wwPDB EM Validation Summary Report (i)

Nov 20, 2022 – 01:42 PM EST

PDB ID	:	7MIS
EMDB ID	:	EMD-23863
Title	:	Cryo-EM structure of SidJ-SdeC-CaM reaction intermediate complex
Authors	:	Osinski, A.; Black, M.H.; Pawlowski, K.; Chen, Z.; Li, Y.; Tagliabracci, V.S.
Deposited on	:	2021-04-17
Resolution	:	2.80 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:

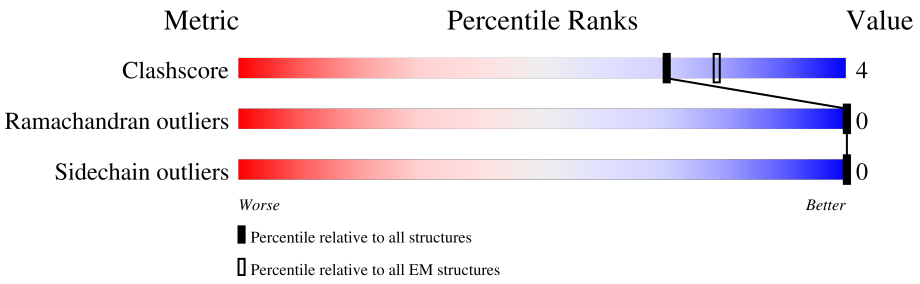
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.31.2

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 2.80 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	756	92% 7%
2	B	150	31% 75% 14% 11%
3	C	997	11% 65% 8% 26%

2 Entry composition (i)

There are 8 unique types of molecules in this entry. The entry contains 25916 atoms, of which 12877 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 Calmodulin-dependent glutamylase SidJ.

Mol	Chain	Residues	Atoms						AltConf	Trace
			Total	C	H	N	O	S		
1	A	749	12185	3910	6073	1036	1151	15	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	96	SER	-	expression tag	UNP Q5ZTK6

- Molecule 2 is a protein called Calmodulin.

Mol	Chain	Residues	Atoms						AltConf	Trace
			Total	C	H	N	O	S		
2	B	133	2047	650	991	172	227	7	0	0

There is a discrepancy between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	0	SER	-	expression tag	UNP P0DP24

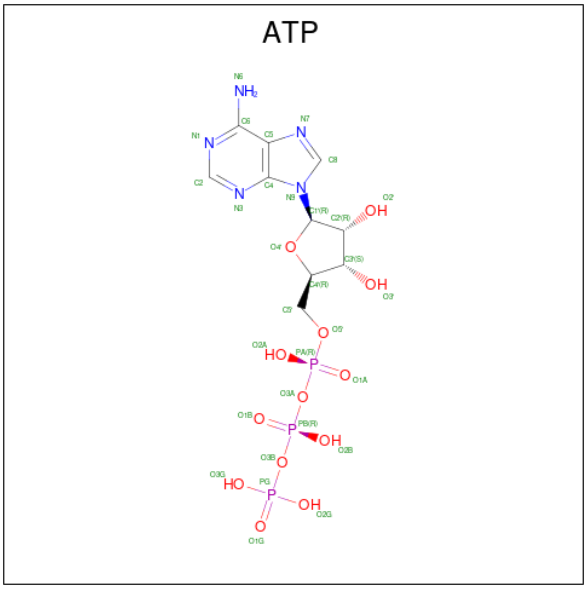
- Molecule 3 is a protein called SdeC.

Mol	Chain	Residues	Atoms						AltConf	Trace
			Total	C	H	N	O	S		
3	C	734	11603	3678	5790	992	1119	24	0	0

There are 5 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
C	226	GLY	-	expression tag	UNP Q6RCQ8
C	227	ALA	-	expression tag	UNP Q6RCQ8
C	228	MET	-	expression tag	UNP Q6RCQ8
C	229	GLY	-	expression tag	UNP Q6RCQ8
C	230	SER	-	expression tag	UNP Q6RCQ8

- 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).



Mol	Chain	Residues	Atoms						AltConf
			Total	C	H	N	O	P	
4	A	1	42	10	11	5	13	3	0

- Molecule 5 is MAGNESIUM ION (three-letter code: MG) (formula: Mg) (labeled as "Ligand of Interest" by depositor).

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

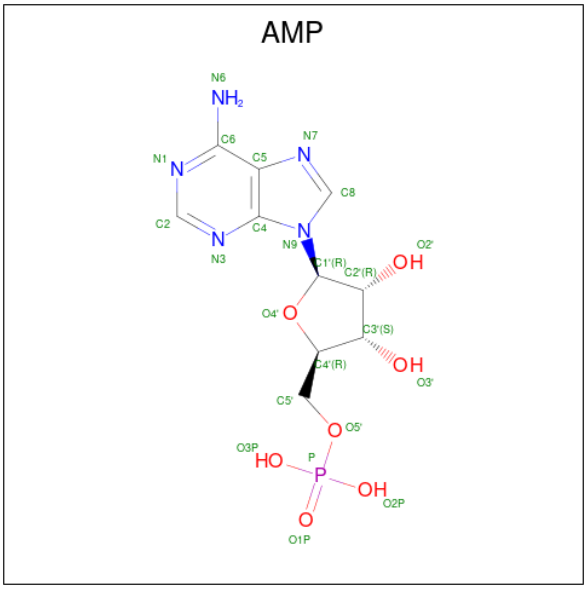
- Molecule 6 is SODIUM ION (three-letter code: NA) (formula: Na) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms		AltConf
			Total	Na	
6	A	1	1	1	0

- Molecule 7 is CALCIUM ION (three-letter code: CA) (formula: Ca) (labeled as "Ligand of Interest" by depositor).

Mol	Chain	Residues	Atoms		AltConf
			Total	Ca	
7	B	1	1	1	0

- Molecule 8 is ADENOSINE MONOPHOSPHATE (three-letter code: AMP) (formula: $C_{10}H_{14}N_5O_7P$) (labeled as "Ligand of Interest" by depositor).



