wwPDB EM Validation Summary Report

Dec 16, 2024 – 06:55 pm GMT

PDB ID	:	8RS9
EMDB ID	:	EMD-19473
Title	:	p97 (VCP) double mutant - F266A F539A
Authors	:	Arie, M.; Matzov, D.; Karmona, R.; Szenkier, N.; Stanhill, A.; Navon, A.
Deposited on	:	2024-01-24
Resolution	:	3.40 Å(reported)
Based on initial model	:	5FTN

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 (i)) were used in the production of this report:

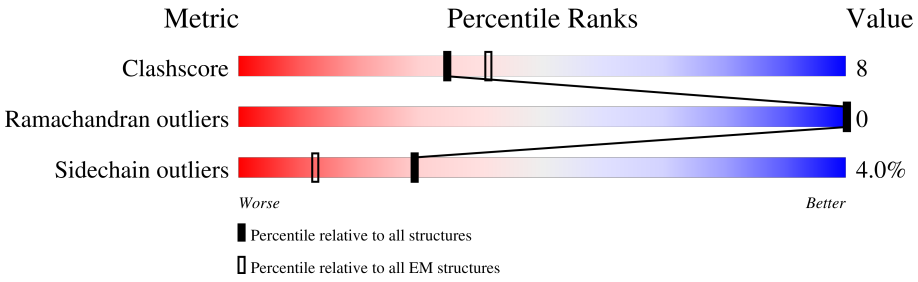
EMDB validation analysis	:	0.0.1.dev113
MolProbity	:	4.02b-467
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
MapQ	:	1.9.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.40

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 3.40 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	210492	15764
Ramachandran outliers	207382	16835
Sidechain outliers	206894	16415

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	806	32% 77% 14% • 8%
1	B	806	32% 77% 15% 8%
1	C	806	33% 77% 14% • 8%
1	D	806	32% 77% 15% 8%
1	E	806	33% 76% 15% 8%
1	F	806	32% 76% 16% 8%

2 Entry composition (i)

There is only 1 type of molecule in this entry. The entry contains 31359 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 Transitional endoplasmic reticulum ATPase.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	743	Total	C	N	O	S	0	0
			5248	3262	948	1015	23		
1	B	743	Total	C	N	O	S	0	0
			5242	3258	947	1014	23		
1	C	743	Total	C	N	O	S	0	0
			5244	3260	947	1014	23		
1	D	743	Total	C	N	O	S	0	0
			5131	3186	931	992	22		
1	E	743	Total	C	N	O	S	0	0
			5246	3260	948	1015	23		
1	F	743	Total	C	N	O	S	0	0
			5248	3262	948	1015	23		

There are 12 discrepancies between the modelled and reference sequences:

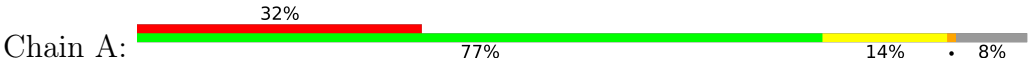
Chain	Residue	Modelled	Actual	Comment	Reference
A	266	ALA	PHE	engineered mutation	UNP P55072
A	539	ALA	PHE	engineered mutation	UNP P55072
B	266	ALA	PHE	engineered mutation	UNP P55072
B	539	ALA	PHE	engineered mutation	UNP P55072
C	266	ALA	PHE	engineered mutation	UNP P55072
C	539	ALA	PHE	engineered mutation	UNP P55072
D	266	ALA	PHE	engineered mutation	UNP P55072
D	539	ALA	PHE	engineered mutation	UNP P55072
E	266	ALA	PHE	engineered mutation	UNP P55072
E	539	ALA	PHE	engineered mutation	UNP P55072
F	266	ALA	PHE	engineered mutation	UNP P55072
F	539	ALA	PHE	engineered mutation	UNP P55072

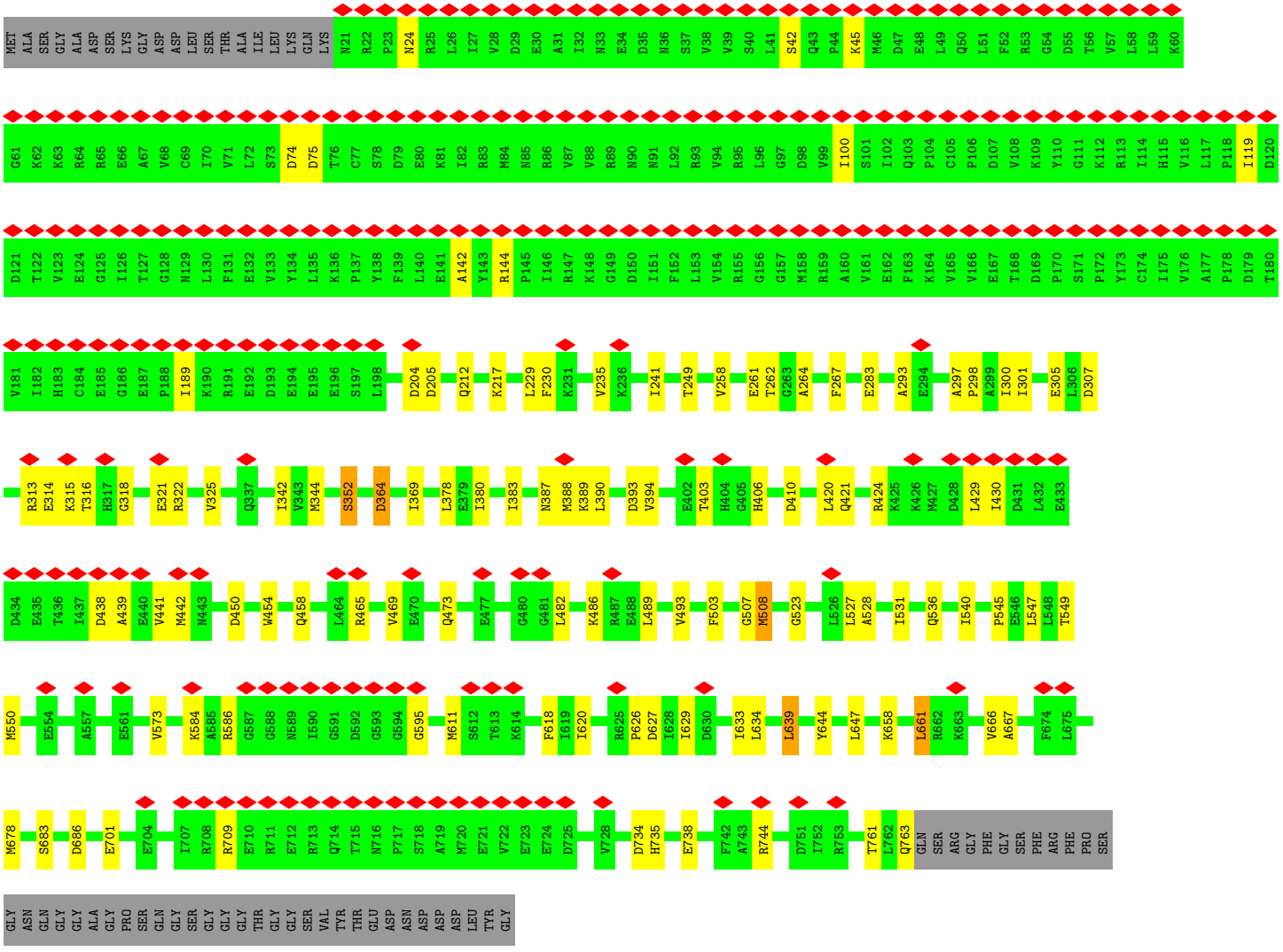
3 Residue-property plots (i)

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: Transitional endoplasmic reticulum ATPase

