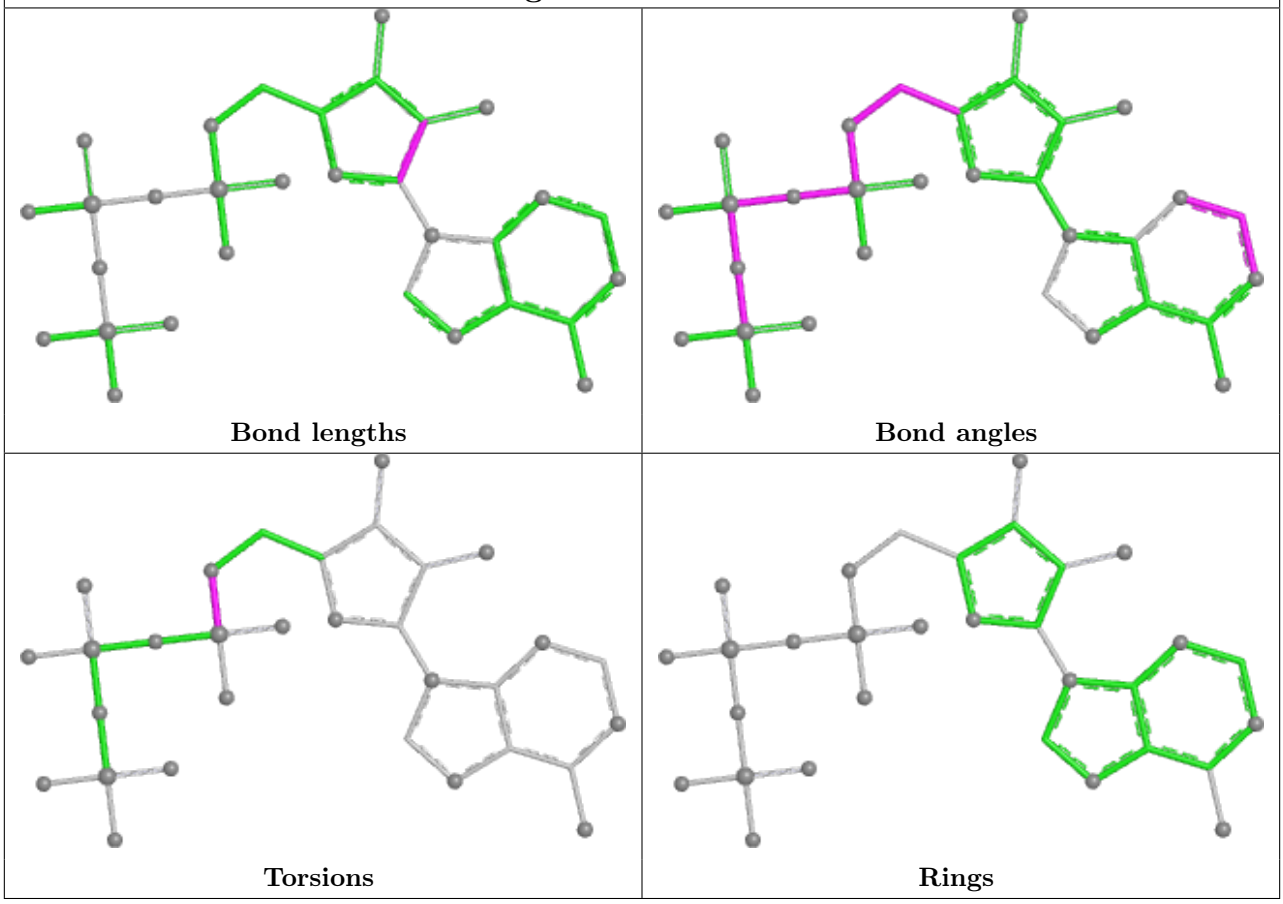
2 monomers are involved in 13 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	602	ATP	5	0
2	A	601	ATP	8	0

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 ATP A 602



Ligand ATP A 601	
 Bond lengths	Bond angles
Torsions	Rings

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.

