



wwPDB EM Validation Summary Report ⓘ

Oct 13, 2024 – 07:02 am BST

PDB ID : 8PIB
EMDB ID : EMD-17679
Title : autoinhibited RfaH bound to E. coli transcription complex paused at ops site (encounter complex)
Authors : Zuber, P.K.; Said, N.; Hilal, T.; Loll, B.; Wahl, M.C.; Knauer, S.H.
Deposited on : 2023-06-21
Resolution : 2.60 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 ⓘ 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.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) : 2.39

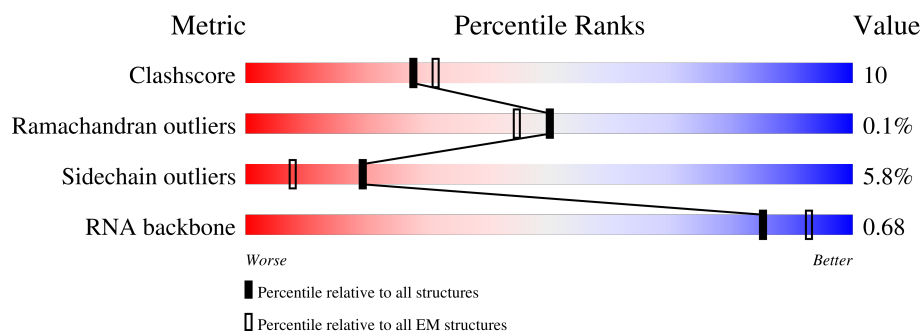
1 Overall quality at a glance

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 2.60 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
RNA backbone	6643	2191

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 $\leq 5\%$. 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	P	164	<div> <div>17%</div> <div>48%</div> <div>28%</div> <div>5%</div> <div>18%</div> </div>
2	I	1342	<div> <div>78%</div> <div>20%</div> <div>2%</div> </div>
3	J	1416	<div> <div>67%</div> <div>25%</div> <div>7%</div> </div>
4	K	91	<div> <div>18%</div> <div>55%</div> <div>33%</div> <div>9%</div> </div>
5	G	329	<div> <div>55%</div> <div>14%</div> <div>29%</div> </div>
5	H	329	<div> <div>49%</div> <div>18%</div> <div>31%</div> </div>
6	R	17	<div> <div>35%</div> <div>29%</div> <div>6%</div> <div>29%</div> </div>

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Mol	Chain	Length	Quality of chain
7	B	40	 28% 72%
8	A	40	 10% 40% 60%

2 Entry composition

There are 10 unique types of molecules in this entry. The entry contains 27848 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 Transcription antitermination protein RfaH.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	P	135	Total	C	N	O	S	0	0
			1073	687	188	191	7		

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
P	-1	GLY	-	expression tag	UNP P0AFW0
P	0	HIS	-	expression tag	UNP P0AFW0
P	51	CYS	PHE	engineered mutation	UNP P0AFW0
P	139	CYS	SER	engineered mutation	UNP P0AFW0

- Molecule 2 is a protein called DNA-directed RNA polymerase subunit beta.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	I	1321	Total	C	N	O	S	0	0
			10423	6539	1816	2024	44		

- Molecule 3 is a protein called DNA-directed RNA polymerase subunit beta'.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	J	1318	Total	C	N	O	S	0	0
			10249	6439	1825	1939	46		

There are 10 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
J	1	VAL	-	expression tag	UNP P0A8T7
J	1408	LEU	-	expression tag	UNP P0A8T7
J	1409	GLU	-	expression tag	UNP P0A8T7
J	1410	VAL	-	expression tag	UNP P0A8T7
J	1411	HIS	-	expression tag	UNP P0A8T7
J	1412	HIS	-	expression tag	UNP P0A8T7
J	1413	HIS	-	expression tag	UNP P0A8T7

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Chain	Residue	Modelled	Actual	Comment	Reference
J	1414	HIS	-	expression tag	UNP P0A8T7
J	1415	HIS	-	expression tag	UNP P0A8T7
J	1416	HIS	-	expression tag	UNP P0A8T7

- Molecule 4 is a protein called DNA-directed RNA polymerase subunit omega.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	K	83	Total	C	N	O	S	0	0
			655	399	123	132	1		

- Molecule 5 is a protein called DNA-directed RNA polymerase subunit alpha.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	G	233	Total	C	N	O	S	1	0
			1811	1127	323	355	6		
5	H	226	Total	C	N	O	S	0	0
			1741	1088	308	339	6		

- Molecule 6 is a RNA chain called RNA.

Mol	Chain	Residues	Atoms					AltConf	Trace
6	R	12	Total	C	N	O	P	0	0
			260	115	47	86	12		

- Molecule 7 is a DNA chain called template DNA.

Mol	Chain	Residues	Atoms					AltConf	Trace
7	B	40	Total	C	N	O	P	0	0
			826	390	165	232	39		

- Molecule 8 is a DNA chain called non-template DNA.

Mol	Chain	Residues	Atoms					AltConf	Trace
8	A	40	Total	C	N	O	P	0	0
			808	386	139	244	39		

- Molecule 9 is ZINC ION (three-letter code: ZN) (formula: Zn).

Mol	Chain	Residues	Atoms		AltConf
9	J	1	Total	Zn	0
			1	1	

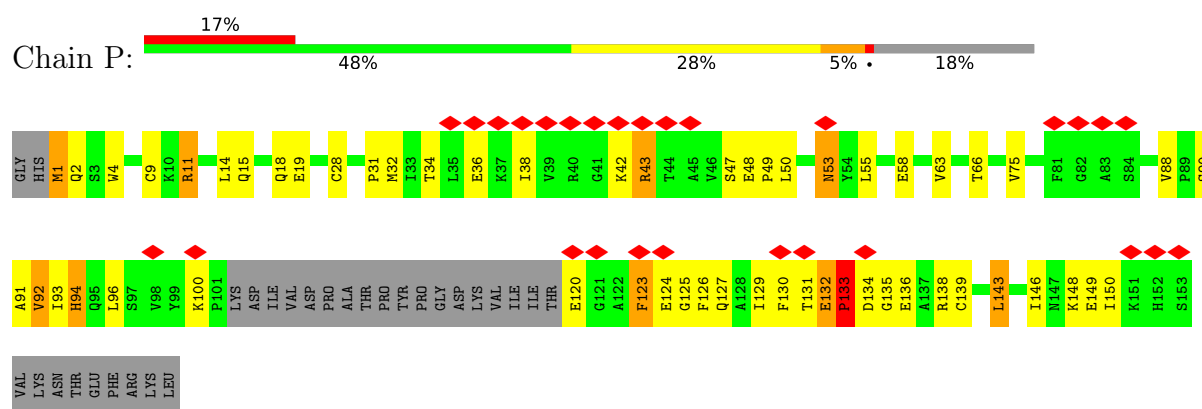
- Molecule 10 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

