Full wwPDB EM Validation Report ⓘ

May 4, 2024 – 04:00 PM EDT

PDB ID	:	8SRS
EMDB ID	:	EMD-40740
Title	:	SpRY-Cas9:gRNA complex targeting TAC PAM DNA
Authors	:	Hibshman, G.N.; Bravo, J.P.K.; Taylor, D.W.
Deposited on	:	2023-05-06
Resolution	:	2.79 Å(reported)

This is a Full wwPDB EM Validation 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 ⓘ 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 ⓘ) were used in the production of this report:

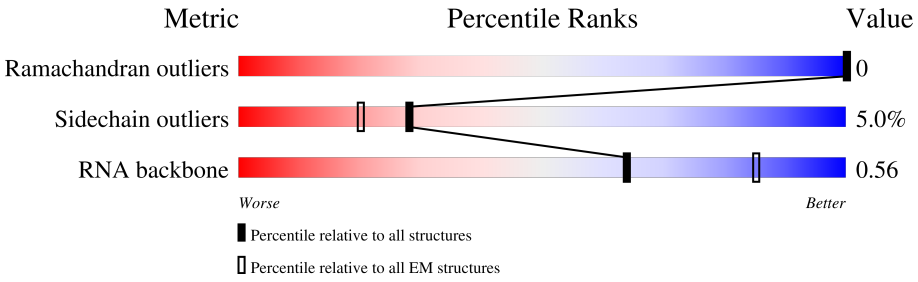
EMDB validation analysis	:	0.0.1.dev92
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36.2

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 2.79 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)
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826
RNA backbone	4643	859

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	1369	10%, 93%, 5%
2	B	98	84%, 16%
3	C	13	100%
4	D	55	35%, 65%
5	c	19	95%, 5%

2 Entry composition

There are 7 unique types of molecules in this entry. The entry contains 14148 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 CRISPR-associated endonuclease Cas9/Csn1.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	1341	Total	C	N	O	S	0	0
			11002	7012	1919	2049	22		

There are 13 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	1	GLU	-	expression tag	UNP Q99ZW2
A	61	ARG	ALA	engineered mutation	UNP Q99ZW2
A	1111	ARG	LEU	engineered mutation	UNP Q99ZW2
A	1135	LEU	ASP	engineered mutation	UNP Q99ZW2
A	1136	TRP	SER	engineered mutation	UNP Q99ZW2
A	1218	LYS	GLY	engineered mutation	UNP Q99ZW2
A	1219	GLN	GLU	engineered mutation	UNP Q99ZW2
A	1317	ARG	ASN	engineered mutation	UNP Q99ZW2
A	1322	ARG	ALA	engineered mutation	UNP Q99ZW2
A	1333	PRO	ARG	engineered mutation	UNP Q99ZW2
A	1335	GLN	ARG	engineered mutation	UNP Q99ZW2
A	1337	ARG	THR	engineered mutation	UNP Q99ZW2
A	1369	GLY	-	expression tag	UNP Q99ZW2

- Molecule 2 is a RNA chain called gRNA.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	B	98	Total	C	N	O	P	0	0
			2100	941	389	673	97		

- Molecule 3 is a DNA chain called TS.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	C	13	Total	C	N	O	P	0	0
			268	128	46	81	13		

- Molecule 4 is a DNA chain called NTS.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	D	19	Total 388	C 184	N 74	O 111	P 19	0	0

- Molecule 5 is a DNA chain called TS.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	c	19	Total 385	C 185	N 61	O 120	P 19	0	0

- Molecule 6 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

Mol	Chain	Residues	Atoms		AltConf
6	A	4	Total 4	Mg 4	0

- Molecule 7 is water.

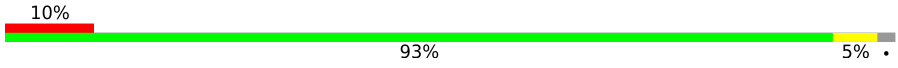
Mol	Chain	Residues	Atoms		AltConf
7	D	1	Total 1	O 1	0

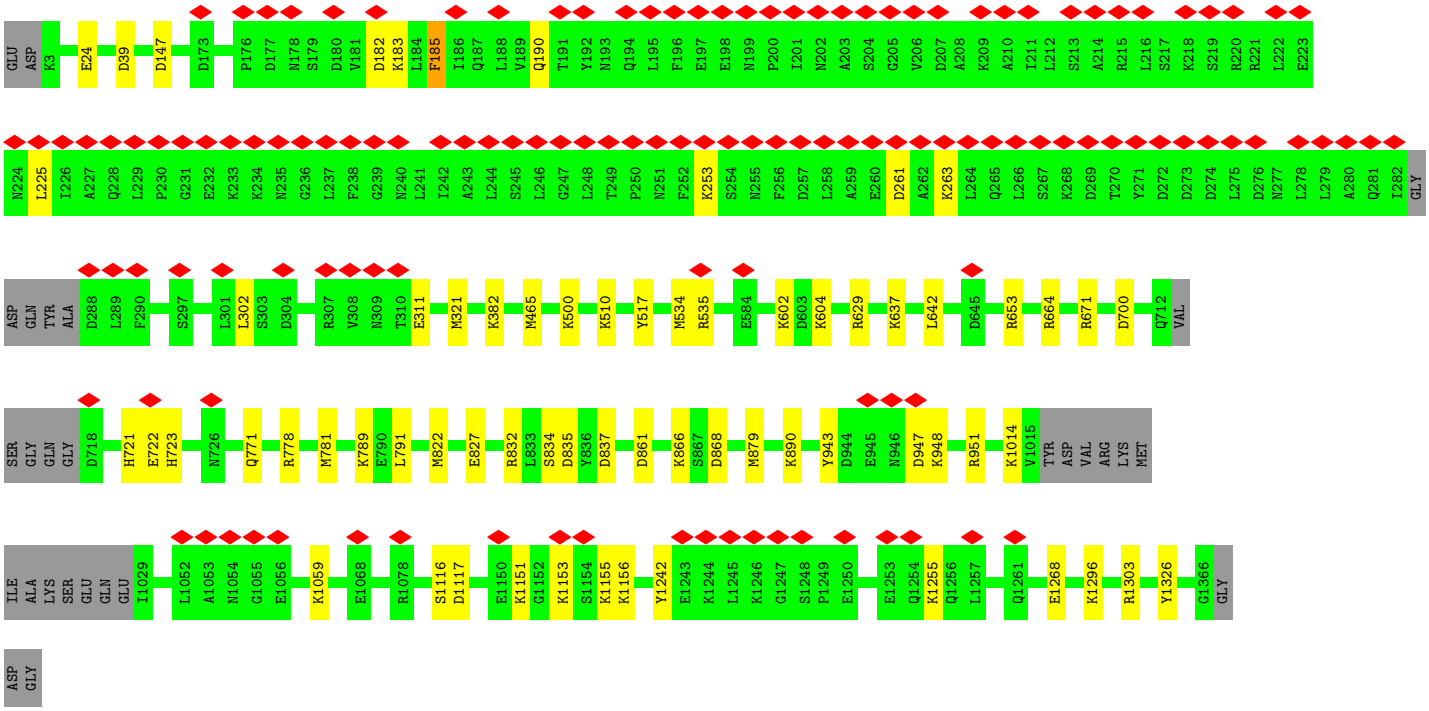
3 Residue-property plots (i)

