wwPDB EM Validation Summary Report (i)

Apr 20, 2024 – 10:24 PM EDT

PDB ID	:	8SWN
EMDB ID	:	EMD-40821
Title	:	Bovine multidrug resistance protein 4 (MRP4) E1202Q mutant bound to ATP in MSP lipid nanodisc
Authors	:	Pourmal, S.; Stroud, R.M.
Deposited on	:	2023-05-19
Resolution	:	3.10 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:

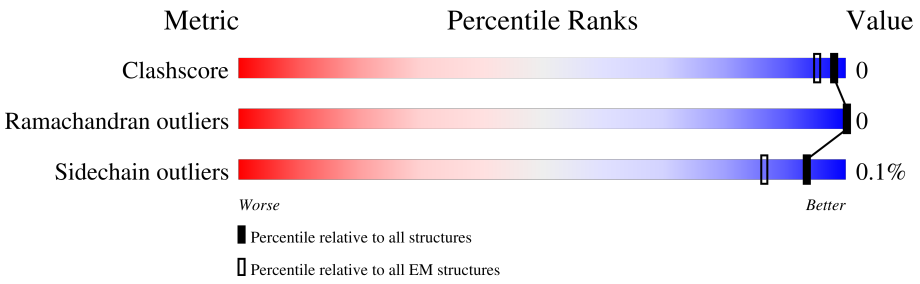
EMDB validation analysis	:	0.0.1.dev92
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36.2

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

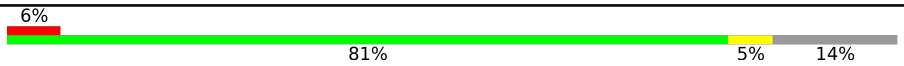
The reported resolution of this entry is 3.10 Å.

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	1325	

2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 18525 atoms, of which 9372 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 ATP binding cassette subfamily C member 4.

Mol	Chain	Residues	Atoms						AltConf	Trace
			Total	C	H	N	O	S		
1	A	1135	18308	5874	9269	1518	1611	36	0	0

There are 76 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	103	GLU	-	insertion	UNP F1MUC1
A	104	SER	-	insertion	UNP F1MUC1
A	105	THR	-	insertion	UNP F1MUC1
A	106	ARG	-	insertion	UNP F1MUC1
A	107	VAL	-	insertion	UNP F1MUC1
A	108	VAL	-	insertion	UNP F1MUC1
A	109	GLN	-	insertion	UNP F1MUC1
A	110	PRO	-	insertion	UNP F1MUC1
A	111	ILE	-	insertion	UNP F1MUC1
A	112	ILE	-	insertion	UNP F1MUC1
A	113	LEU	-	insertion	UNP F1MUC1
A	114	GLY	-	insertion	UNP F1MUC1
A	115	LYS	-	insertion	UNP F1MUC1
A	116	ILE	-	insertion	UNP F1MUC1
A	117	ILE	-	insertion	UNP F1MUC1
A	118	GLY	-	insertion	UNP F1MUC1
A	119	TYR	-	insertion	UNP F1MUC1
A	120	PHE	-	insertion	UNP F1MUC1
A	121	GLU	-	insertion	UNP F1MUC1
A	122	ASN	-	insertion	UNP F1MUC1
A	123	TYR	-	insertion	UNP F1MUC1
A	124	ASP	-	insertion	UNP F1MUC1
A	125	PRO	-	insertion	UNP F1MUC1
A	126	SER	-	insertion	UNP F1MUC1
A	127	ASP	-	insertion	UNP F1MUC1
A	128	SER	-	insertion	UNP F1MUC1
A	129	ALA	-	insertion	UNP F1MUC1
A	130	ALA	-	insertion	UNP F1MUC1

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Chain	Residue	Modelled	Actual	Comment	Reference
A	131	LEU	-	insertion	UNP F1MUC1
A	132	TYR	-	insertion	UNP F1MUC1
A	133	GLU	-	insertion	UNP F1MUC1
A	134	ALA	-	insertion	UNP F1MUC1
A	135	HIS	-	insertion	UNP F1MUC1
A	136	GLY	-	insertion	UNP F1MUC1
A	137	TYR	-	insertion	UNP F1MUC1
A	138	ALA	-	insertion	UNP F1MUC1
A	139	GLY	-	insertion	UNP F1MUC1
A	140	VAL	-	insertion	UNP F1MUC1
A	141	LEU	-	insertion	UNP F1MUC1
A	142	SER	-	insertion	UNP F1MUC1
A	143	ALA	-	insertion	UNP F1MUC1
A	144	CYS	-	insertion	UNP F1MUC1
A	145	THR	-	insertion	UNP F1MUC1
A	146	LEU	-	insertion	UNP F1MUC1
A	147	VAL	-	insertion	UNP F1MUC1
A	148	LEU	-	insertion	UNP F1MUC1
A	149	ALA	-	insertion	UNP F1MUC1
A	150	ILE	-	insertion	UNP F1MUC1
A	151	LEU	-	insertion	UNP F1MUC1
A	152	HIS	-	insertion	UNP F1MUC1
A	153	HIS	-	insertion	UNP F1MUC1
A	154	LEU	-	insertion	UNP F1MUC1
A	155	TYR	-	insertion	UNP F1MUC1
A	156	PHE	-	insertion	UNP F1MUC1
A	157	TYR	-	insertion	UNP F1MUC1
A	158	HIS	-	insertion	UNP F1MUC1
A	159	VAL	-	insertion	UNP F1MUC1
A	160	GLN	-	insertion	UNP F1MUC1
A	161	CYS	-	insertion	UNP F1MUC1
A	162	ALA	-	insertion	UNP F1MUC1
A	163	GLY	-	insertion	UNP F1MUC1
A	164	MET	-	insertion	UNP F1MUC1
A	165	ARG	-	insertion	UNP F1MUC1
A	166	LEU	-	insertion	UNP F1MUC1
A	167	ARG	-	insertion	UNP F1MUC1
A	168	VAL	-	insertion	UNP F1MUC1
A	169	ALA	-	insertion	UNP F1MUC1
A	170	MET	-	insertion	UNP F1MUC1
A	171	CYS	-	insertion	UNP F1MUC1
A	172	HIS	-	insertion	UNP F1MUC1

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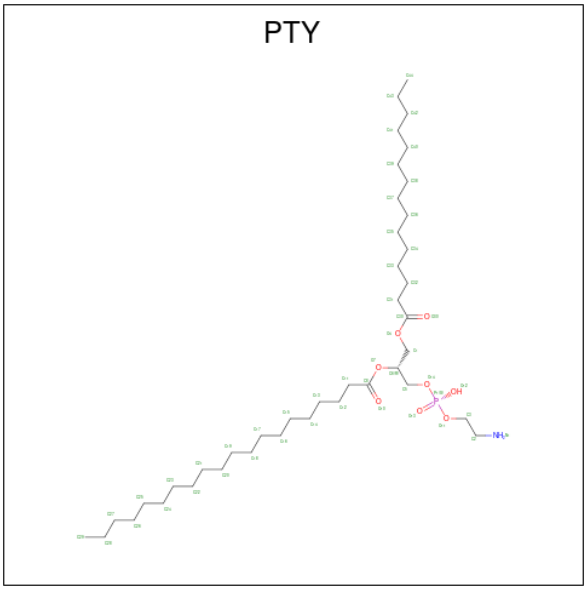
Chain	Residue	Modelled	Actual	Comment	Reference
A	173	MET	-	insertion	UNP F1MUC1
A	174	ILE	-	insertion	UNP F1MUC1
A	175	TYR	-	insertion	UNP F1MUC1
A	176	ARG	-	insertion	UNP F1MUC1
A	177	LYS	-	insertion	UNP F1MUC1
A	1202	GLN	GLU	engineered mutation	UNP F1MUC1

- Molecule 2 is MAGNESIUM ION (three-letter code: MG) (formula: Mg) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms		AltConf
			Total	Mg	
2	A	2	2	2	0

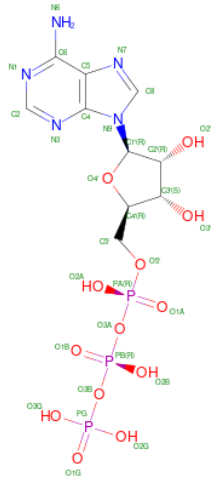
- Molecule 3 is PHOSPHATIDYLETHANOLAMINE (three-letter code: PTY) (formula: $C_{40}H_{80}NO_8P$).