Mol	Chain	Residues	Atoms		AltConf
10	J	1	Total	Mg	0
			1	1	

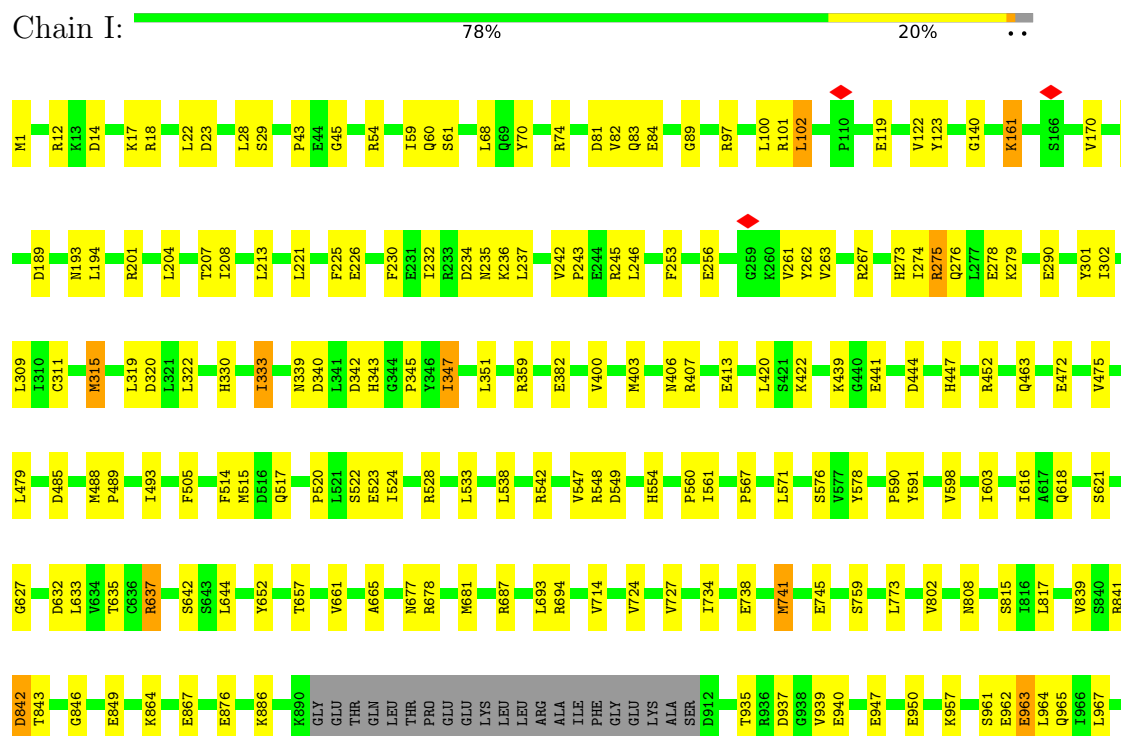
3 Residue-property plots

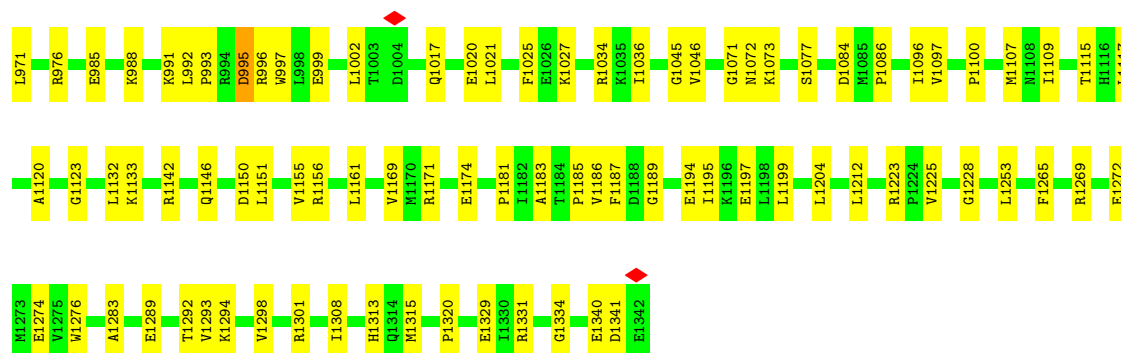
These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and atom inclusion 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: Transcription antitermination protein RfaH

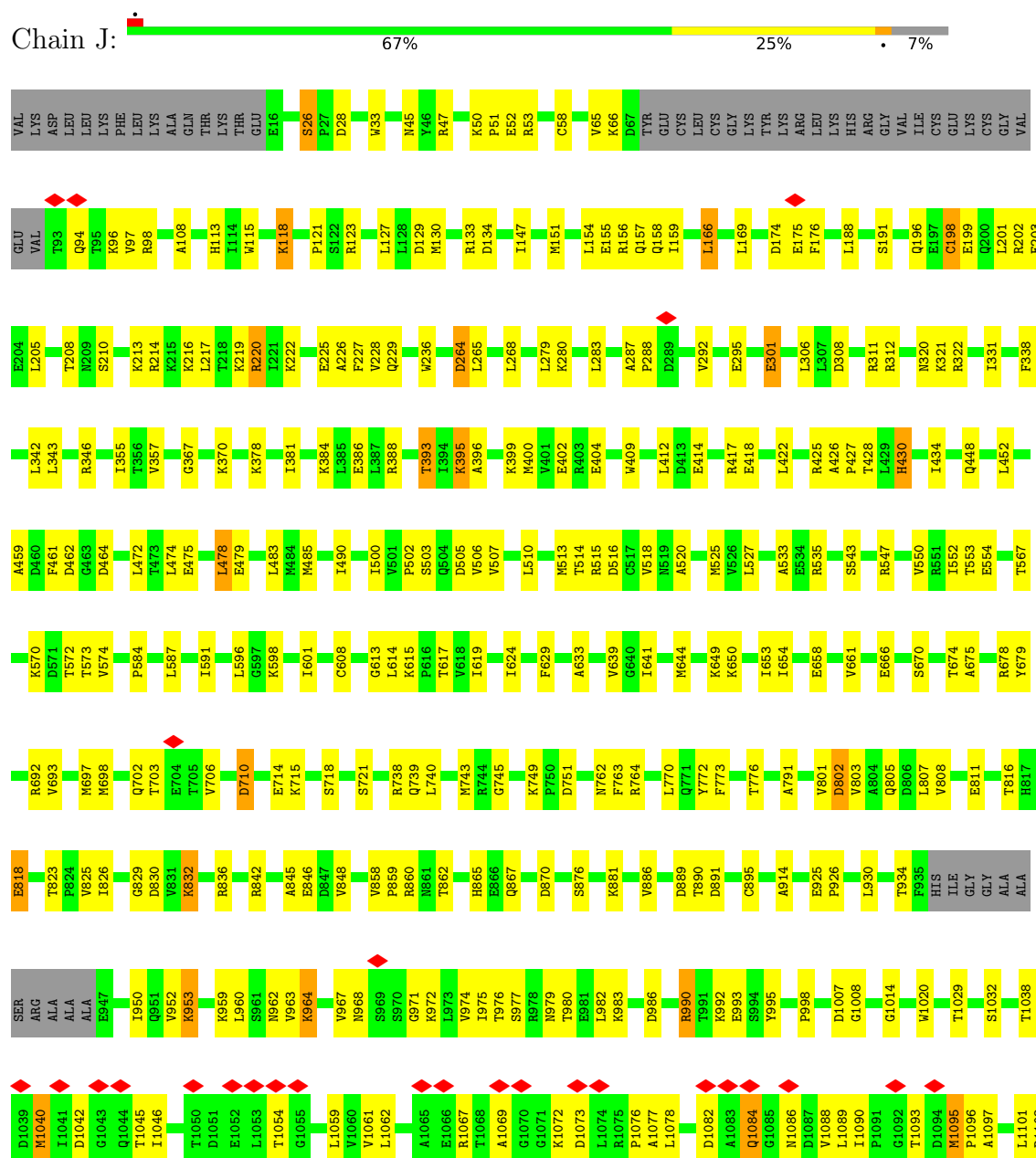


- Molecule 2: DNA-directed RNA polymerase subunit beta



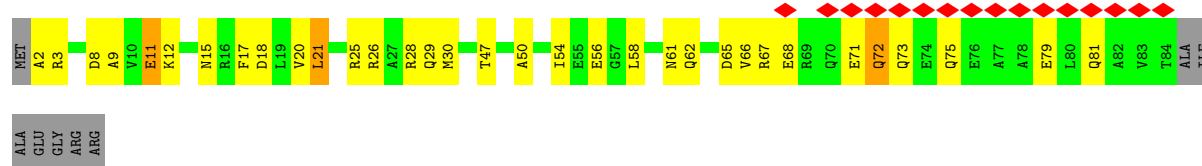


• Molecule 3: DNA-directed RNA polymerase subunit beta'