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: CRISPR-associated endonuclease Cas9/Csn1

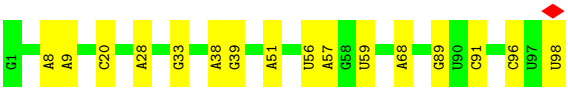
Chain A:





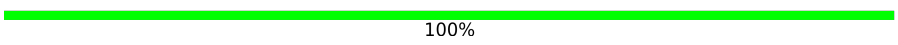
• Molecule 2: gRNA

Chain B:



• Molecule 3: TS

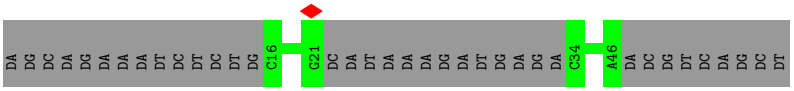
Chain C:



There are no outlier residues recorded for this chain.

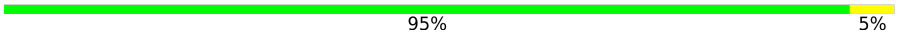
• Molecule 4: NTS

Chain D:



• Molecule 5: TS

Chain c:



4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	89331	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 ($e^-/Å^2$)	80	Depositor
Minimum defocus (nm)	1500	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	0.839	Depositor
Minimum map value	-0.299	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.012	Depositor
Recommended contour level	0.125	Depositor
Map size (Å)	426.5984, 426.5984, 426.5984	wwPDB
Map dimensions	512, 512, 512	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.8332, 0.8332, 0.8332	Depositor

5 Model quality

5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: 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	0.29	0/11198	0.61	7/15042 (0.0%)
2	B	0.26	0/2354	0.88	0/3669
3	C	0.60	0/299	1.04	0/460
4	D	0.63	0/434	0.94	0/664
5	c	0.84	1/428 (0.2%)	1.03	0/656
All	All	0.34	1/14713 (0.0%)	0.71	7/20491 (0.0%)

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	1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
5	c	0	DT	OP3-P	-10.68	1.48	1.61

All (7) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	182	ASP	CB-CG-OD1	7.73	125.26	118.30
1	A	835	ASP	CB-CG-OD1	6.56	124.20	118.30
1	A	185	PHE	CB-CG-CD1	6.13	125.09	120.80
1	A	791	LEU	CA-CB-CG	6.02	129.14	115.30
1	A	39	ASP	CB-CG-OD1	5.58	123.32	118.30
1	A	302	LEU	CA-CB-CG	5.38	127.68	115.30
1	A	948	LYS	CB-CG-CD	5.06	124.76	111.60

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	653	ARG	Sidechain

5.2 Too-close contacts (i)

Due to software issues we are unable to calculate clashes - this section is therefore empty.

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	1333/1369 (97%)	1293 (97%)	40 (3%)	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	1209/1230 (98%)	1149 (95%)	60 (5%)	24	56

All (60) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	24	GLU

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Mol	Chain	Res	Type
1	A	147	ASP
1	A	183	LYS
1	A	185	PHE
1	A	190	GLN
1	A	225	LEU
1	A	253	LYS
1	A	261	ASP
1	A	263	LYS
1	A	311	GLU
1	A	321	MET
1	A	382	LYS
1	A	465	MET
1	A	500	LYS
1	A	510	LYS
1	A	517	TYR
1	A	534	MET
1	A	535	ARG
1	A	602	LYS
1	A	604	LYS
1	A	629	ARG
1	A	637	LYS
1	A	642	LEU
1	A	664	ARG
1	A	671	ARG
1	A	700	ASP
1	A	721	HIS
1	A	722	GLU
1	A	723	HIS
1	A	771	GLN
1	A	778	ARG
1	A	781	MET
1	A	789	LYS
1	A	822	MET
1	A	827	GLU
1	A	832	ARG
1	A	834	SER
1	A	837	ASP
1	A	861	ASP
1	A	866	LYS
1	A	868	ASP
1	A	879	MET
1	A	890	LYS

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Mol	Chain	Res	Type
1	A	943	TYR
1	A	947	ASP
1	A	951	ARG
1	A	1014	LYS
1	A	1059	LYS
1	A	1116	SER
1	A	1117	ASP
1	A	1151	LYS
1	A	1153	LYS
1	A	1155	LYS
1	A	1156	LYS
1	A	1242	TYR
1	A	1255	LYS
1	A	1268	GLU
1	A	1296	LYS
1	A	1303	ARG
1	A	1326	TYR

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (1) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	1261	GLN

5.3.3 RNA (i)

Mol	Chain	Analysed	Backbone Outliers	Pucker Outliers
2	B	97/98 (98%)	15 (15%)	1 (1%)

All (15) RNA backbone outliers are listed below:

Mol	Chain	Res	Type
2	B	8	A
2	B	9	A
2	B	20	C
2	B	28	A
2	B	33	G
2	B	39	G
2	B	51	A
2	B	56	U
2	B	57	A

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Mol	Chain	Res	Type
2	B	59	U
2	B	68	A
2	B	89	G
2	B	91	C
2	B	96	C
2	B	98	U

All (1) RNA pucker outliers are listed below:

Mol	Chain	Res	Type
2	B	38	A

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, 4 are monoatomic - leaving 0 for Mogul analysis.

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

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.

