wwPDB EM Validation Summary Report (i)

Oct 10, 2022 – 04:00 pm BST

PDB ID	:	8AA5
EMDB ID	:	EMD-15294
Title	:	Cryo-EM structure of the strand transfer complex of the TnsB transposase (type V-K CRISPR-associated transposon)
Authors	:	Tenjo-Castano, F.; Sofos, N.; Lopez-Mendez, B.; Stutzke, L.S.; Fuglsang, A.; Stella, S.; Montoya, G.
Deposited on	:	2022-06-30
Resolution	:	2.46 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 (i)) were used in the production of this report:

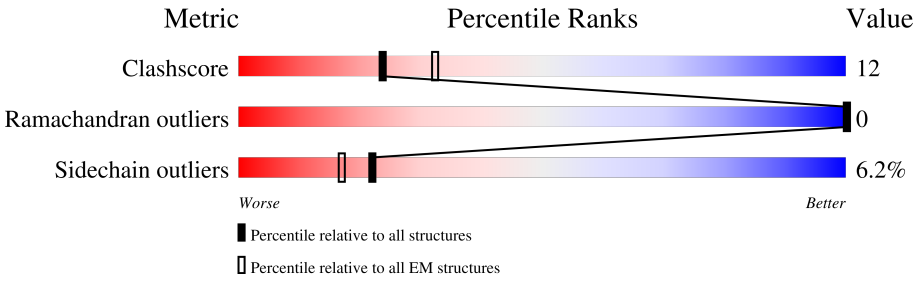
EMDB validation analysis	:	0.0.1.dev43
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.31.2

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 2.46 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	AP1	596	53% 20% 25%
1	BP1	596	50% 22% 25%
1	CP1	596	35% 14% 50%
1	DP1	596	33% 17% 50%
2	I	79	27% 32% 42%
3	J	74	11% 27% 62%
4	K	15	33% 60% 7%
5	L	80	28% 28% 45%

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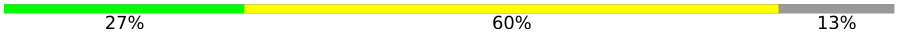
Mol	Chain	Length	Quality of chain
6	M	75	
7	N	15	

2 Entry composition (i)

There are 8 unique types of molecules in this entry. The entry contains 15505 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 TnsB.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	AP1	445	Total	C	N	O	S	0	0
			3587	2248	659	668	12		
1	BP1	445	Total	C	N	O	S	0	0
			3583	2246	659	666	12		
1	CP1	297	Total	C	N	O	S	0	0
			2393	1502	433	448	10		
1	DP1	299	Total	C	N	O	S	0	0
			2419	1518	442	449	10		

- Molecule 2 is a DNA chain called RE_Target.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	I	46	Total	C	N	O	P	0	0
			945	450	180	269	46		

- Molecule 3 is a DNA chain called RE_PolyA.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	J	28	Total	C	N	O	P	0	0
			577	277	101	171	28		

- Molecule 4 is a DNA chain called Target_1.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	K	14	Total	C	N	O	P	0	0
			281	136	47	85	13		

- Molecule 5 is a DNA chain called LE_Target.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	L	44	Total	C	N	O	P	0	0
			900	431	163	263	43		

- Molecule 6 is a DNA chain called LE_PolyA.

Mol	Chain	Residues	Atoms					AltConf	Trace
6	M	27	Total	C	N	O	P	0	0
			553	265	98	163	27		

- Molecule 7 is a DNA chain called Target_2.

Mol	Chain	Residues	Atoms					AltConf	Trace
7	N	13	Total	C	N	O	P	0	0
			265	126	51	75	13		

- Molecule 8 is water.

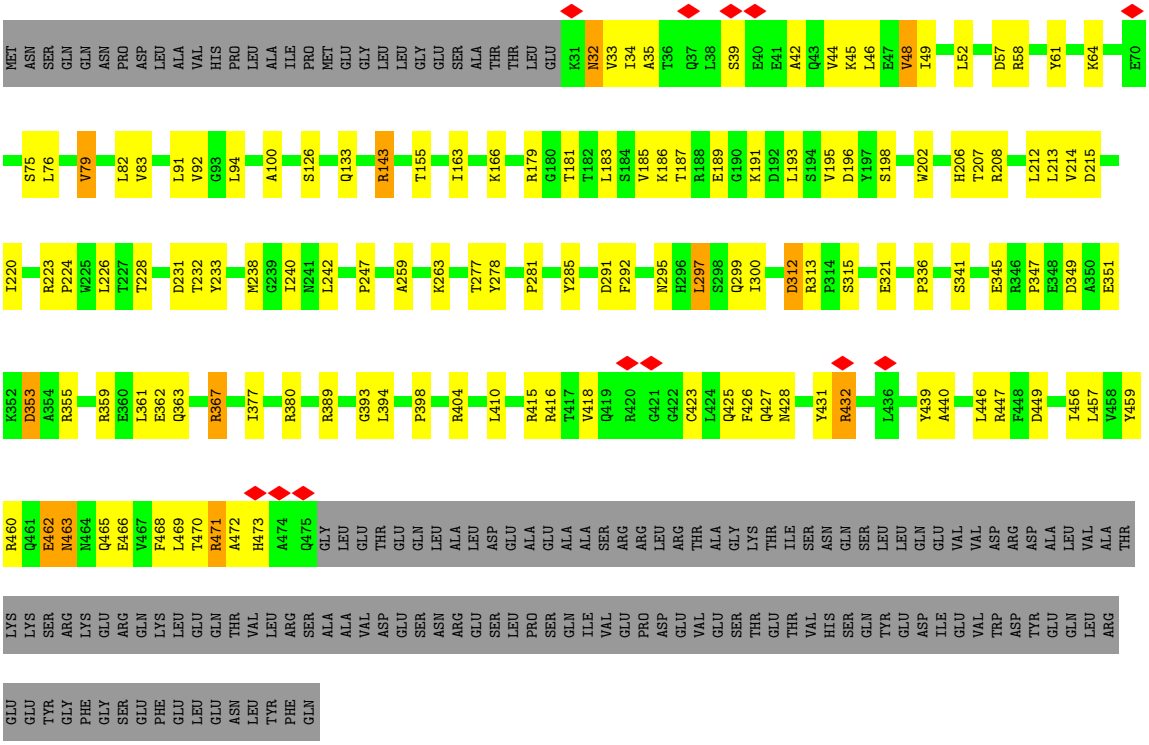
Mol	Chain	Residues	Atoms		AltConf
8	BP1	1	Total	O	0
			1	1	
8	N	1	Total	O	0
			1	1	

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.