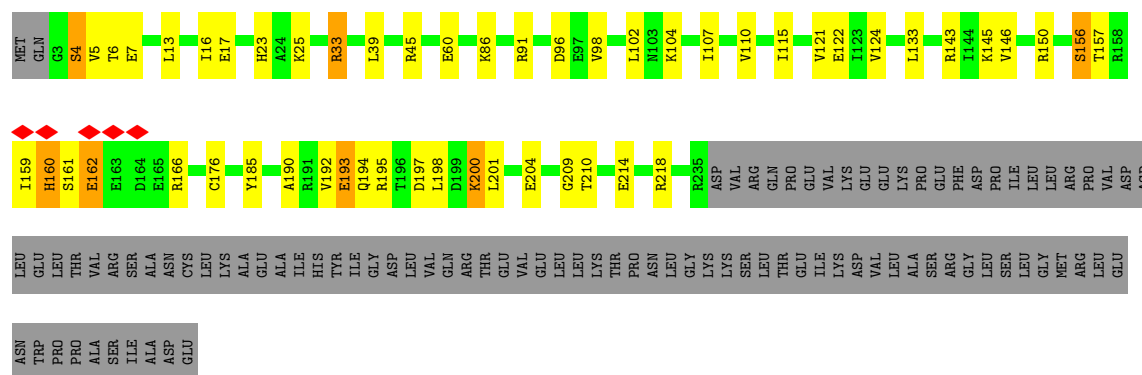




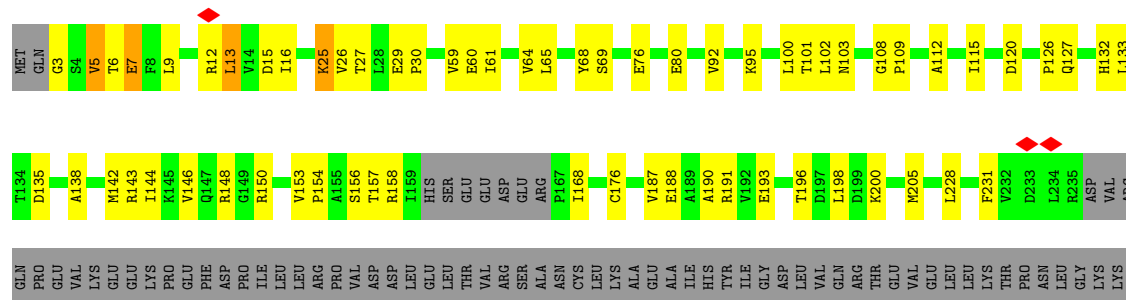
• Molecule 4: DNA-directed RNA polymerase subunit omega



• Molecule 5: DNA-directed RNA polymerase subunit alpha



• Molecule 5: DNA-directed RNA polymerase subunit alpha



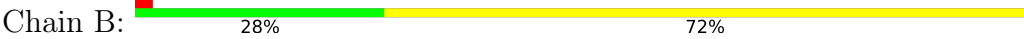
SER	LEU	THR	GLU	ILE	LYS	ASP	VAL	LEU	ALA	SER	ARG	GLY	LEU	SER	LEU	GLY	MET	ARG	LEU	GLU	ASN	TRP	PRO	PRO	ALA	SER	ILE	ALA	ASP	GLU
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● Molecule 6: RNA



U	U	C	U	U	U6	G7	G8	G9	G10	G11	G16	U17
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● Molecule 7: template DNA



G1	G2	A3	A4	G9	A10	A11	A12	A13	A14	A15	G16	C17	A18	C19	G20	G21	T22	A23	C24	C25	G26	C29	G30	C31	G32	T33	G34	G35	T36	G37	G38	T39	G40
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● Molecule 8: non-template DNA



C1	A2	C3	C4	A5	C6	C7	A8	C9	G10	C11	G12	G13	G14	C15	A19	G20	C21	G22	T29	T30	T31	C32	C36	T37	T38	C39	C40
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4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	848008	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 ($e^-/\text{\AA}^2$)	42	Depositor
Minimum defocus (nm)	800	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	96000	Depositor
Image detector	FEI FALCON III (4k x 4k)	Depositor
Maximum map value	2.524	Depositor
Minimum map value	-0.000	Depositor
Average map value	0.007	Depositor
Map value standard deviation	0.057	Depositor
Recommended contour level	0.24	Depositor
Map size (Å)	319.488, 319.488, 319.488	wwPDB
Map dimensions	384, 384, 384	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.832, 0.832, 0.832	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: ZN, 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	P	0.30	0/1097	0.64	2/1485 (0.1%)
2	I	0.25	0/10589	0.50	1/14286 (0.0%)
3	J	0.25	0/10403	0.51	1/14049 (0.0%)
4	K	0.28	0/657	0.62	0/886
5	G	0.25	0/1836	0.55	0/2487
5	H	0.25	0/1761	0.55	1/2385 (0.0%)
6	R	0.20	0/290	0.85	0/451
7	B	0.50	0/930	0.84	0/1435
8	A	0.50	0/902	0.92	0/1389
All	All	0.28	0/28465	0.56	5/38853 (0.0%)

There are no bond length outliers.

All (5) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
5	H	13	LEU	CA-CB-CG	6.38	129.97	115.30
1	P	133	PRO	CA-N-CD	-6.15	102.89	111.50
3	J	166	LEU	CA-CB-CG	6.08	129.28	115.30
2	I	488	MET	CB-CG-SD	5.35	128.44	112.40
1	P	143	LEU	CA-CB-CG	5.34	127.59	115.30

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	P	1073	0	1080	38	0
2	I	10423	0	10436	164	0
3	J	10249	0	10459	228	0
4	K	655	0	663	17	0
5	G	1811	0	1843	28	0
5	H	1741	0	1783	38	0
6	R	260	0	130	3	0
7	B	826	0	447	31	0
8	A	808	0	453	17	0
9	J	1	0	0	0	0
10	J	1	0	0	0	0
All	All	27848	0	27294	525	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 10.

The worst 5 of 525 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)
5:H:29:GLU:HA	5:H:200:LYS:HG3	1.56	0.88
3:J:697:MET:HE1	3:J:738:ARG:HA	1.58	0.84
2:I:618:GLN:HE22	3:J:770:LEU:HB2	1.48	0.78
1:P:132:GLU:OE1	1:P:138:ARG:HG2	1.87	0.74
2:I:940:GLU:OE2	2:I:940:GLU:N	2.20	0.73

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	P	131/164 (80%)	123 (94%)	7 (5%)	1 (1%)	16	34
2	I	1317/1342 (98%)	1284 (98%)	33 (2%)	0	100	100
3	J	1310/1416 (92%)	1278 (98%)	32 (2%)	0	100	100
4	K	81/91 (89%)	80 (99%)	1 (1%)	0	100	100
5	G	232/329 (70%)	226 (97%)	5 (2%)	1 (0%)	30	52
5	H	222/329 (68%)	213 (96%)	9 (4%)	0	100	100
All	All	3293/3671 (90%)	3204 (97%)	87 (3%)	2 (0%)	50	71

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	P	133	PRO
5	G	193	GLU

5.3.2 Protein sidechains ⓘ

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	P	117/143 (82%)	101 (86%)	16 (14%)	3	5
2	I	1140/1157 (98%)	1096 (96%)	44 (4%)	27	53
3	J	1104/1177 (94%)	1037 (94%)	67 (6%)	15	34
4	K	70/75 (93%)	58 (83%)	12 (17%)	1	2
5	G	201/286 (70%)	189 (94%)	12 (6%)	16	35
5	H	193/286 (68%)	179 (93%)	14 (7%)	11	25
All	All	2825/3124 (90%)	2660 (94%)	165 (6%)	19	36

5 of 165 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
3	J	1054	THR
5	G	96	ASP
3	J	1095	MET

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Mol	Chain	Res	Type
4	K	18	ASP
5	G	200	LYS

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (2) such sidechains are listed below:

Mol	Chain	Res	Type
2	I	517	GLN
2	I	618	GLN

5.3.3 RNA [i](#)

Mol	Chain	Analysed	Backbone Outliers	Pucker Outliers
6	R	11/17 (64%)	2 (18%)	0

All (2) RNA backbone outliers are listed below:

Mol	Chain	Res	Type
6	R	7	G
6	R	17	U

There are no RNA pucker outliers to report.

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](#)

Of 2 ligands modelled in this entry, 2 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.

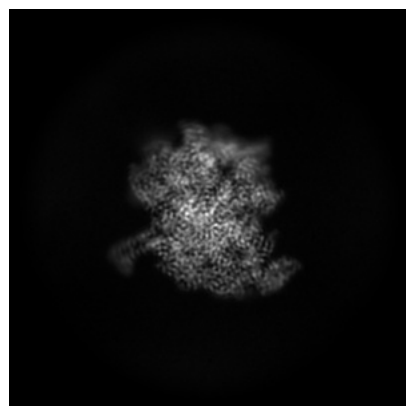
6 Map visualisation [i](#)

This section contains visualisations of the EMDB entry EMD-17679. These allow visual inspection of the internal detail of the map and identification of artifacts.