Mol	Chain	Residues	Atoms						AltConf
			Total	C	H	N	O	P	
3	A	1	129	40	79	1	8	1	0

- Molecule 4 is ADENOSINE-5'-TRIPHOSPHATE (three-letter code: ATP) (formula: $C_{10}H_{16}N_5O_{13}P_3$) (labeled as "Ligand of Interest" by depositor).

ATP



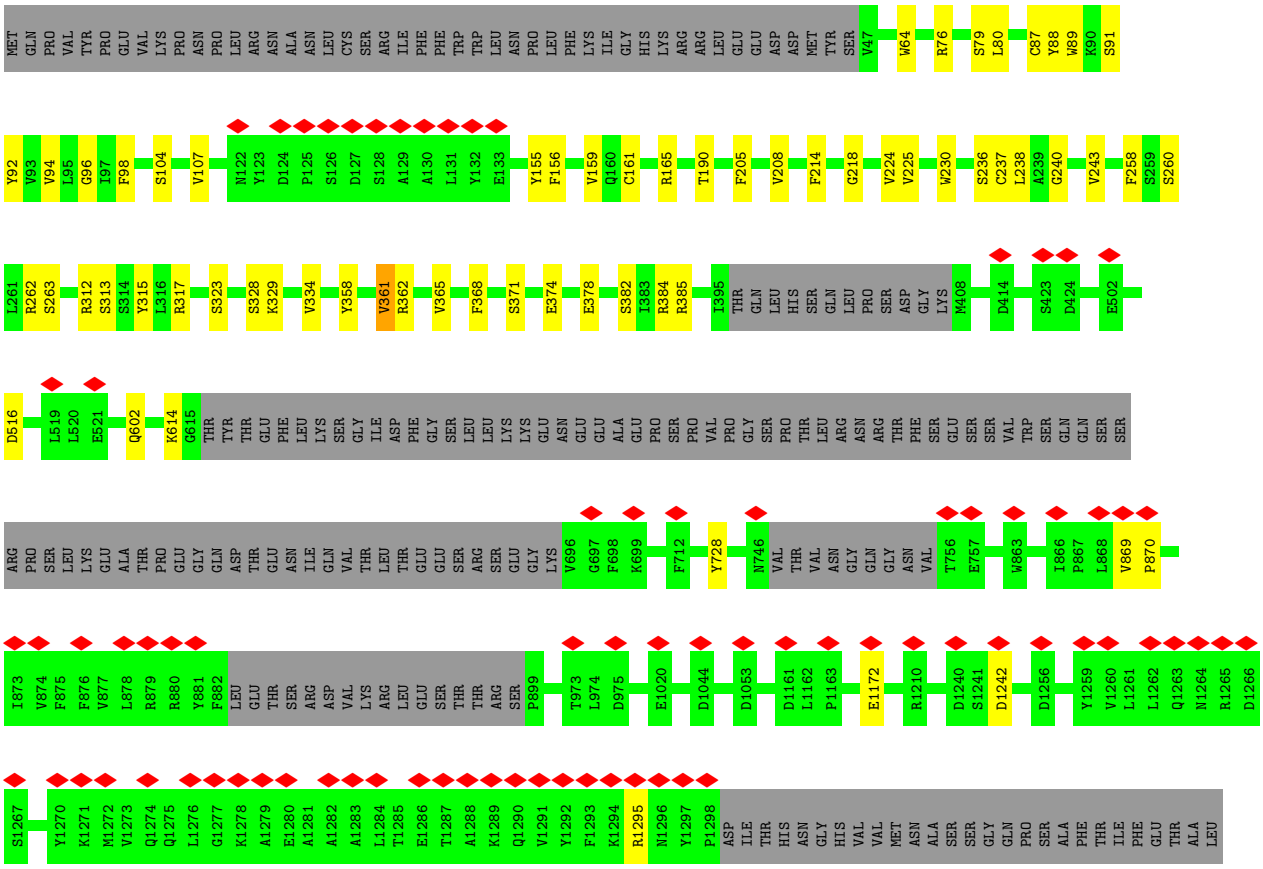
Mol	Chain	Residues	Atoms						AltConf
			Total	C	H	N	O	P	
4	A	1	43	10	12	5	13	3	0
			Total	C	H	N	O	P	
4	A	1	43	10	12	5	13	3	0

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: ATP binding cassette subfamily C member 4

Chain A: 6% 81% 5% 14%



4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	104051	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	TFS KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	66	Depositor
Minimum defocus (nm)	500	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	105000	Depositor
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor
Maximum map value	3.122	Depositor
Minimum map value	-1.800	Depositor
Average map value	0.003	Depositor
Map value standard deviation	0.075	Depositor
Recommended contour level	0.55	Depositor
Map size (Å)	250.2, 250.2, 250.2	wwPDB
Map dimensions	300, 300, 300	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.834, 0.834, 0.834	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: PTY, MG, ATP

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	0.92	58/9230 (0.6%)	0.89	22/12511 (0.2%)

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

The worst 5 of 58 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	362	ARG	CZ-NH2	-7.68	1.23	1.33
1	A	385	ARG	CZ-NH2	-7.65	1.23	1.33
1	A	165	ARG	CZ-NH2	-7.56	1.23	1.33
1	A	262	ARG	CZ-NH2	-7.51	1.23	1.33
1	A	317	ARG	CZ-NH2	-7.49	1.23	1.33

The worst 5 of 22 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	315	TYR	CB-CG-CD2	8.29	125.97	121.00
1	A	358	TYR	CB-CG-CD2	7.36	125.42	121.00
1	A	385	ARG	CD-NE-CZ	7.07	133.50	123.60
1	A	88	TYR	CB-CG-CD2	7.03	125.22	121.00
1	A	368	PHE	CB-CG-CD2	6.84	125.59	120.80

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	76	ARG	Peptide
1	A	92	TYR	Sidechain

5.2 Too-close contacts (i)

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

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	9039	9269	9262	6	0
2	A	2	0	0	0	0
3	A	50	79	79	0	0
4	A	62	24	24	0	0
All	All	9153	9372	9365	6	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 0.

The worst 5 of 6 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:190:THR:HA	1:A:1172:GLU:OE2	1.88	0.74
1:A:1242:ASP:OD1	1:A:1295:ARG:NH1	2.48	0.47
1:A:329:LYS:HE2	1:A:728:TYR:CE2	2.50	0.46
1:A:602:GLN:OE1	1:A:614:LYS:HE2	2.19	0.43
1:A:869:VAL:HB	1:A:870:PRO:HD3	2.01	0.41

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	1125/1325 (85%)	1111 (99%)	14 (1%)	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	983/1156 (85%)	982 (100%)	1 (0%)	93	98

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

Mol	Chain	Res	Type
1	A	516	ASP

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

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 5 ligands modelled in this entry, 2 are monoatomic - leaving 3 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	$\#\|Z\| > 2$
4	ATP	A	1404	2	26,33,33	1.12	3 (11%)	31,52,52	1.81	7 (22%)
4	ATP	A	1405	2	26,33,33	1.12	2 (7%)	31,52,52	1.71	5 (16%)
3	PTY	A	1403	-	49,49,49	0.73	0	52,54,54	0.55	0

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
4	ATP	A	1404	2	-	2/18/38/38	0/3/3/3
4	ATP	A	1405	2	-	3/18/38/38	0/3/3/3
3	PTY	A	1403	-	-	16/53/53/53	-

All (5) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
4	A	1405	ATP	O4'-C1'	2.62	1.44	1.41
4	A	1405	ATP	C8-N7	-2.45	1.30	1.34
4	A	1404	ATP	C8-N7	-2.44	1.30	1.34
4	A	1404	ATP	O4'-C1'	2.33	1.44	1.41
4	A	1404	ATP	C4-N3	-2.25	1.32	1.35

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

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
4	A	1404	ATP	PA-O3A-PB	-5.54	113.83	132.83
4	A	1405	ATP	PA-O3A-PB	-4.92	115.96	132.83
4	A	1404	ATP	PB-O3B-PG	-4.91	115.96	132.83
4	A	1405	ATP	PB-O3B-PG	-4.85	116.17	132.83
4	A	1405	ATP	N3-C2-N1	-4.18	122.15	128.68

There are no chirality outliers.

