wwPDB EM Validation Summary Report (i)

May 12, 2024 – 02:23 PM EDT

PDB ID	:	8F75
EMDB ID	:	EMD-28895
Title	:	LRRC8A(T48D):C conformation 2 LRR focus
Authors	:	Kern, D.M.; Brohawn, S.G.
Deposited on	:	2022-11-18
Resolution	:	4.00 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:

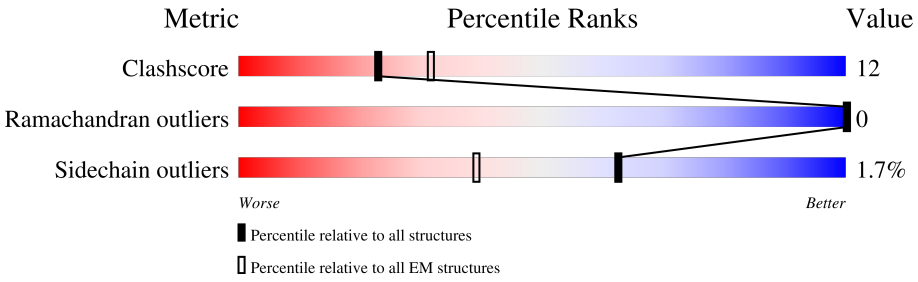
EMDB validation analysis	:	0.0.1.dev92
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36.2

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 4.00 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	158937	4297
Ramachandran outliers	154571	4023
Sidechain outliers	154315	3826

The table below summarises the geometric issues observed across the polymeric chains and their fit to the map. The red, orange, yellow and green segments of the bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5% The upper red bar (where present) indicates the fraction of residues that have poor fit to the EM map (all-atom inclusion < 40%). The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	729	16% 39% 16% · 44%
1	B	729	30% 38% 17% 44%
2	F	813	34% 34% 16% 50%

2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 9899 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 Volume-regulated anion channel subunit LRRC8A.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	407	Total	C	N	O	S	0	0
			3321	2135	583	595	8		
1	B	407	Total	C	N	O	S	0	0
			3321	2135	583	595	8		

There are 18 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	811	SER	-	expression tag	UNP Q80WG5
A	812	ASN	-	expression tag	UNP Q80WG5
A	813	SER	-	expression tag	UNP Q80WG5
A	814	LEU	-	expression tag	UNP Q80WG5
A	815	GLU	-	expression tag	UNP Q80WG5
A	816	VAL	-	expression tag	UNP Q80WG5
A	817	LEU	-	expression tag	UNP Q80WG5
A	818	PHE	-	expression tag	UNP Q80WG5
A	819	GLN	-	expression tag	UNP Q80WG5
B	811	SER	-	expression tag	UNP Q80WG5
B	812	ASN	-	expression tag	UNP Q80WG5
B	813	SER	-	expression tag	UNP Q80WG5
B	814	LEU	-	expression tag	UNP Q80WG5
B	815	GLU	-	expression tag	UNP Q80WG5
B	816	VAL	-	expression tag	UNP Q80WG5
B	817	LEU	-	expression tag	UNP Q80WG5
B	818	PHE	-	expression tag	UNP Q80WG5
B	819	GLN	-	expression tag	UNP Q80WG5

- Molecule 2 is a protein called Volume-regulated anion channel subunit LRRC8C.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	F	404	Total	C	N	O	S	0	0
			3257	2099	547	595	16		