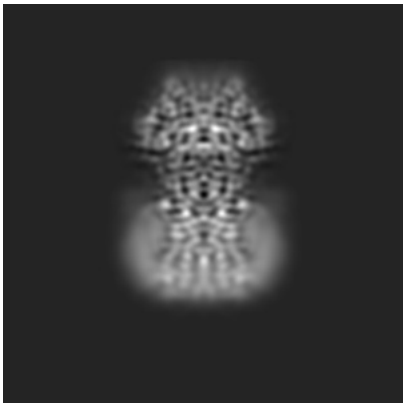
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-4749. These allow visual inspection of the internal detail of the map and identification of artifacts.

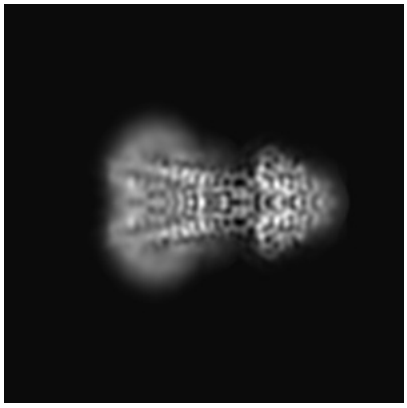
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections (i)

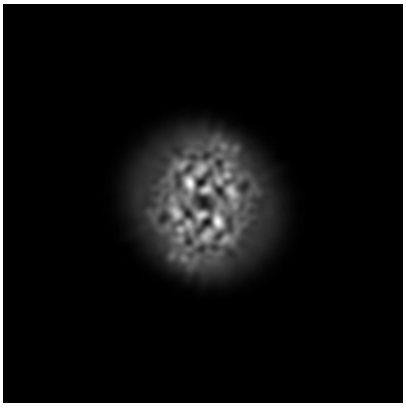
6.1.1 Primary map



X



Y



Z

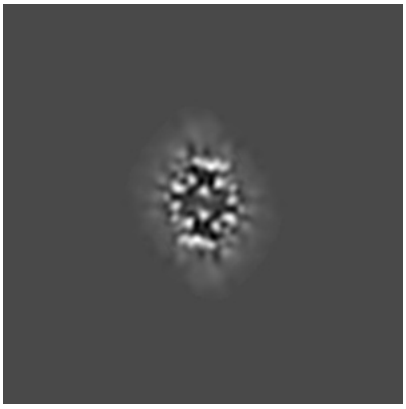
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

6.2.1 Primary map

X Index: 100

Y Index: 100



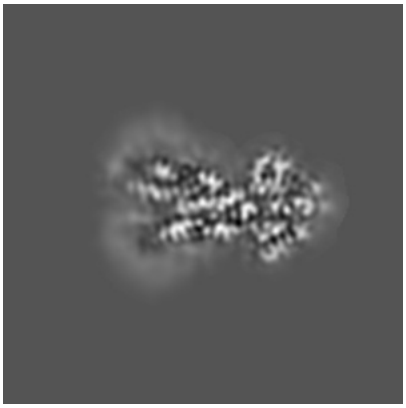
Z Index: 100

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

6.3 Largest variance slices (i)

6.3.1 Primary map

X Index: 107



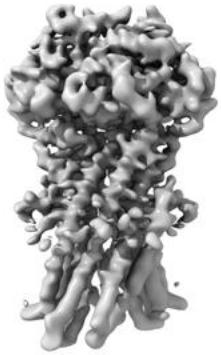
Y Index: 94

Z Index: 136

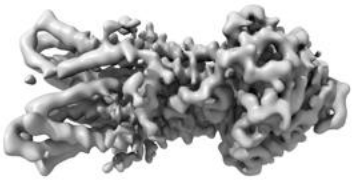
The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal surface views (i)

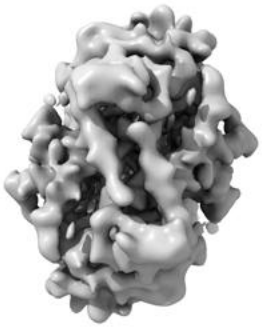
6.4.1 Primary map



X



Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.33. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