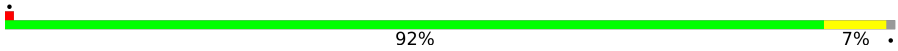
Mol	Chain	Residues	Atoms						AltConf
			Total	C	H	N	O	P	
8	C	1	35	10	12	5	7	1	0

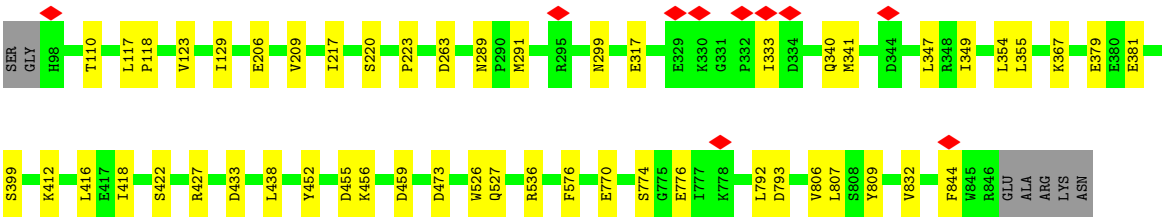
3 Residue-property plots (i)

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: Calmodulin-dependent glutamylase SidJ

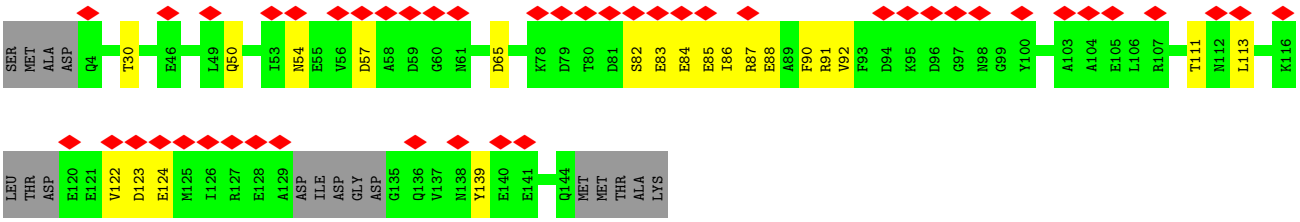
Chain A:





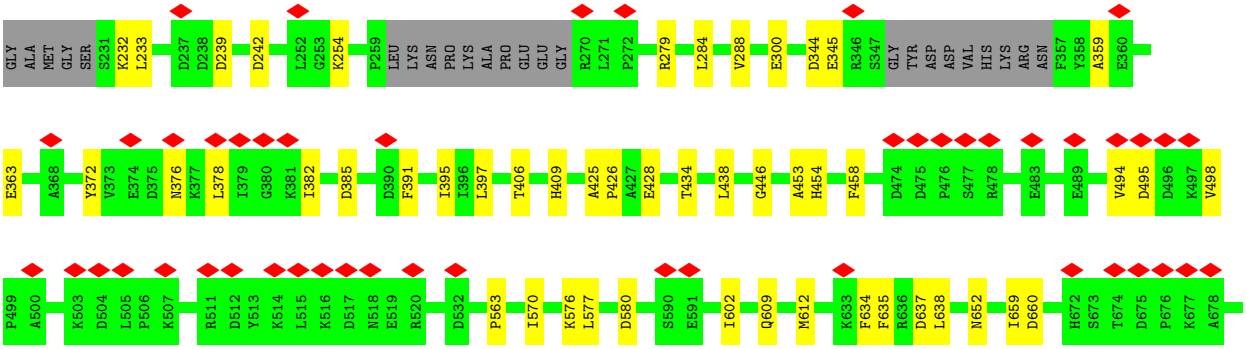
- Molecule 2: Calmodulin

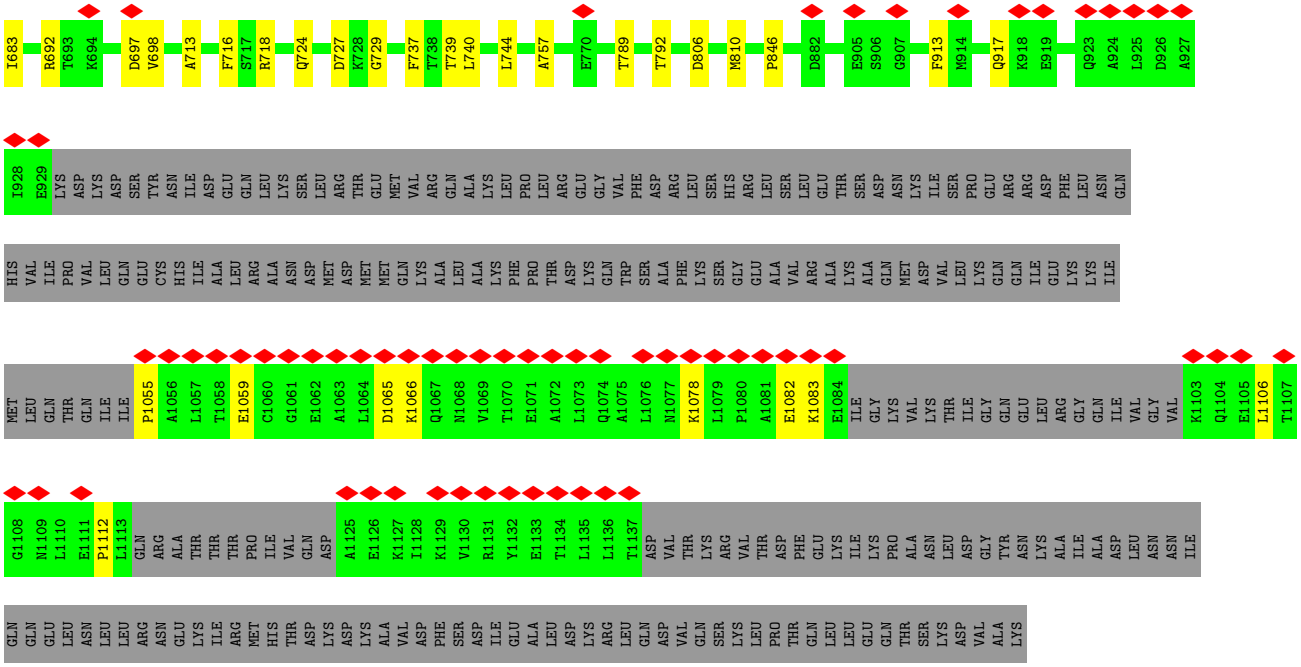
Chain B:



- Molecule 3: SdeC

Chain C:




I683 R692 T693 K694 D697 V698 A713 F716 S717 R718 Q724 D727 K728 G729 F737 T738 T739 L740 L744 A757 E770 T789 T792 D806 M810 P846 D882 E905 S906 G907 F913 M914 Q917 K918 E919 Q923 A924 L925 D926 A927
I928 E929 LYS ASP LYS ASP SER TYR ASN ILE ASP GLU GLN LEU LYS SER LEU ARG THR GLU MET VAL ARG GLN ALA LYS LEU PRO LEU ARG GLU GLY VAL PHE ASP ARG LEU SER HIS ARG LEU SER LEU GLU THR SER ASP ASN LYS ILE SER PRO GLU ARG ARG ASP PHE LEU ASN GLN
HIS VAL ILE PRO VAL LEU GLN GLU CYS HIS ILE ALA LEU ARG ALA ASN ASP MET ASP MET MET GLN LYS ALA LEU ALA LYS PHE PRO THR ASP LYS GLN TRP SER ALA PHE LYS SER GLY GLU ALA VAL ARG ALA LYS ALA GLN MET ASP VAL LEU LYS GLN GLN ILE GLU LYS LYS ILE
MET LEU GLN THR GLN ILE ILE P1055 A1056 L1057 T1058 E1059 C1060 G1061 E1062 A1063 L1064 D1065 K1066 Q1067 N1068 V1069 T1070 E1071 A1072 L1073 Q1074 A1075 L1076 N1077 K1078 L1079 P1080 A1081 E1082 K1083 E1084 ILE GLY LYS VAL LYS THR ILE GLY GLN GLU LEU ARG GLY GLN ILE VAL GLY VAL K1103 Q1104 E1105 L1106 T1107
G1108 N1109 L1110 E1111 P1112 L1113 GLN ARG ALA THR THR THR PRO ILE VAL GLN ASP A1125 E1126 K1127 I1128 K1129 V1130 R1131 Y1132 E1133 T1134 L1135 L1136 T1137 ASP VAL THR LYS ARG VAL THR ASP PHE GLU LYS ILE LYS PRO ALA ASN LEU ASP GLY TYR ASN LYS ALA ILE ALA ASP LEU ASN ASN ILE
GLN GLN GLU LEU ASN LEU LEU ARG ASN GLU LYS ILE ARG MET HIS THR ASP LYS ASP LYS ALA VAL ASP PHE SER ASP ILE GLU ALA LEU ASP LYS ARG LEU GLN ASP VAL GLN SER LYS LEU PRO THR GLN LEU LEU GLU GLN THR SER LYS ASP VAL ALA LYS

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	152589	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 ($e^-/Å^2$)	50	Depositor
Minimum defocus (nm)	Not provided	
Maximum defocus (nm)	Not provided	
Magnification	Not provided	
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor
Maximum map value	0.204	Depositor
Minimum map value	-0.091	Depositor
Average map value	-0.000	Depositor
Map value standard deviation	0.005	Depositor
Recommended contour level	0.03	Depositor
Map size (Å)	432.00003, 432.00003, 432.00003	wwPDB
Map dimensions	400, 400, 400	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.08, 1.08, 1.08	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, MG, ATP, NA, AMP

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	0.27	0/6254	0.47	0/8456
2	B	0.25	0/1066	0.50	0/1427
3	C	0.25	0/5926	0.46	0/8005
All	All	0.26	0/13246	0.47	0/17888

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts (i)

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

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	6112	6073	6072	33	0
2	B	1056	991	991	15	0
3	C	5813	5790	5789	48	0
4	A	31	11	12	3	0
5	A	2	0	0	0	0
6	A	1	0	0	0	0
7	B	1	0	0	0	0
8	C	23	12	12	0	0
All	All	13039	12877	12876	94	0

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including

hydrogen atoms). The all-atom clashscore for this structure is 4.

The worst 5 of 94 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:774:SER:OG	1:A:776:GLU:OE1	1.98	0.79
3:C:789:THR:OG1	3:C:792:THR:O	2.01	0.79
1:A:220:SER:OG	1:A:793:ASP:OD2	2.01	0.78
2:B:88:GLU:OE1	2:B:91:ARG:NH2	2.20	0.74
3:C:254:LYS:O	3:C:279:ARG:NH1	2.23	0.71

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	747/756 (99%)	728 (98%)	19 (2%)	0	100	100
2	B	127/150 (85%)	124 (98%)	3 (2%)	0	100	100
3	C	722/997 (72%)	684 (95%)	38 (5%)	0	100	100
All	All	1596/1903 (84%)	1536 (96%)	60 (4%)	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	679/684 (99%)	679 (100%)	0	100	100
2	B	114/128 (89%)	114 (100%)	0	100	100
3	C	634/868 (73%)	634 (100%)	0	100	100
All	All	1427/1680 (85%)	1427 (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. All (2) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	527	GLN
3	C	454	HIS

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	$\#\|Z\| > 2$
4	ATP	A	1001	5	26,33,33	0.64	0	31,52,52	0.50	0

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\#\|Z\| > 2$	Counts	RMSZ	$\#\|Z\| > 2$
8	AMP	C	2000	3	22,25,25	0.76	0	25,38,38	0.74	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	1001	5	-	2/18/38/38	0/3/3/3
8	AMP	C	2000	3	-	1/6/26/26	0/3/3/3

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
4	A	1001	ATP	C5'-O5'-PA-O2A
4	A	1001	ATP	C5'-O5'-PA-O3A
8	C	2000	AMP	C5'-O5'-P-O1P