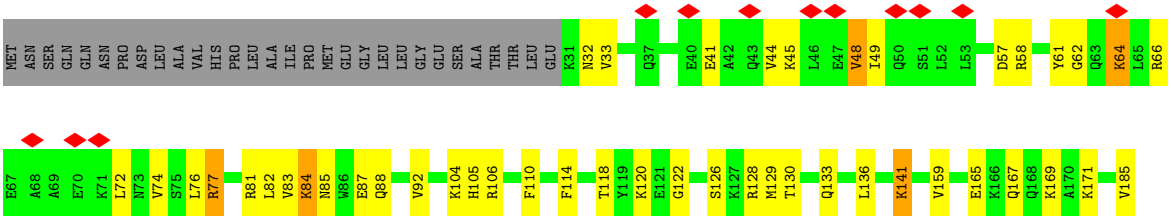
- Molecule 1: TnsB

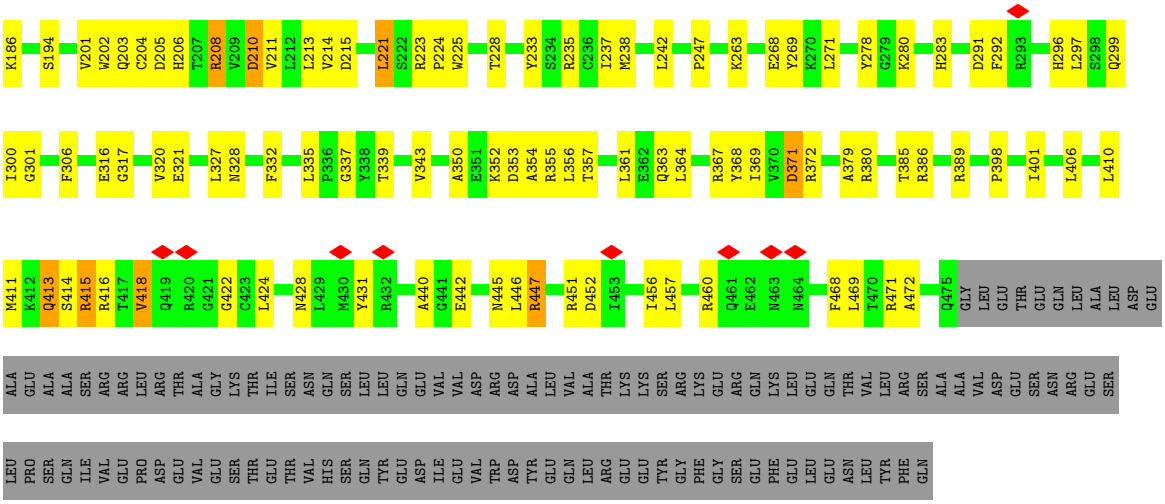
Chain AP1:



- Molecule 1: TnsB

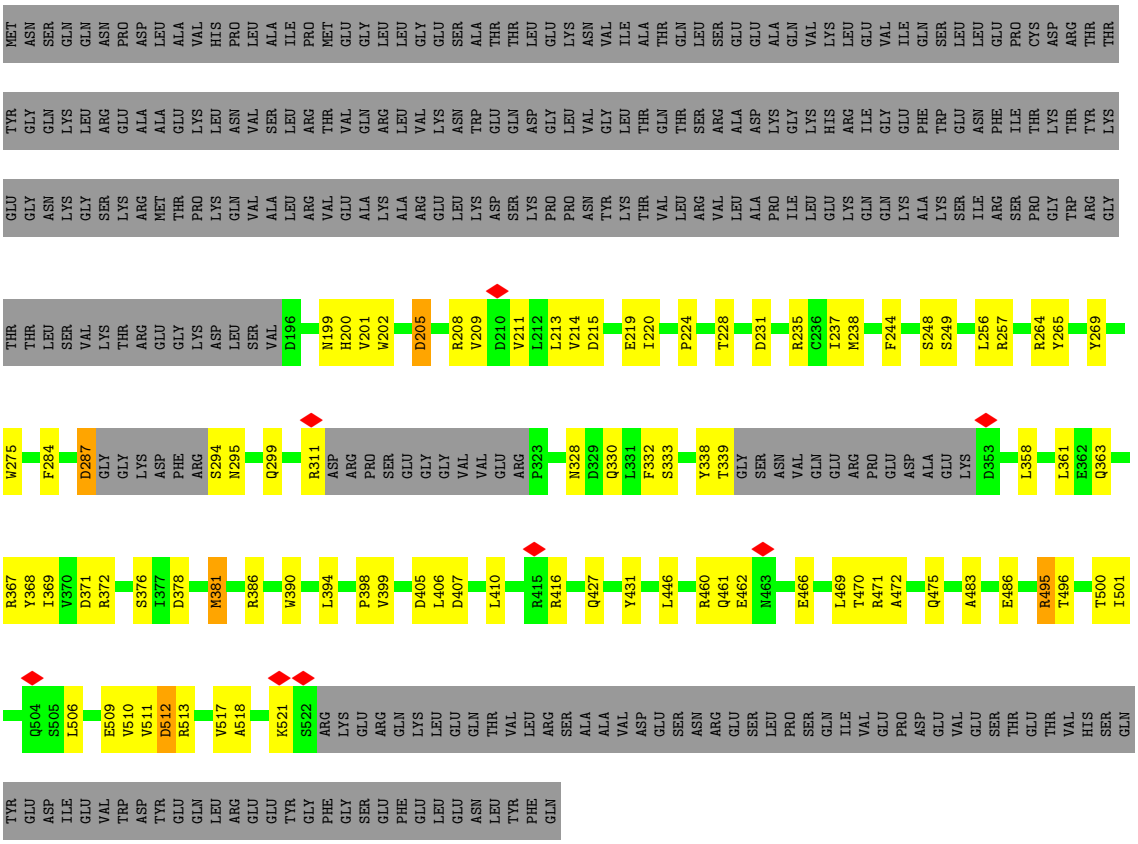
Chain BP1:





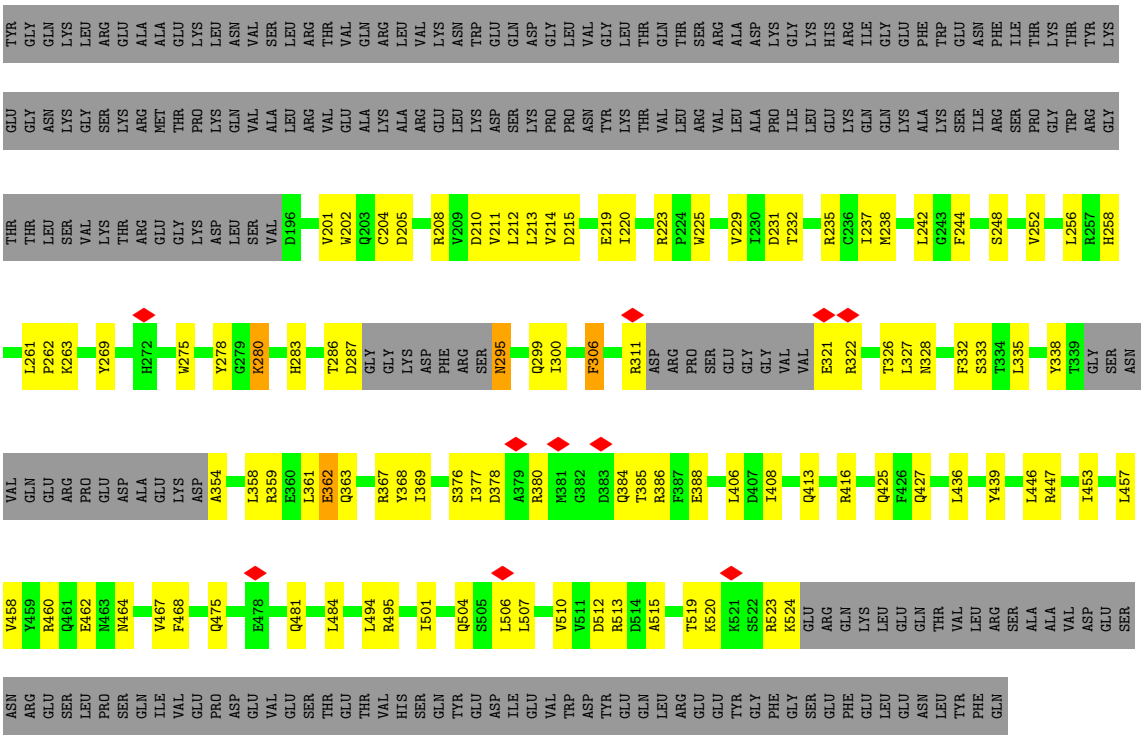
• Molecule 1: TnsB

Chain CP1:



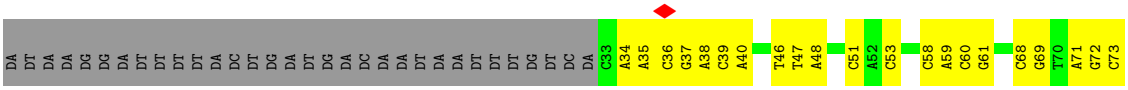
• Molecule 1: TnsB

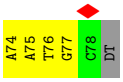
Chain DP1:



- Molecule 2: RE_Target

Chain I:



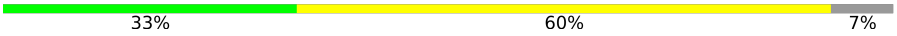


- Molecule 3: RE_PolyA

Chain J:

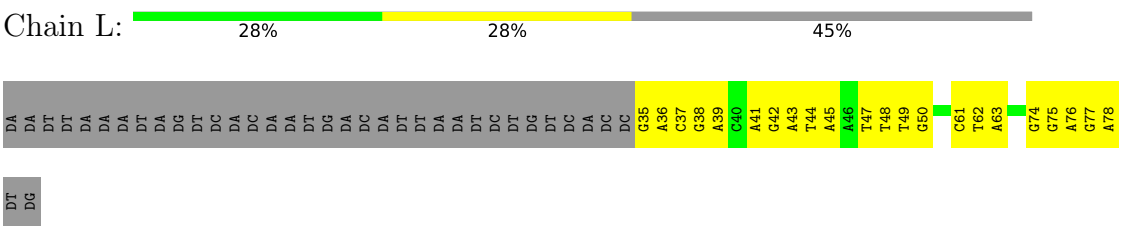
- Molecule 4: Target_1

Chain K:



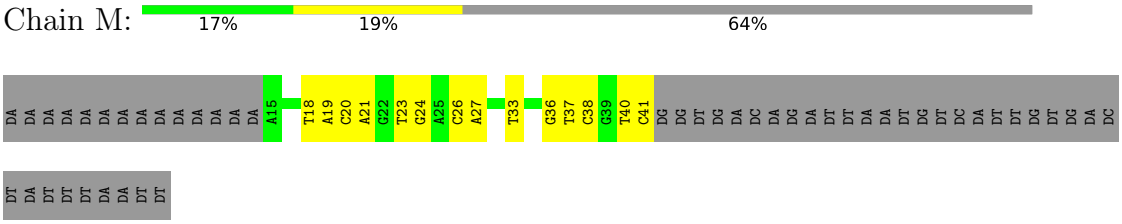
- Molecule 5: LE_Target

Chain L:



• Molecule 6: LE_PolyA

Chain M:



• Molecule 7: Target_2

Chain N:

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	208000	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	TFS KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	40	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	1.824	Depositor
Minimum map value	-0.002	Depositor
Average map value	0.016	Depositor
Map value standard deviation	0.080	Depositor
Recommended contour level	0.06	Depositor
Map size (Å)	95.68, 118.144005, 148.09601	wwPDB
Map dimensions	178, 142, 115	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.832, 0.832, 0.832	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	AP1	0.27	0/3657	0.53	0/4941
1	BP1	0.27	0/3653	0.52	0/4936
1	CP1	0.27	0/2439	0.53	1/3302 (0.0%)
1	DP1	0.26	0/2465	0.55	0/3335
2	I	0.54	0/1062	0.91	0/1636
3	J	0.55	0/646	0.98	0/996
4	K	0.53	0/313	0.97	0/481
5	L	0.58	0/1009	0.96	0/1556
6	M	0.57	0/619	0.94	0/953
7	N	0.56	0/297	0.85	0/455
All	All	0.36	0/16160	0.67	1/22591 (0.0%)

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	CP1	381	MET	CA-CB-CG	5.03	121.85	113.30

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	AP1	3587	0	3595	85	0
1	BP1	3583	0	3591	99	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	CP1	2393	0	2355	54	0
1	DP1	2419	0	2390	66	0
2	I	945	0	517	24	0
3	J	577	0	320	13	0
4	K	281	0	161	8	0
5	L	900	0	499	20	0
6	M	553	0	307	16	0
7	N	265	0	146	9	0
8	BP1	1	0	0	1	0
8	N	1	0	0	0	0
All	All	15505	0	13881	353	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 12.

The worst 5 of 353 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:DP1:261:LEU:HD21	1:DP1:406:LEU:HD13	1.57	0.85
1:BP1:33:VAL:HG22	1:DP1:467:VAL:HG12	1.66	0.77
6:M:36:DG:H2"	6:M:37:DT:H5"	1.65	0.76
1:CP1:237:ILE:HG21	1:CP1:369:ILE:HD13	1.69	0.75
1:DP1:213:LEU:HD21	1:DP1:361:LEU:HD12	1.69	0.74

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	AP1	443/596 (74%)	423 (96%)	20 (4%)	0	100	100
1	BP1	443/596 (74%)	423 (96%)	20 (4%)	0	100	100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	CP1	289/596 (48%)	281 (97%)	8 (3%)	0	100	100
1	DP1	291/596 (49%)	279 (96%)	12 (4%)	0	100	100
All	All	1466/2384 (62%)	1406 (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	AP1	390/523 (75%)	364 (93%)	26 (7%)	16	20
1	BP1	389/523 (74%)	367 (94%)	22 (6%)	20	26
1	CP1	259/523 (50%)	245 (95%)	14 (5%)	22	28
1	DP1	261/523 (50%)	243 (93%)	18 (7%)	15	18
All	All	1299/2092 (62%)	1219 (94%)	80 (6%)	22	23

5 of 80 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	CP1	461	GLN
1	DP1	326	THR
1	CP1	475	GLN
1	DP1	280	LYS
1	DP1	425	GLN

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

5.3.3 RNA (i)

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

5.6 Ligand geometry (i)

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.