5 of 21 torsion outliers are listed below:

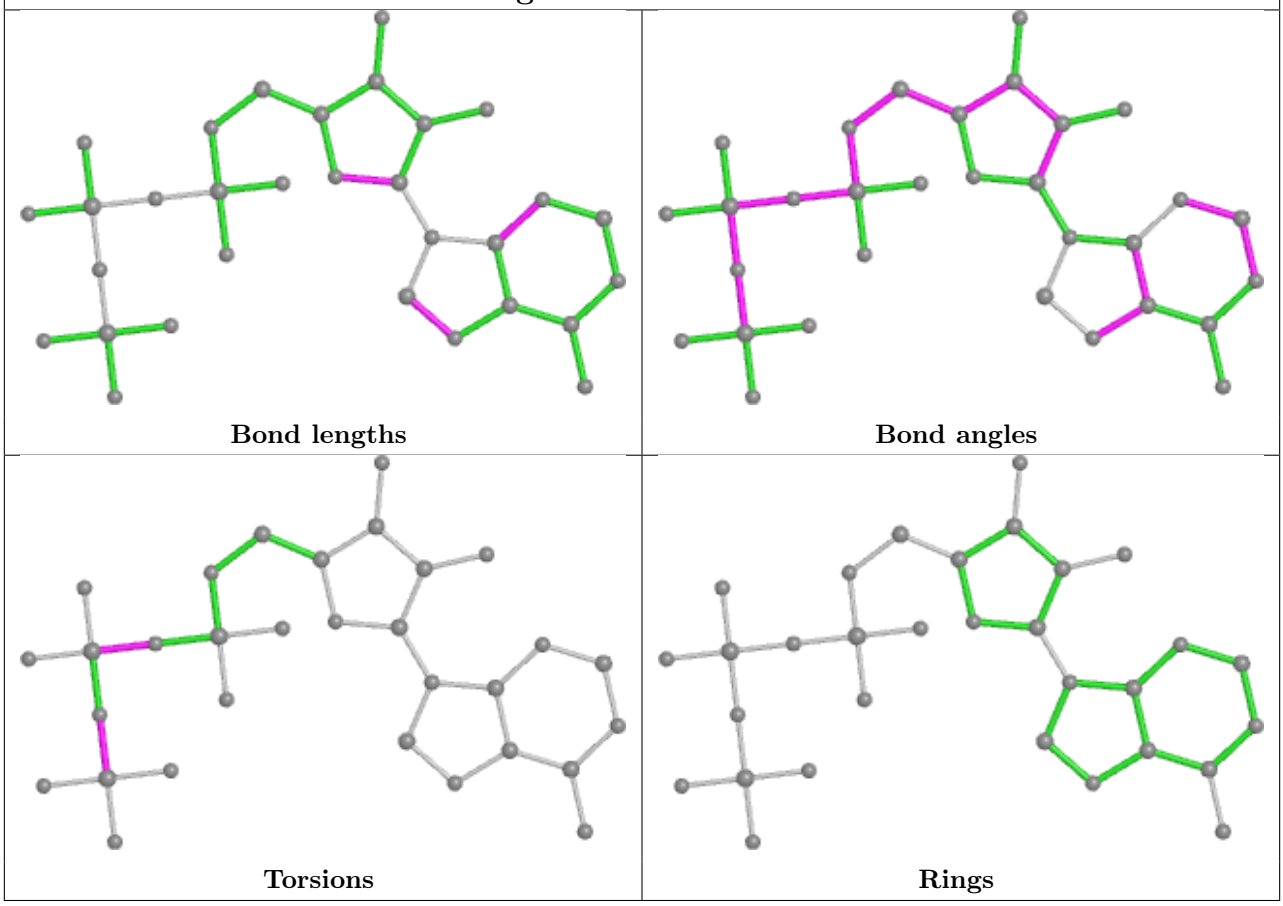
Mol	Chain	Res	Type	Atoms
3	A	1403	PTY	C2-C3-O11-P1
3	A	1403	PTY	O10-C8-O7-C6
3	A	1403	PTY	C11-C8-O7-C6
3	A	1403	PTY	C3-O11-P1-O13
4	A	1405	ATP	PB-O3B-PG-O2G

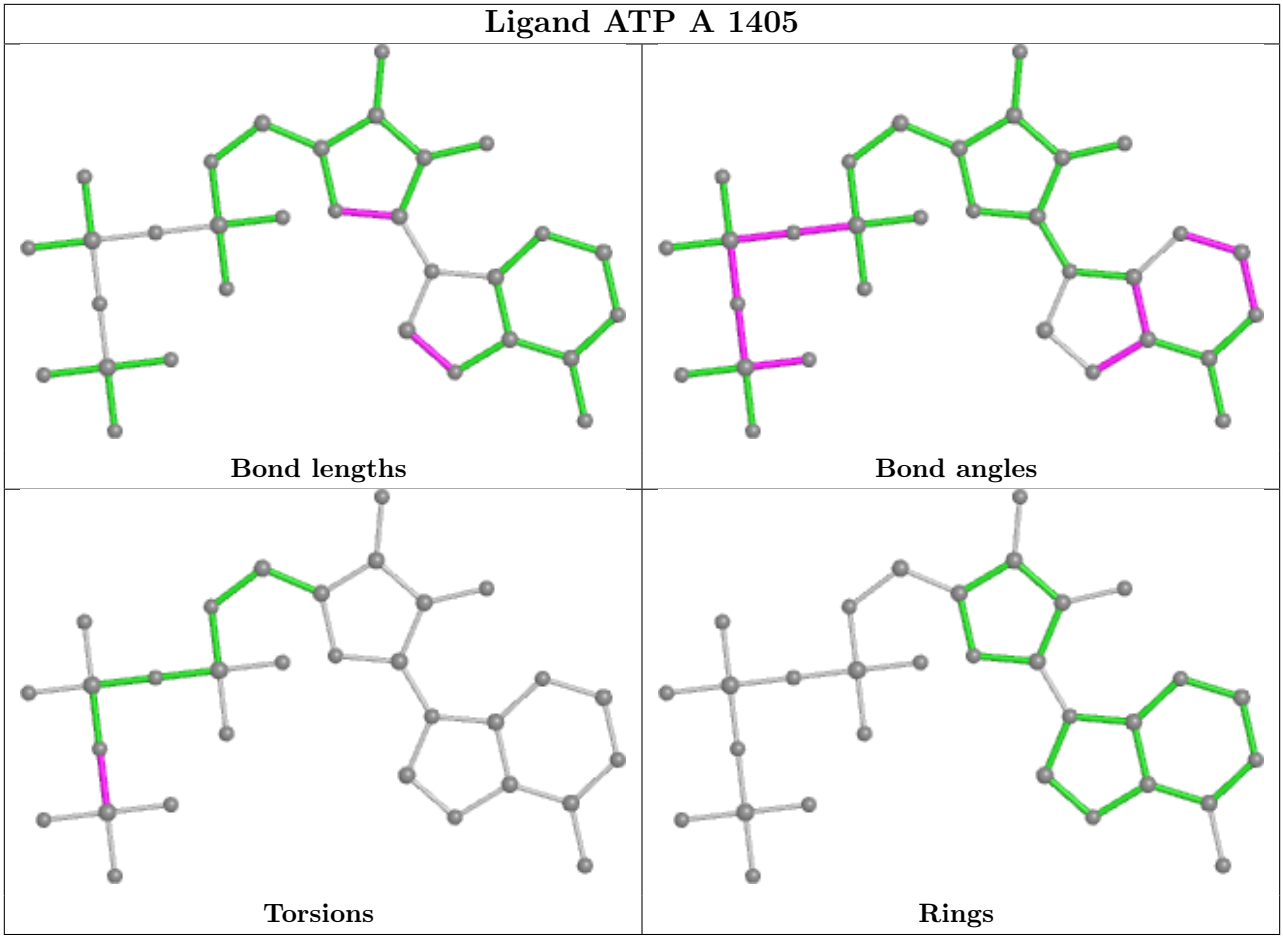
There are no ring outliers.

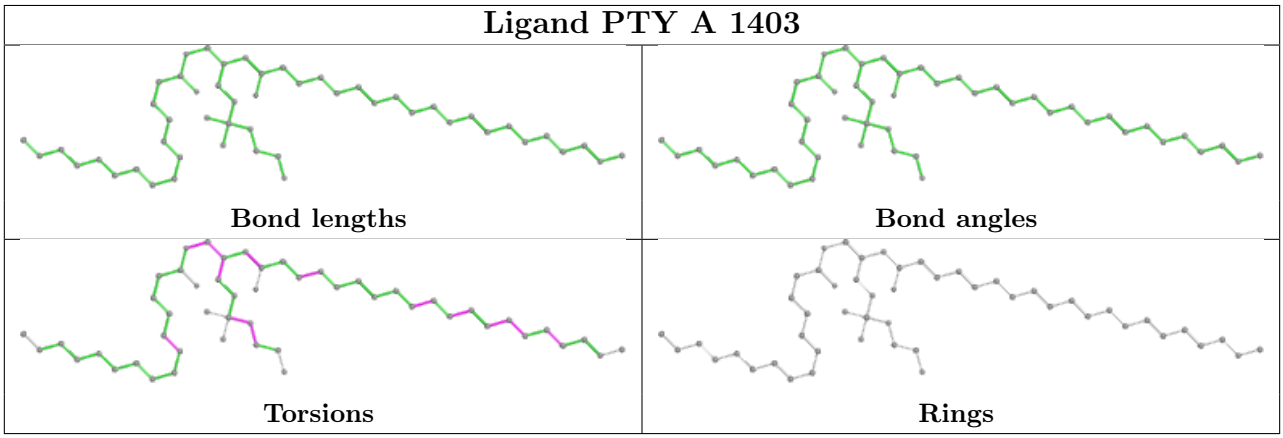
No monomer is involved in short contacts.