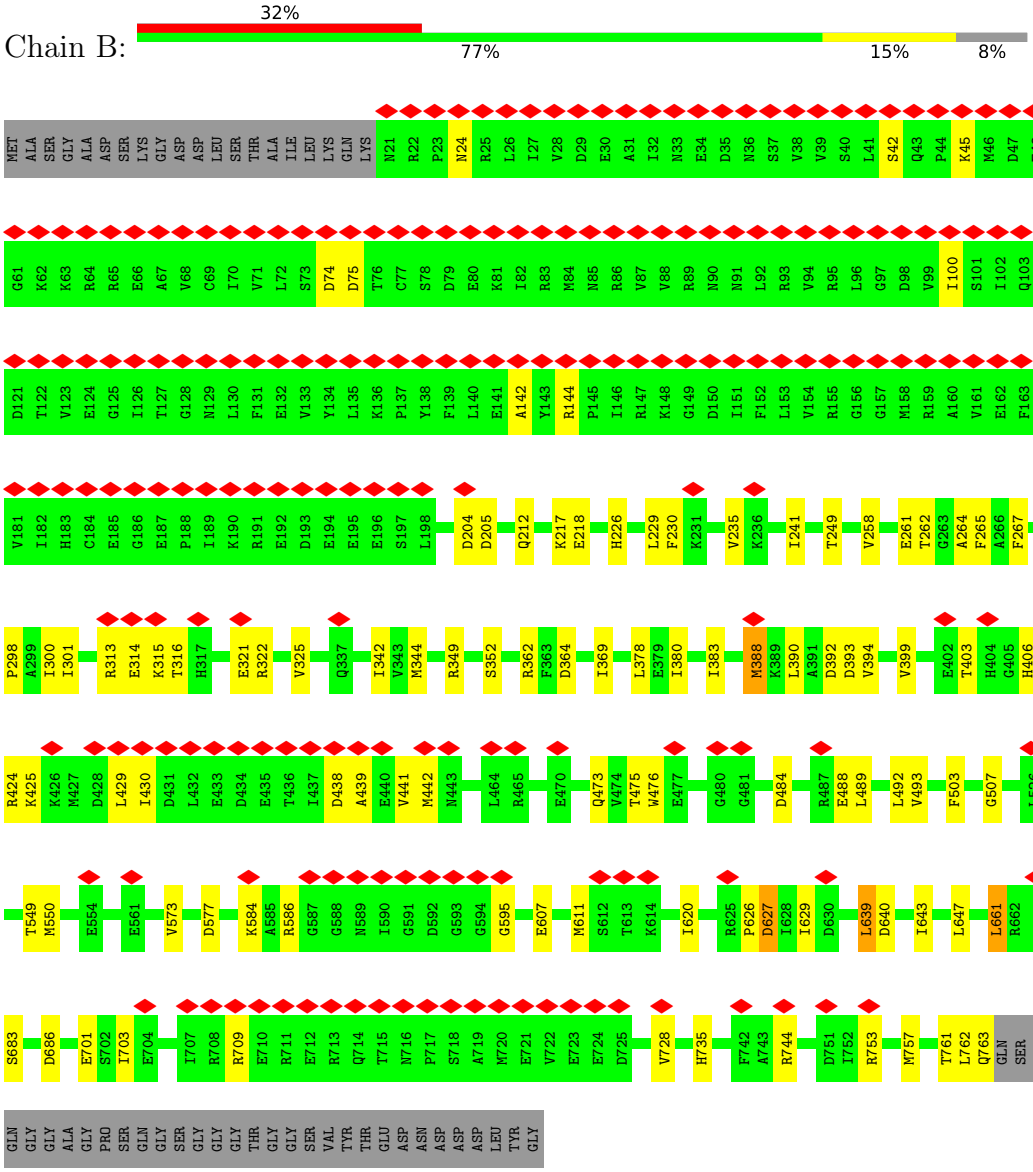
Chain A:





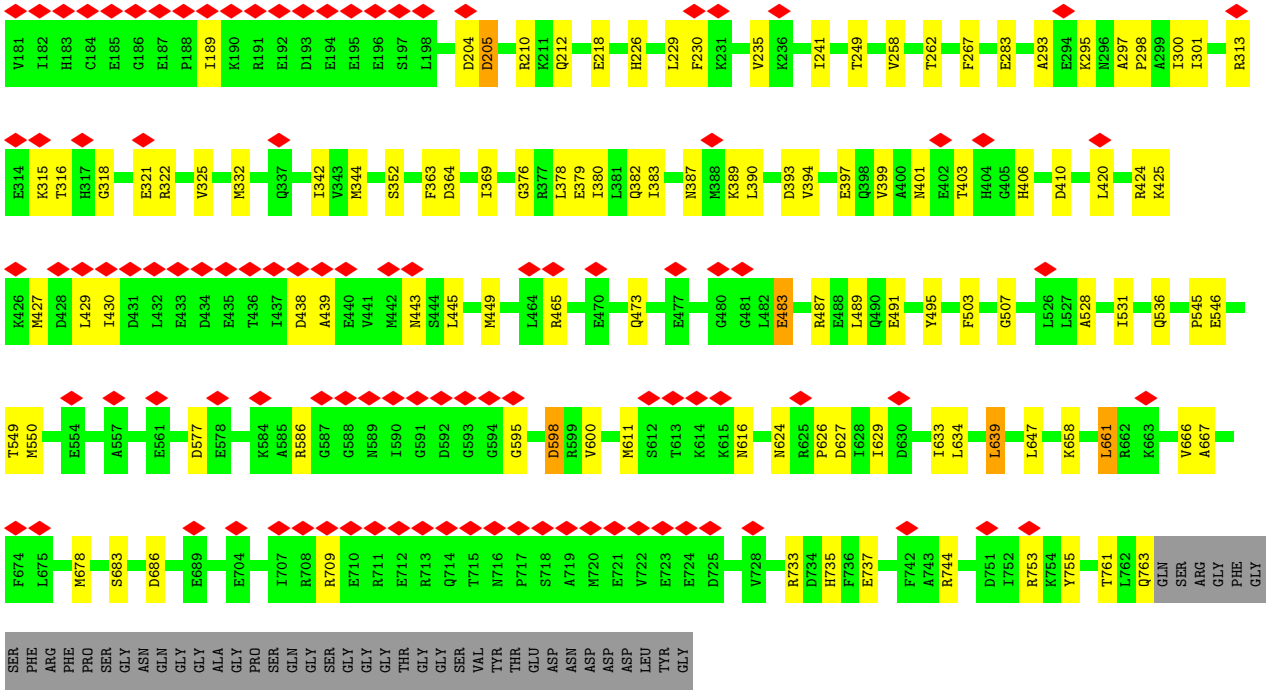
• Molecule 1: Transitional endoplasmic reticulum ATPase

Chain B:



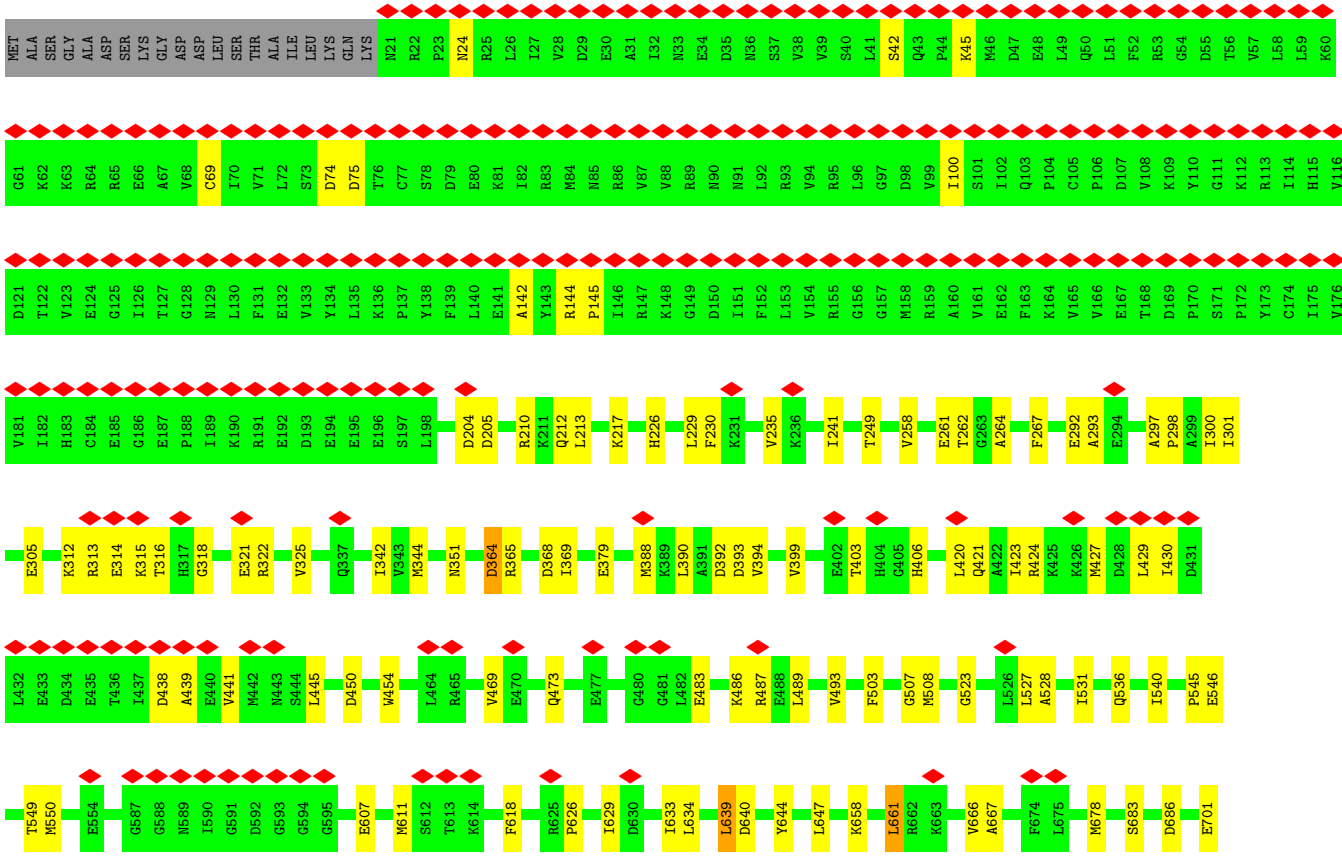
• Molecule 1: Transitional endoplasmic reticulum ATPase