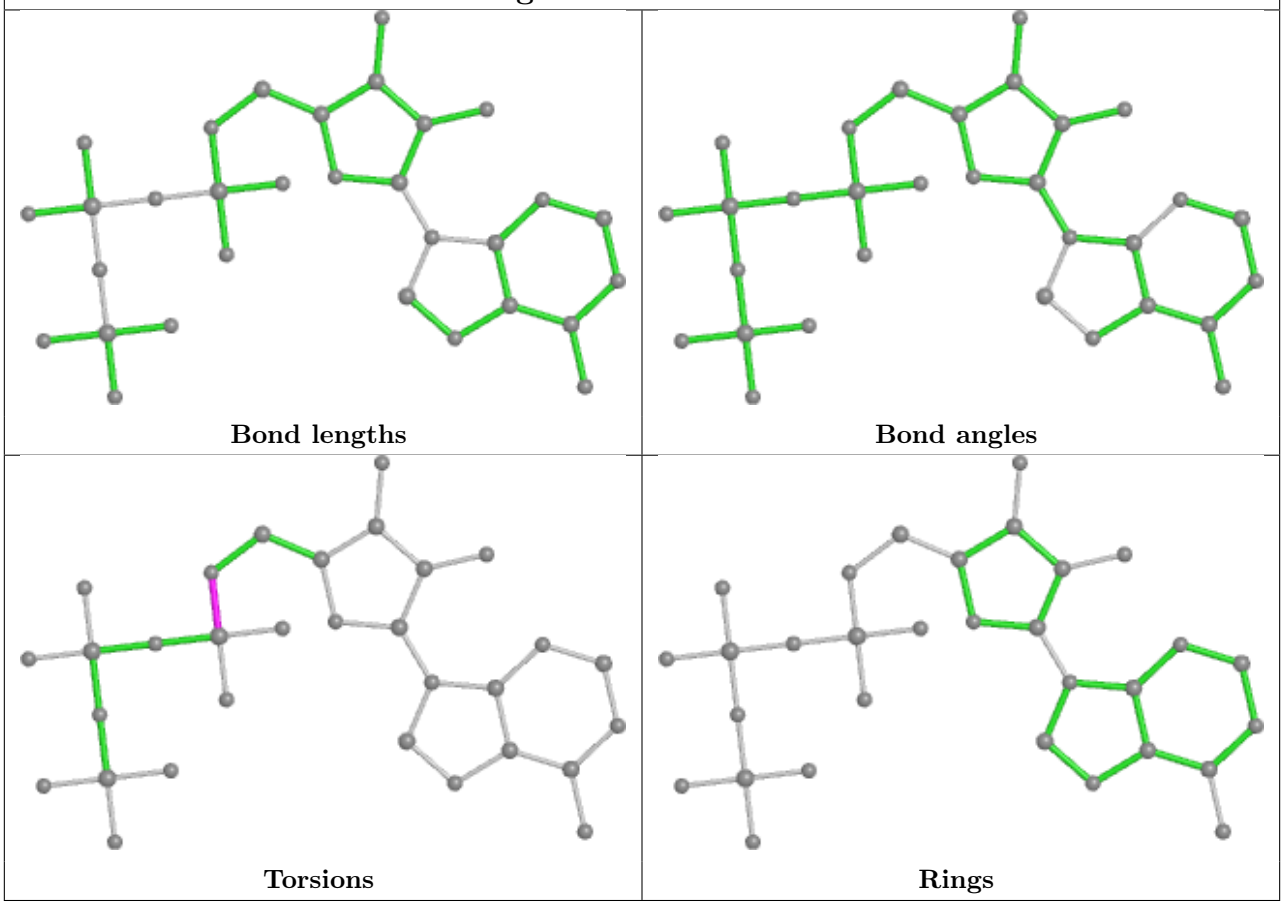
There are no ring outliers.

1 monomer is involved in 3 short contacts:

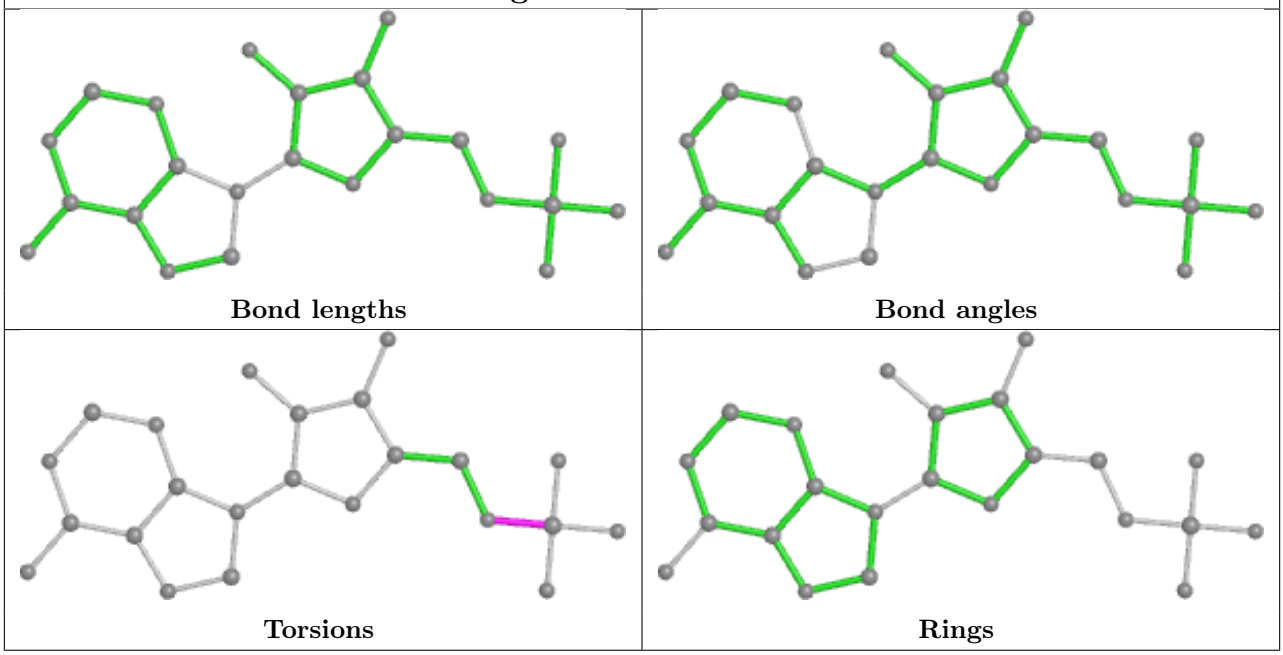
Mol	Chain	Res	Type	Clashes	Symm-Clashes
4	A	1001	ATP	3	0

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 1001



Ligand AMP C 2000



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.

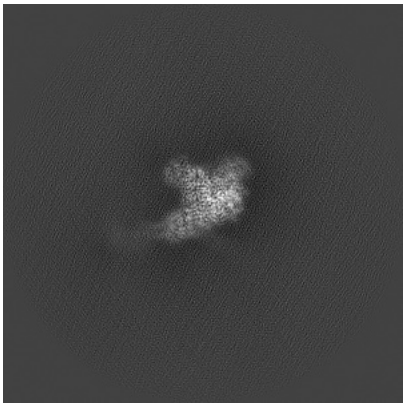
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-23863. These allow visual inspection of the internal detail of the map and identification of artifacts.