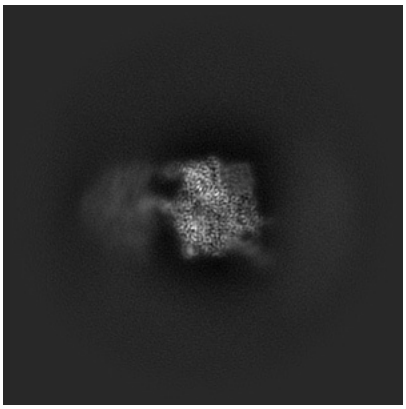
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-40740. These allow visual inspection of the internal detail of the map and identification of artifacts.

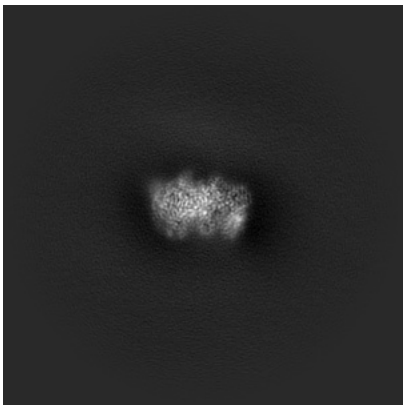
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections

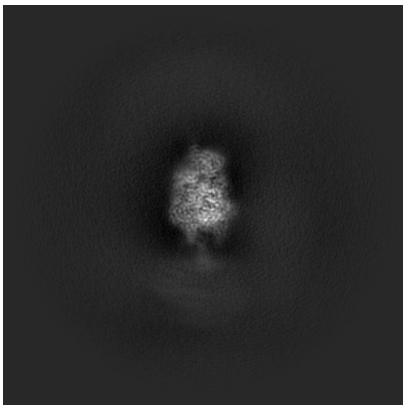
6.1.1 Primary map



X

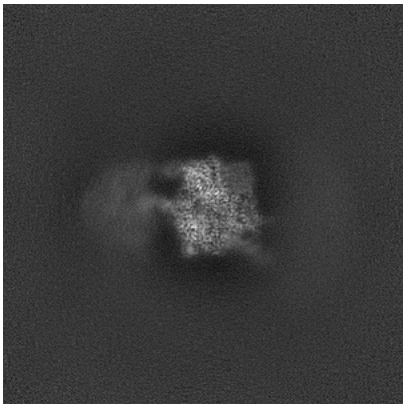


Y

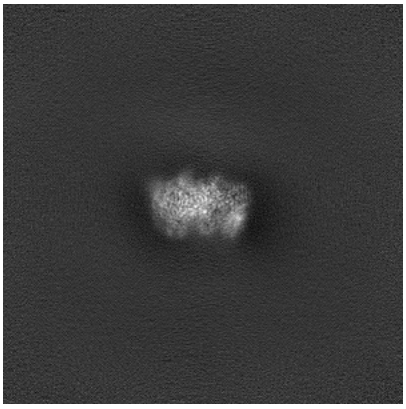


Z

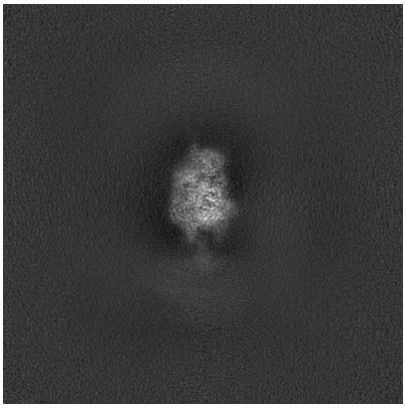
6.1.2 Raw map



X



Y

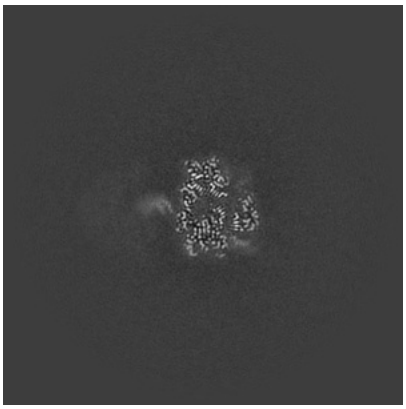


Z

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

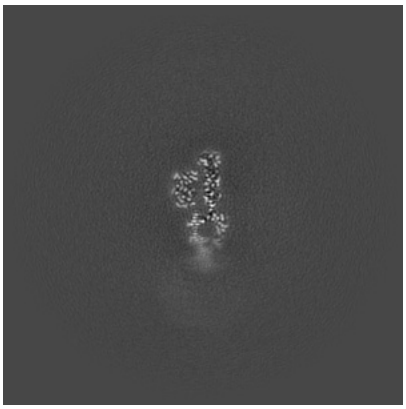
6.2 Central slices (i)