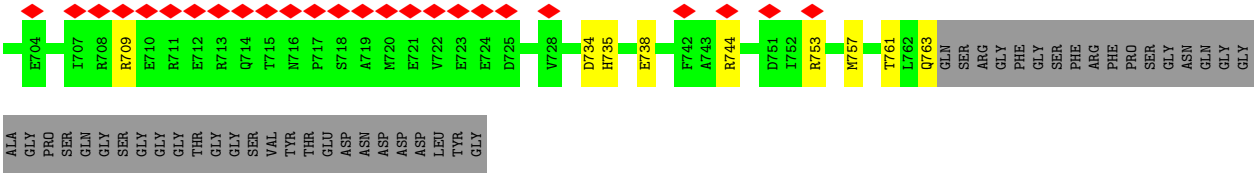
Chain C:



• Molecule 1: Transitional endoplasmic reticulum ATPase

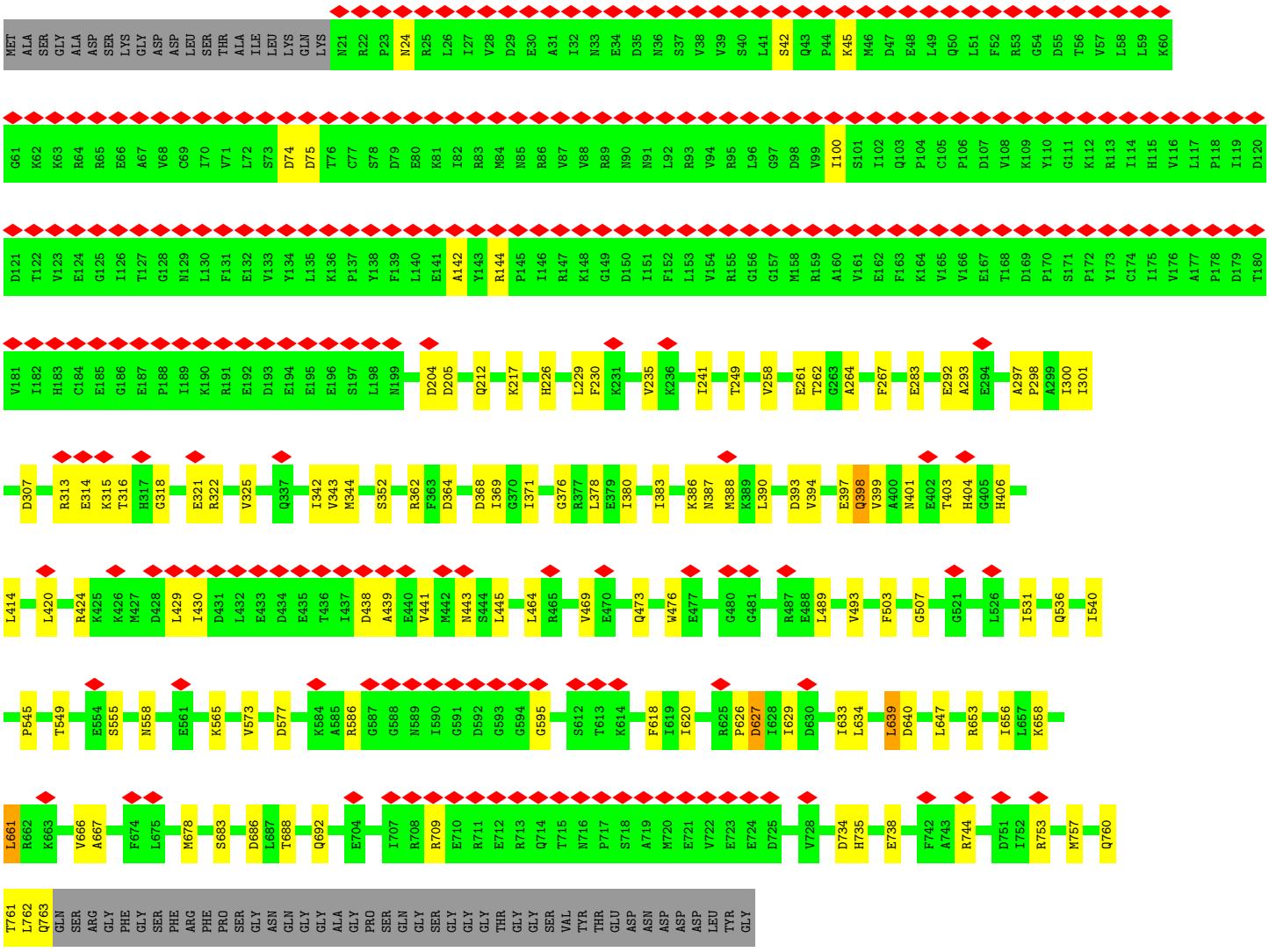
Chain D:





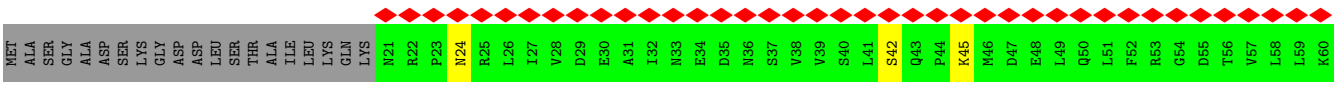
• Molecule 1: Transitional endoplasmic reticulum ATPase

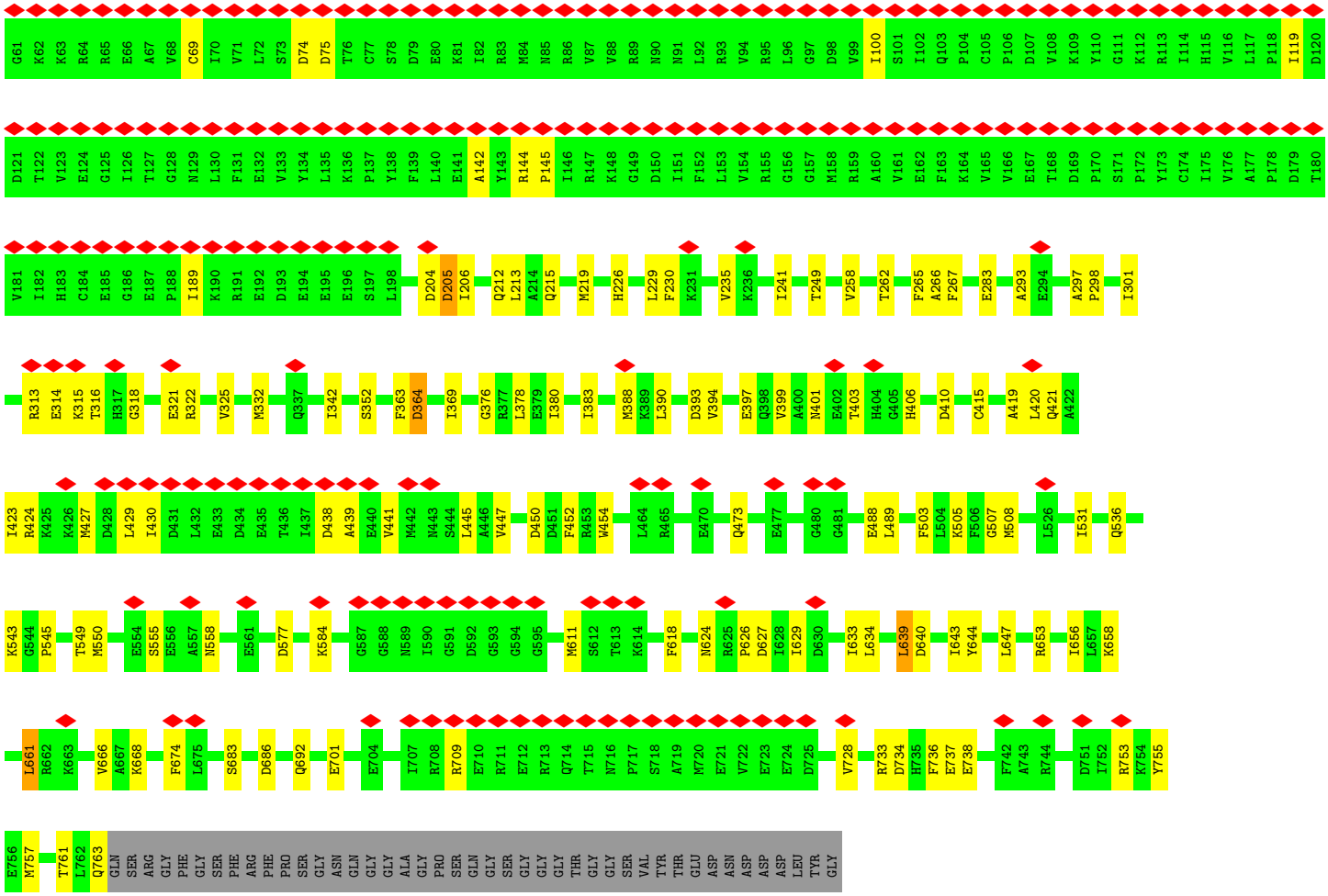
Chain E:



• Molecule 1: Transitional endoplasmic reticulum ATPase

Chain F:




G61 K62 K63 R64 R65 E66 A67 V68 C69 I70 V71 L72 S73 D74 D75 T76 C77 S78 D79 E80 K81 I82 R83 M84 N85 R86 V87 V88 R89 N90 N91 L92 R93 V94 R95 L96 G97 D98 V99 I100 S101 I102 Q103 P104 C105 P106 D107 V108 K109 Y110 G111 K112 R113 I114 H115 V116 L117 P118 I119 D120
D121 T122 V123 E124 G125 I126 T127 G128 N129 L130 F131 E132 V133 Y134 L135 K136 P137 Y138 F139 L140 E141 A142 Y143 R144 P145 I146 R147 K148 G149 D150 I151 F152 L153 V154 R155 G156 G157 M158 R159 A160 V161 E162 F163 K164 V165 V166 E167 T168 D169 P170 S171 P172 Y173 C174 I175 V176 A177 P178 D179 T180
V181 I182 H183 C184 E185 G186 E187 P188 I189 K190 R191 E192 D193 E194 E195 E196 S197 L198 D204 D205 I206 Q212 L213 A214 Q215 M219 H226 L229 F230 K231 V235 K236 I241 T249 V258 T262 F265 A266 F267 E283 A293 E294 A297 P298 I301
R313 E314 K315 T316 H317 G318 E321 R322 V325 M332 Q337 I342 S352 F363 D364 I369 G376 R377 L378 E379 I380 I383 M388 K389 L390 D393 V394 E397 Q398 V399 A400 N401 E402 T403 H404 G405 H406 D410 C415 A419 L420 Q421 A422
I423 R424 K425 K426 M427 D428 L429 I430 D431 L432 E433 D434 E435 T436 I437 D438 A439 E440 V441 M442 N443 S444 L445 A446 V447 D450 D451 F452 R453 W454 L464 R465 E470 Q473 E477 G480 G481 E488 L489 F503 L504 K505 F506 G507 M508 L526 I531 Q536
K543 G544 P545 T549 M550 E554 S555 E556 A557 N558 E561 D577 K584 G587 G588 N589 I590 G591 D592 G593 G594 G595 M611 S612 T613 K614 F618 N624 R625 P626 D627 I628 I629 D630 I633 L634 L639 D640 I643 Y644 L647 R653 I656 L657 K658
L661 R662 K663 V666 A667 K668 F674 L675 S683 D686 Q692 E701 E704 I707 R708 R709 E710 R711 E712 R713 Q714 T715 N716 P717 S718 A719 M720 E721 V722 E723 E724 D725 V728 R733 D734 H735 F736 E737 E738 F742 A743 R744 D751 T752 R753 K754 Y755
E756 M757 T761 L762 Q763 GLN SER ARG GLY PHE GLY SER PHE ARG PHE PRO SER GLY ASN GLN GLY GLY GLY ALA GLY PRO SER GLN GLY SER GLY GLY GLY GLY THR GLY GLY GLY SER VAL TYR THR GLU ASP ASN ASP ASP ASP ASP LEU TYR GLY

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C6	Depositor
Number of particles used	79612	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 ($e^-/Å^2$)	38	Depositor
Minimum defocus (nm)	800	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	0.979	Depositor
Minimum map value	-0.555	Depositor
Average map value	0.007	Depositor
Map value standard deviation	0.053	Depositor
Recommended contour level	0.244	Depositor
Map size (Å)	240.8, 240.8, 240.8	wwPDB
Map dimensions	280, 280, 280	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.86, 0.86, 0.86	Depositor

5 Model quality

5.1 Standard geometry

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	0.26	0/5318	0.53	0/7222
1	B	0.26	0/5312	0.53	0/7214
1	C	0.26	0/5314	0.53	0/7217
1	D	0.26	0/5197	0.53	0/7069
1	E	0.26	0/5316	0.54	0/7219
1	F	0.26	0/5318	0.54	0/7222
All	All	0.26	0/31775	0.53	0/43163

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

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	5248	0	4751	81	0
1	B	5242	0	4738	89	0
1	C	5244	0	4745	79	0
1	D	5131	0	4543	81	0
1	E	5246	0	4744	85	0
1	F	5248	0	4751	85	0
All	All	31359	0	28272	471	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 8.

The worst 5 of 471 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:E:386:LYS:NZ	1:E:387:ASN:OD1	2.01	0.93
1:A:465:ARG:NH2	1:B:607:GLU:OE2	2.05	0.89
1:C:744:ARG:NH1	1:D:763:GLN:OE1	2.10	0.84
1:C:763:GLN:N	1:C:763:GLN:OE1	2.10	0.84
1:A:458:GLN:OE1	1:A:458:GLN:N	2.11	0.83

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	741/806 (92%)	670 (90%)	71 (10%)	0	100	100
1	B	741/806 (92%)	670 (90%)	71 (10%)	0	100	100
1	C	741/806 (92%)	672 (91%)	69 (9%)	0	100	100
1	D	741/806 (92%)	671 (91%)	70 (9%)	0	100	100
1	E	741/806 (92%)	671 (91%)	70 (9%)	0	100	100
1	F	741/806 (92%)	667 (90%)	74 (10%)	0	100	100
All	All	4446/4836 (92%)	4021 (90%)	425 (10%)	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	463/676 (68%)	444 (96%)	19 (4%)	26	51
1	B	461/676 (68%)	445 (96%)	16 (4%)	31	56
1	C	462/676 (68%)	444 (96%)	18 (4%)	27	53
1	D	430/676 (64%)	410 (95%)	20 (5%)	22	49
1	E	462/676 (68%)	443 (96%)	19 (4%)	26	51
1	F	463/676 (68%)	444 (96%)	19 (4%)	26	51
All	All	2741/4056 (68%)	2630 (96%)	111 (4%)	29	52

5 of 111 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	D	365	ARG
1	F	709	ARG
1	D	709	ARG
1	F	674	PHE
1	F	415	CYS

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 oligosaccharides in this entry.

5.6 Ligand geometry (i)

There are no ligands in this entry.

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.

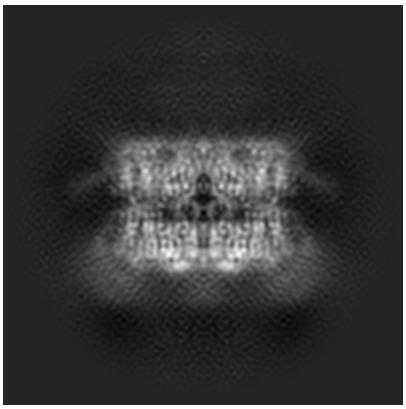
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-19473. These allow visual inspection of the internal detail of the map and identification of artifacts.

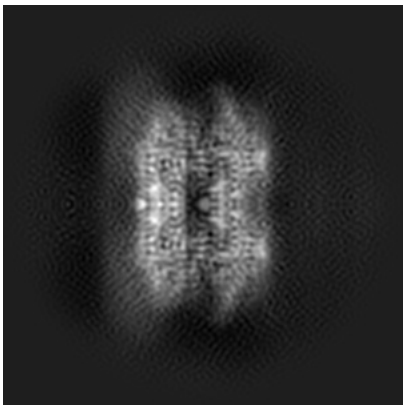
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections

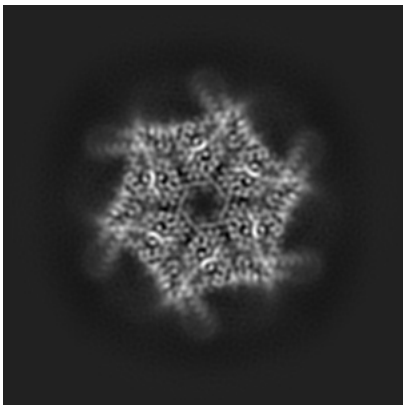
6.1.1 Primary map



X

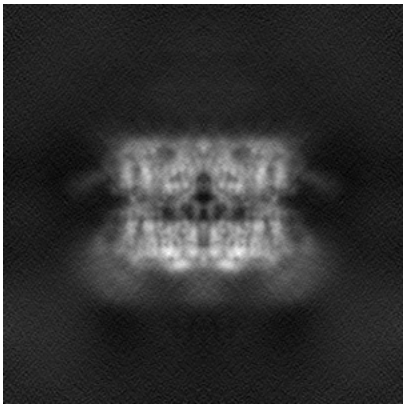


Y

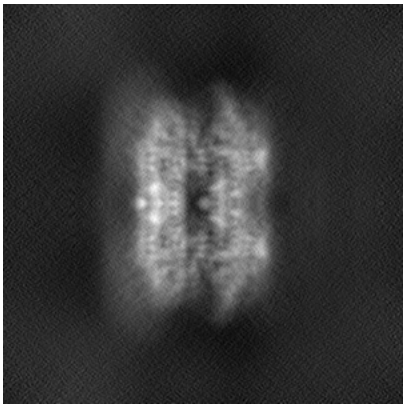


Z

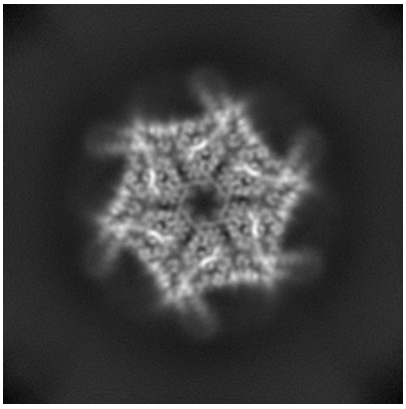
6.1.2 Raw map



X



Y

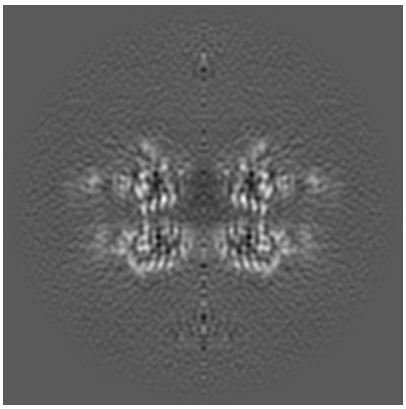


Z

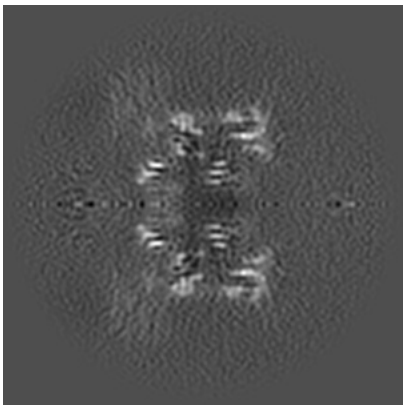
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