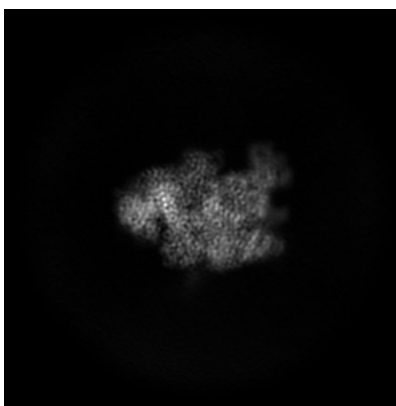
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections [i](#)

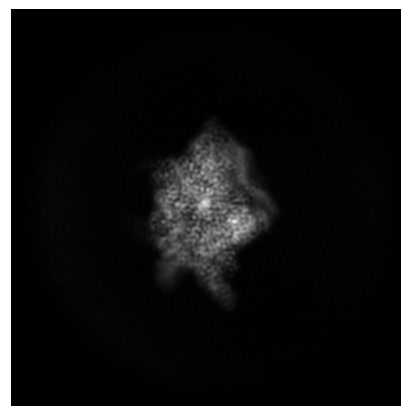
6.1.1 Primary map



X

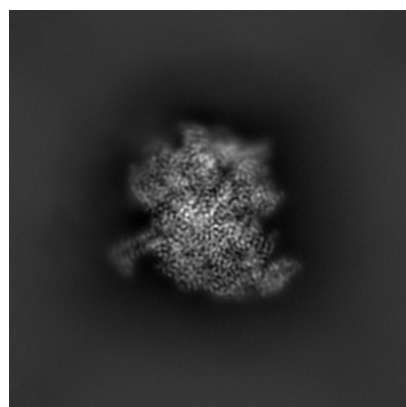


Y

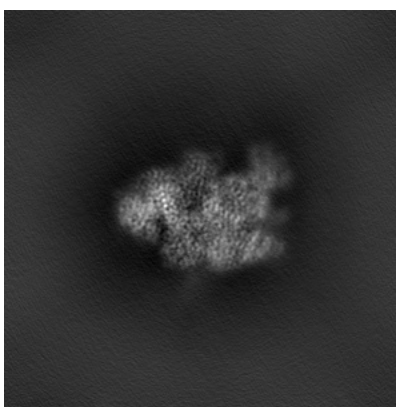


Z

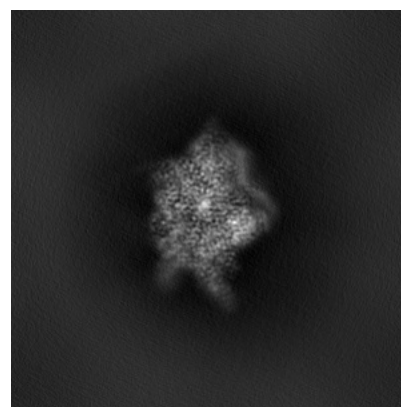
6.1.2 Raw map



X



Y

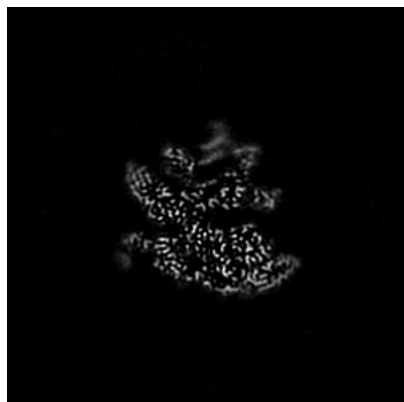


Z

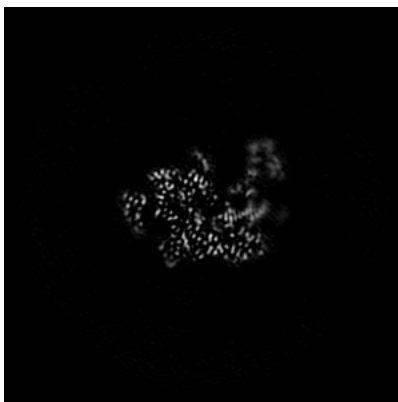
The images above show the map projected in three orthogonal directions.

6.2 Central slices [i](#)

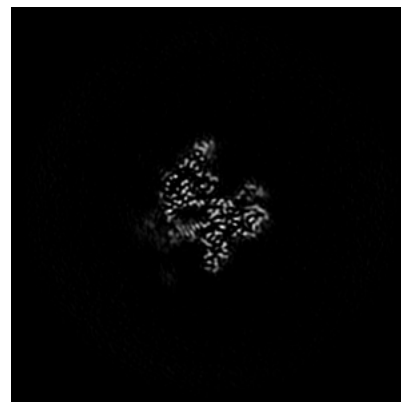
6.2.1 Primary map



X Index: 192

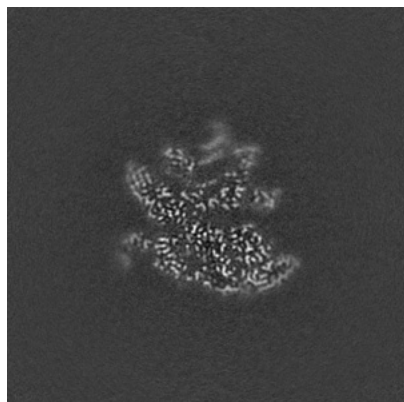


Y Index: 192

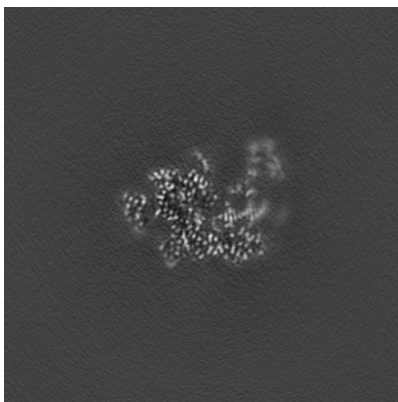


Z Index: 192

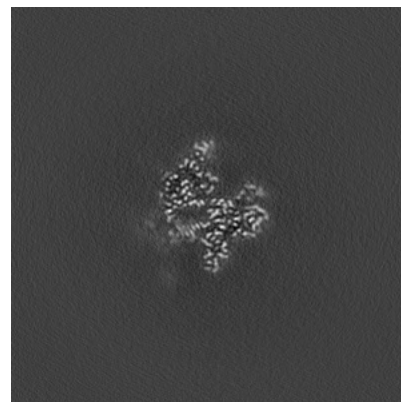
6.2.2 Raw map



X Index: 192



Y Index: 192

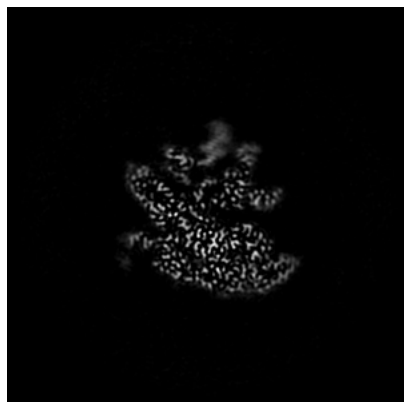


Z Index: 192

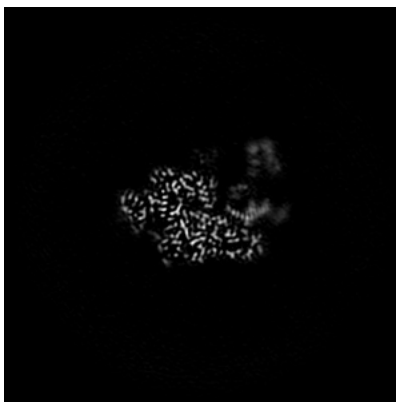
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices [i](#)