6.2.1 Primary map



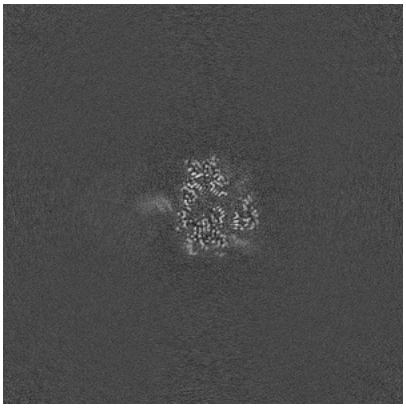
X Index: 256

Y Index: 256

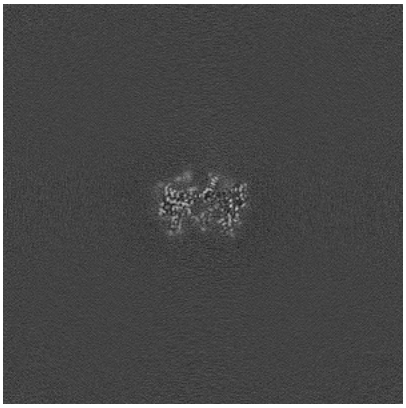


Z Index: 256

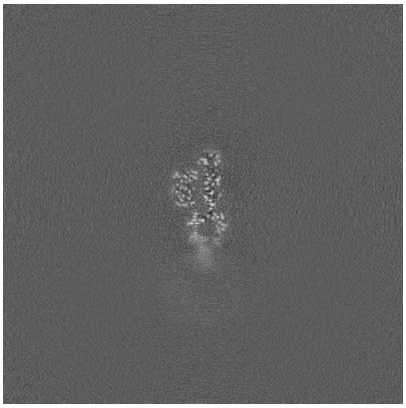
6.2.2 Raw map



X Index: 256



Y Index: 256

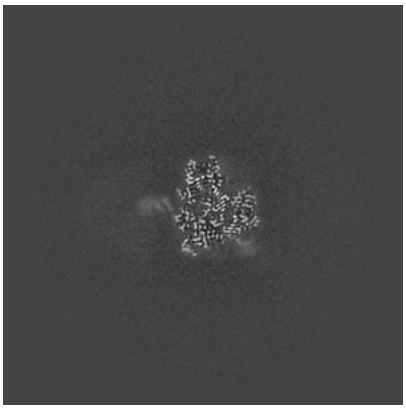


Z Index: 256

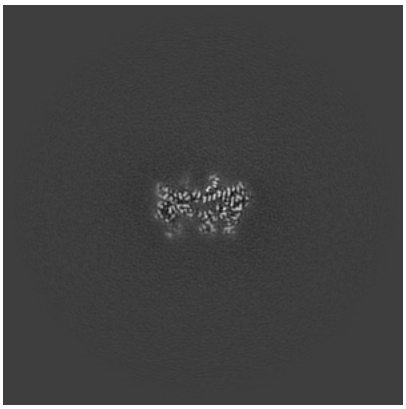
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

6.3.1 Primary map



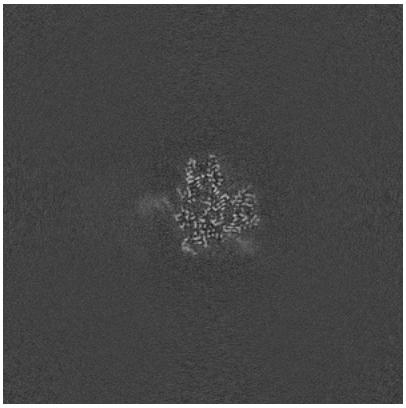
X Index: 260



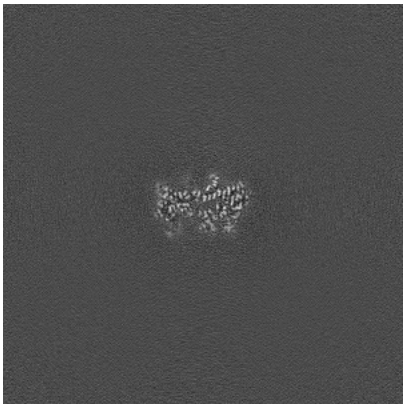
Y Index: 261

Z Index: 227

6.3.2 Raw map



X Index: 260



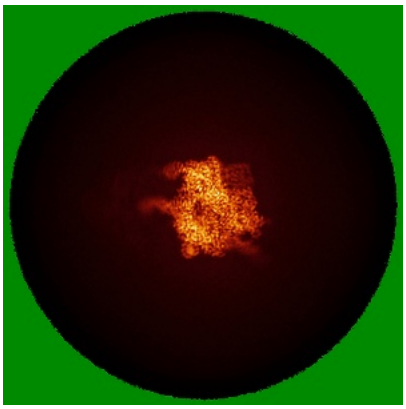
Y Index: 261

Z Index: 259

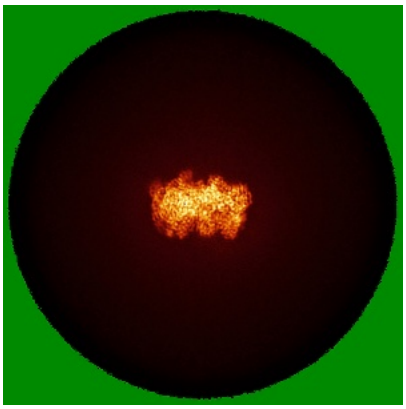
The images above show the largest variance slices of the map in three orthogonal directions.

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

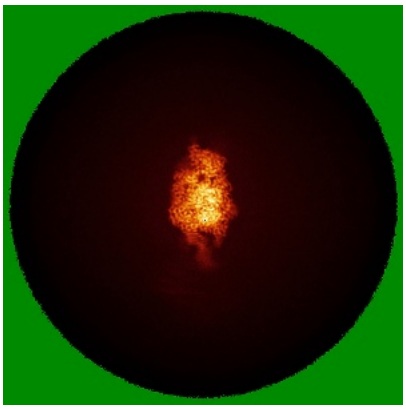
6.4.1 Primary map



X

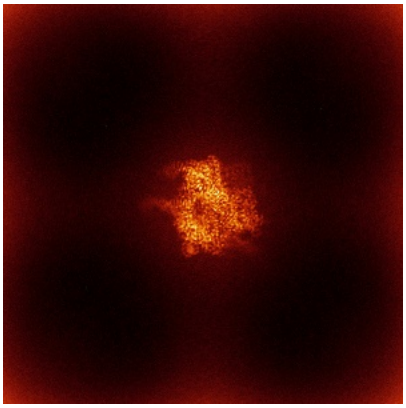


Y

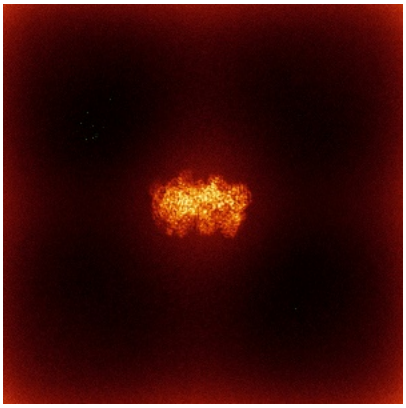


Z

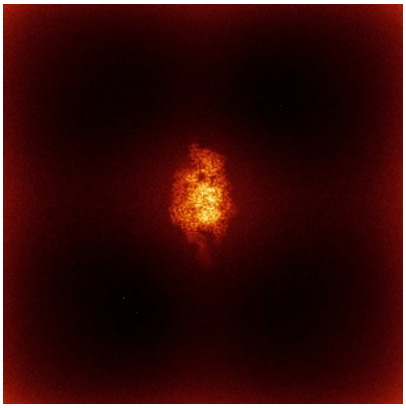
6.4.2 Raw map



X



Y

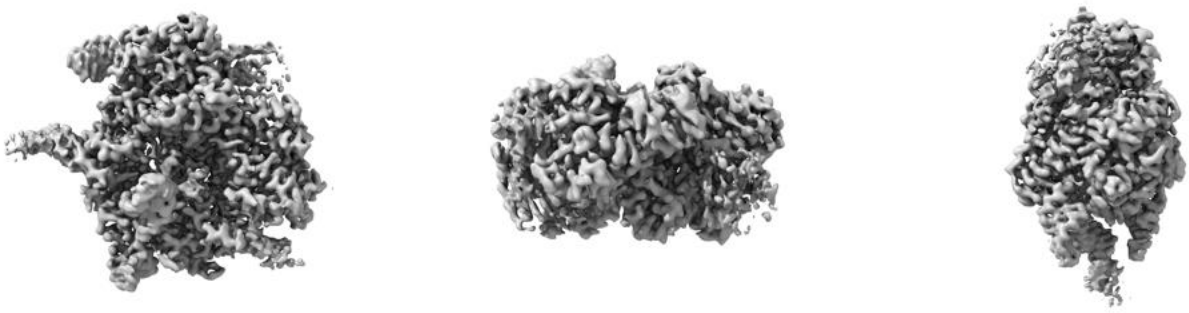


Z

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 0.125. 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.