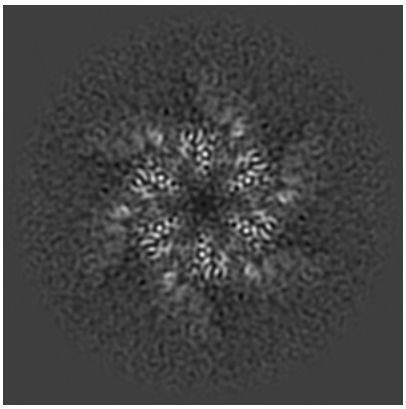
6.2.1 Primary map



X Index: 140

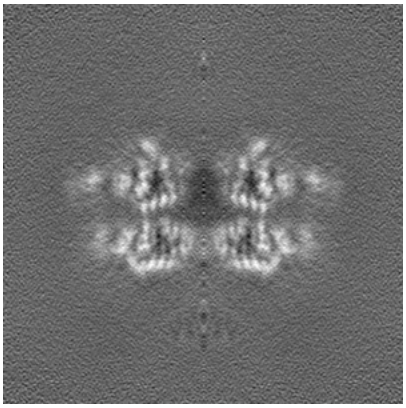


Y Index: 140

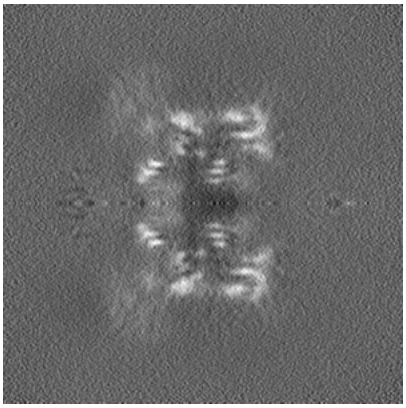


Z Index: 140

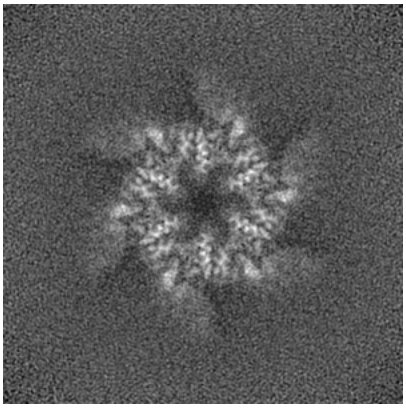
6.2.2 Raw map



X Index: 140



Y Index: 140

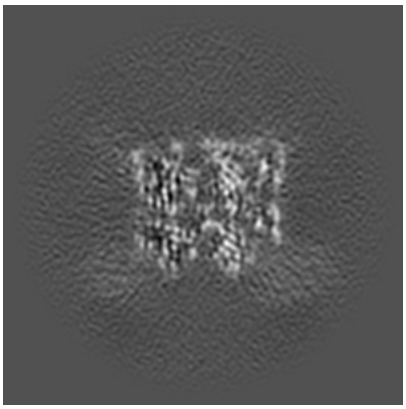


Z Index: 140

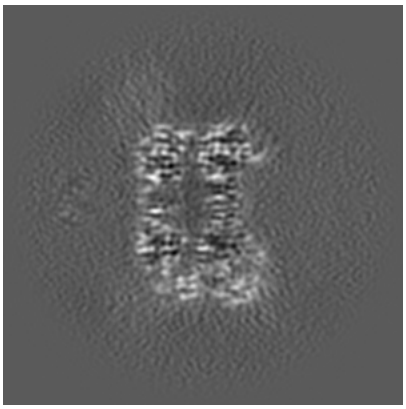
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

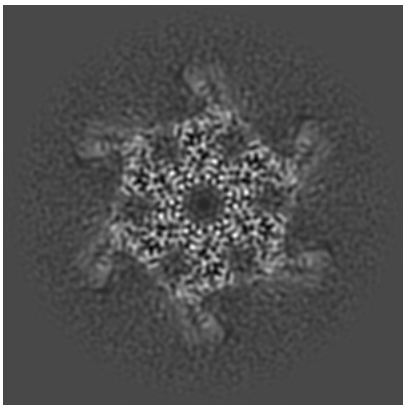
6.3.1 Primary map



X Index: 104

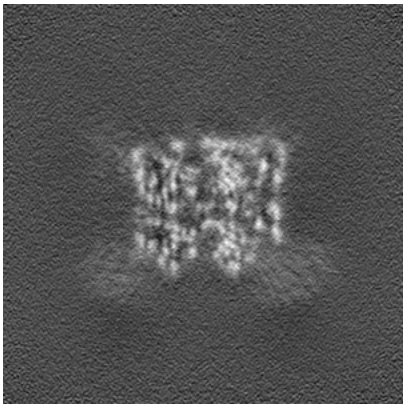


Y Index: 123

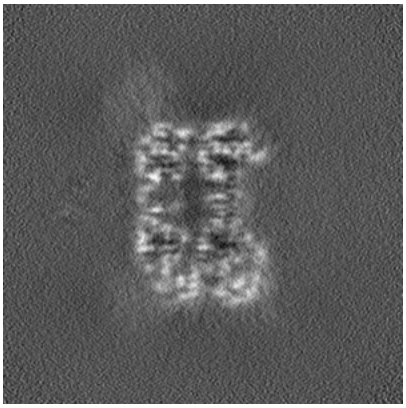


Z Index: 152

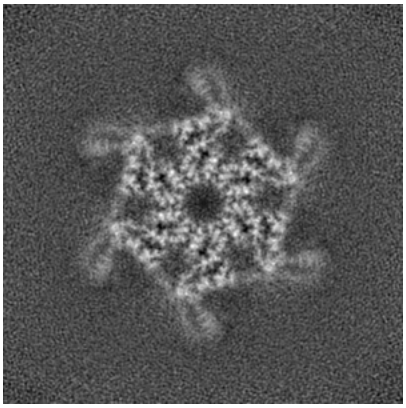
6.3.2 Raw map



X Index: 104



Y Index: 123

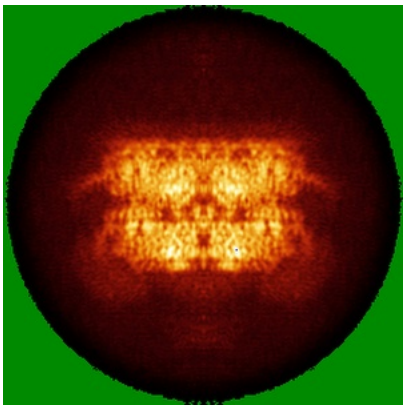


Z Index: 152

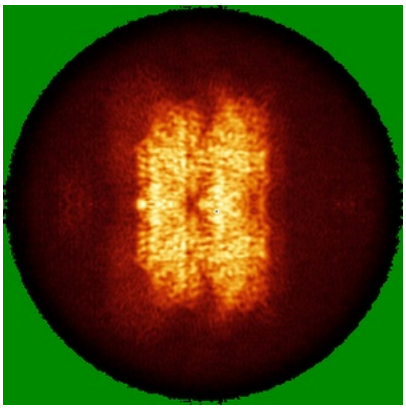
The images above show the largest variance slices of the map in three orthogonal directions.