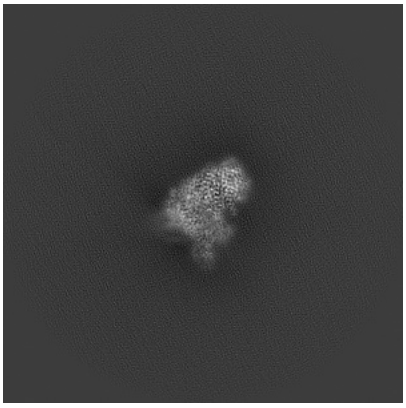
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections (i)

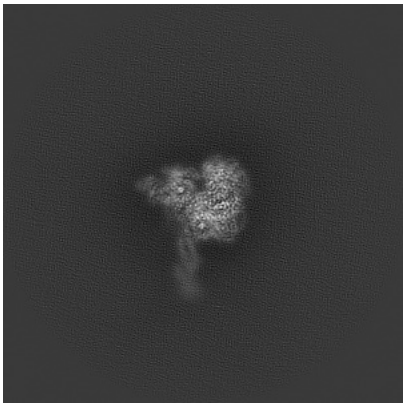
6.1.1 Primary map



X



Y

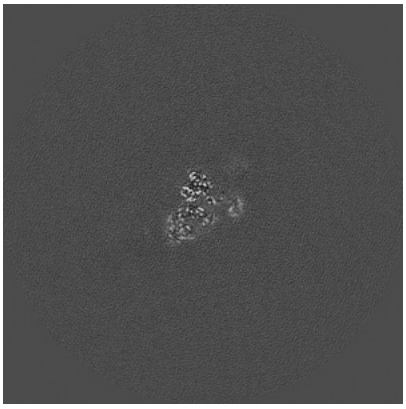


Z

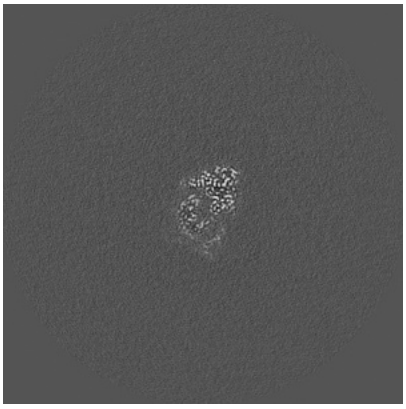
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

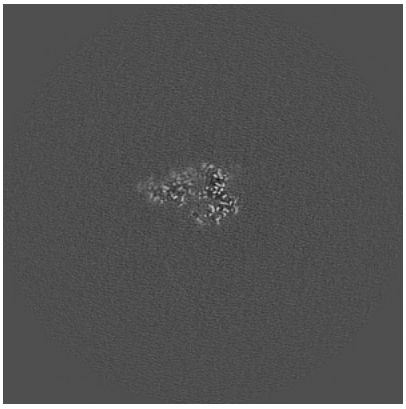
6.2.1 Primary map



X Index: 200



Y Index: 200

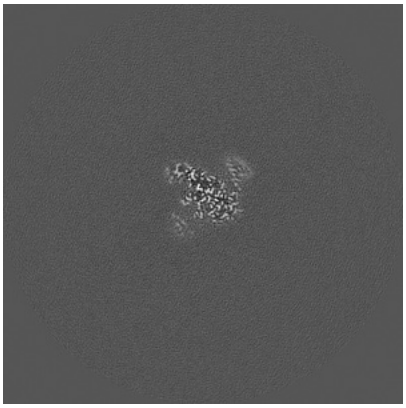


Z Index: 200

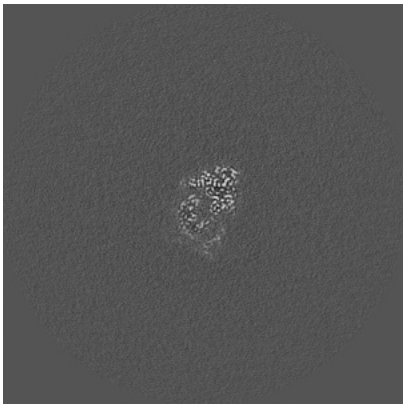
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

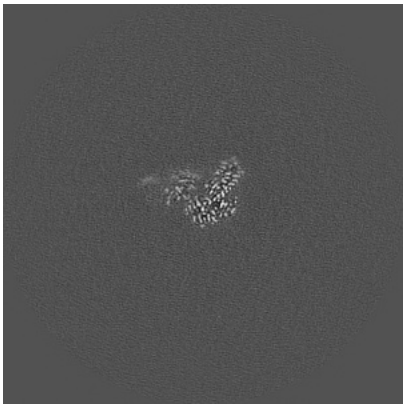
6.3.1 Primary map



X Index: 215



Y Index: 200



Z Index: 211

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 0.03. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

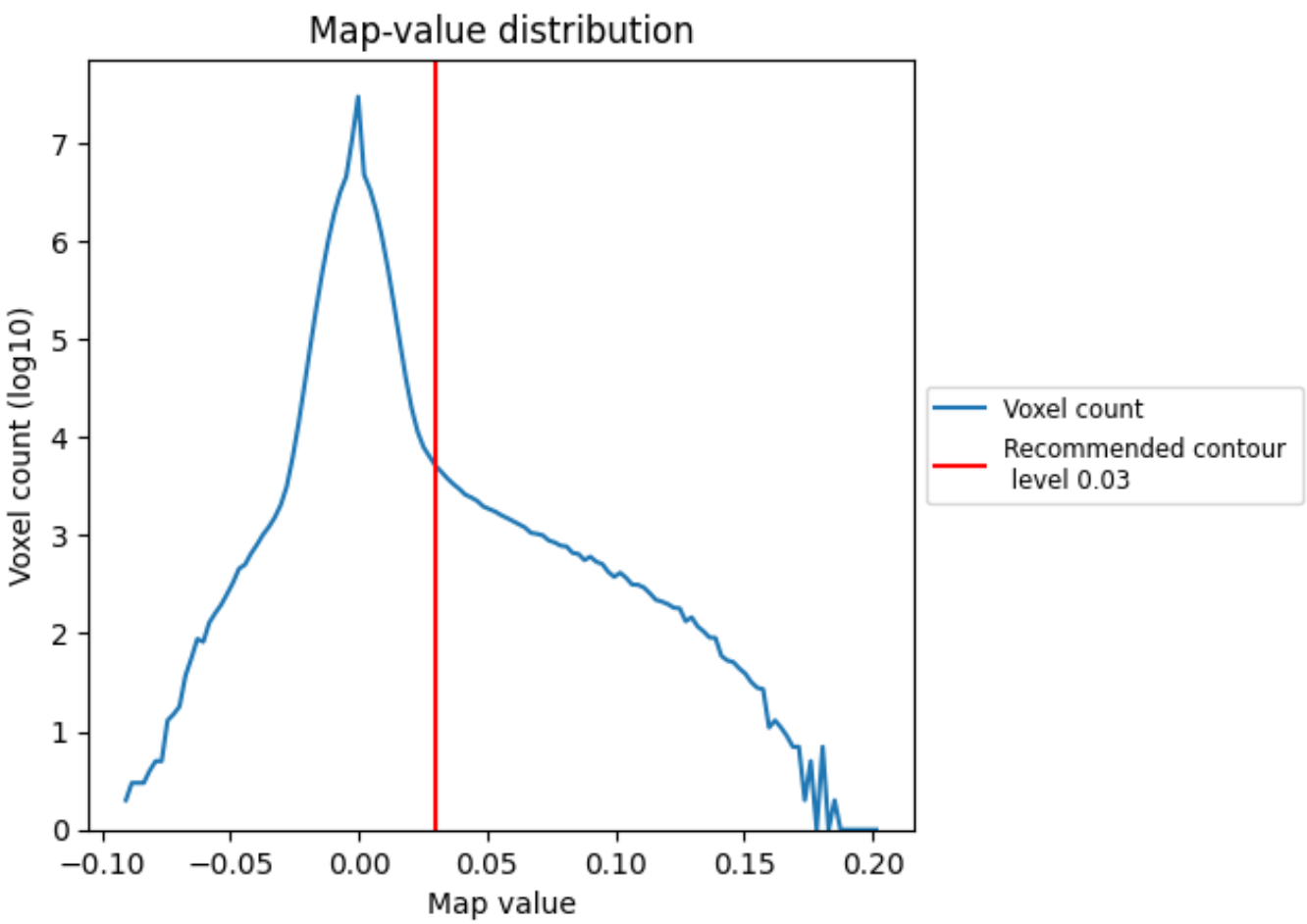
6.5 Mask visualisation (i)