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 ATP A 1404







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.

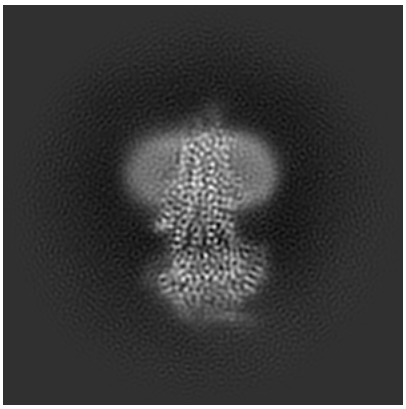
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-40821. These allow visual inspection of the internal detail of the map and identification of artifacts.

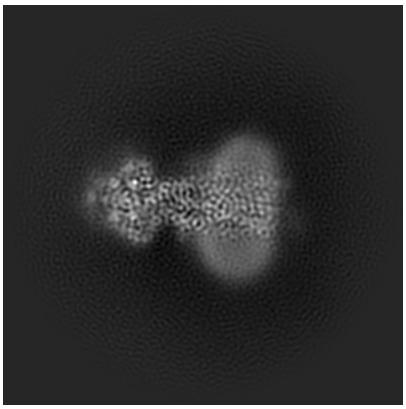
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections (i)

6.1.1 Primary map



X



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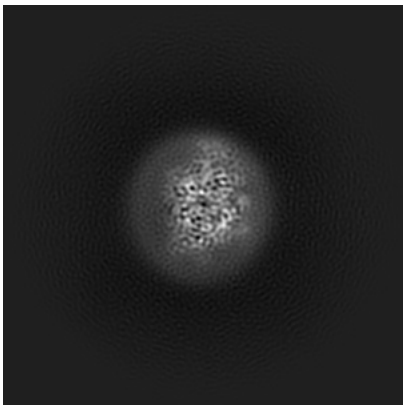
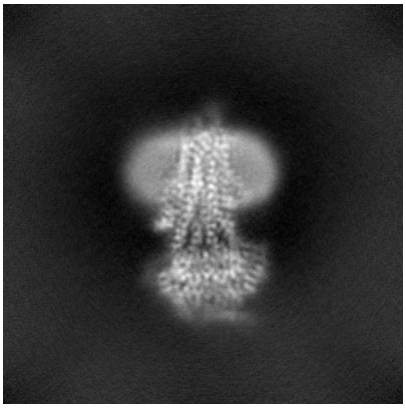
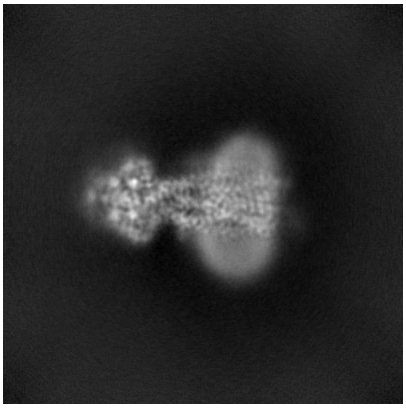


Z

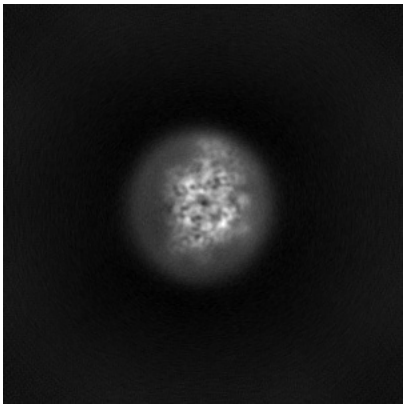
6.1.2 Raw map



X



Y

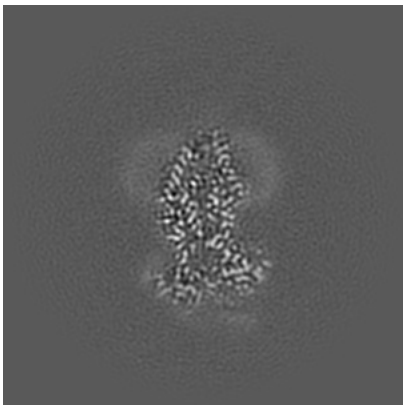


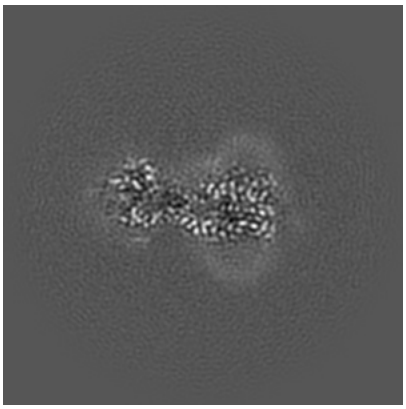
Z

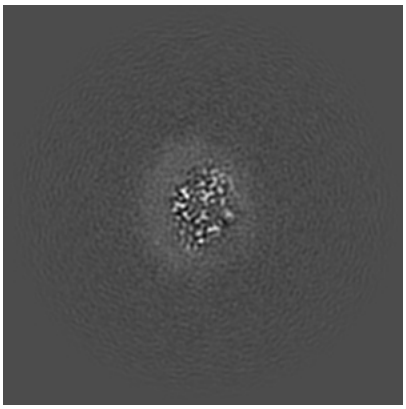
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

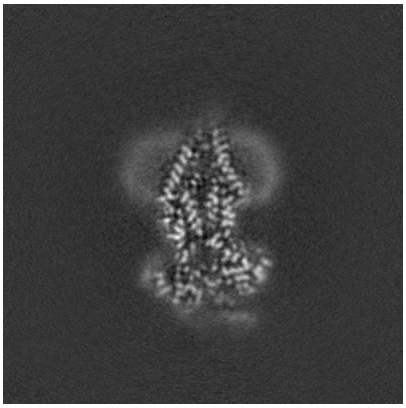
6.2.1 Primary map

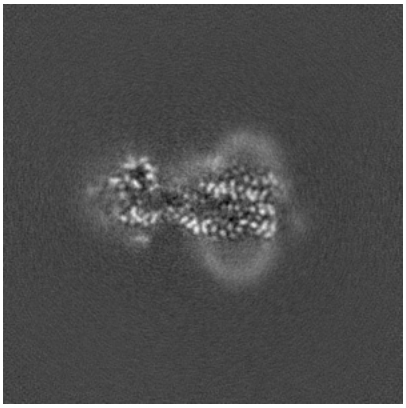

X Index: 150

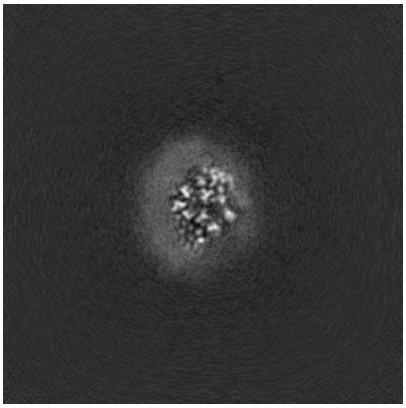

Y Index: 150


Z Index: 150

6.2.2 Raw map


X Index: 150

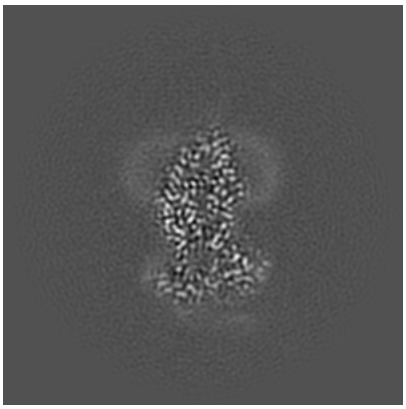

Y Index: 150


Z Index: 150

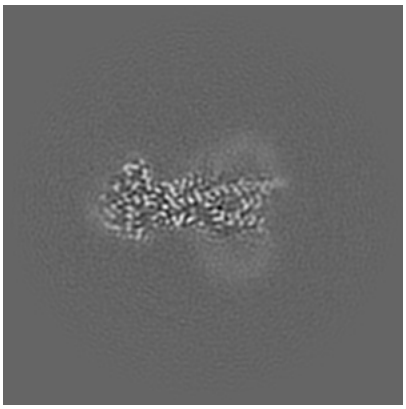
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