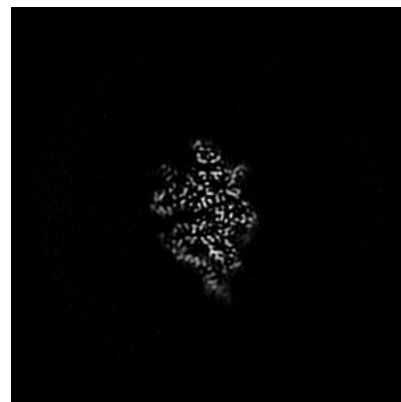
6.3.1 Primary map



X Index: 190

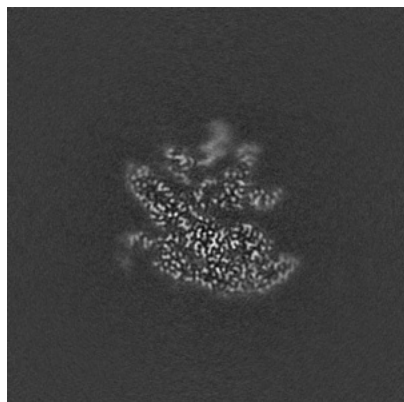


Y Index: 195

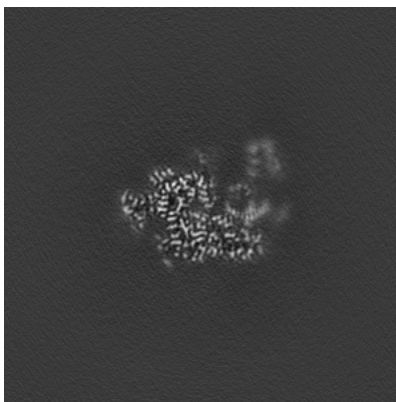


Z Index: 160

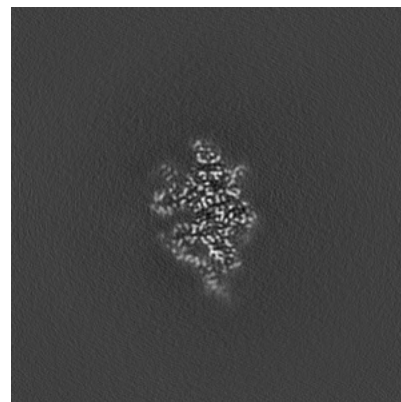
6.3.2 Raw map



X Index: 190



Y Index: 194

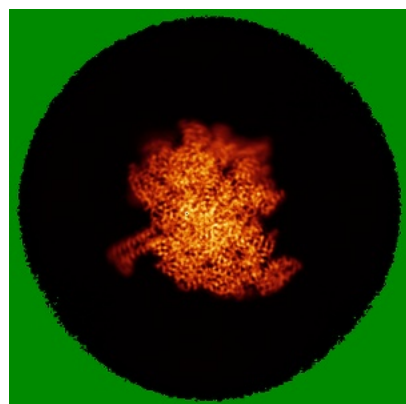


Z Index: 160

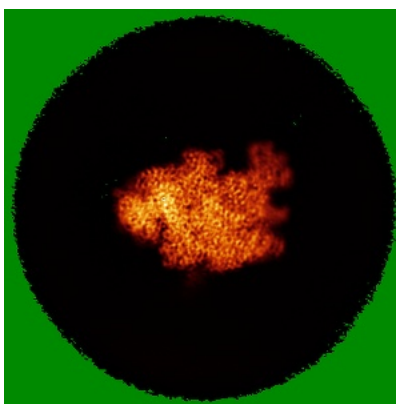
The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal standard-deviation projections (False-color) [i](#)

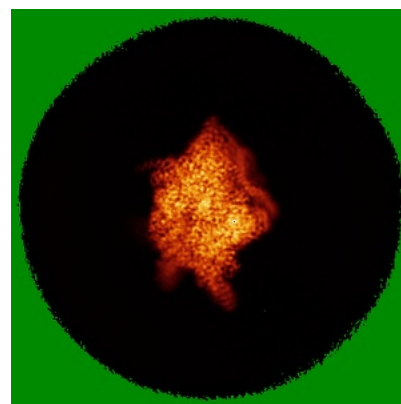
6.4.1 Primary map



X

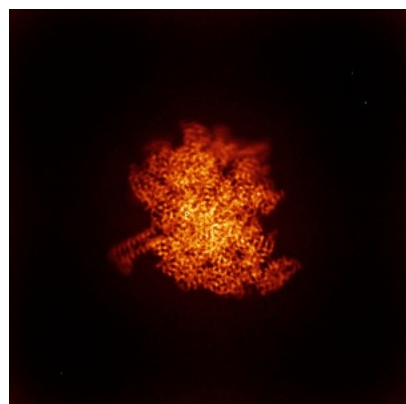


Y

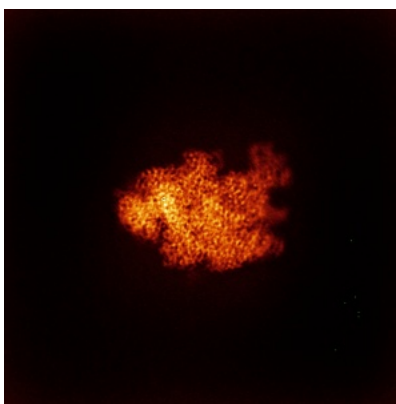


Z

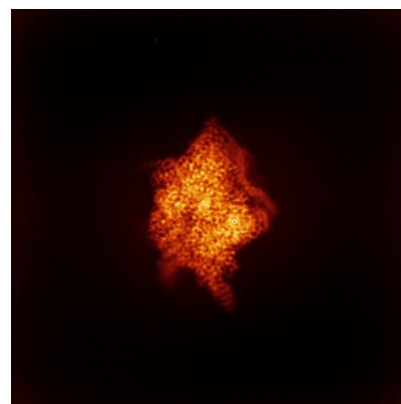
6.4.2 Raw map



X



Y

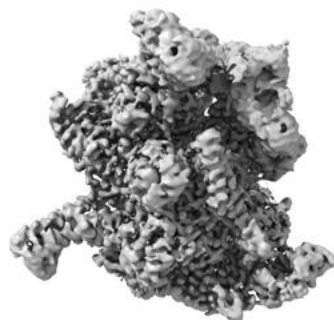


Z

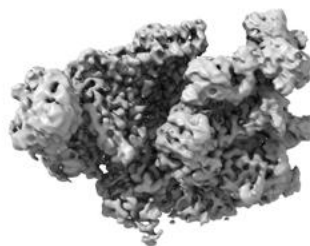
The images above show the map standard deviation projections with false color in three orthogonal directions. Minimum values are shown in green, max in blue, and dark to light orange shades represent small to large values respectively.

6.5 Orthogonal surface views [i](#)

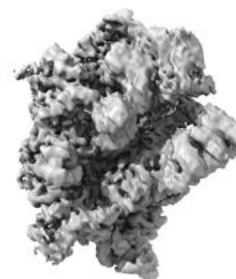
6.5.1 Primary map



X



Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.24. 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.