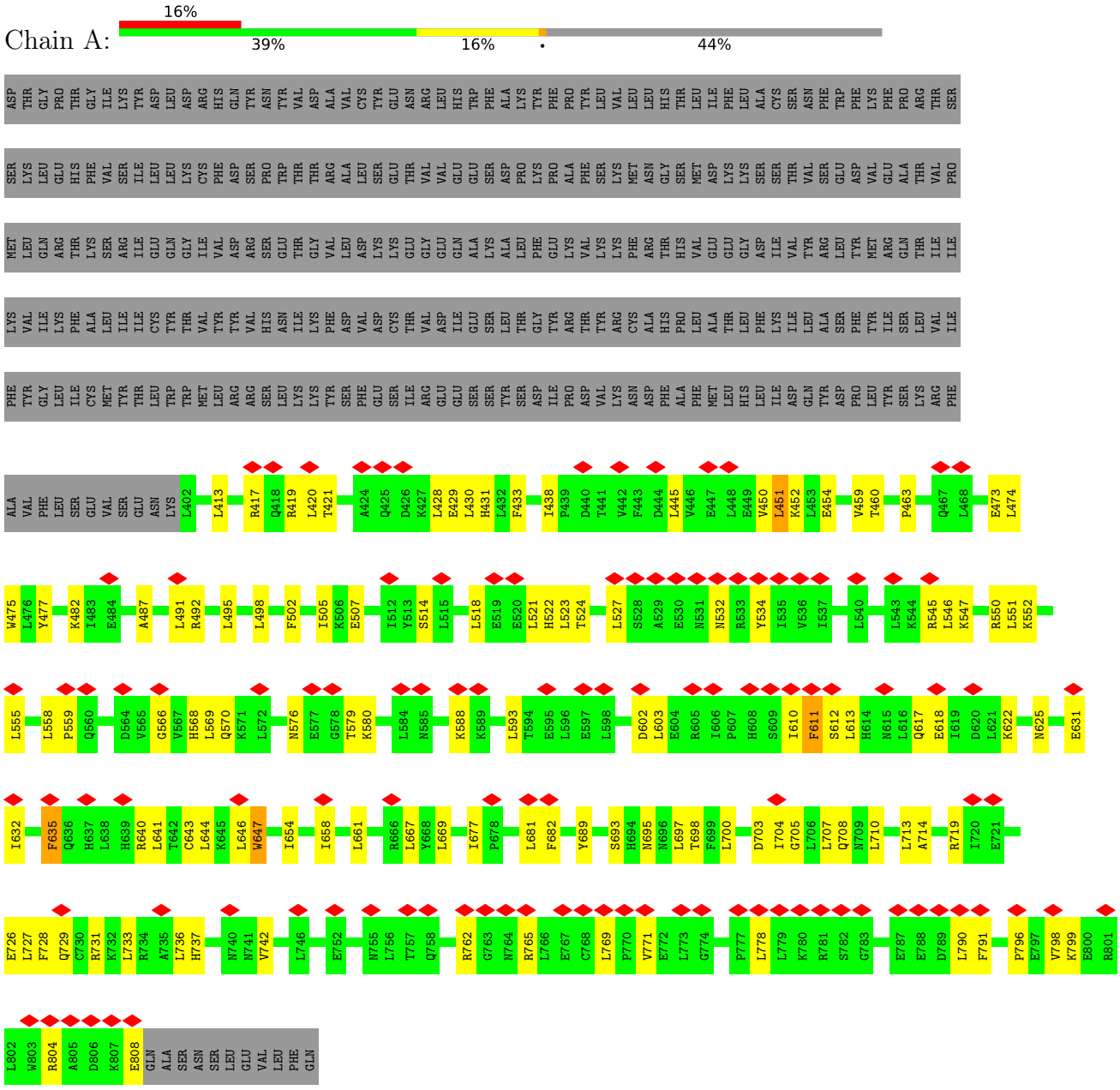
There are 10 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
F	804	SER	-	expression tag	UNP Q8R502
F	805	ASN	-	expression tag	UNP Q8R502
F	806	SER	-	expression tag	UNP Q8R502
F	807	GLU	-	expression tag	UNP Q8R502
F	808	ASN	-	expression tag	UNP Q8R502
F	809	LEU	-	expression tag	UNP Q8R502
F	810	TYR	-	expression tag	UNP Q8R502
F	811	PHE	-	expression tag	UNP Q8R502
F	812	GLN	-	expression tag	UNP Q8R502
F	813	GLY	-	expression tag	UNP Q8R502