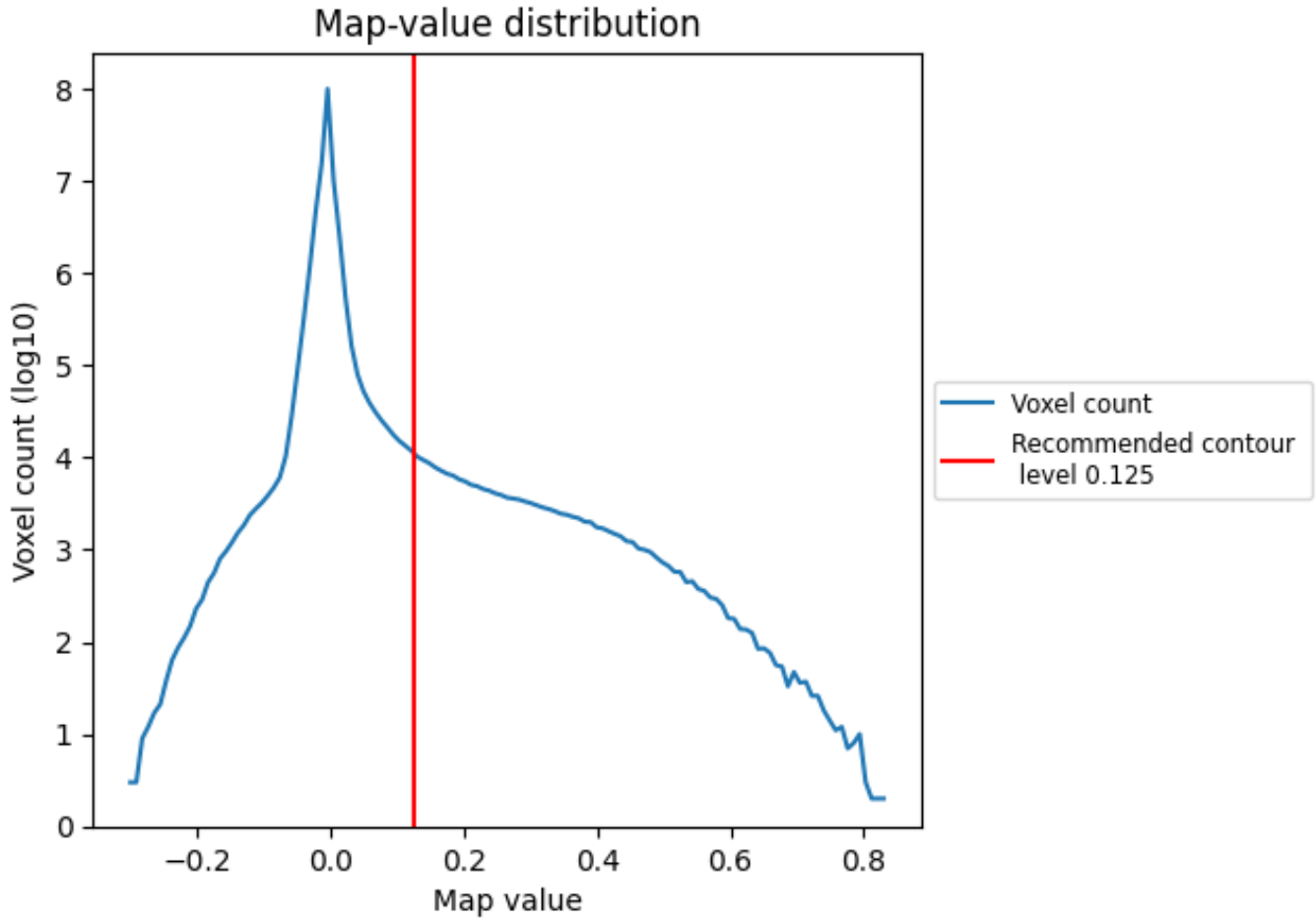
6.6 Mask visualisation (i)