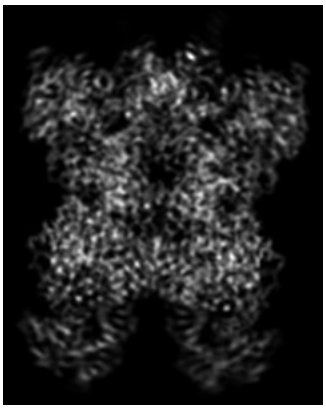
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-15294. These allow visual inspection of the internal detail of the map and identification of artifacts.

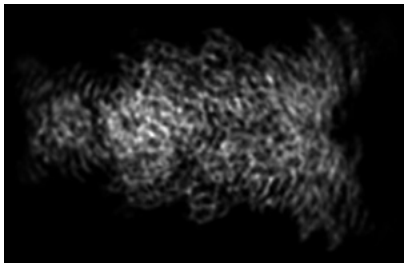
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections

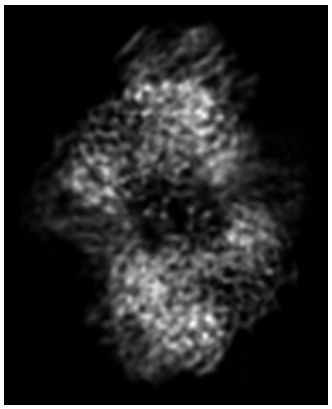
6.1.1 Primary map



X

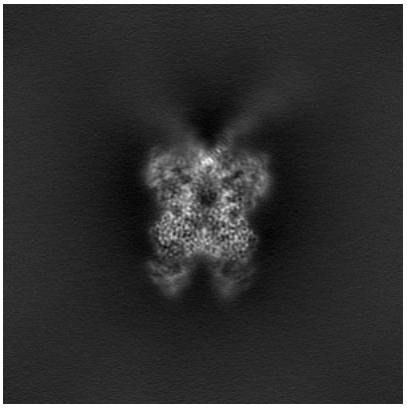


Y

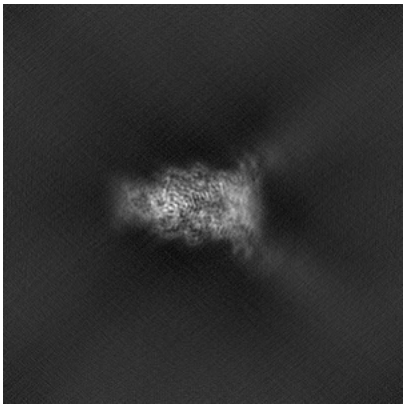


Z

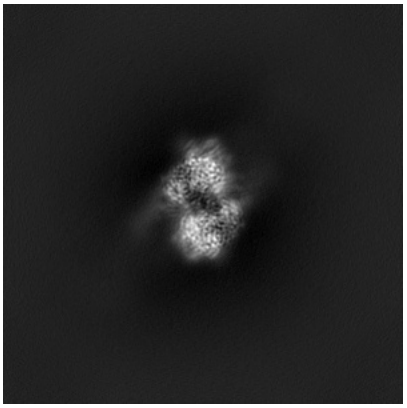
6.1.2 Raw map



X



Y



Z

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

6.2 Central slices (i)

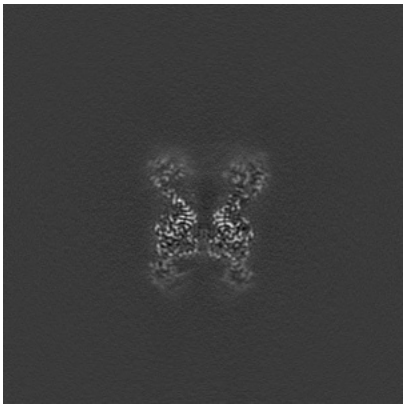
6.2.1 Primary map

X Index: 57

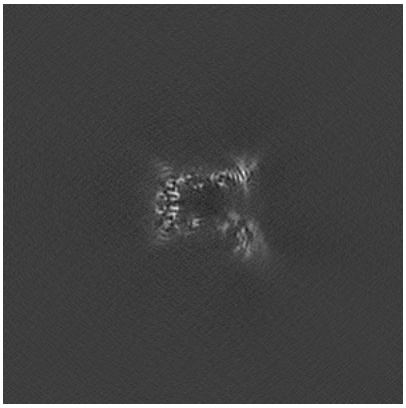
Y Index: 71

Z Index: 89

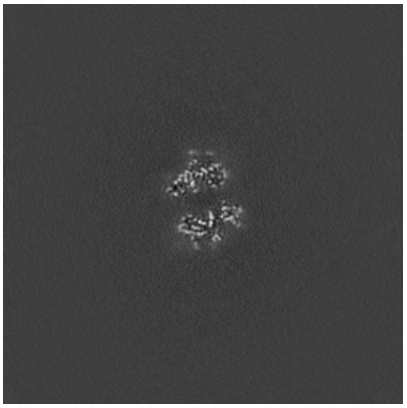
6.2.2 Raw map



X Index: 208



Y Index: 208



Z Index: 208

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

6.3 Largest variance slices (i)

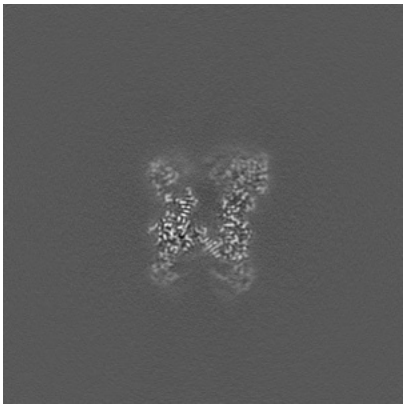
6.3.1 Primary map

X Index: 67