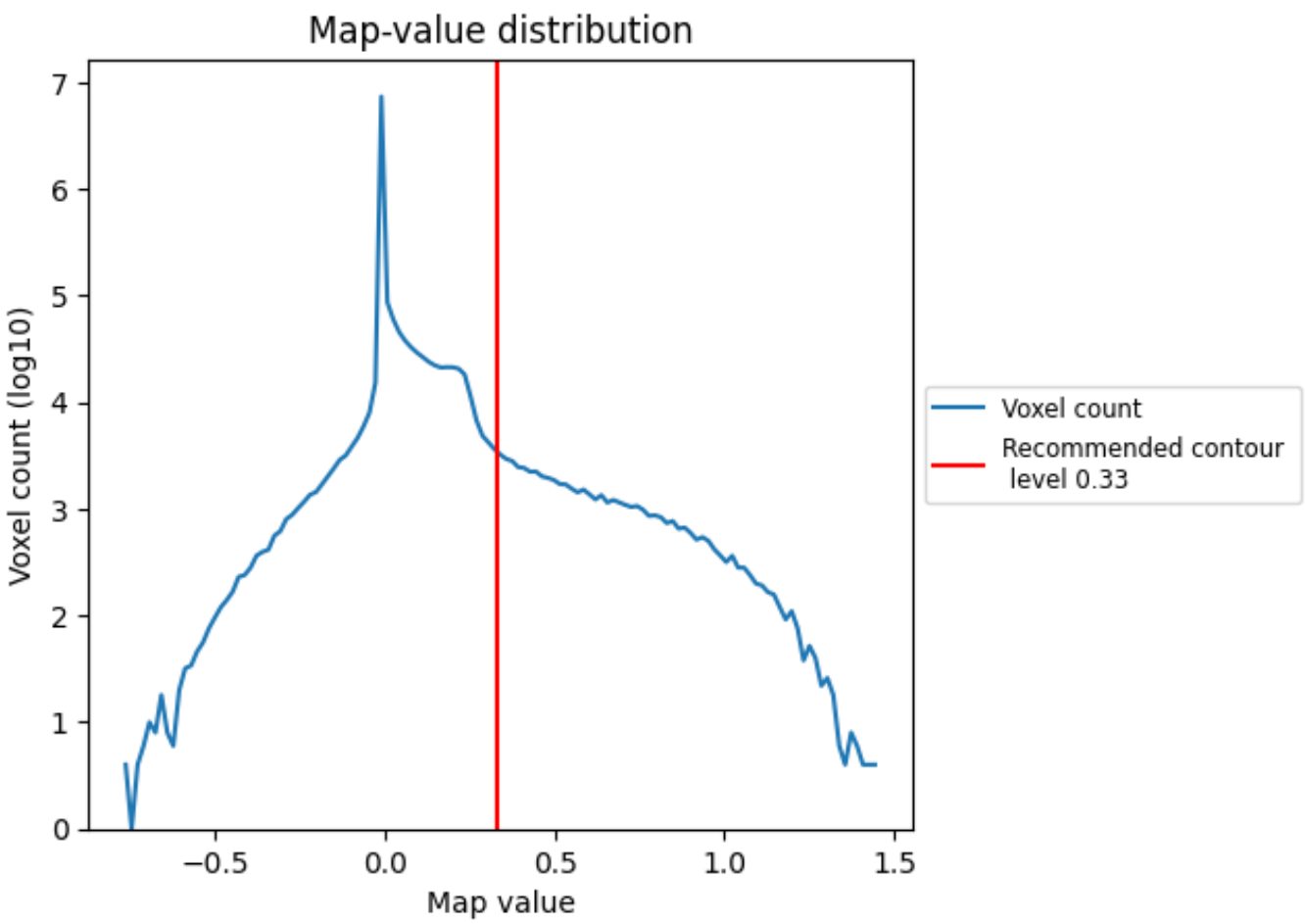
6.5 Mask visualisation (i)