3 Residue-property plots (i)

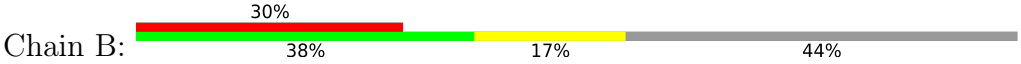
These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and atom inclusion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

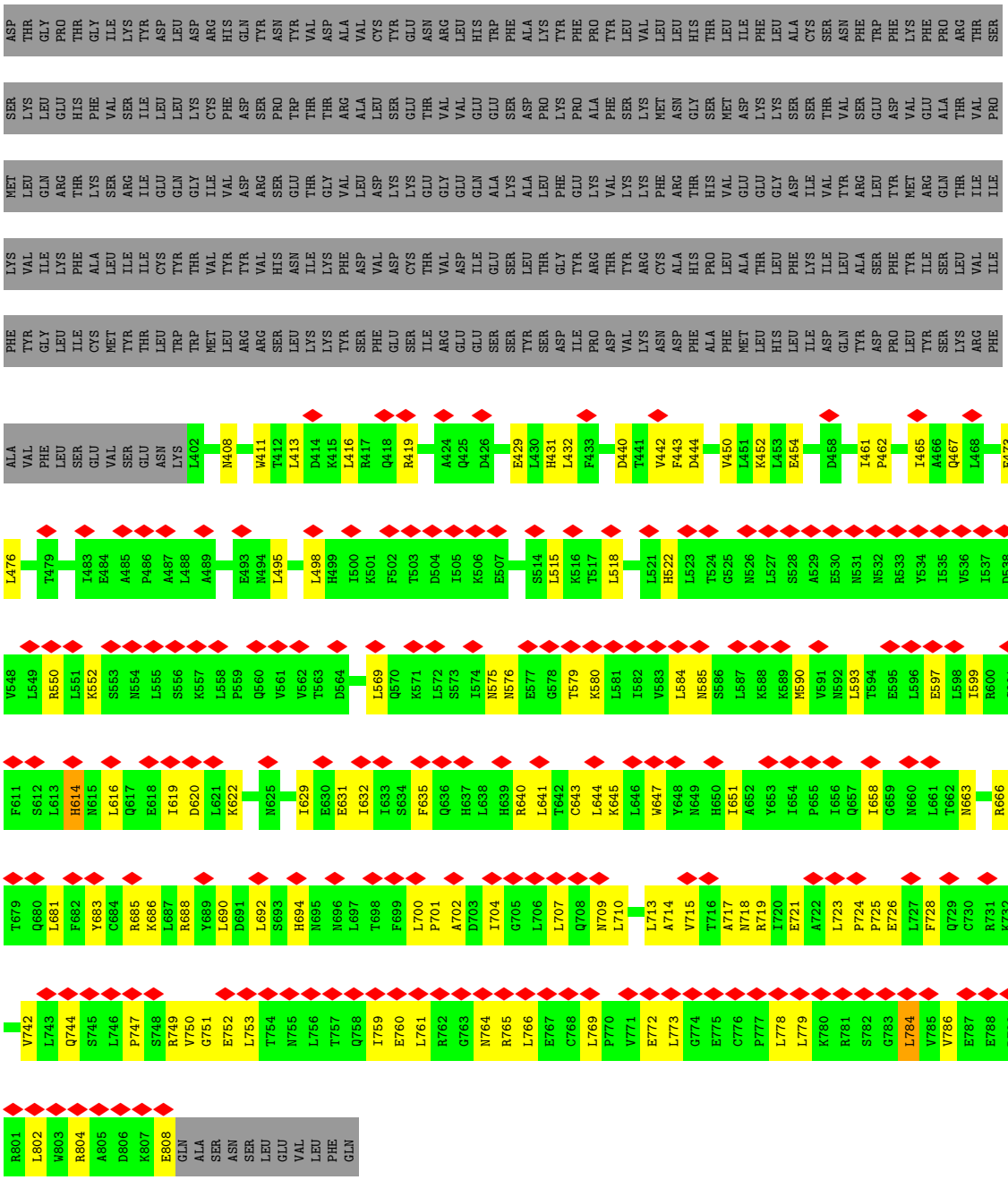
- Molecule 1: Volume-regulated anion channel subunit LRRC8A