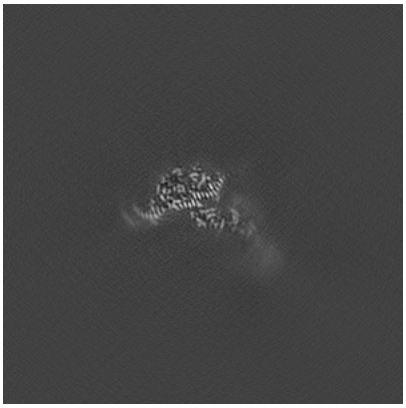
Y Index: 30

Z Index: 64

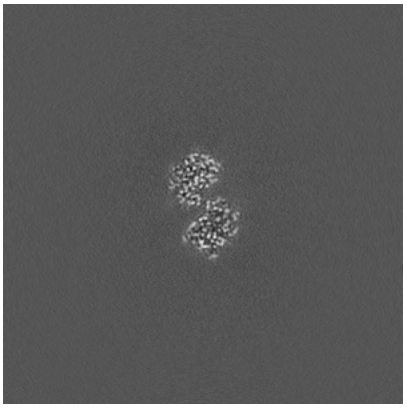
6.3.2 Raw map



X Index: 214



Y Index: 192

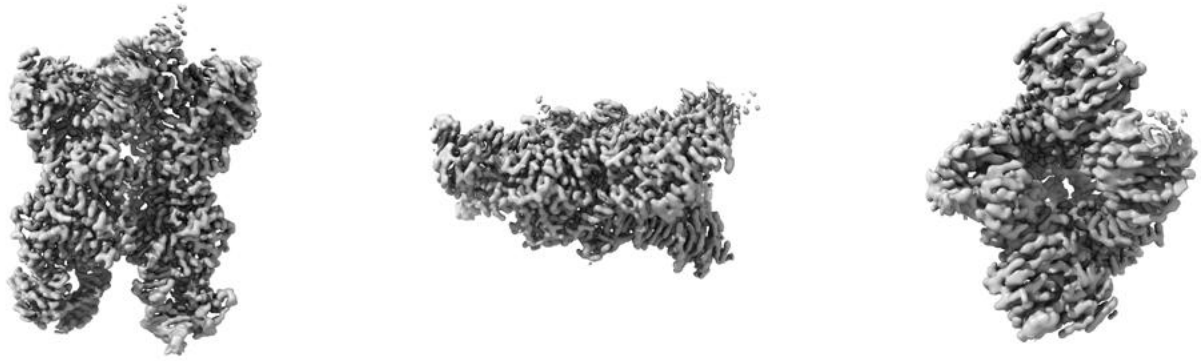


Z Index: 178

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

6.4 Orthogonal surface views (i)

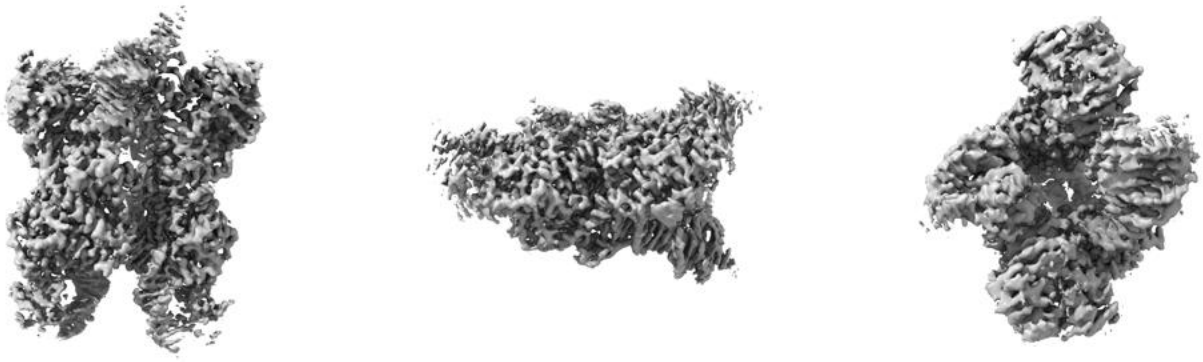
6.4.1 Primary map



X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.06. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.4.2 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.

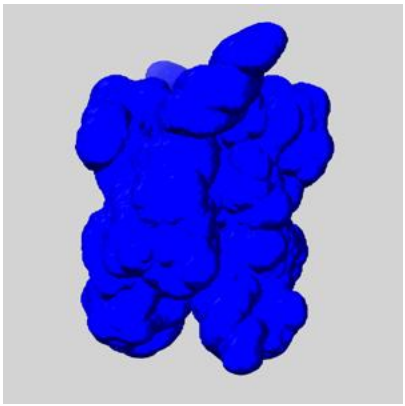
6.5 Mask visualisation (i)

This section shows the 3D surface view of the primary map at 50% transparency overlaid with the specified mask at 0% transparency

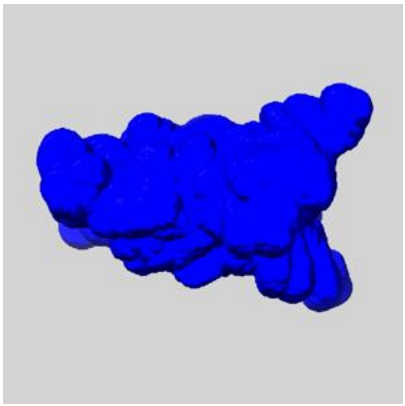
A mask typically either:

- Encompasses the whole structure
- Separates out a domain, a functional unit, a monomer or an area of interest from a larger structure

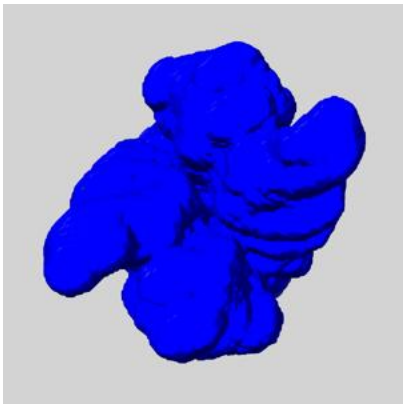
6.5.1 emd_15294_msk_1.map (i)