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

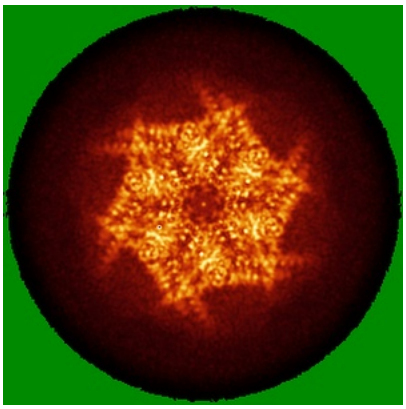
6.4.1 Primary map



X

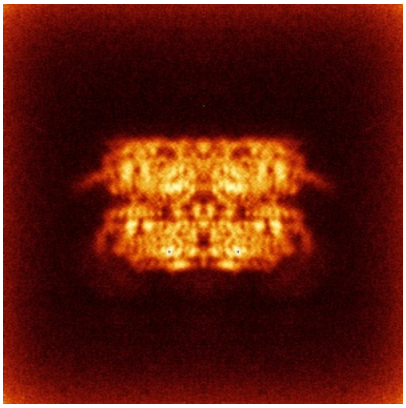


Y

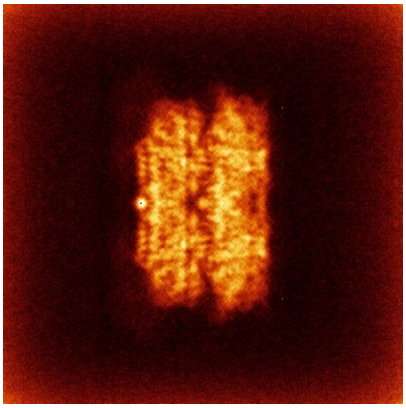


Z

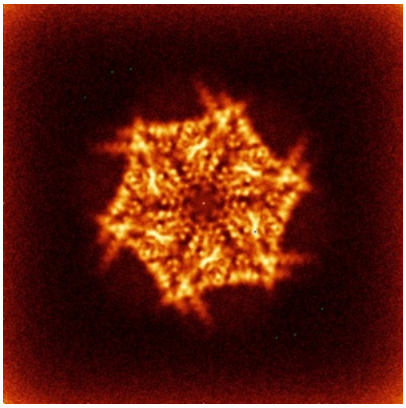
6.4.2 Raw map



X



Y

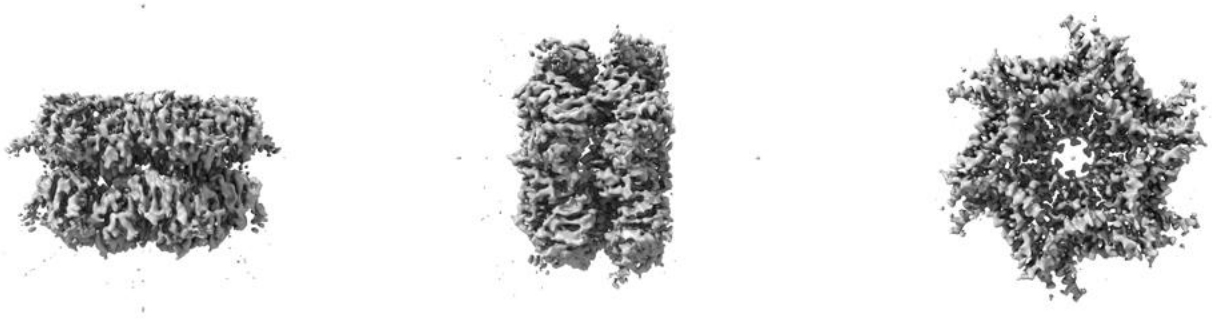


Z

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 0.244. 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.

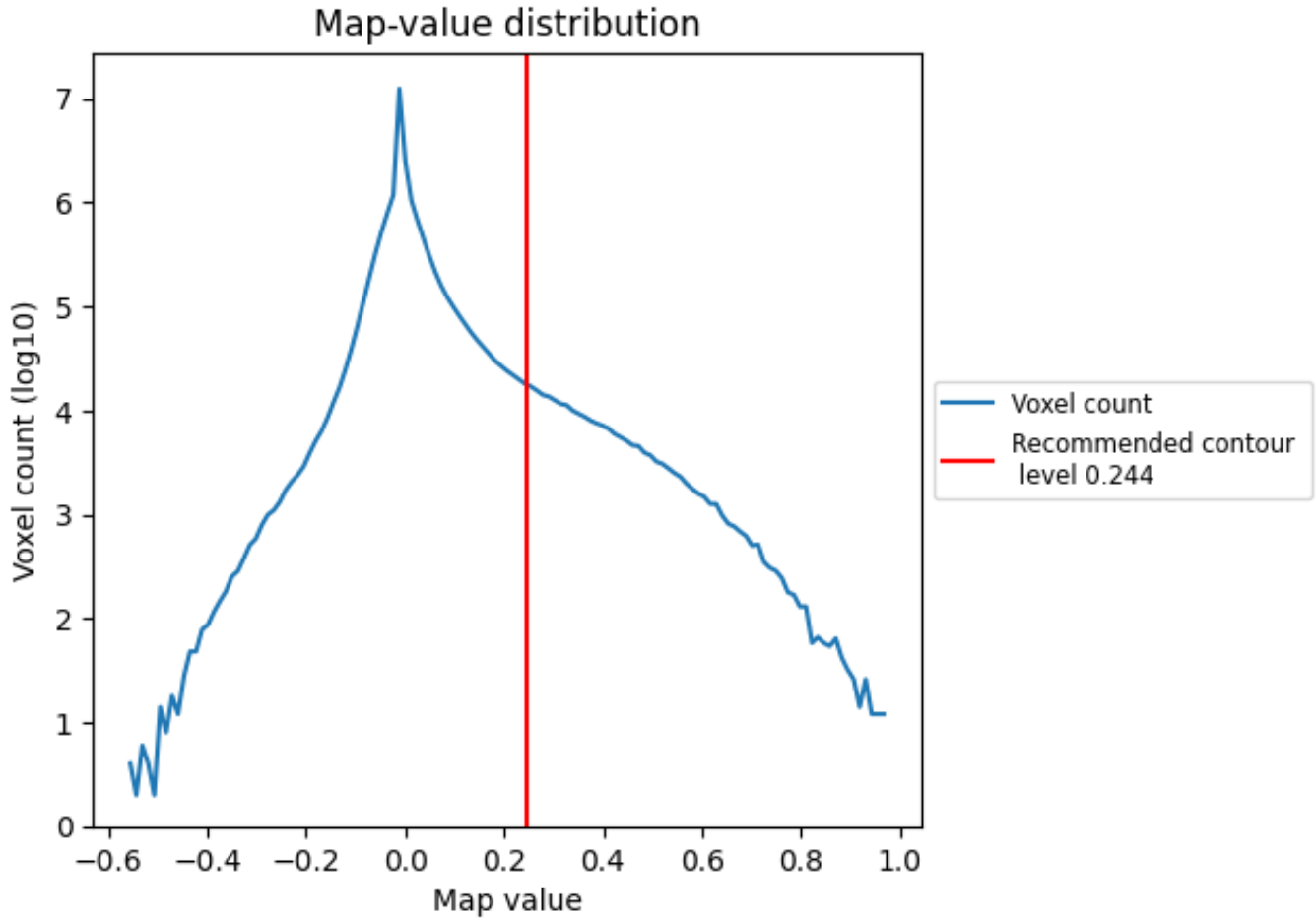
6.6 Mask visualisation (i)

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

7 Map analysis

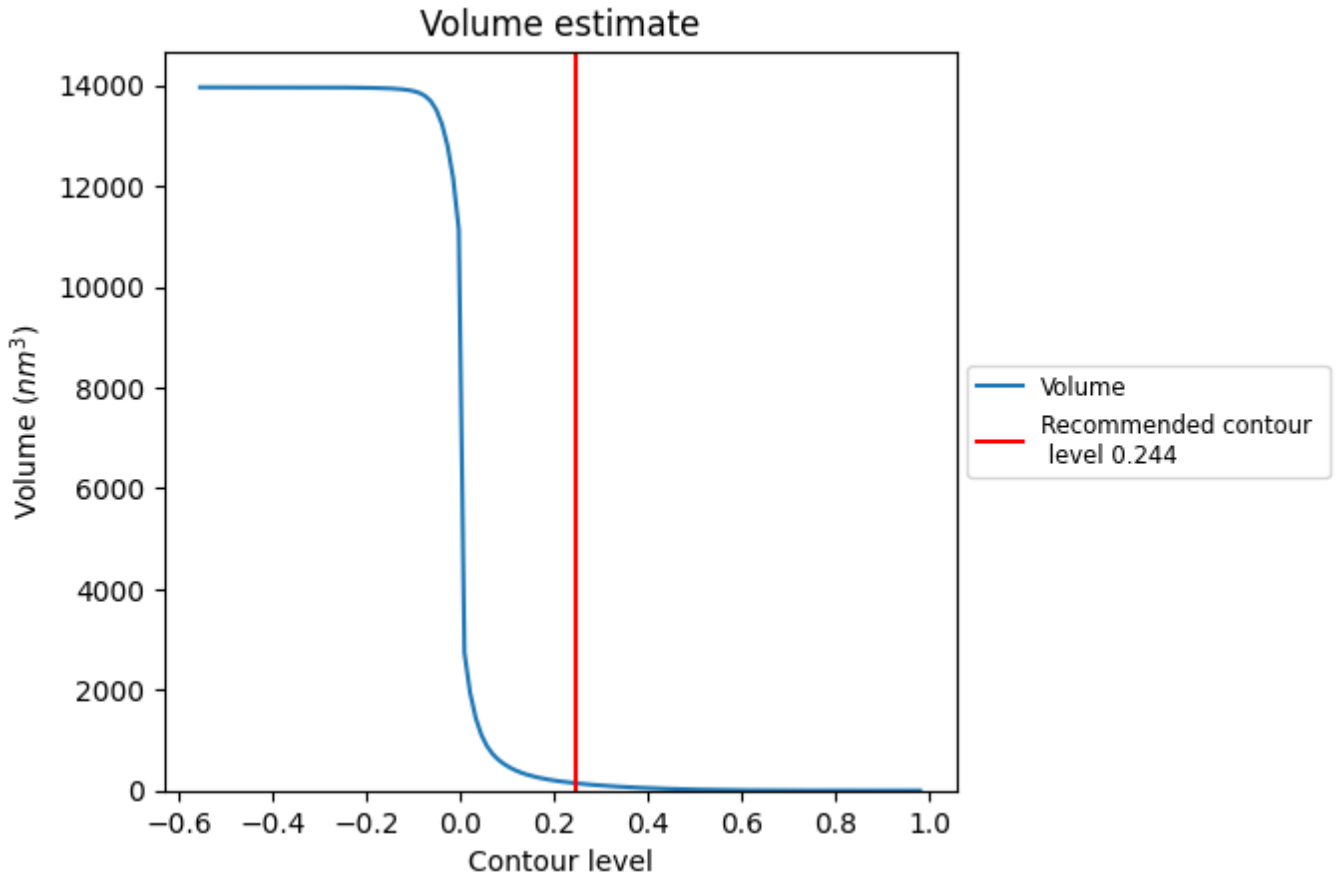
This section contains the results of statistical analysis of the map.