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

7 Map analysis (i)

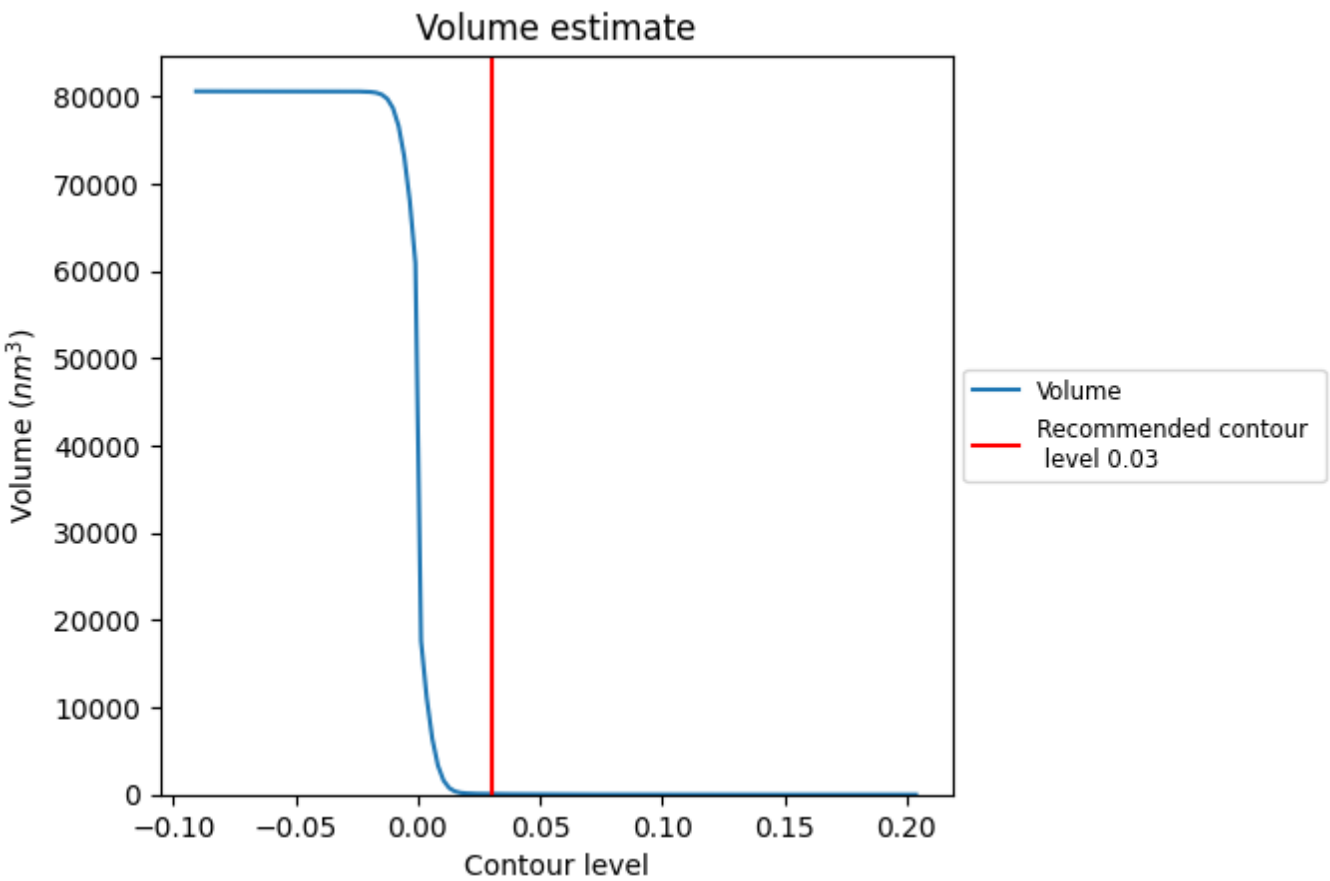
This section contains the results of statistical analysis of the map.

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.