X



Y

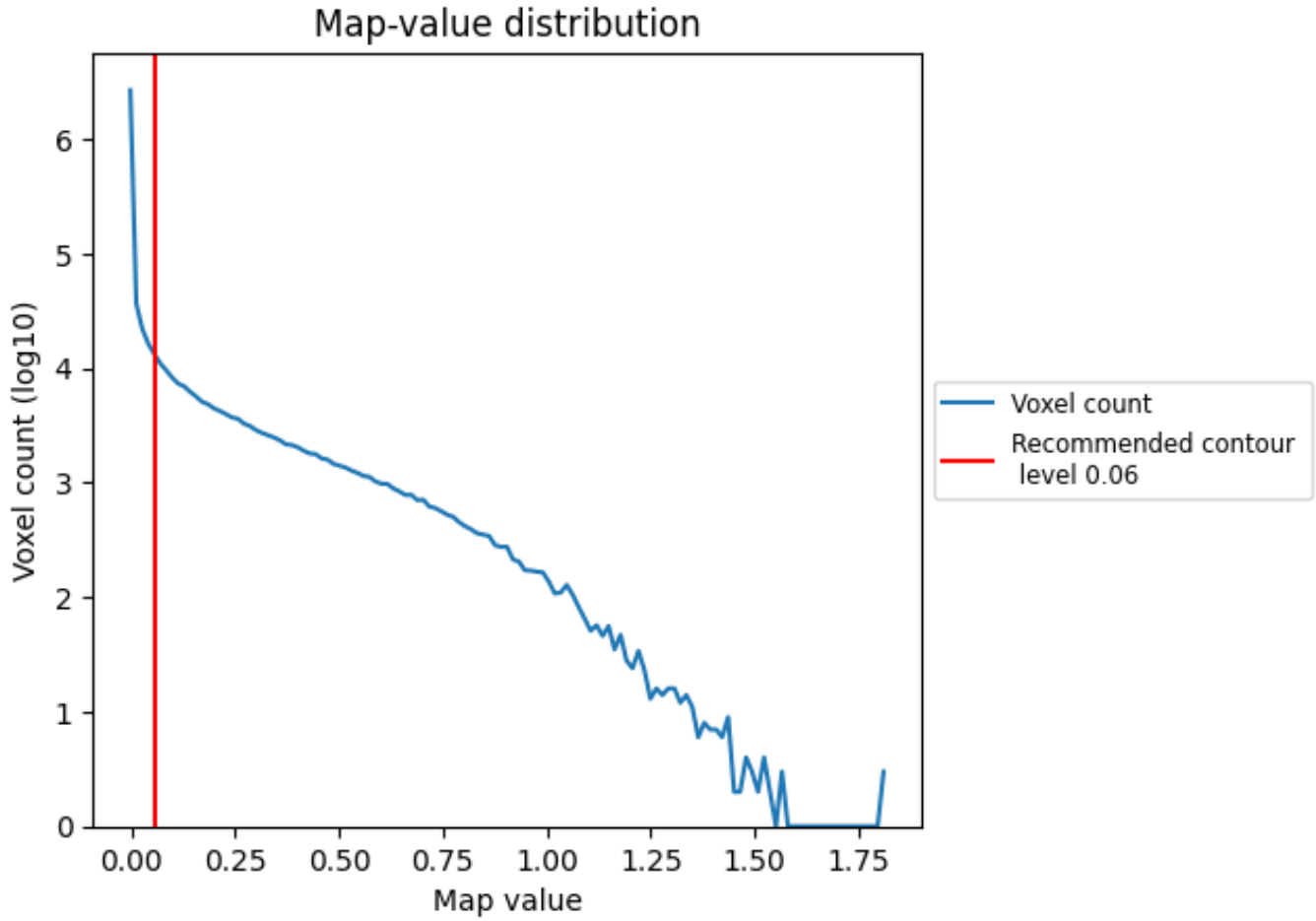


Z

7 Map analysis

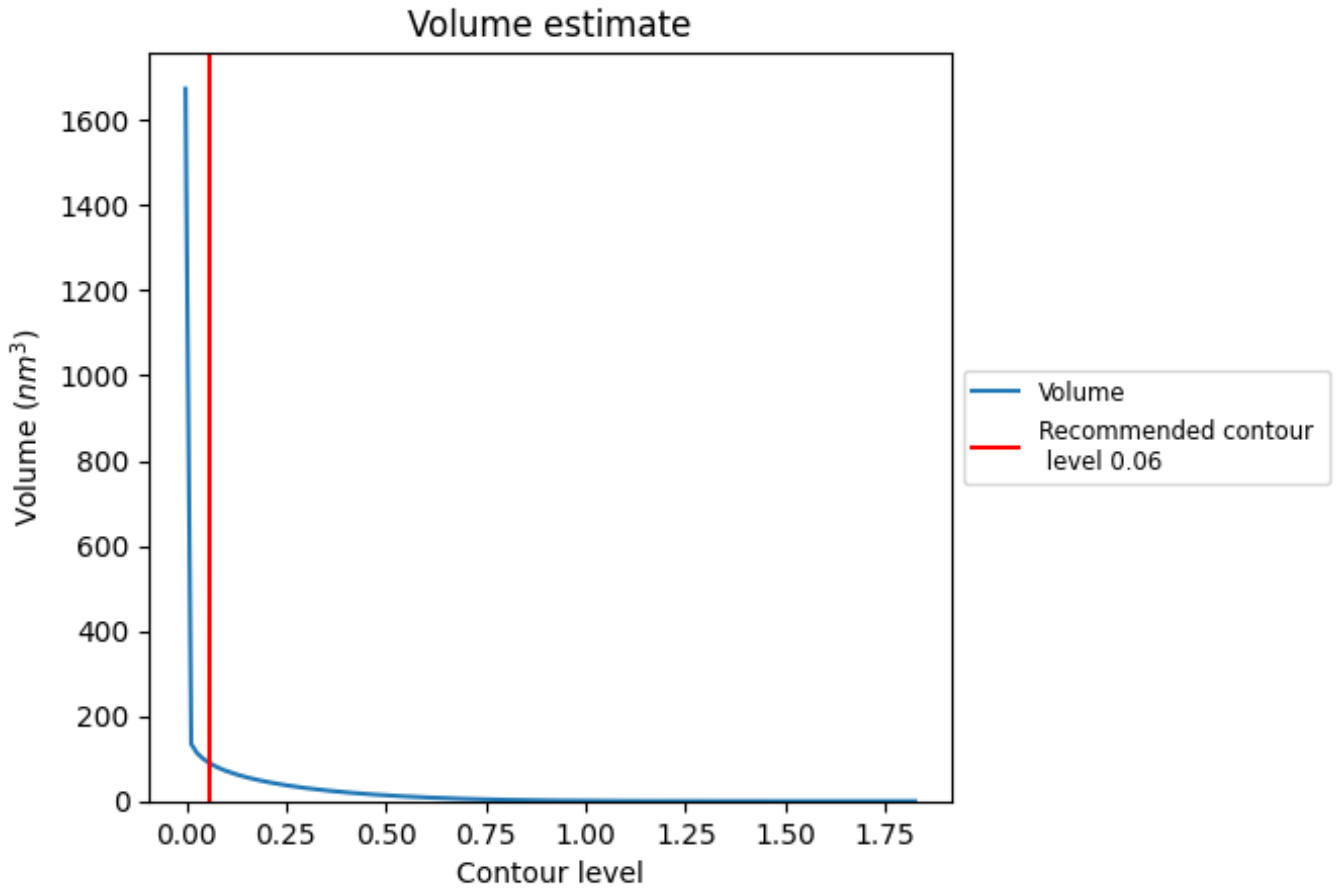
This section contains the results of statistical analysis of the map.

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 89 nm^3; this corresponds to an approximate mass of 80 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.