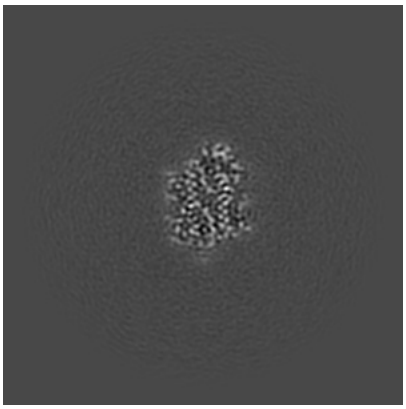
6.3.1 Primary map



X Index: 149

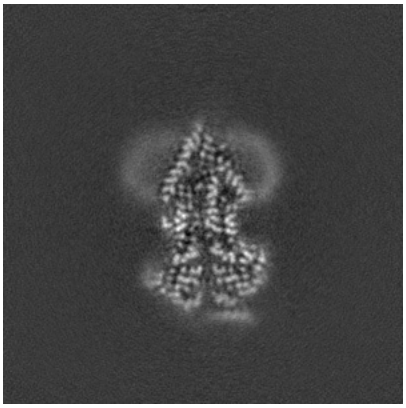


Y Index: 135

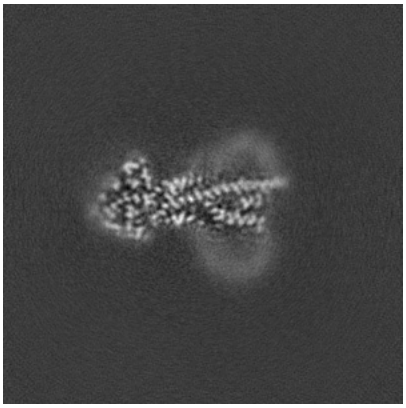


Z Index: 98

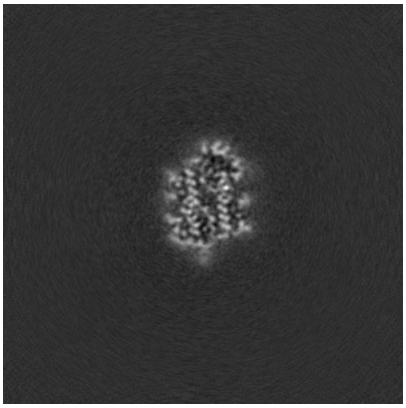
6.3.2 Raw map



X Index: 153



Y Index: 135

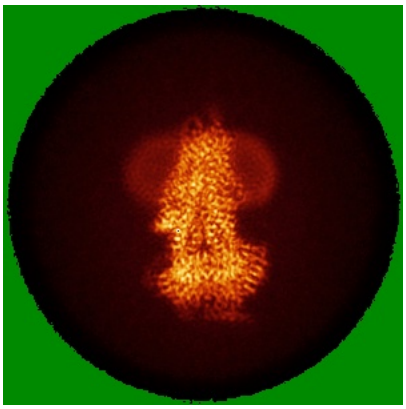


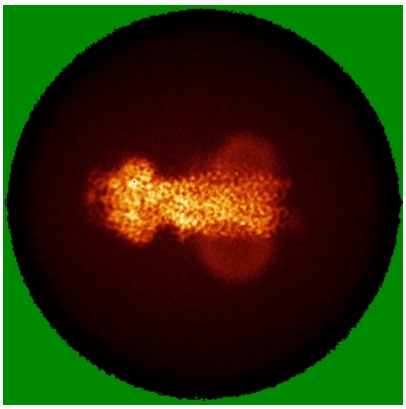
Z Index: 97

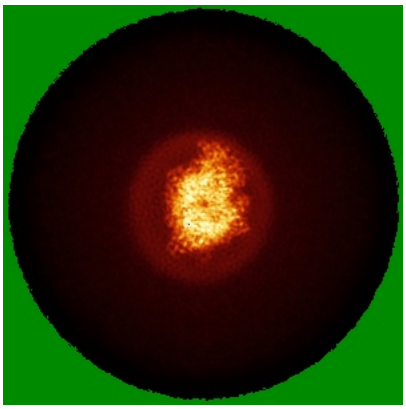
The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal standard-deviation projections (False-color) (i)

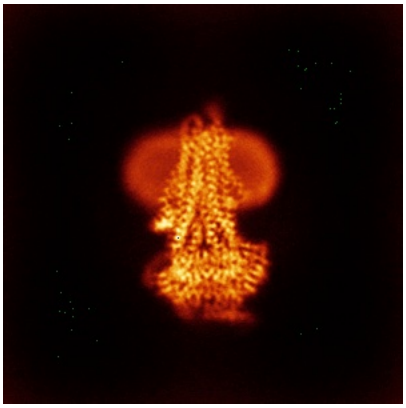
6.4.1 Primary map

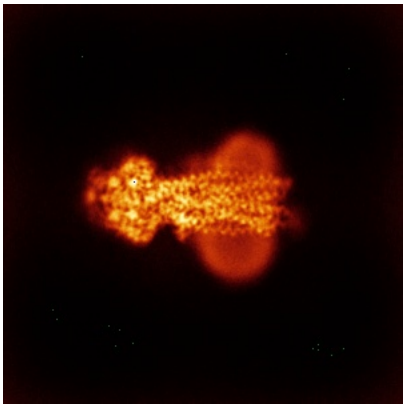

X

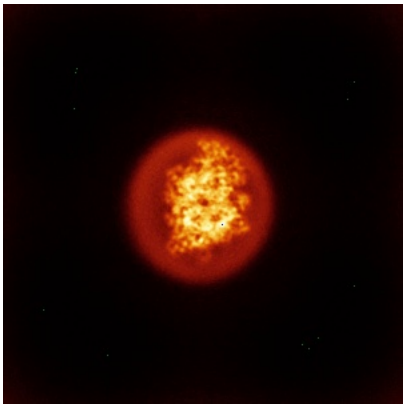

Y


Z

6.4.2 Raw map


X

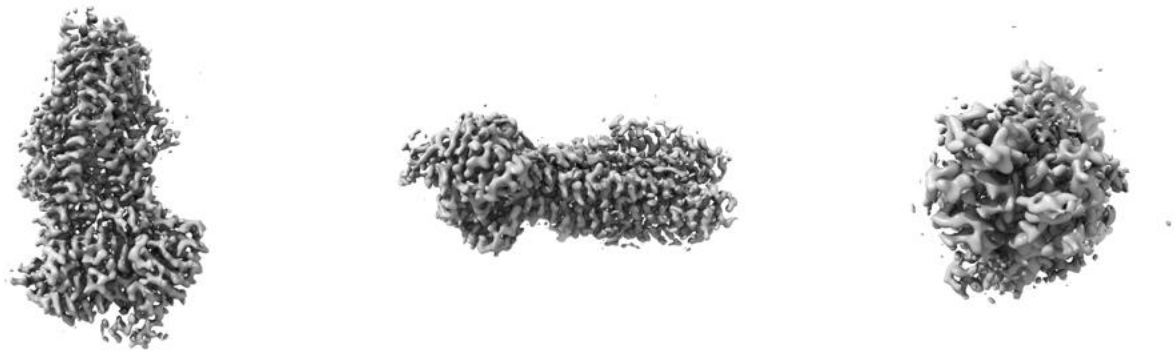

Y


Z

The images above show the map standard deviation projections with false color in three orthogonal directions. Minimum values are shown in green, max in blue, and dark to light orange shades represent small to large values respectively.