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

7 Map analysis

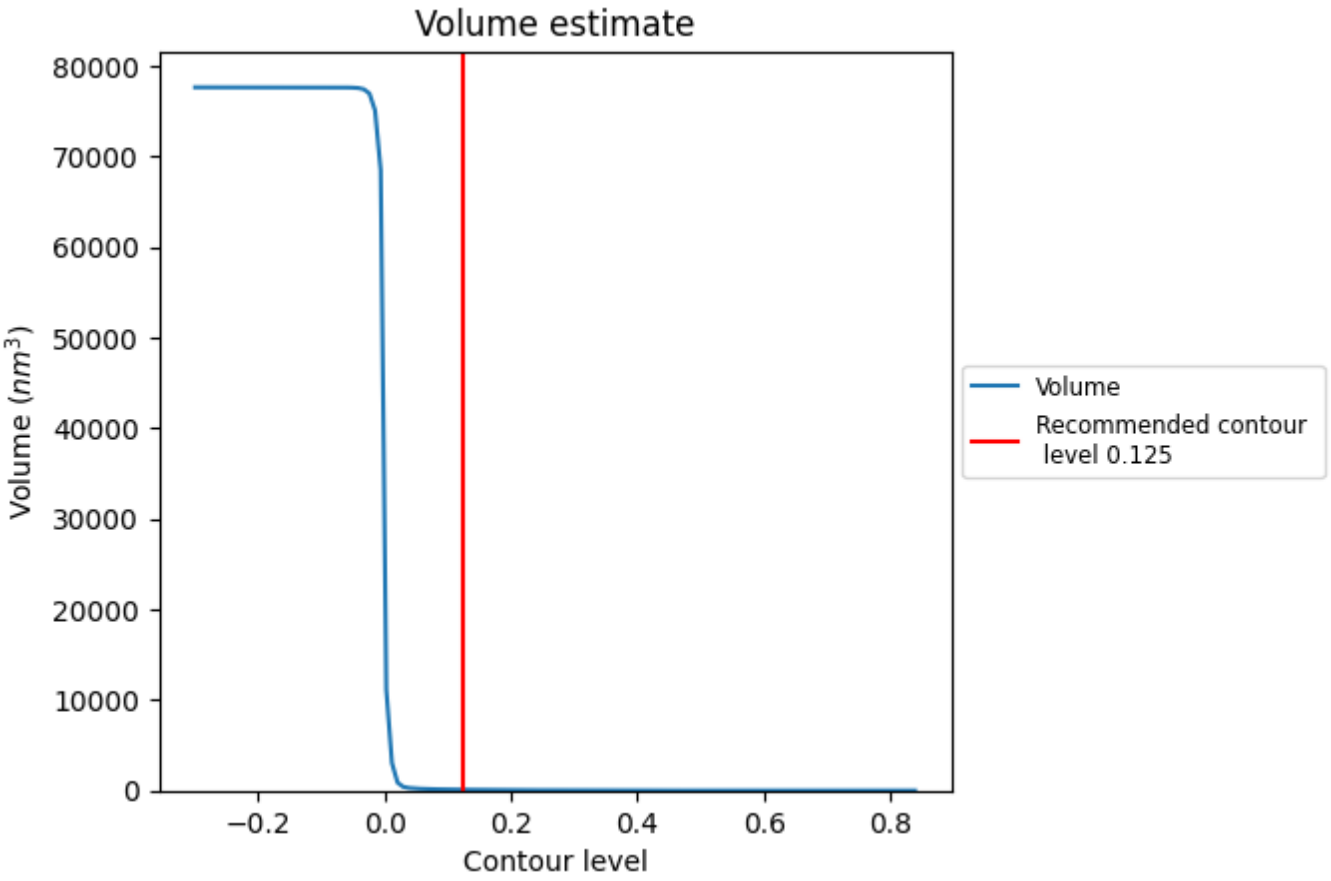
This section contains the results of statistical analysis of the map.

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.

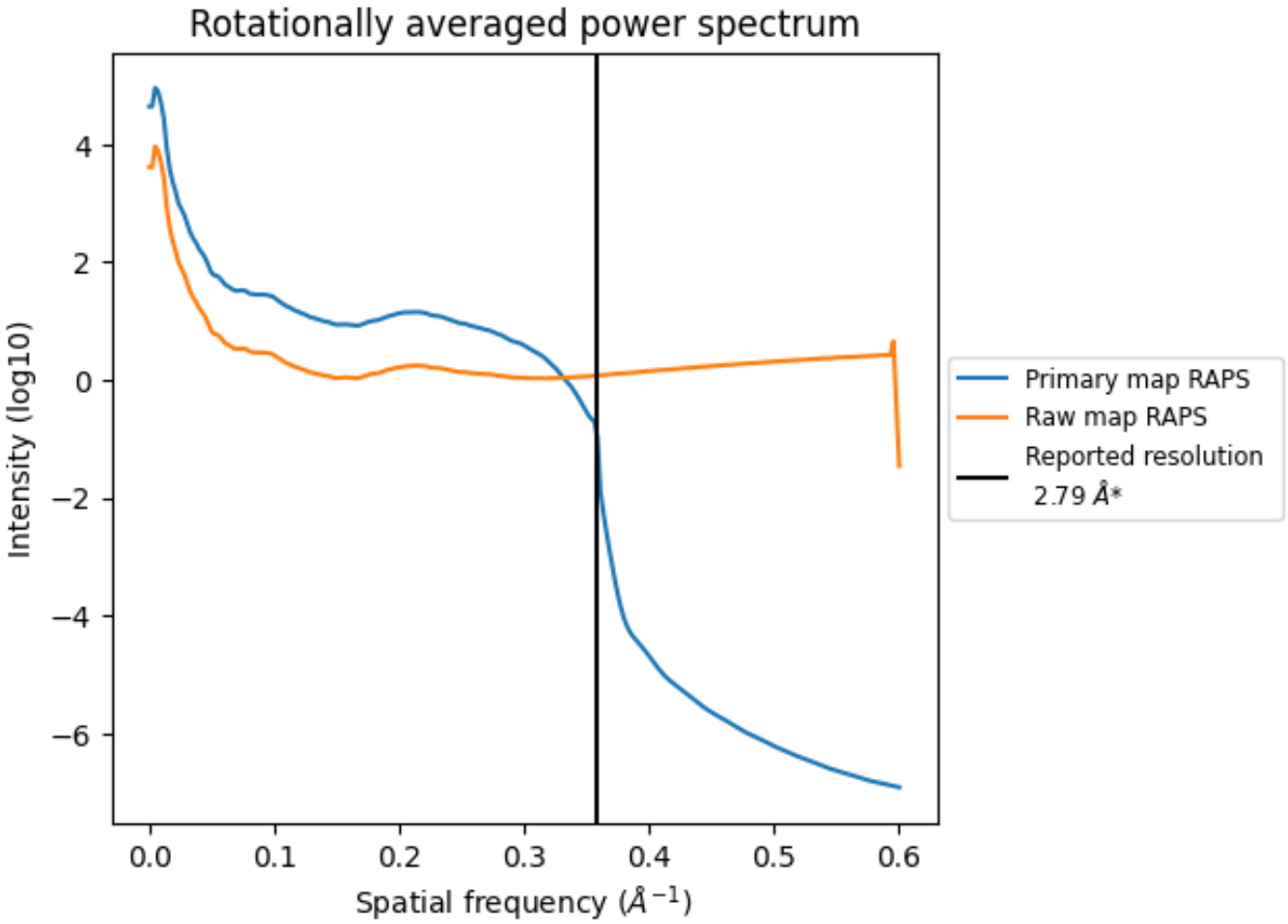
7.2 Volume estimate (i)



The volume at the recommended contour level is 95 nm^3; this corresponds to an approximate mass of 86 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)