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

7 Map analysis (i)

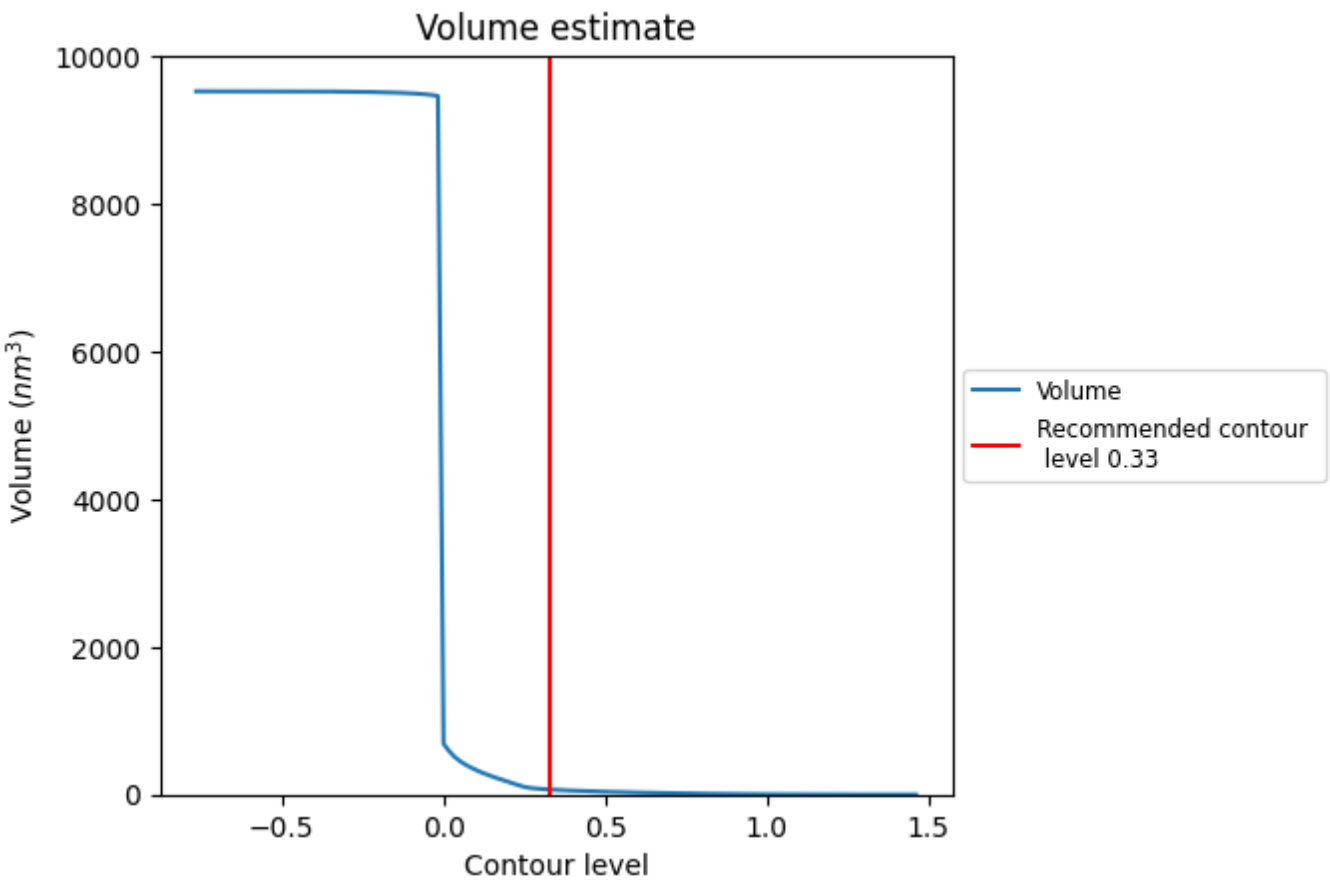
This section contains the results of statistical analysis of the map.

7.1 Map-value distribution (i)



The map-value distribution is plotted in 128 intervals along the x-axis. The y-axis is logarithmic. A spike in this graph at zero usually indicates that the volume has been masked.