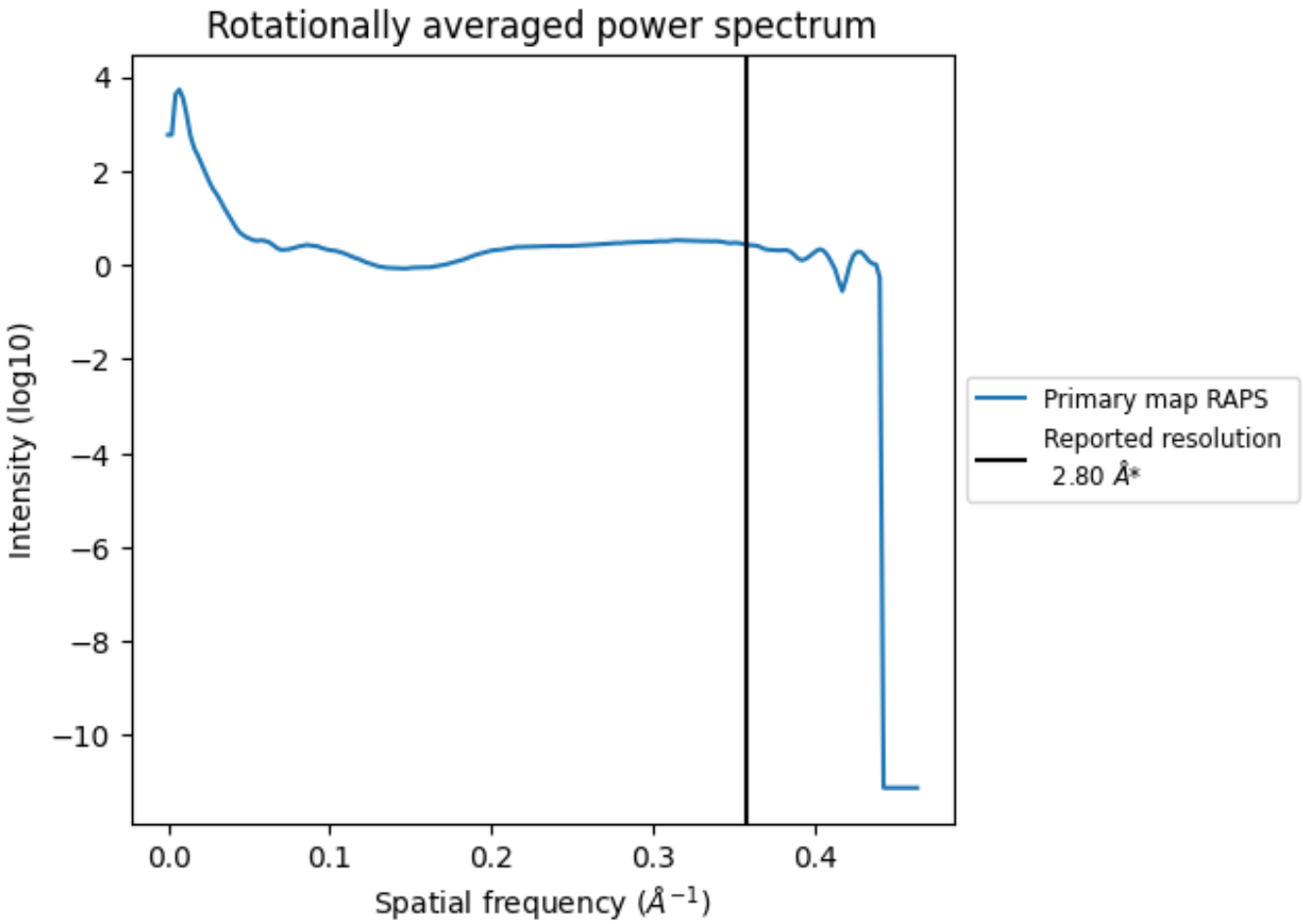
7.2 Volume estimate (i)



The volume at the recommended contour level is 68 nm^3; this corresponds to an approximate mass of 62 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 0.357 Å^{-1}

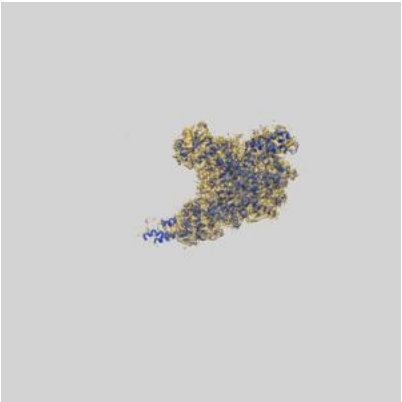
8 Fourier-Shell correlation (i)

This section was not generated. No FSC curve or half-maps provided.

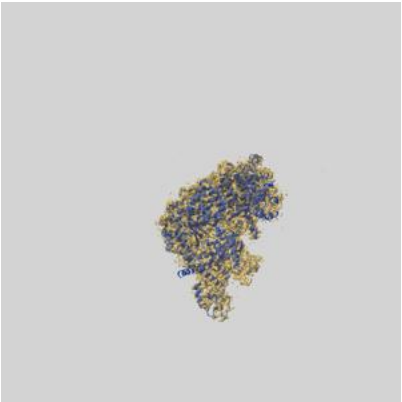
9 Map-model fit (i)

This section contains information regarding the fit between EMDB map EMD-23863 and PDB model 7MIS. Per-residue inclusion information can be found in section 3 on page 6.

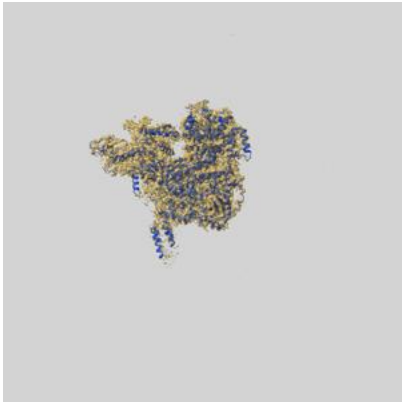
9.1 Map-model overlay (i)