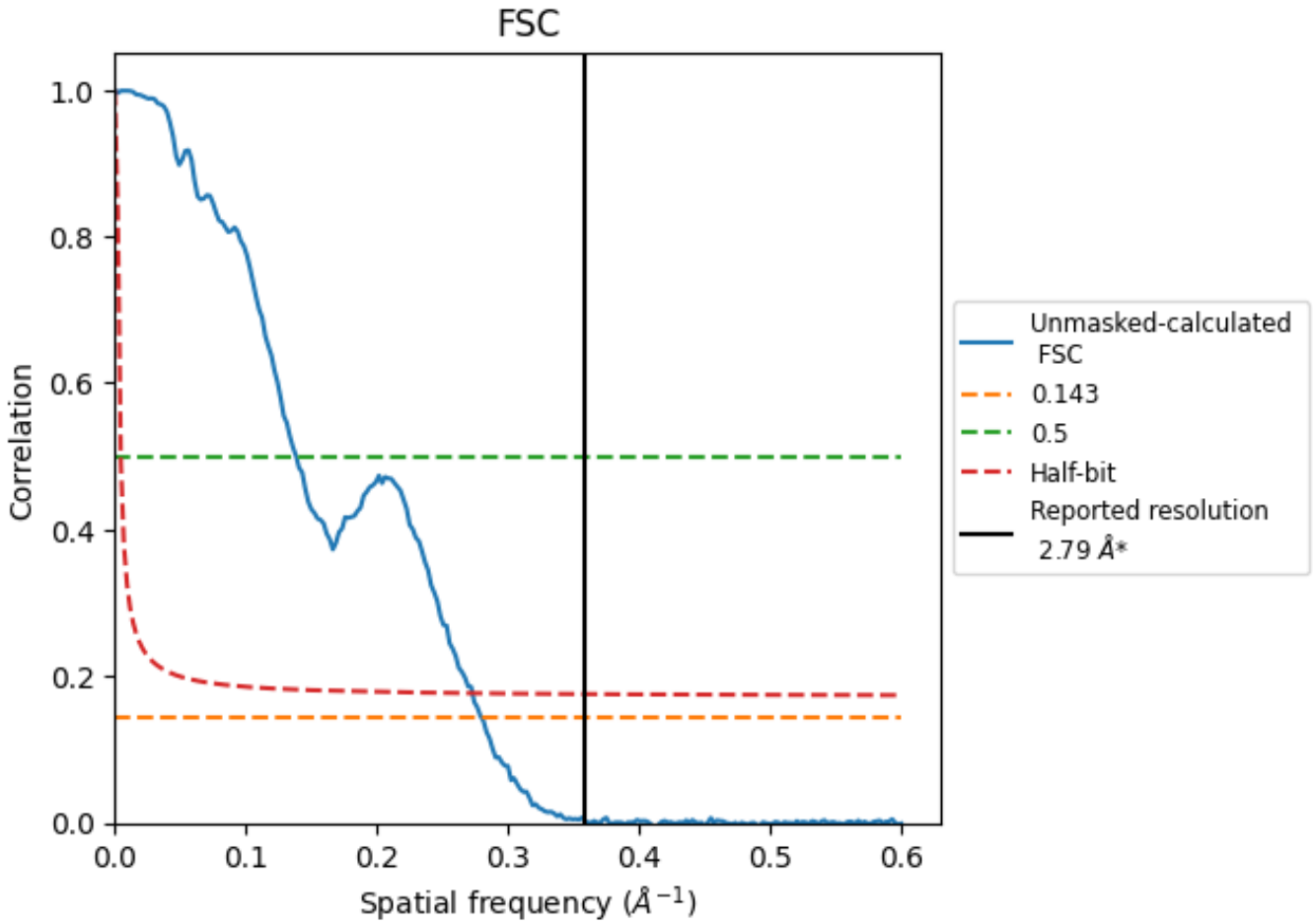


*Reported resolution corresponds to spatial frequency of 0.358 Å^{-1}

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 0.358 Å^{-1}

8.2 Resolution estimates (i)

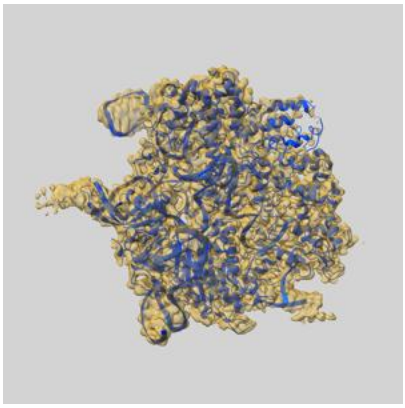
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	2.79	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	3.57	7.23	3.66

*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 3.57 differs from the reported value 2.79 by more than 10 %

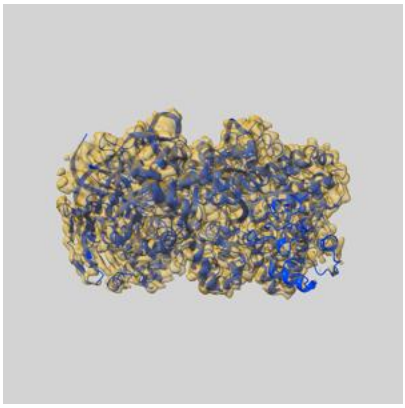
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-40740 and PDB model 8SRS. Per-residue inclusion information can be found in section 3 on page 5.

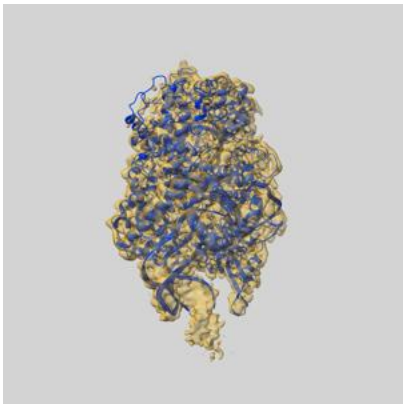
9.1 Map-model overlay



X



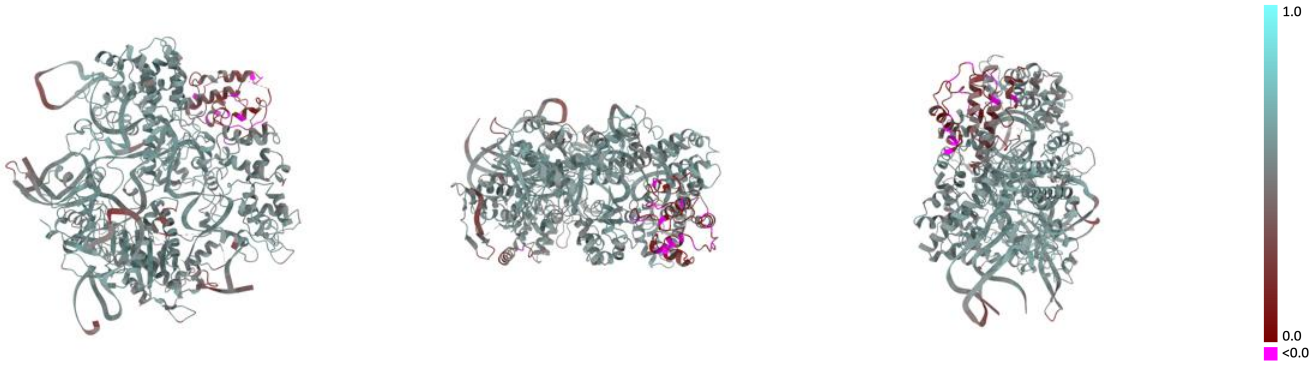
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.125 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.