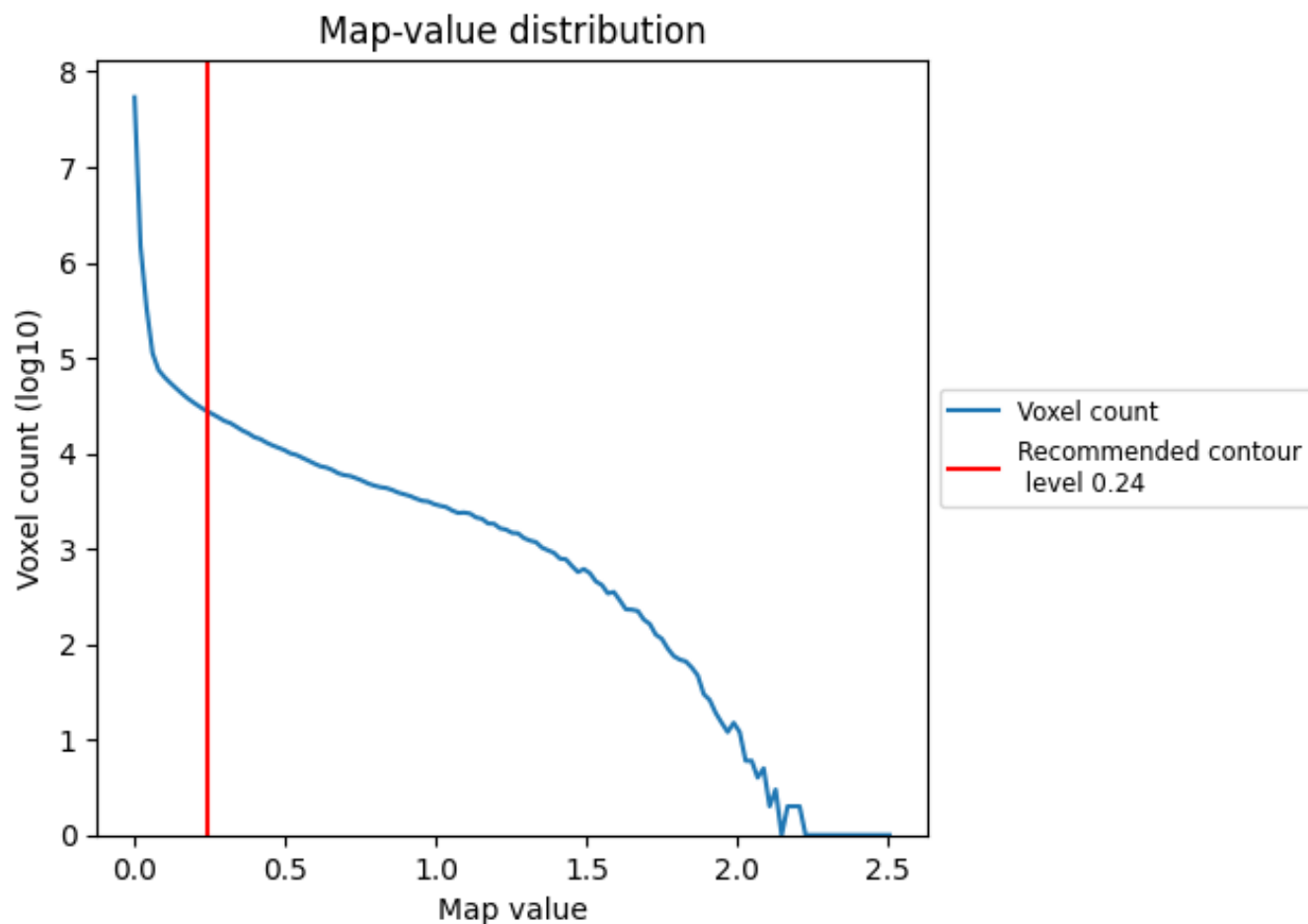
6.6 Mask visualisation [i](#)

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

7 Map analysis [i](#)

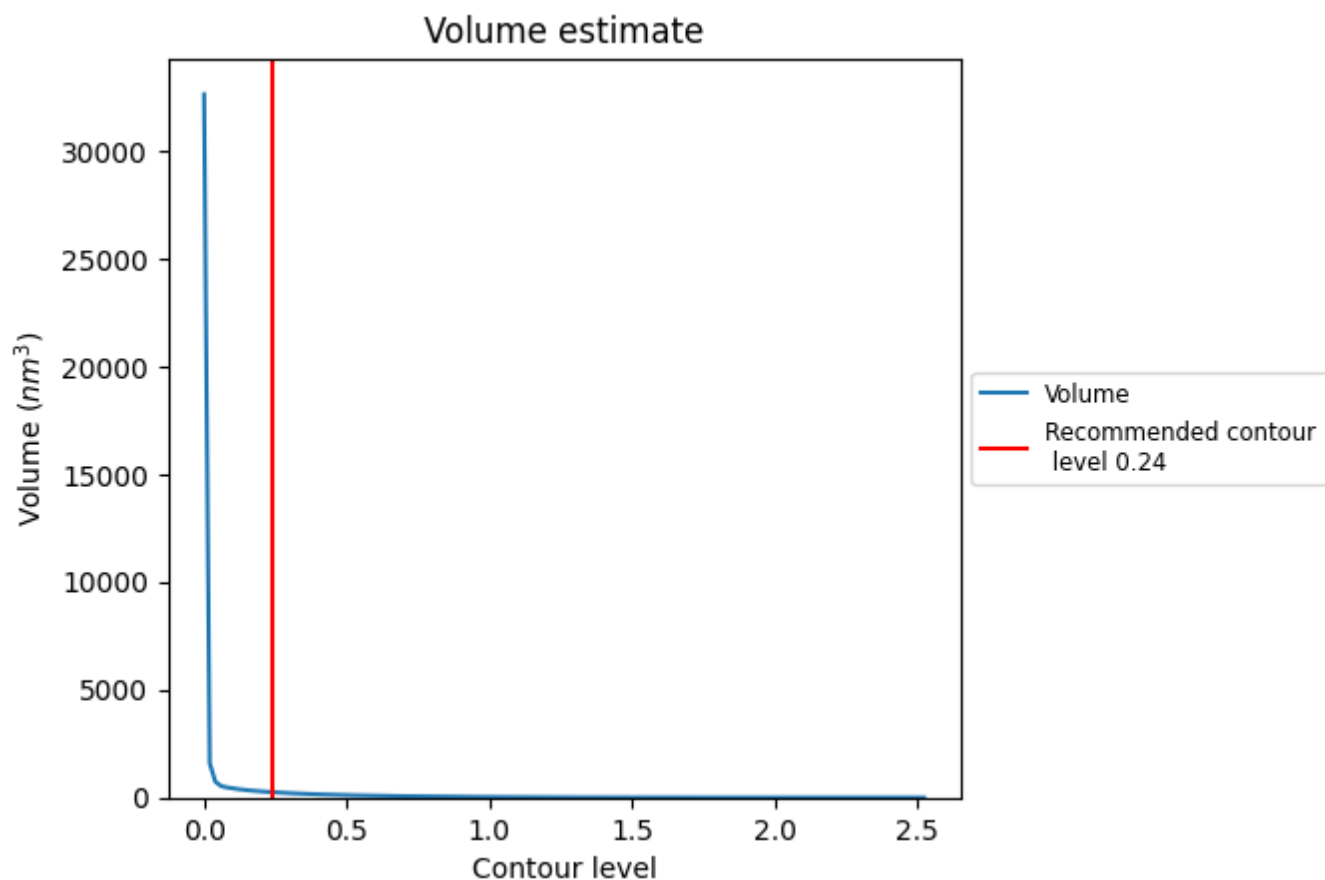
This section contains the results of statistical analysis of the map.

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.

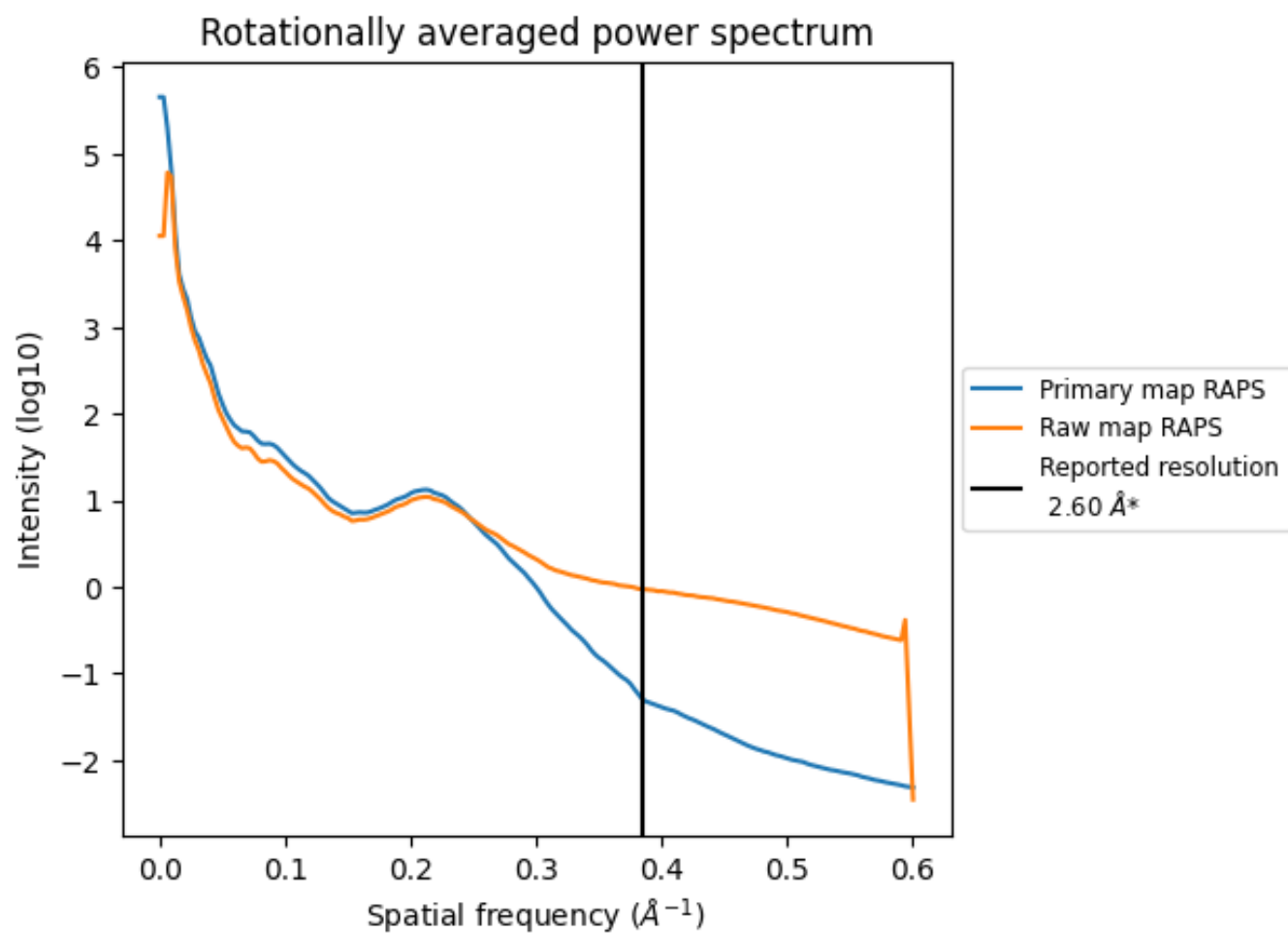
7.2 Volume estimate [i](#)