• Molecule 1: Volume-regulated anion channel subunit LRRC8A

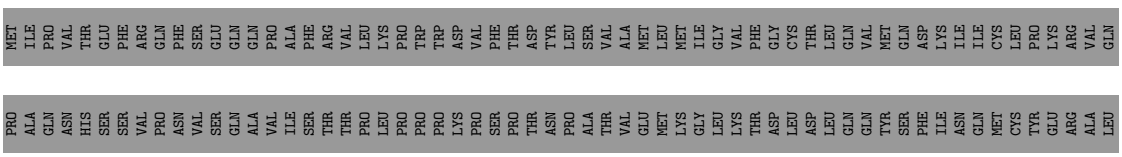
Chain B:

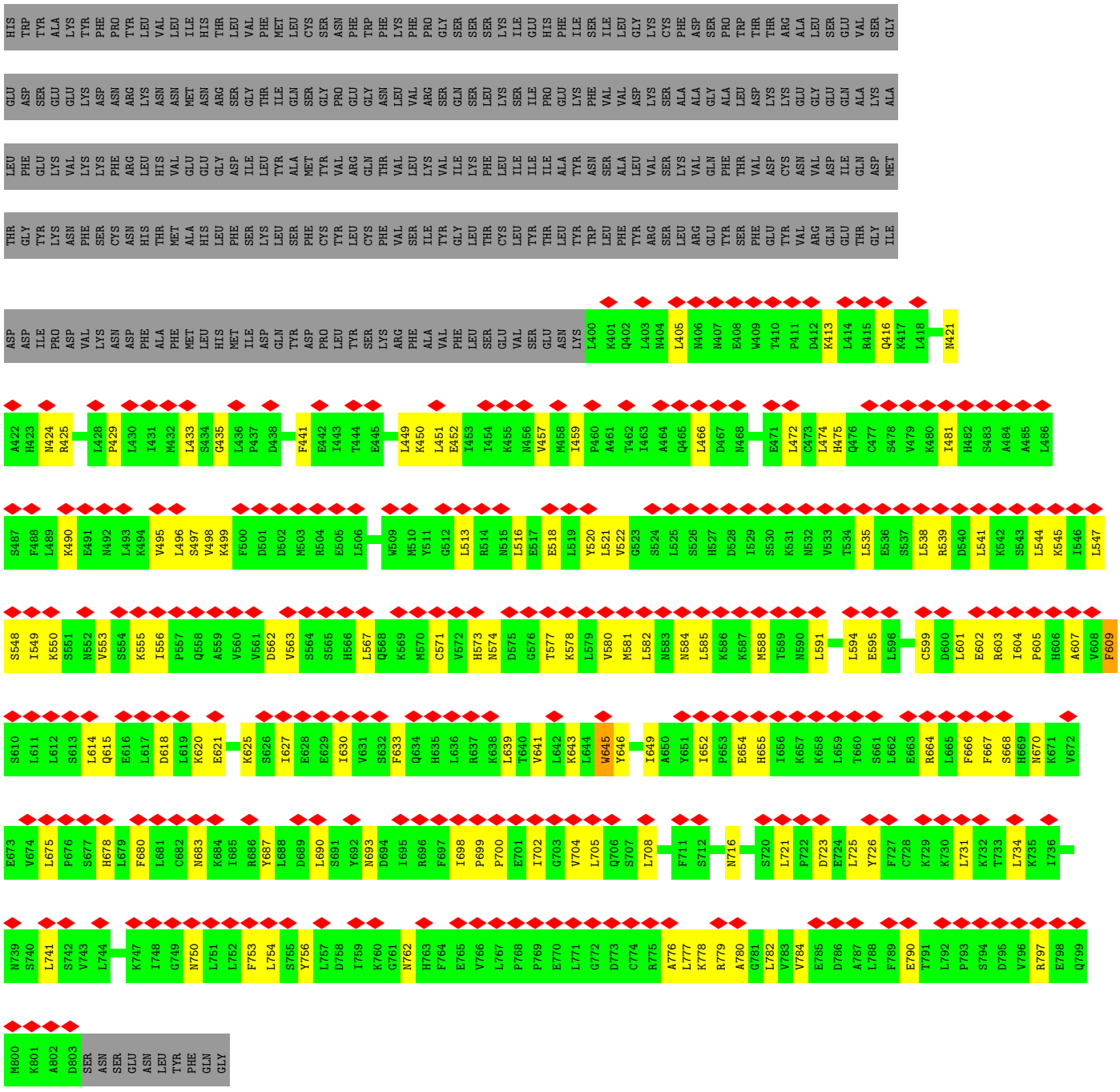




• Molecule 2: Volume-regulated anion channel subunit LRRC8C

Chain F:




HIS TRP TYR ALA LYS TYR PHE PRO TYR LEU VAL LEU ILE HIS THR LEU VAL PHE MET LEU CYS SER ASN PHE TRP PHE LYS PHE PRO GLY SER SER SER LYS ILE GLU HIS PHE ILE SER ILE LEU GLY LYS CYS PHE ASP SER PRO TRP THR THR ARG ALA LEU SER GLU VAL SER GLY
GLU ASP SER GLU GLU LYS ASP ASN ARG LYS ASN ASN MET ASN ARG SER GLY THR ILE GLN SER GLY VAL GLU GLU LYS PHE VAL VAL ASP LYS SER ALA ALA GLY ALA LEU ASP LYS LYS GLU GLY GLU GLN ALA LYS ALA
LEU PHE GLU LYS VAL LYS LYS PHE ARG LEU HIS VAL GLU GLU GLY ASP ILE LEU TYR ALA MET TYR VAL ARG GLN THR VAL LEU LYS VAL ILE LYS PHE LEU ILE ILE ILE ILE ALA TYR ASN SER ALA LEU VAL SER LYS VAL GLN PHE THR VAL ASP CYS ASN VAL ASP ILE GLN ASP MET
THR GLY TYR LYS ASN PHE SER CYS ASN HIS THR MET ALA HIS LEU PHE SER LYS LEU SER PHE CYS TYR VAL LEU CYS PHE VAL SER ILE TYR GLY LEU THR CYS LEU TYR THR LEU TYR TRP LEU PHE TYR ARG SER LEU ARG GLU TYR SER PHE GLU TYR VAL ARG GLN GLU GLY THR GLY ILE
ASP ASP ILE PRO ASP VAL LYS ASN ASP PHE ALA PHE MET LEU HIS MET ILE ASP GLN TYR ASP PRO LEU TYR SER LYS ARG PHE ALA VAL PHE LEU SER GLU VAL SER GLU ASN LYS L400 K401 Q402 L403 N404 L405 N406 N407 E408 W409 T410 P411 D412 K413 L414 R415 Q416 K417 L418 N421
A422 H423 N424 R425 L428 P429 L430 I431 M432 L433 S434 G435 L436 P437 D438 F441 E442 I443 T444 E445 L449 K450 L451 E452 I453 I454 K455 N456 V457 M458 I459 P460 A461 T462 I463 A464 Q465 L466 D467 N468 E471 L472 C473 L474 H475 Q476 C477 S478 V479 K480 I481 H482 S483 A484 A485 L486