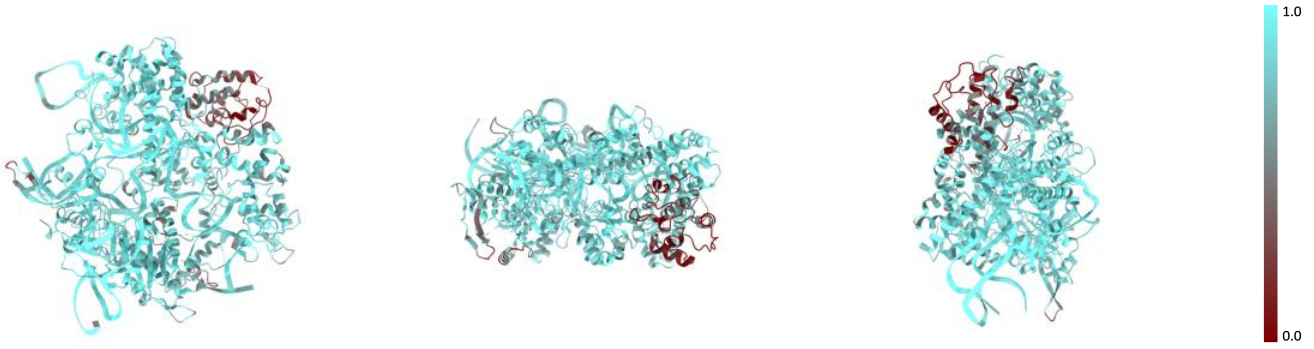
9.2 Q-score mapped to coordinate model



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.

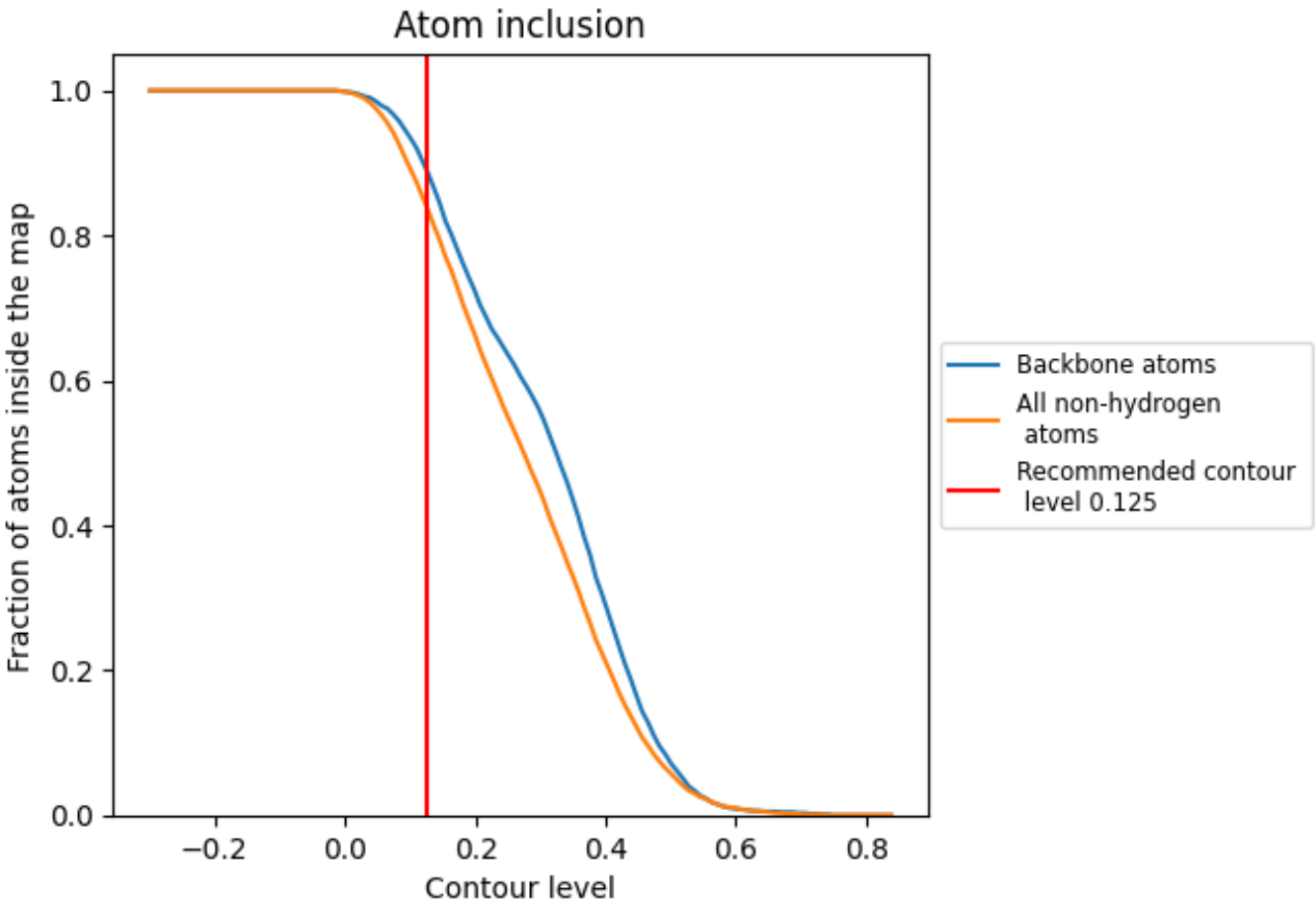
9.3 Atom inclusion mapped to coordinate model



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 (0.125).

9.4 Atom inclusion (i)



At the recommended contour level, 89% of all backbone atoms, 84% 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 (0.125) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.8410	0.5210
A	0.8120	0.5160
B	0.9510	0.5390
C	0.9630	0.5540
D	0.8400	0.4780
c	0.9710	0.5810