X



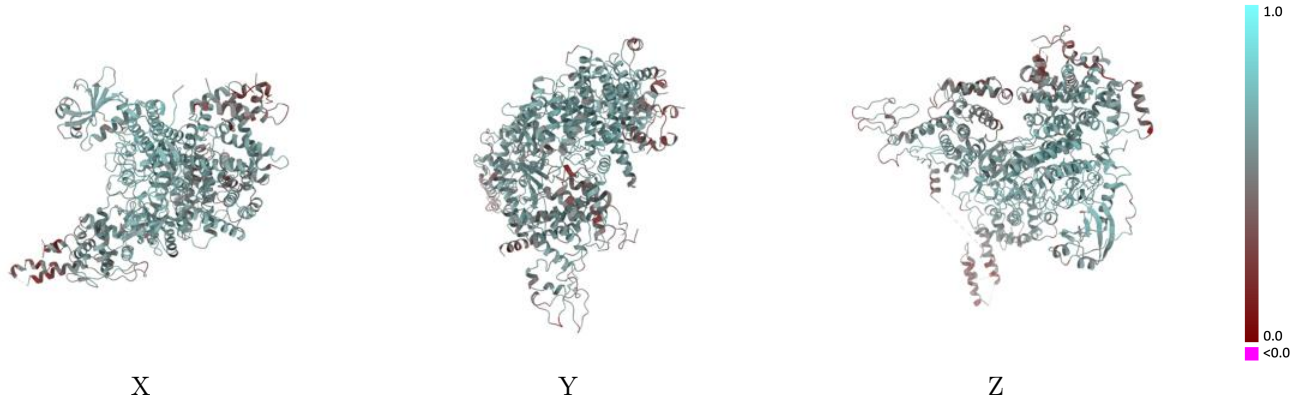
Y



Z

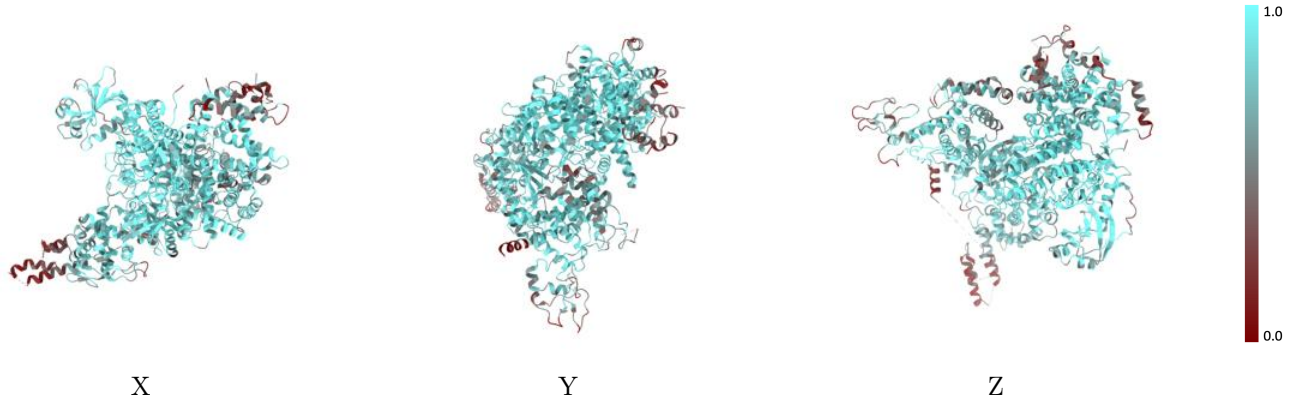
The images above show the 3D surface view of the map at the recommended contour level 0.03 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

9.2 Q-score mapped to coordinate model (i)



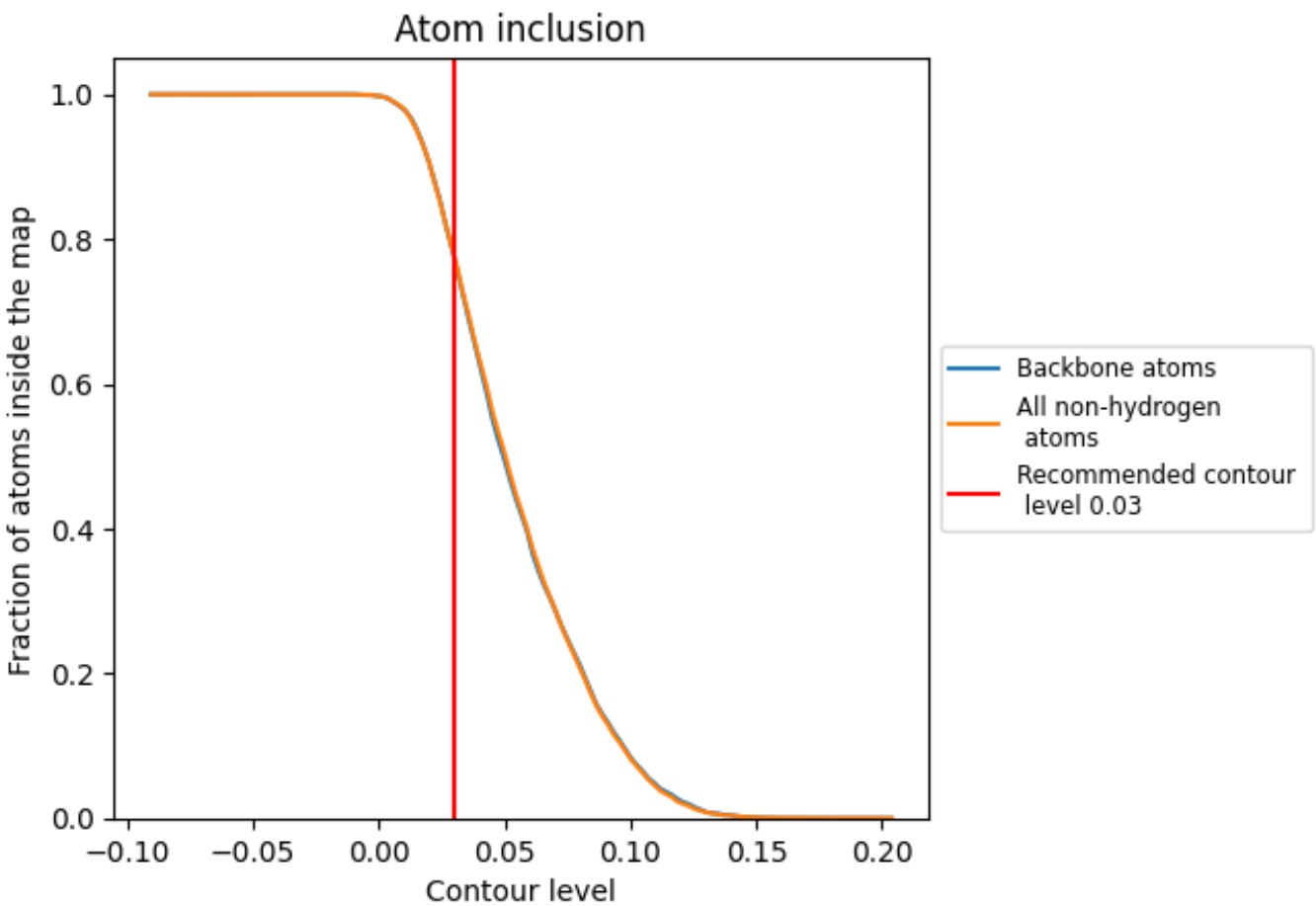
The images above show the model with each residue coloured according its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions 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)



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 (0.03).

9.4 Atom inclusion (i)



At the recommended contour level, 77% 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 (0.03) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.7759	0.5650
A	0.8827	0.6150
B	0.5638	0.4680
C	0.7089	0.5300