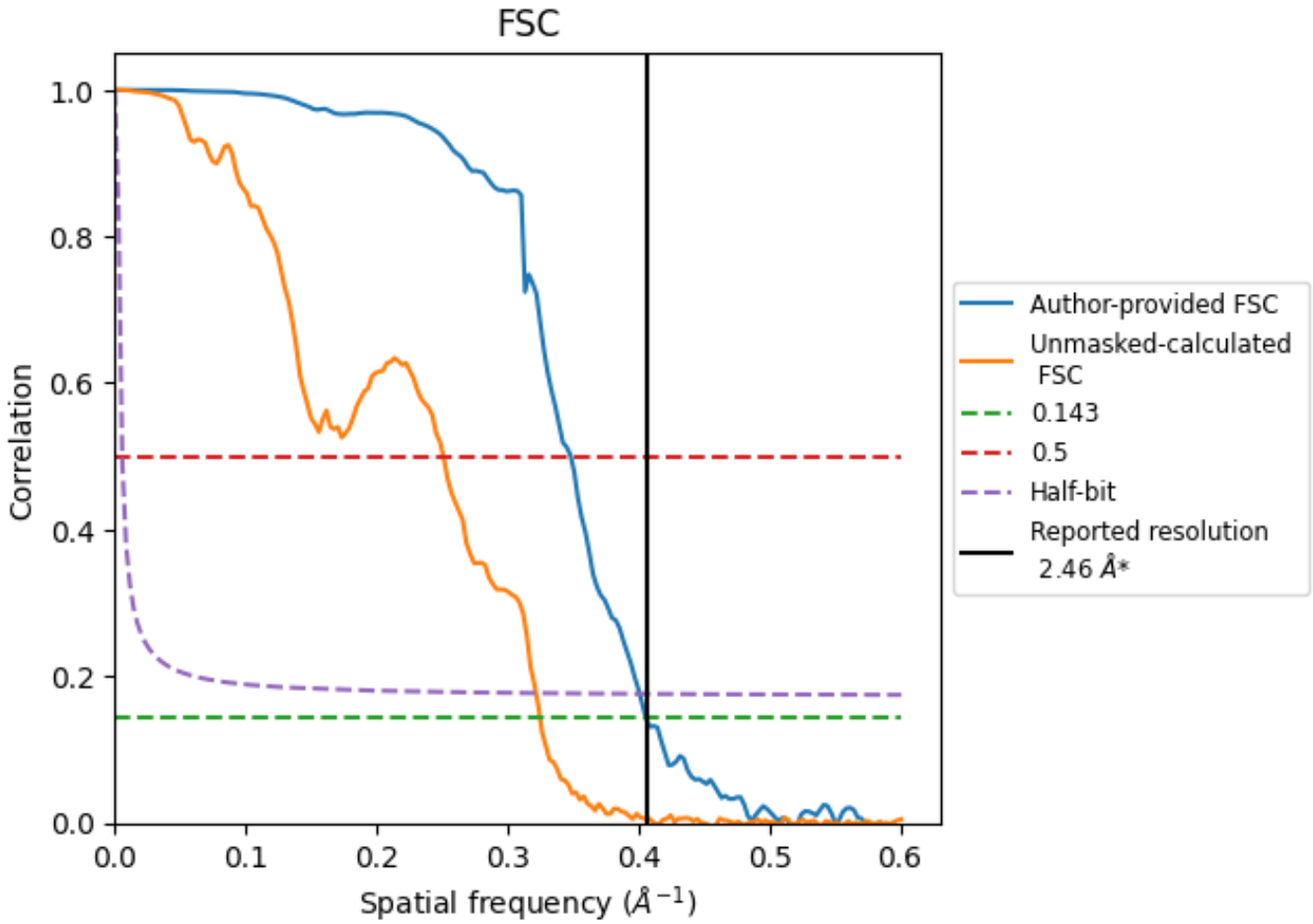
7.3 Rotationally averaged power spectrum (i)

This section was not generated. The rotationally averaged power spectrum is only generated for cubic maps.

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 0.407 Å^{-1}

8.2 Resolution estimates (i)

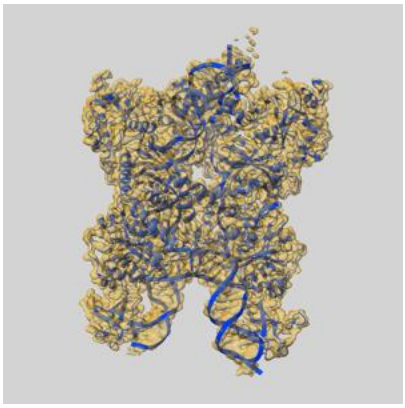
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	2.46	-	-
Author-provided FSC curve	2.46	2.87	2.49
Unmasked-calculated*	3.08	3.98	3.11

*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 3.08 differs from the reported value 2.46 by more than 10 %

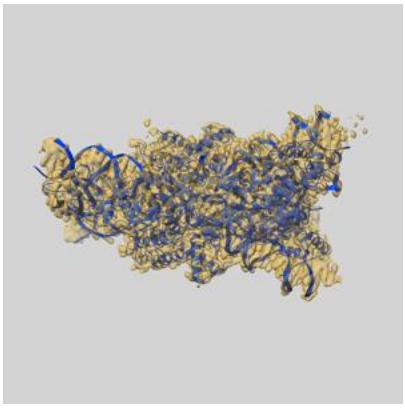
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-15294 and PDB model 8AA5. Per-residue inclusion information can be found in section 3 on page 6.

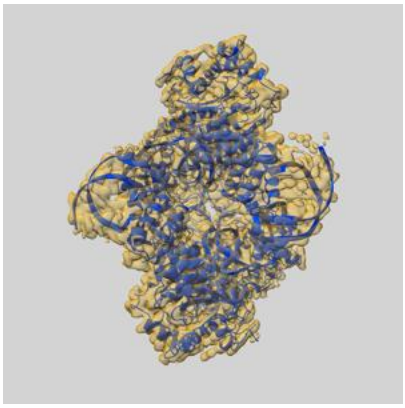
9.1 Map-model overlay



X



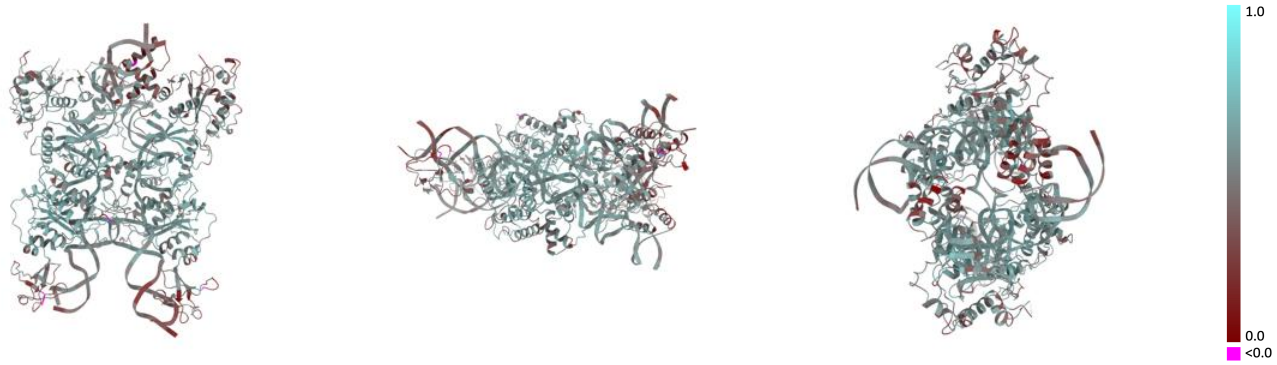
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.06 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