6.5 Orthogonal surface views (i)

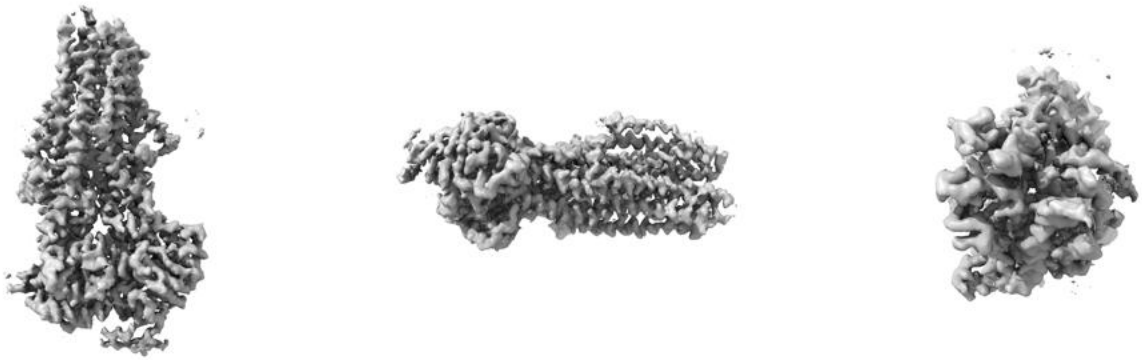
6.5.1 Primary map



X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.55. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.5.2 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.

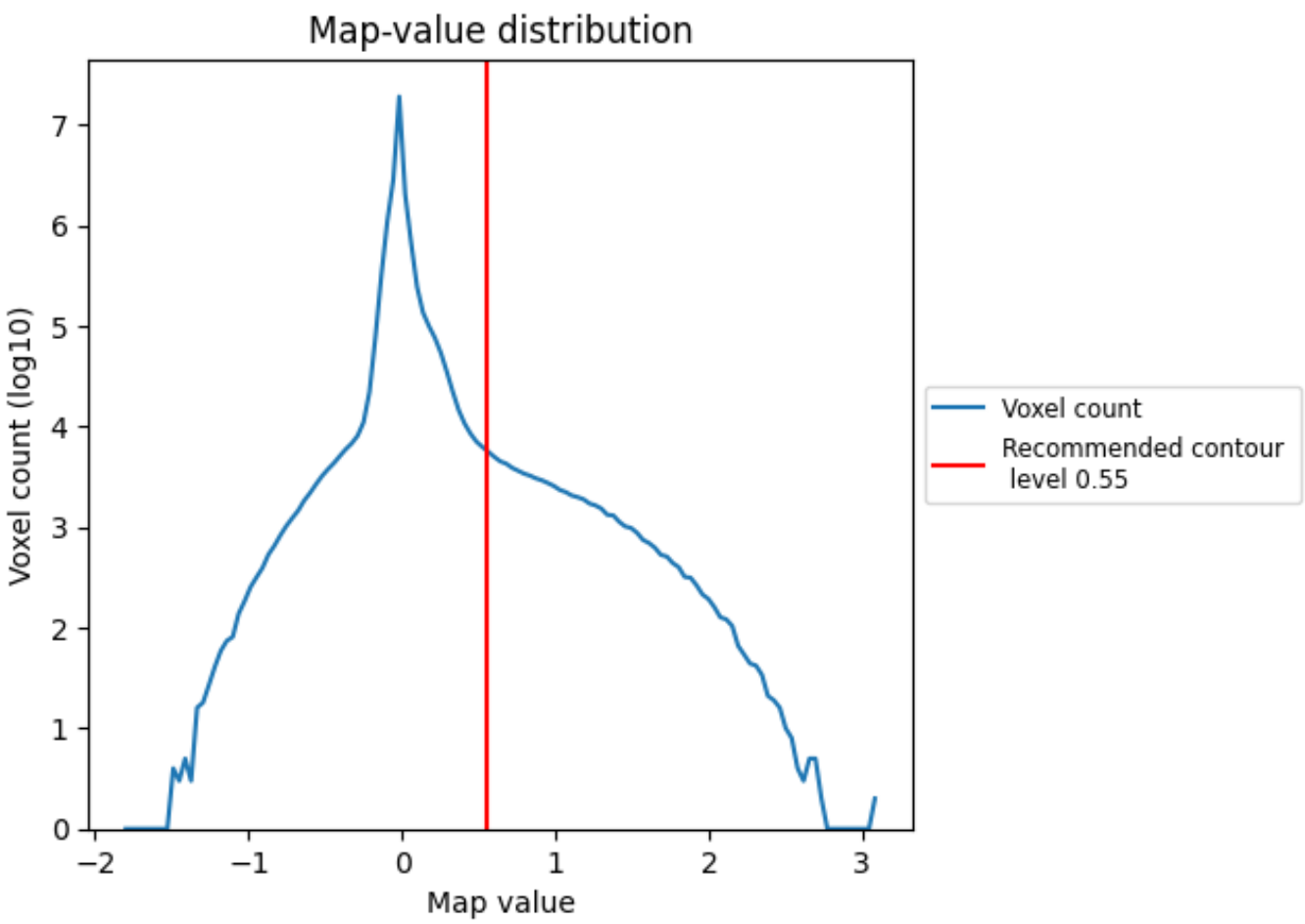
6.6 Mask visualisation (i)

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

7 Map analysis (i)

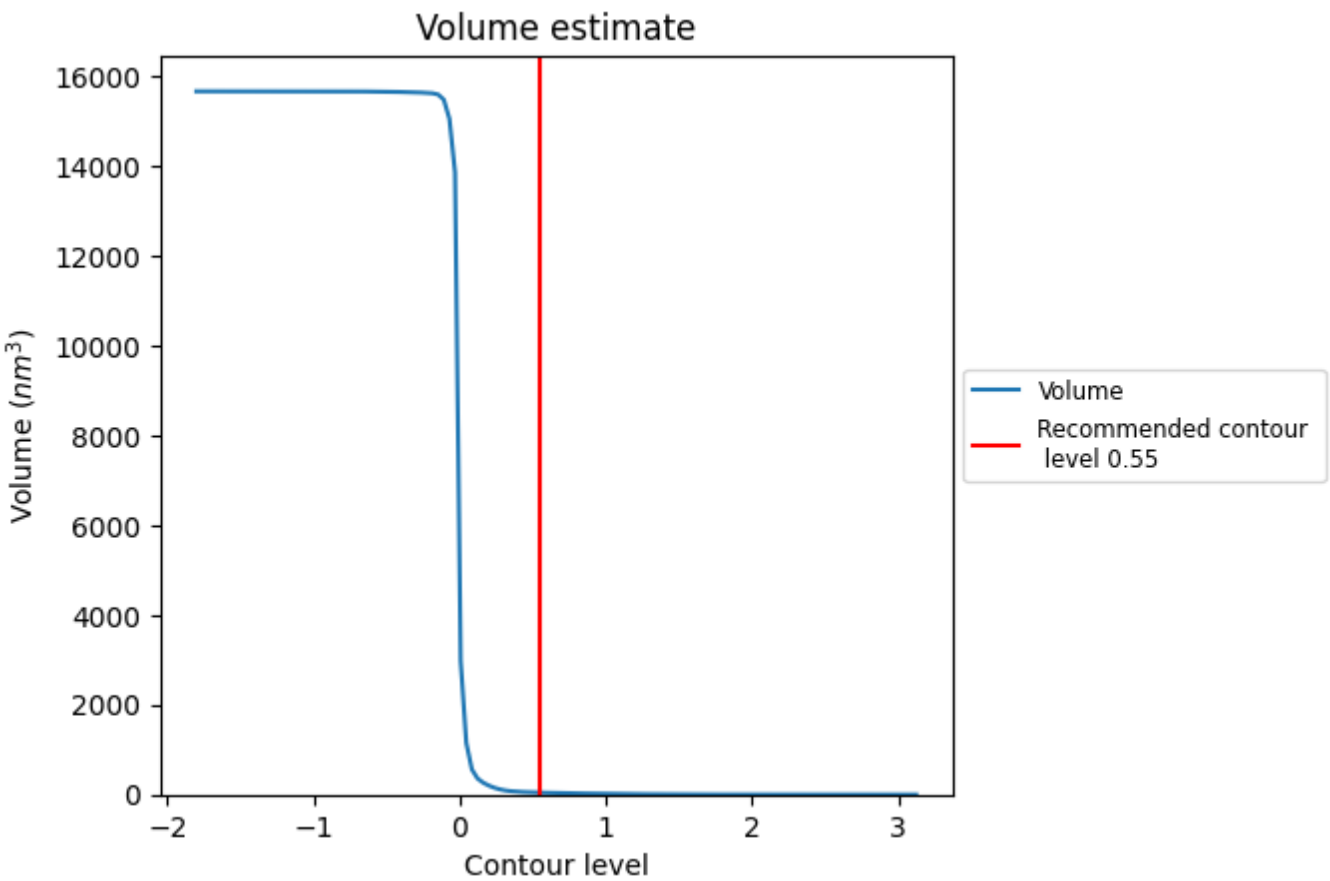
This section contains the results of statistical analysis of the map.