7.1 Map-value distribution



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.

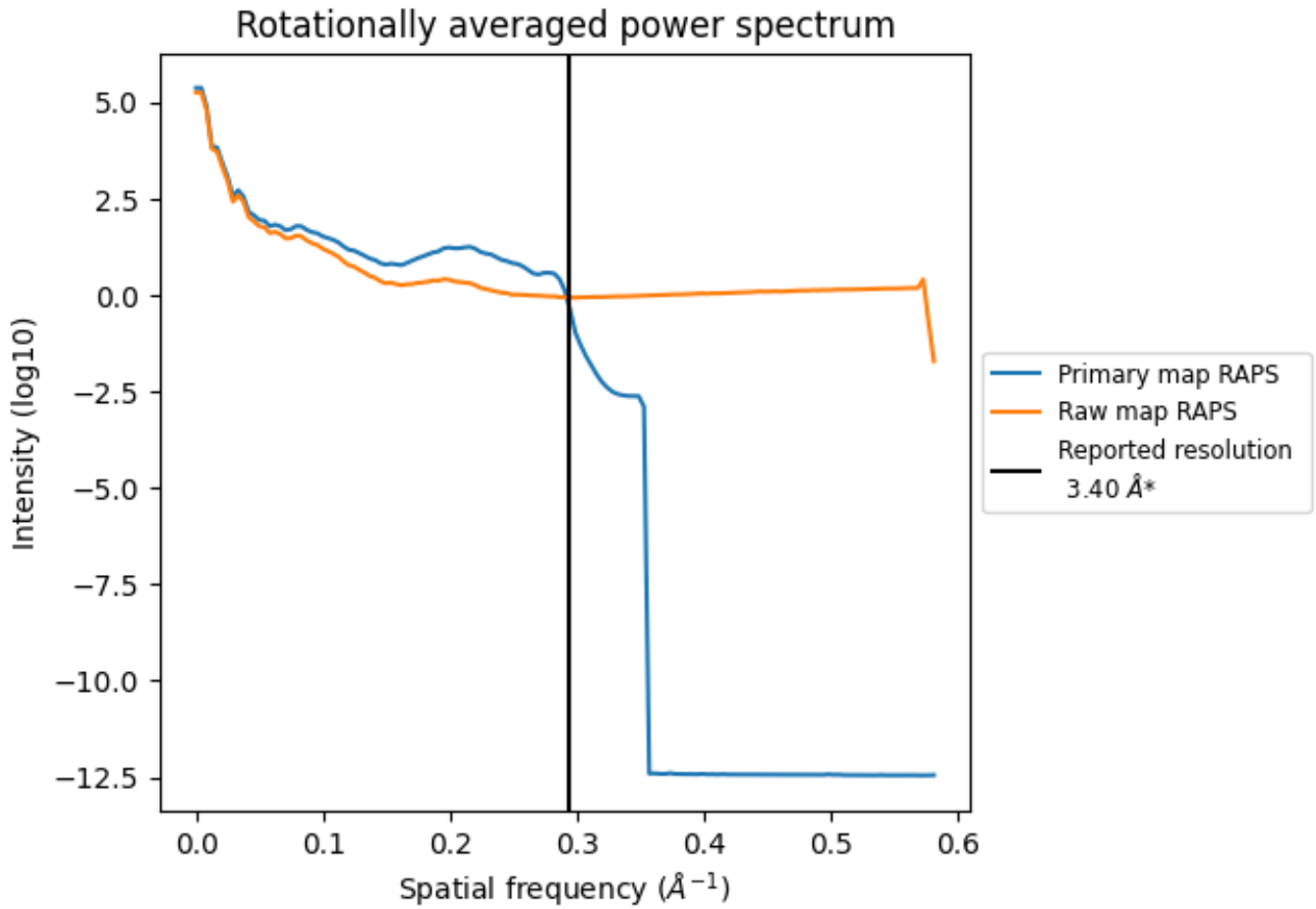
7.2 Volume estimate (i)



The volume at the recommended contour level is 147 nm^3; this corresponds to an approximate mass of 133 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)

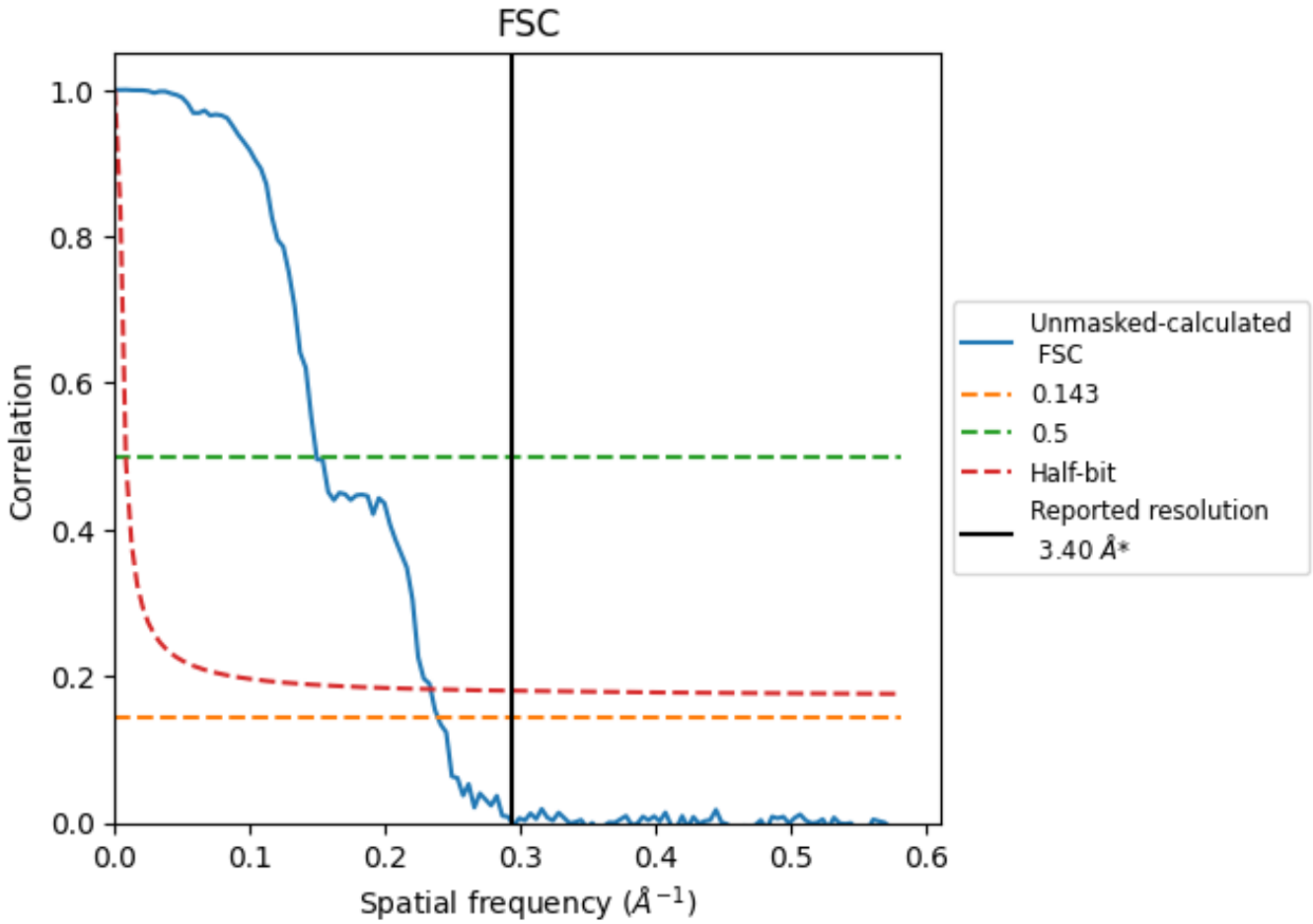


*Reported resolution corresponds to spatial frequency of 0.294 $Å^{-1}$

8 Fourier-Shell correlation

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



*Reported resolution corresponds to spatial frequency of 0.294 Å^{-1}

8.2 Resolution estimates (i)

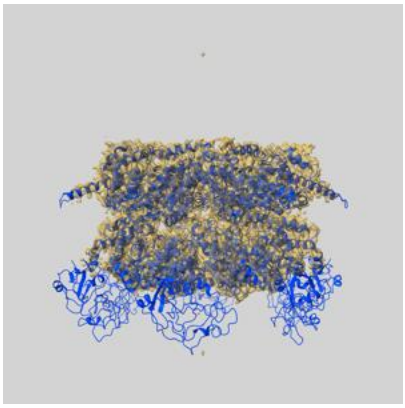
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.40	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	4.18	6.70	4.28

*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 4.18 differs from the reported value 3.4 by more than 10 %

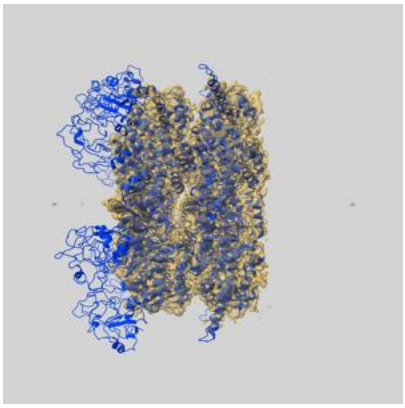
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-19473 and PDB model 8RS9. Per-residue inclusion information can be found in section 3 on page 4.