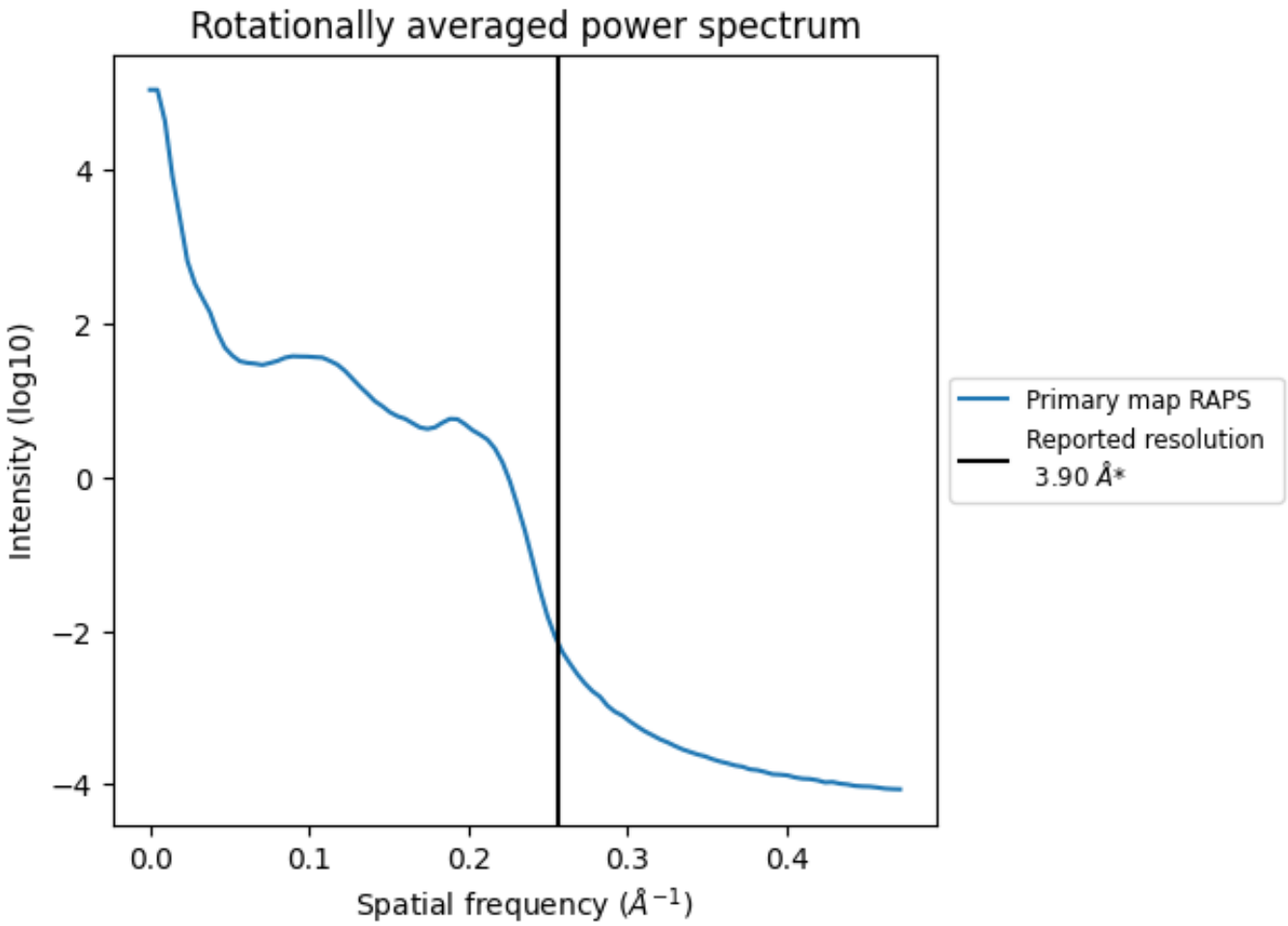
7.2 Volume estimate (i)



The volume at the recommended contour level is 68 nm^3; this corresponds to an approximate mass of 61 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)