S487 F488 L489 K490 E491 N492 L493 K494 V495 L496 S497 V498 K499 F500 D501 D502 N503 R504 E505 L506 W509 M510 Y511 G512 L513 R514 N515 L516 E517 E518 L519 Y520 L521 V522 G523 S524 L525 S526 H527 D528 I529 S530 K531 N532 V533 T534 L535 E536 S537 L538 R539 D540 L541 K542 S543 L544 K545 I546 L547
S548 I549 K550 S551 N552 V553 S554 K555 I556 P557 Q558 A559 V560 V561 D562 V563 S564 S565 H566 L567 Q568 K569 M570 C571 V572 H573 N574 D575 G576 T577 K578 L579 V580 M581 L582 N583 N584 L585 K586 K587 M588 T589 N590 L591 L594 E595 L596 C599 D600 L601 E602 R603 I604 P605 H606 A607 V608 F609
S610 L611 L612 S613 L614 Q615 E616 L617 D618 L619 K620 E621 K625 S626 I627 E628 E629 I630 V631 S632 F633 Q634 H635 L636 R637 K638 L639 T640 V641 L642 K643 L644 W645 Y646 I649 A650 Y651 I652 P653 E654 H655 I656 K657 K658 L659 T660 S661 L662 E663 R664 L665 F666 F667 S668 H669 N670 K671 V672
E673 V674 L675 P676 S677 H678 L679 F680 L681 C682 N683 K684 I685 R686 Y687 L688 D689 L690 S691 Y692 N693 D694 I695 R696 F697 I698 P699 P700 E701 I702 G703 V704 L705 Q706 S707 L708 F711 S712 N716 S720 L721 P722 D723 E724 L725 Y726 F727 C728 K729 K730 L731 K732 T733 L734 K735 I736
N739 S740 L741 S742 V743 L744 K747 I748 G749 N750 L751 L752 F753 L754 S755 Y756 L757 D758 I759 K760 G761 N762 H763 F764 E765 V766 L767 P768 P769 E770 L771 G772 D773 C774 R775 A776 L777 K778 R779 A780 G781 L782 V783 V784 E785 D786 A787 L788 F789 E790 T791 L792 P793 S794 D795 V796 R797 E798 Q799
M800 K801 A802 D803 SER ASN SER GLU ASN LEU TYR PHE GLN GLY