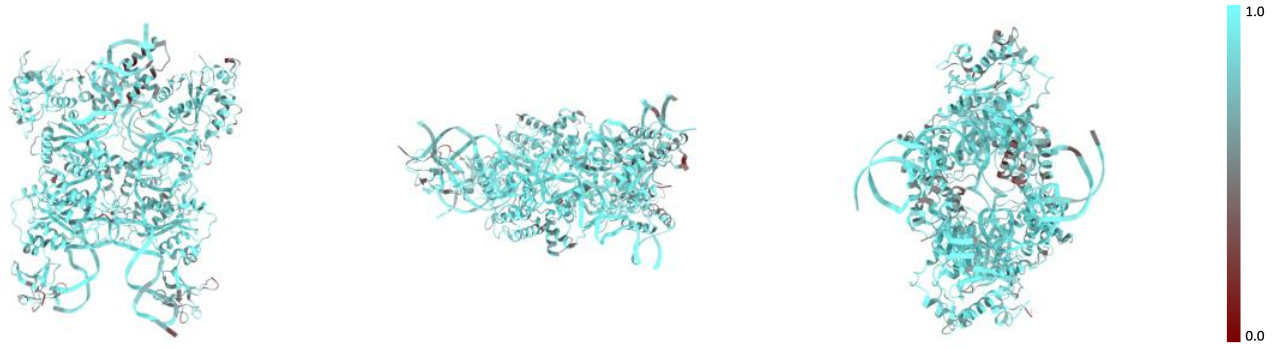
9.2 Q-score mapped to coordinate model (i)



X Y Z

The images above show the model with each residue coloured according its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions at which atoms can be resolved. Low Q-score values may therefore be expected for many entries.

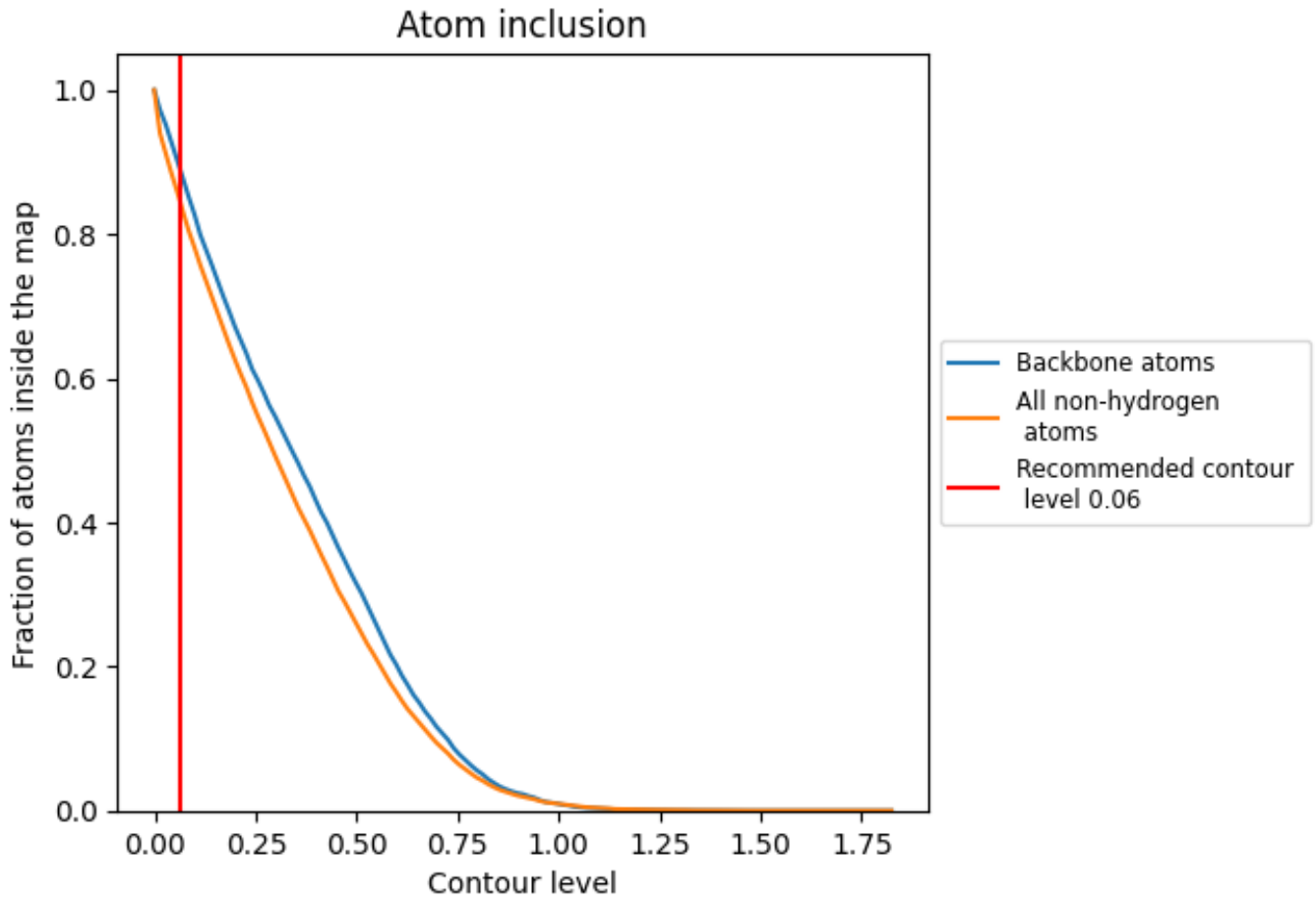
9.3 Atom inclusion mapped to coordinate model (i)



X Y Z

The images above show the model with each residue coloured according to its atom inclusion. This shows to what extent they are inside the map at the recommended contour level (0.06).

9.4 Atom inclusion (i)



At the recommended contour level, 89% of all backbone atoms, 85% 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 (0.06) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.8501	0.5190
AP1	0.8471	0.5330
BP1	0.8222	0.5070
CP1	0.8623	0.5320
DP1	0.8269	0.4940
I	0.8825	0.5140
J	0.8995	0.5470
K	0.8007	0.4420
L	0.9211	0.5330
M	0.9476	0.5630
N	0.8679	0.4880