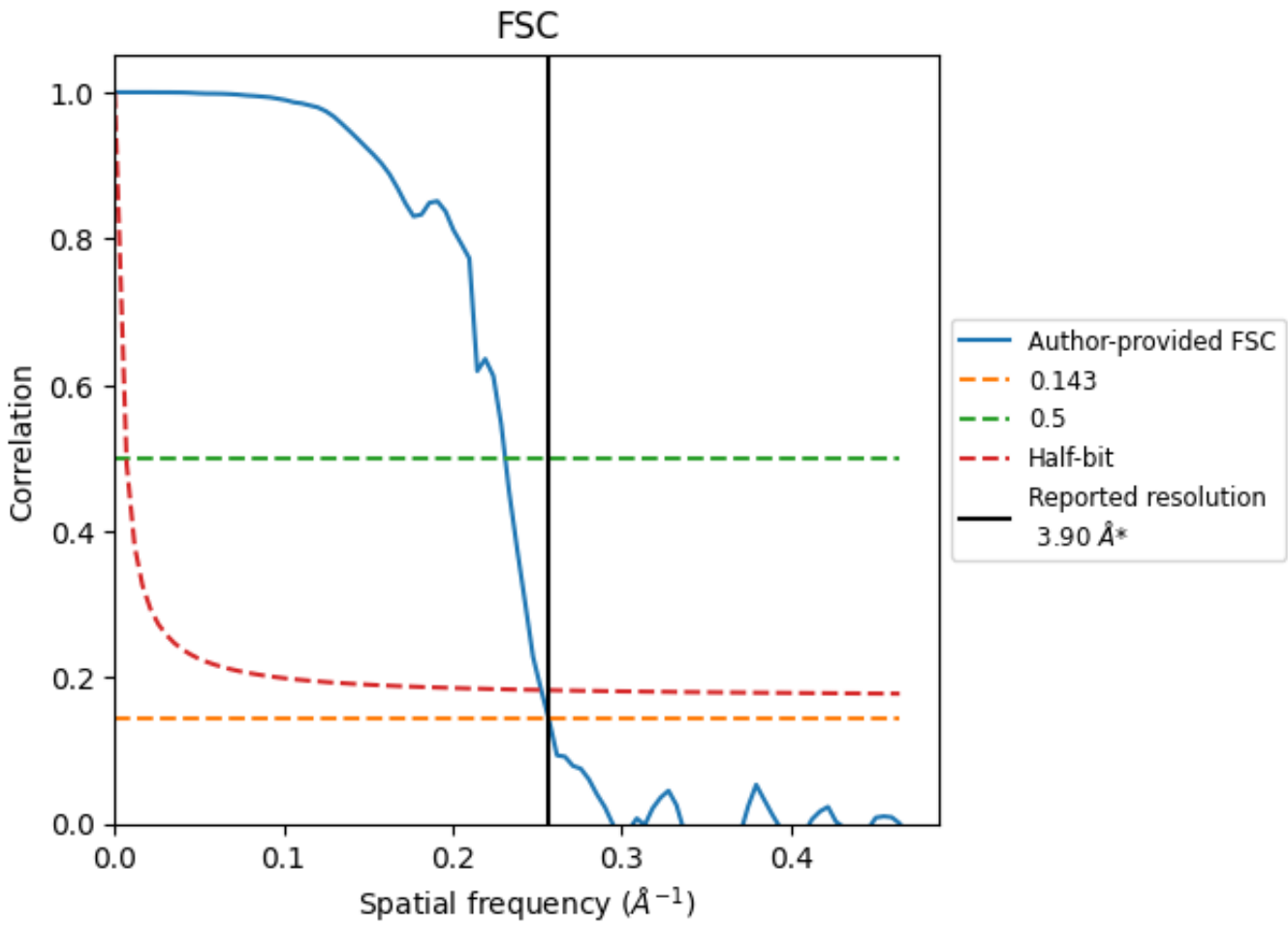


*Reported resolution corresponds to spatial frequency of 0.256 Å^{-1}

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 0.256 Å^{-1}

8.2 Resolution estimates (i)

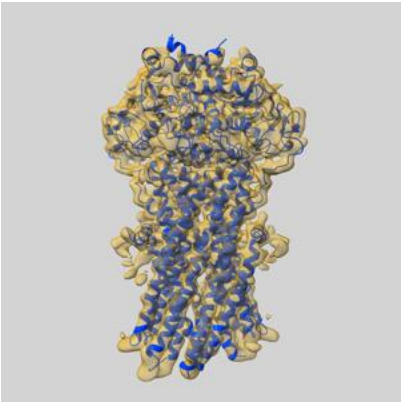
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.90	-	-
Author-provided FSC curve	3.89	4.33	3.96
Unmasked-calculated*	-	-	-

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

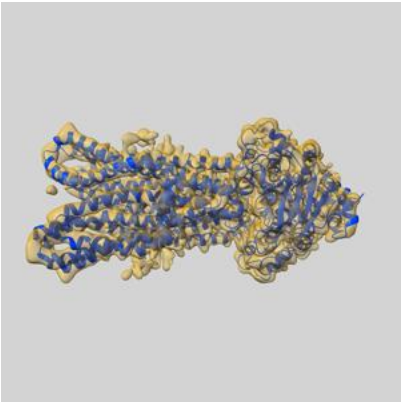
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-4749 and PDB model 6R81. Per-residue inclusion information can be found in section 3 on page 5.