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	71198	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 ($e^-/Å^2$)	50	Depositor
Minimum defocus (nm)	0.6	Depositor
Maximum defocus (nm)	2	Depositor
Magnification	Not provided	
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor
Maximum map value	2.978	Depositor
Minimum map value	-2.213	Depositor
Average map value	0.003	Depositor
Map value standard deviation	0.044	Depositor
Recommended contour level	0.45	Depositor
Map size (Å)	435.968, 435.968, 435.968	wwPDB
Map dimensions	416, 416, 416	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.048, 1.048, 1.048	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	0/3378	0.57	0/4580
1	B	0.29	0/3378	0.58	0/4580
2	F	0.27	0/3315	0.54	0/4482
All	All	0.27	0/10071	0.56	0/13642

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	3321	0	3491	85	0
1	B	3321	0	3491	86	0
2	F	3257	0	3405	93	0
All	All	9899	0	10387	252	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 252 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:762:ARG:HD2	2:F:424:ASN:CG	1.57	1.22

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Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:762:ARG:HD2	2:F:424:ASN:ND2	1.69	1.06
1:A:640:ARG:NH2	1:B:719:ARG:HG2	1.83	0.94
1:A:640:ARG:HH21	1:B:719:ARG:HG2	1.41	0.86
1:B:686:LYS:HA	1:B:709:ASN:HB2	1.65	0.78

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	405/729 (56%)	386 (95%)	19 (5%)	0	100	100
1	B	405/729 (56%)	375 (93%)	30 (7%)	0	100	100
2	F	402/813 (49%)	383 (95%)	19 (5%)	0	100	100
All	All	1212/2271 (53%)	1144 (94%)	68 (6%)	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	378/676 (56%)	370 (98%)	8 (2%)	53	72
1	B	378/676 (56%)	372 (98%)	6 (2%)	62	79
2	F	382/756 (50%)	377 (99%)	5 (1%)	69	82

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Mol	Chain	Analysed	Rotameric	Outliers	Percentiles
All	All	1138/2108 (54%)	1119 (98%)	19 (2%)	62 78

5 of 19 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	F	449	LEU
2	F	753	PHE
2	F	756	TYR
2	F	645	TRP
1	B	429	GLU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 7 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	522	HIS
1	B	764	ASN
2	F	558	GLN
2	F	515	ASN
1	B	409	ASN

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.