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.

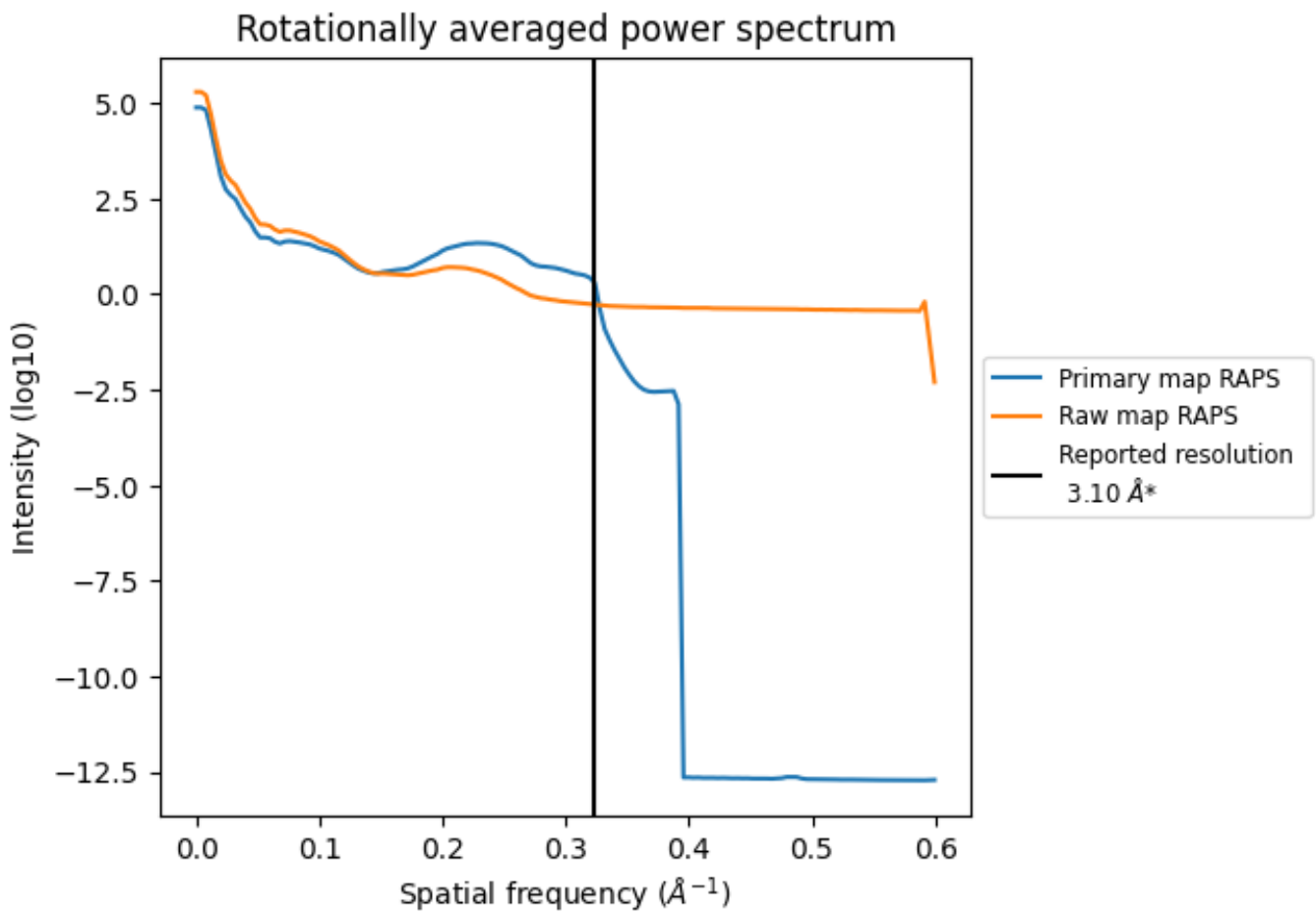
7.2 Volume estimate (i)



The volume at the recommended contour level is 44 nm^3; this corresponds to an approximate mass of 39 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)

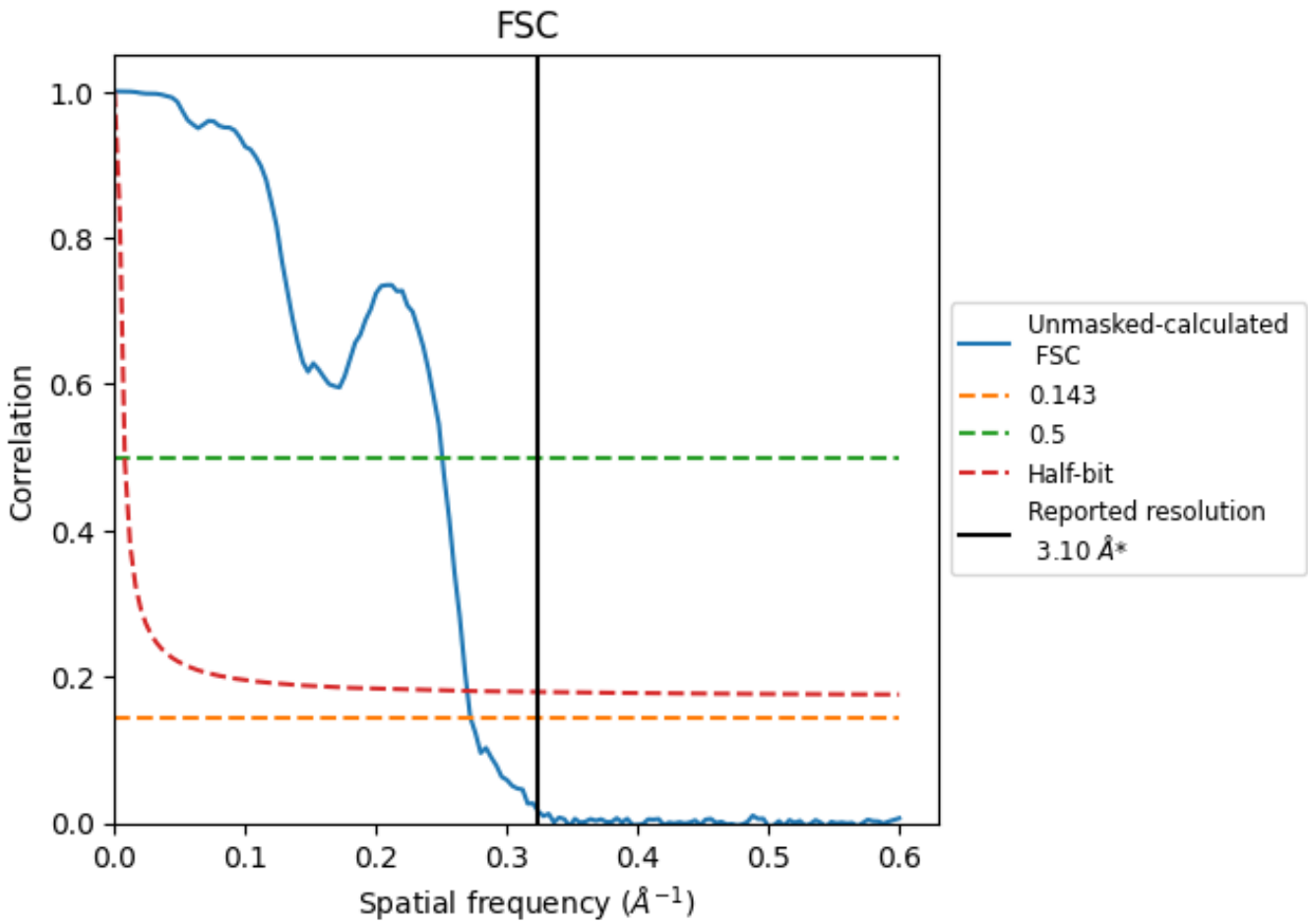


*Reported resolution corresponds to spatial frequency of 0.323 Å^{-1}

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 0.323 Å^{-1}

8.2 Resolution estimates (i)

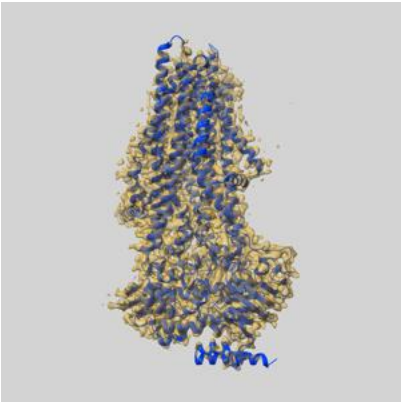
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.10	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	3.68	3.99	3.71

*Resolution estimate based on FSC curve calculated by comparison of deposited half-maps. The value from deposited half-maps intersecting FSC 0.143 CUT-OFF 3.68 differs from the reported value 3.1 by more than 10 %

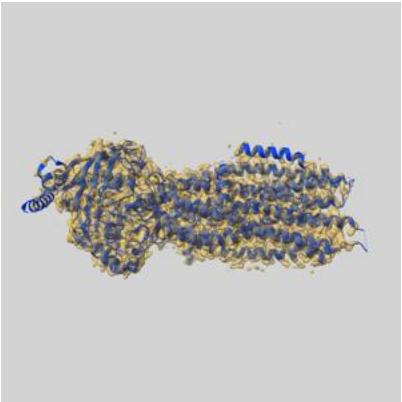
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-40821 and PDB model 8SWN. Per-residue inclusion information can be found in section 3 on page 7.