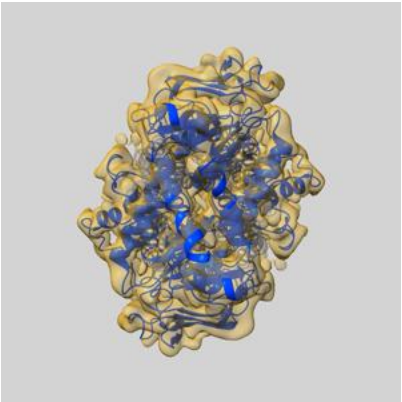
9.1 Map-model overlay (i)



X



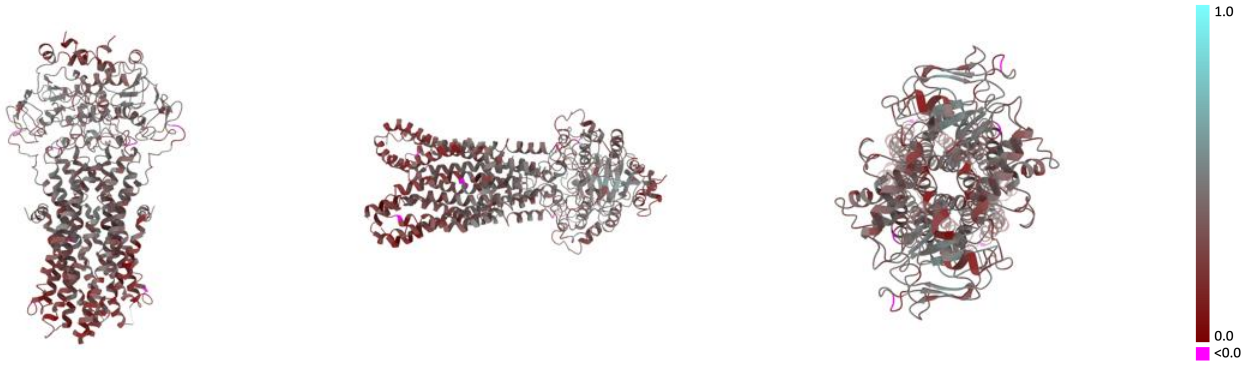
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.33 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.

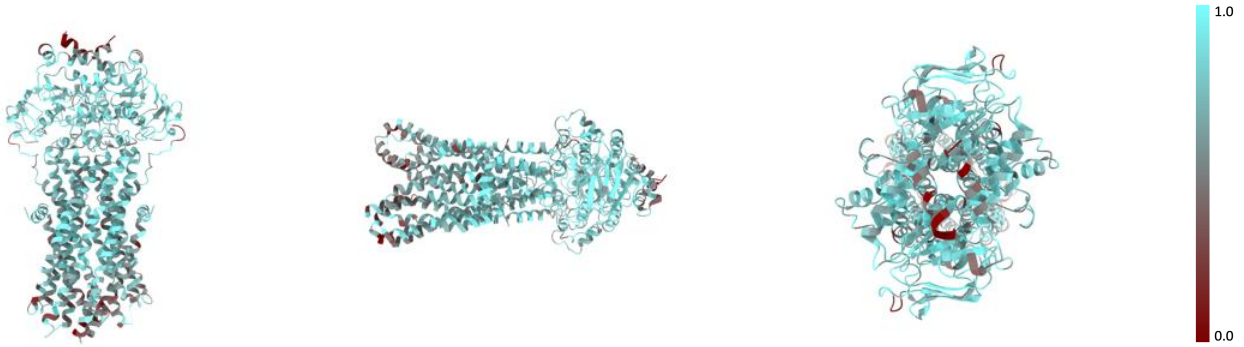
9.2 Q-score mapped to coordinate model (i)



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.