The volume at the recommended contour level is 248 nm^3 ; this corresponds to an approximate mass of 224 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 ⓘ

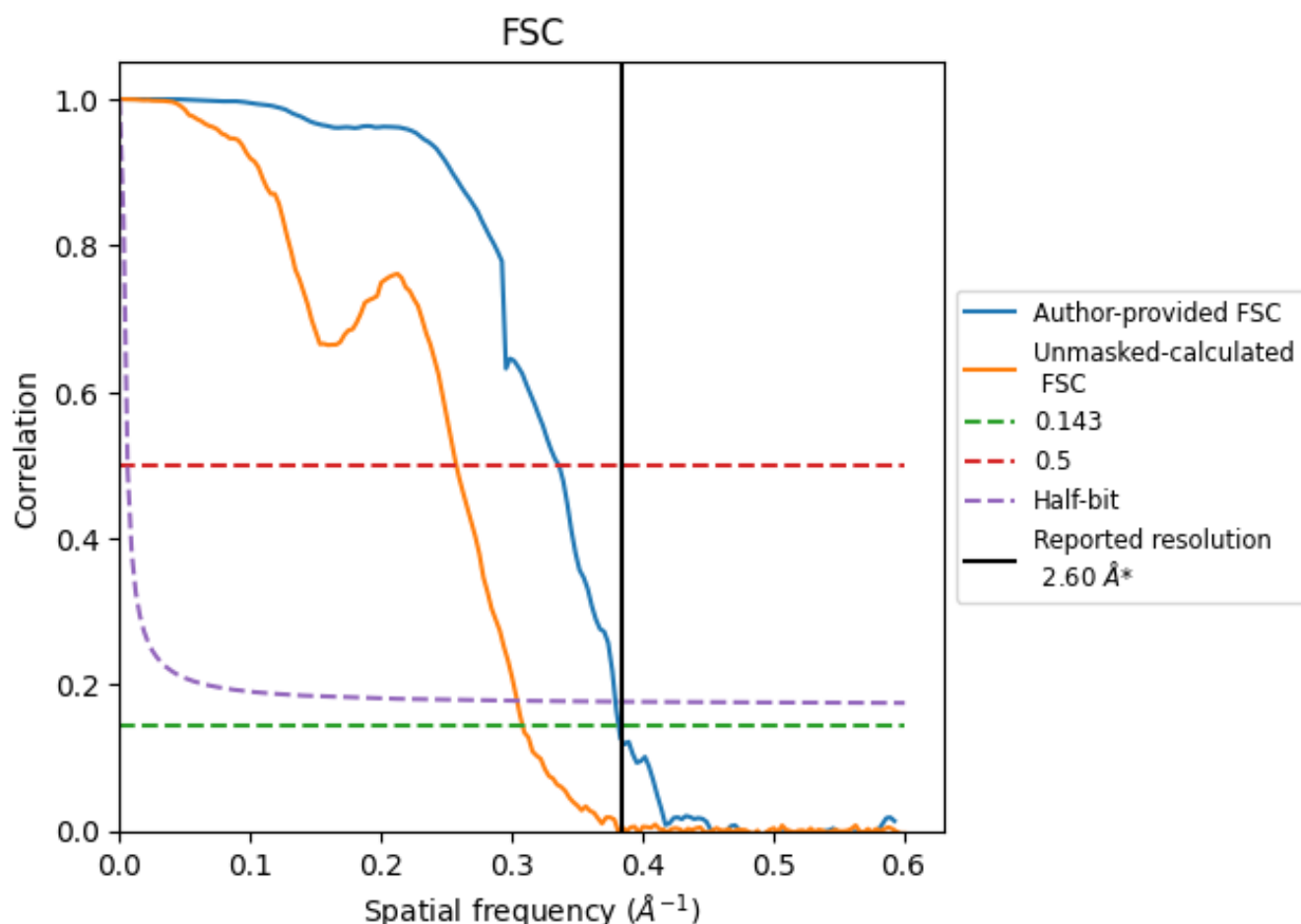


*Reported resolution corresponds to spatial frequency of 0.385 Å⁻¹

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 0.385 \AA^{-1}

8.2 Resolution estimates [i](#)

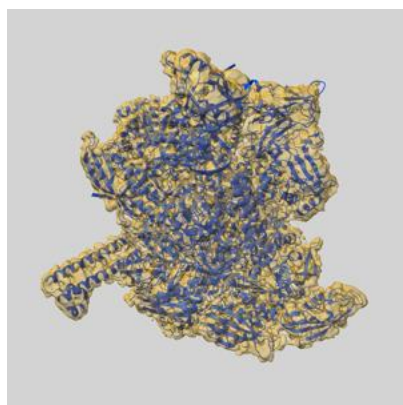
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	2.60	-	-
Author-provided FSC curve	2.62	2.98	2.63
Unmasked-calculated*	3.24	3.88	3.29

*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 3.24 differs from the reported value 2.6 by more than 10 %

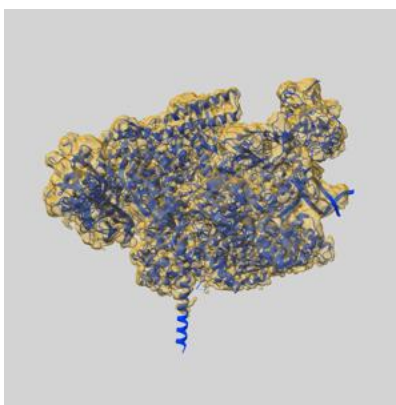
9 Map-model fit [i](#)

This section contains information regarding the fit between EMDB map EMD-17679 and PDB model 8PIB. Per-residue inclusion information can be found in section [3](#) on page [7](#).