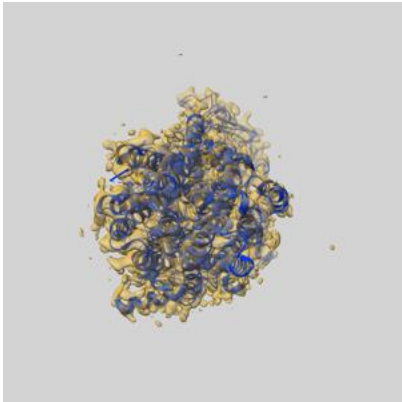
9.1 Map-model overlay (i)



X



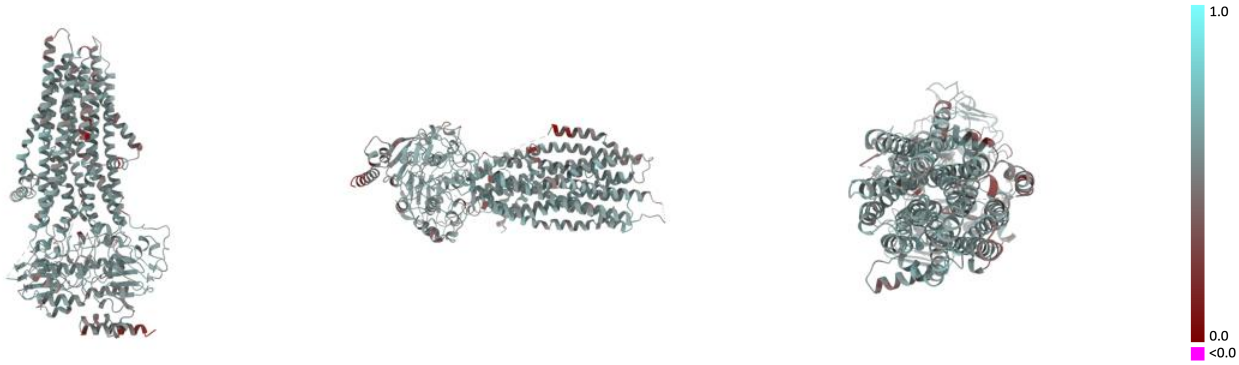
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.55 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.

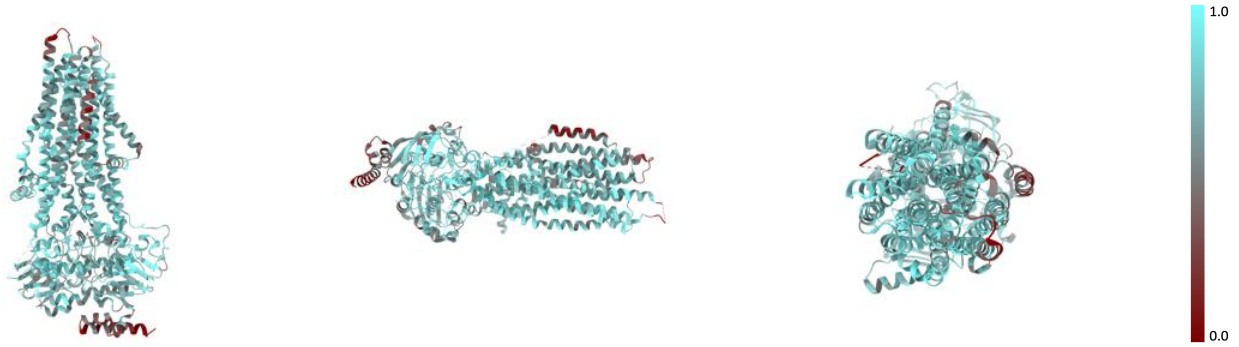
9.2 Q-score mapped to coordinate model (i)



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.

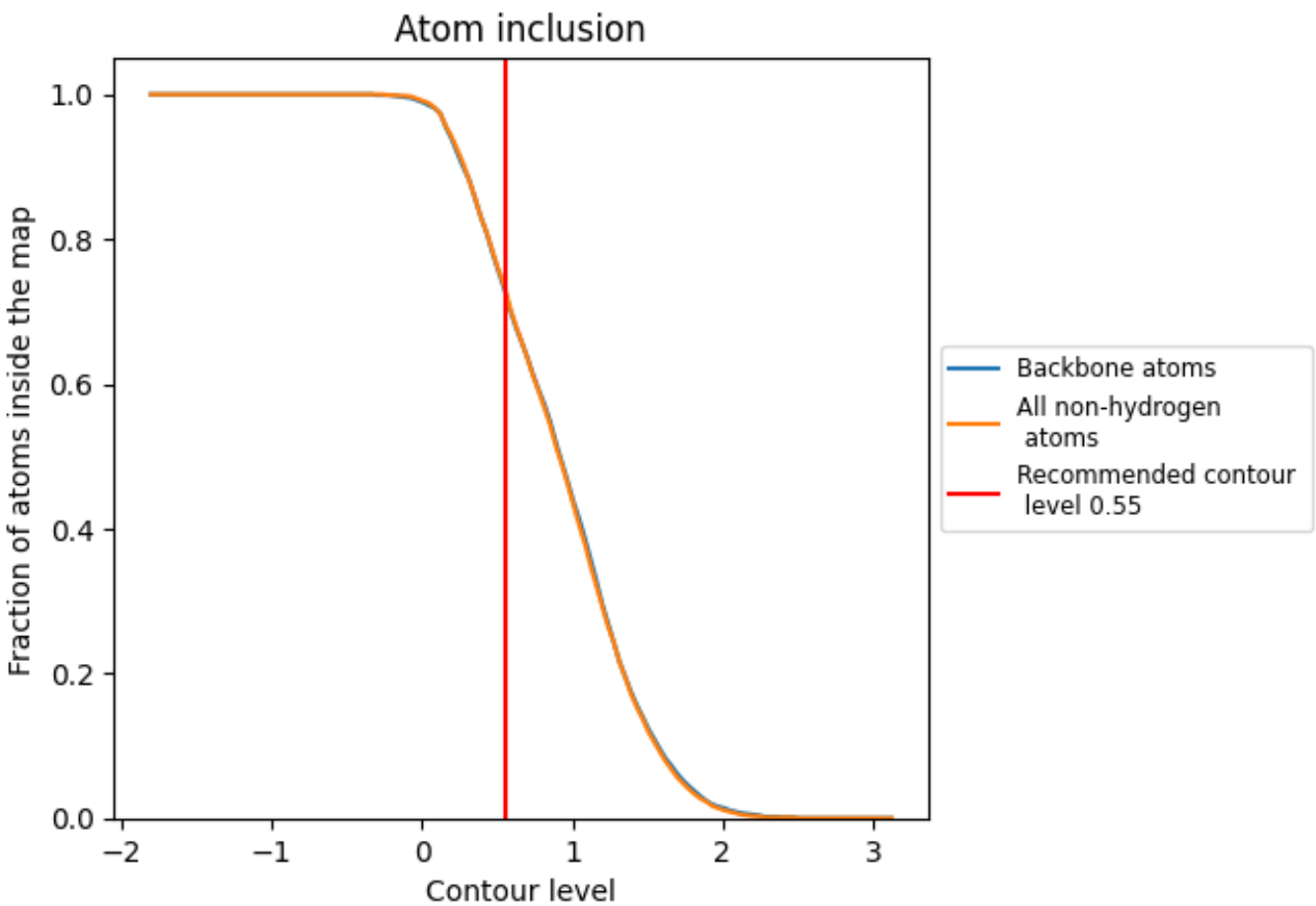
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.55).

9.4 Atom inclusion (i)



At the recommended contour level, 73% 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 (0.55) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.7340	0.5350
A	0.7340	0.5350