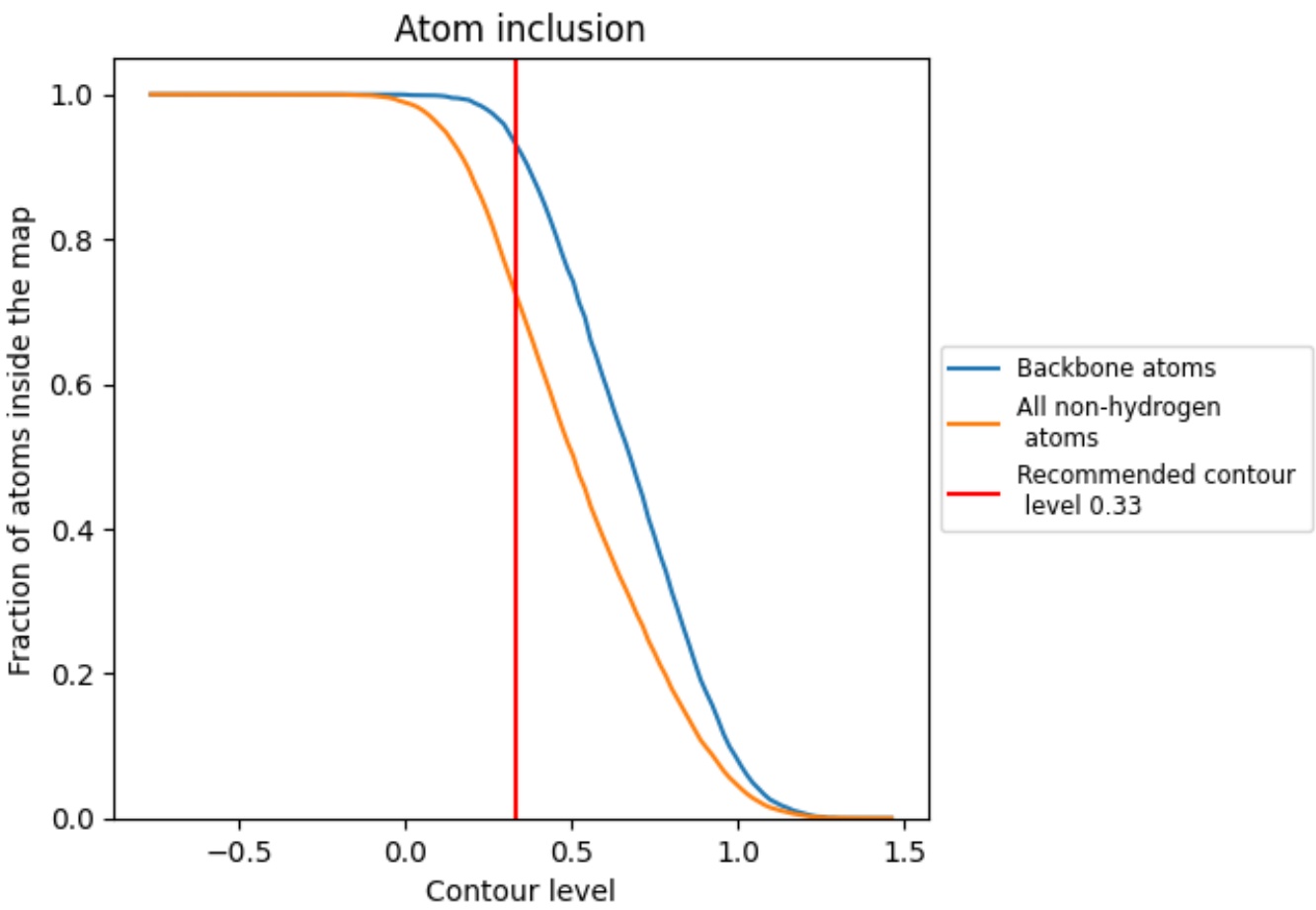
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.33).

9.4 Atom inclusion (i)



At the recommended contour level, 93% of all backbone atoms, 73% 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 (0.33) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.7264	0.3590
A	0.7265	0.3590
B	0.7263	0.3580