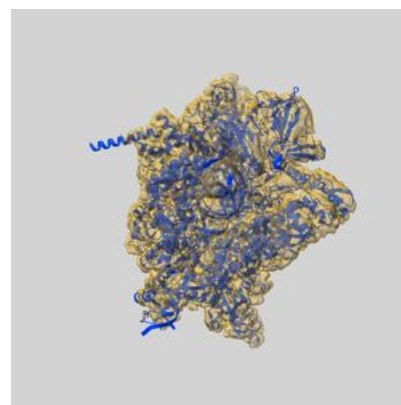
9.1 Map-model overlay [i](#)



X



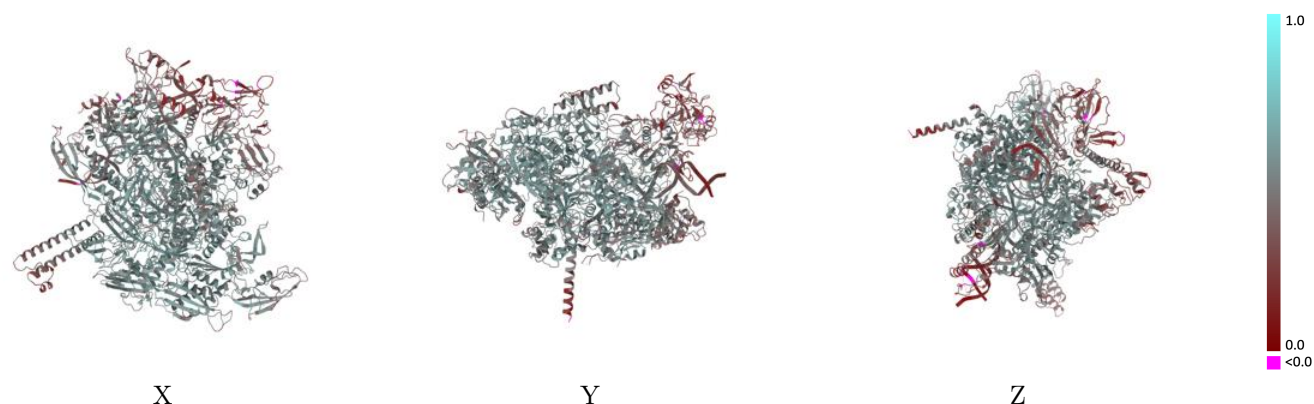
Y



Z

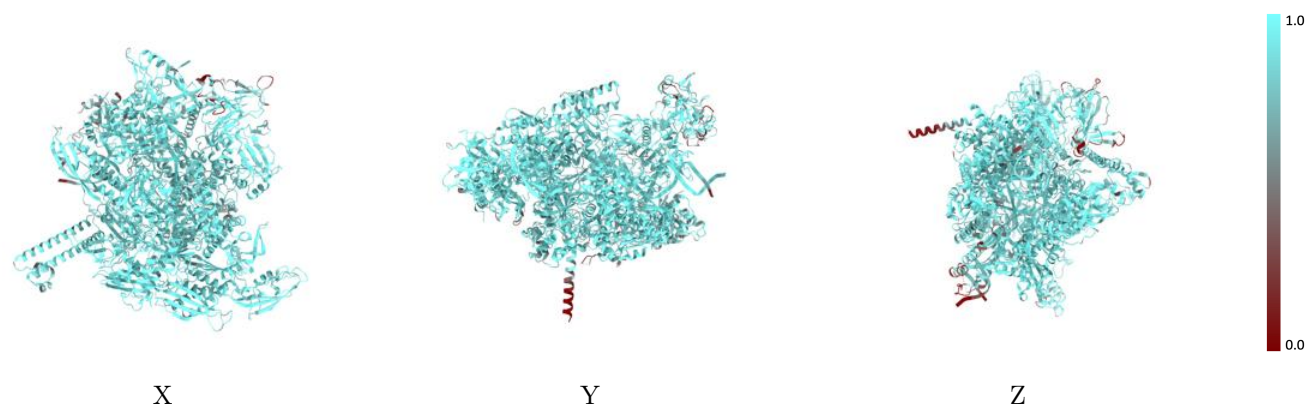
The images above show the 3D surface view of the map at the recommended contour level 0.24 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

9.2 Q-score mapped to coordinate model [i](#)



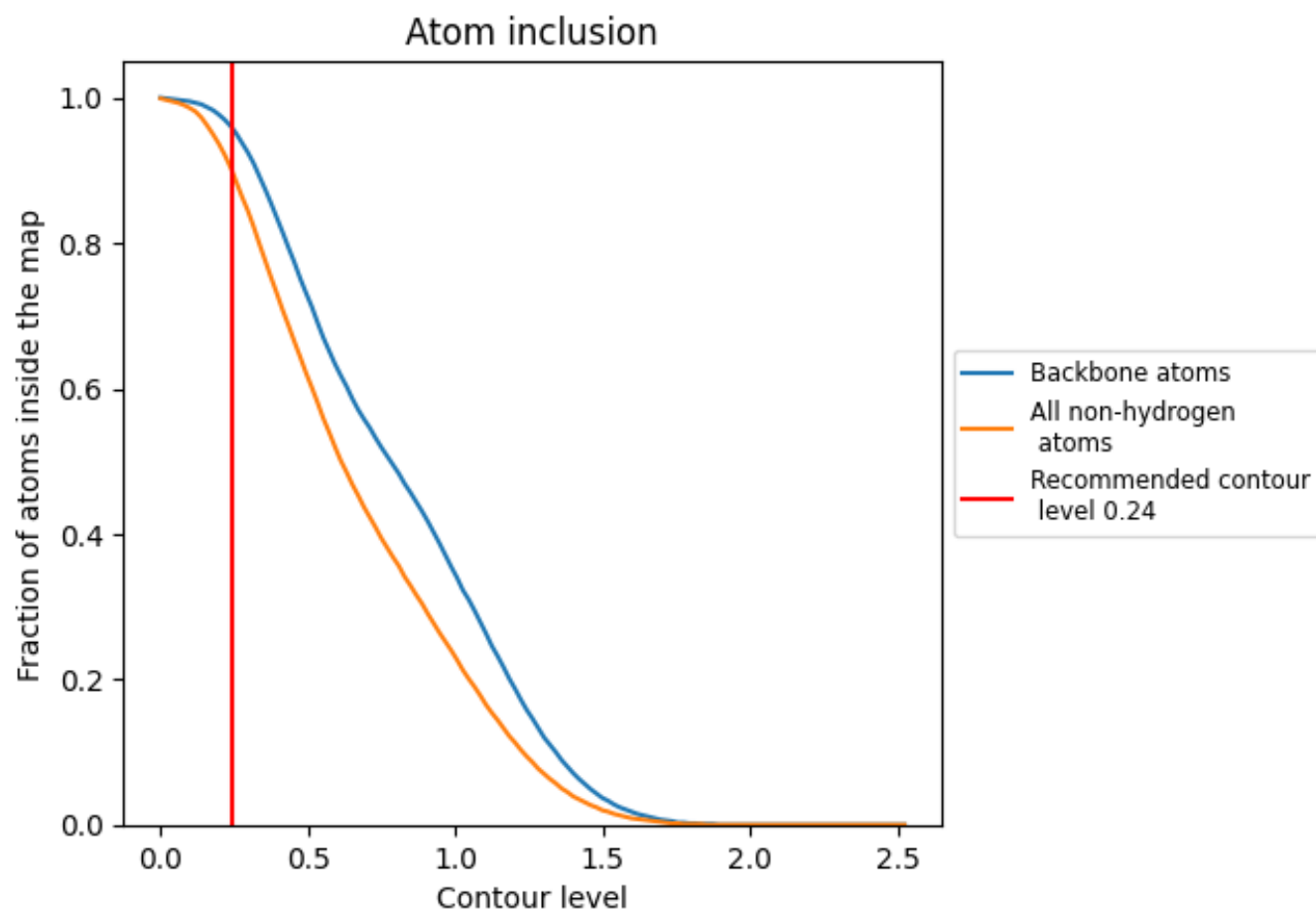
The images above show the model with each residue coloured according to its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions 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 (0.24).

9.4 Atom inclusion [i](#)



At the recommended contour level, 96% of all backbone atoms, 90% 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 (0.24) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	<div></div> 0.9020	<div></div> 0.4940
A	<div></div> 0.8540	<div></div> 0.3690
B	<div></div> 0.8980	<div></div> 0.3840
G	<div></div> 0.9220	<div></div> 0.5320
H	<div></div> 0.9100	<div></div> 0.4960
I	<div></div> 0.9290	<div></div> 0.5210
J	<div></div> 0.9040	<div></div> 0.4930
K	<div></div> 0.7310	<div></div> 0.4640
P	<div></div> 0.7110	<div></div> 0.3730
R	<div></div> 0.9390	<div></div> 0.5200

1.0

0.0

<0.0