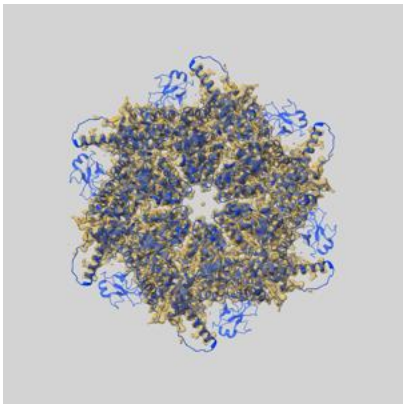
9.1 Map-model overlay



X



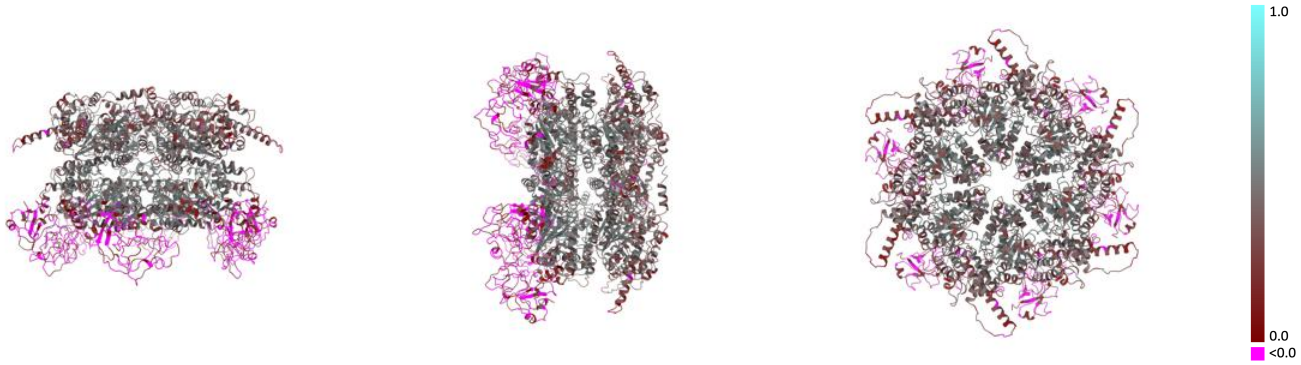
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.244 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.

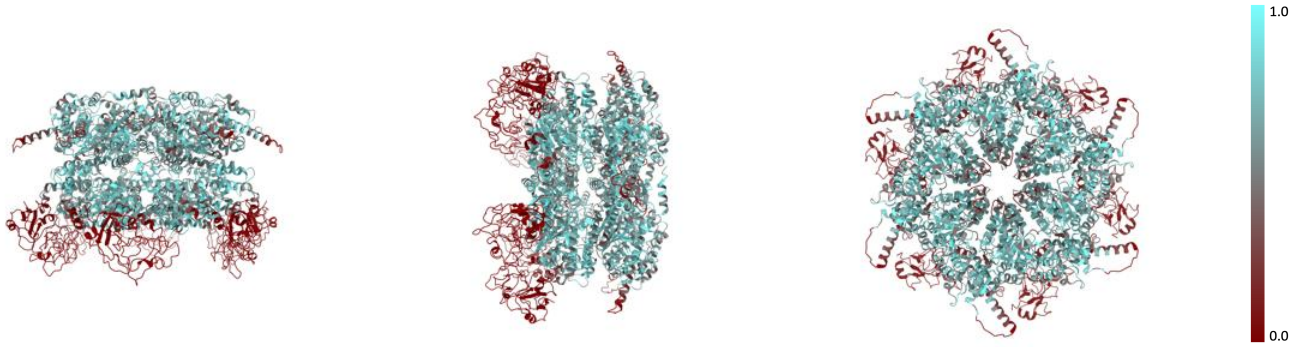
9.2 Q-score mapped to coordinate model



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.

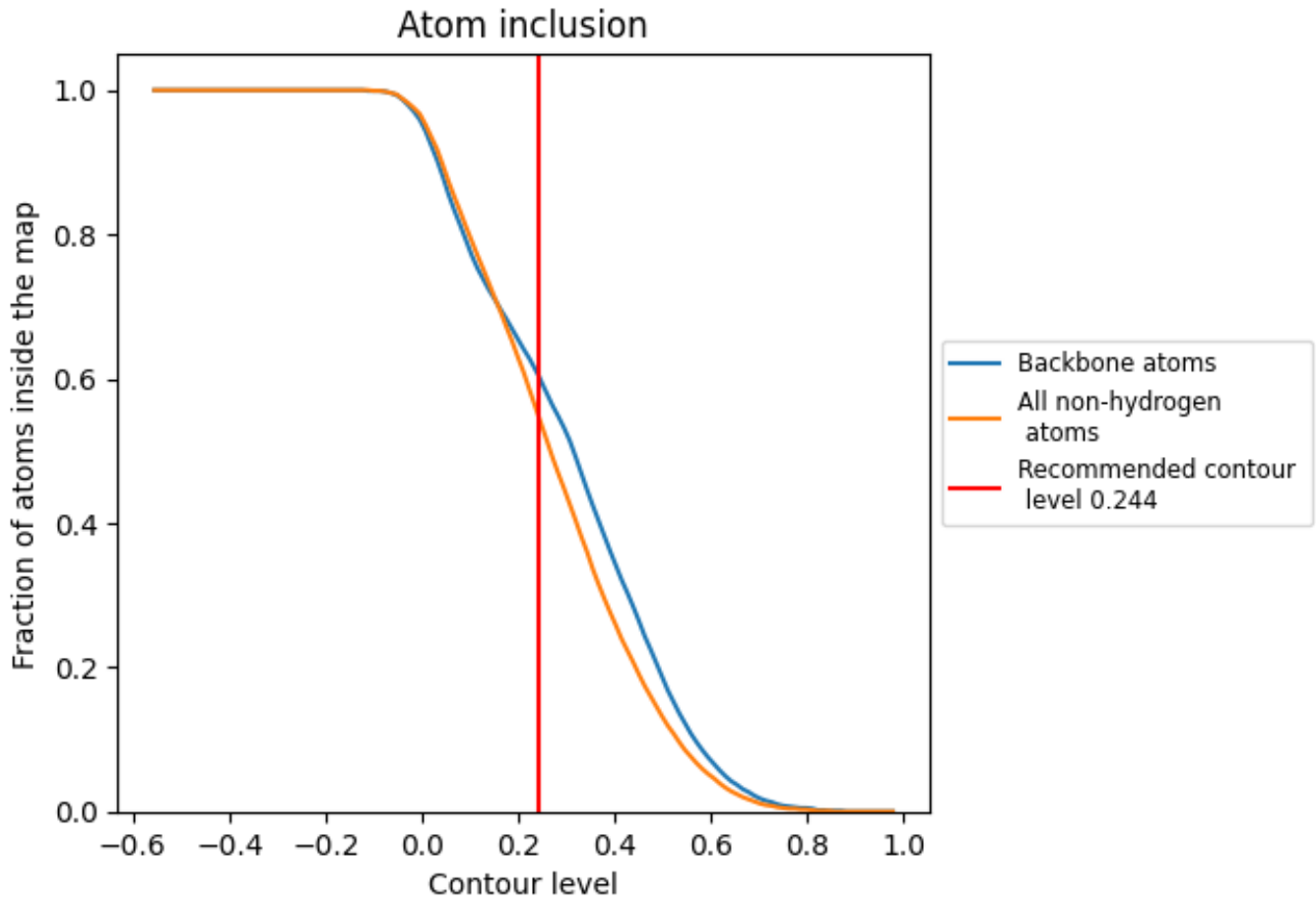
9.3 Atom inclusion mapped to coordinate model



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 (0.244).

9.4 Atom inclusion (i)



At the recommended contour level, 60% of all backbone atoms, 55% of all non-hydrogen atoms, are inside the map.

9.5 Map-model fit summary

The table lists the average atom inclusion at the recommended contour level (0.244) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.5460	0.3350
A	0.5460	0.3350
B	0.5450	0.3340
C	0.5440	0.3360
D	0.5500	0.3340
E	0.5460	0.3360
F	0.5460	0.3340