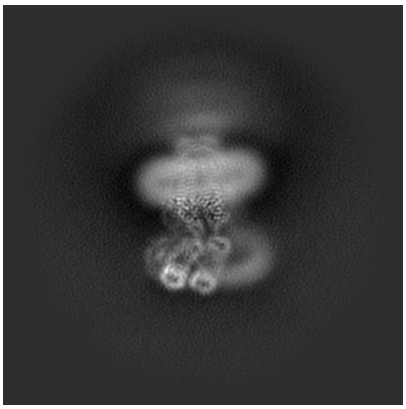
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-28895. These allow visual inspection of the internal detail of the map and identification of artifacts.

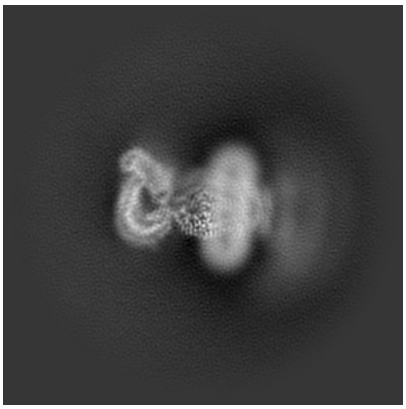
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections

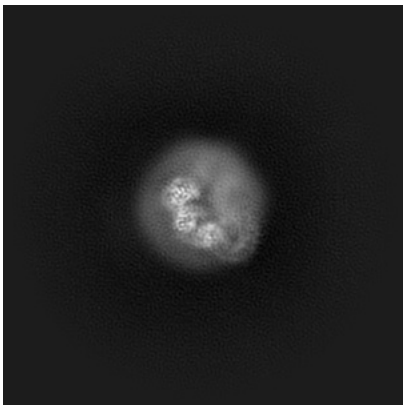
6.1.1 Primary map



X

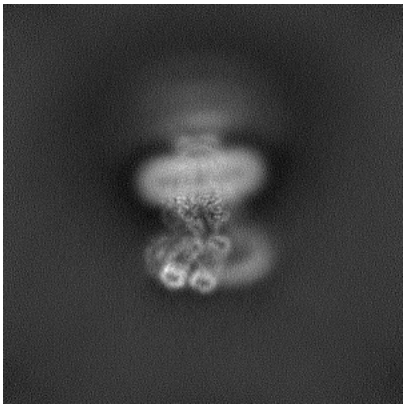


Y

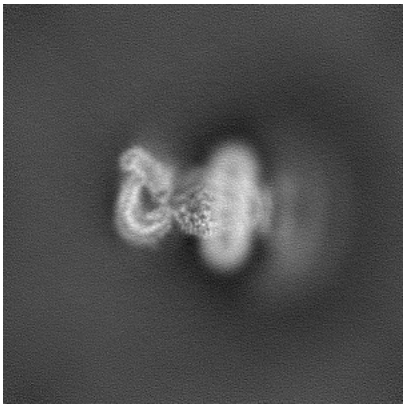


Z

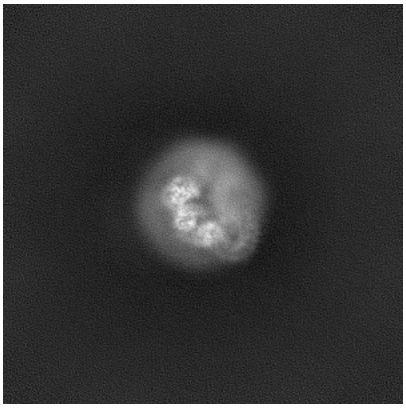
6.1.2 Raw map



X



Y

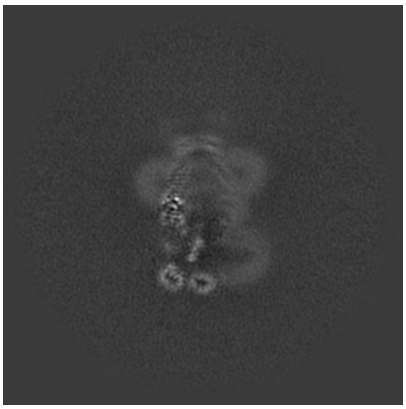


Z

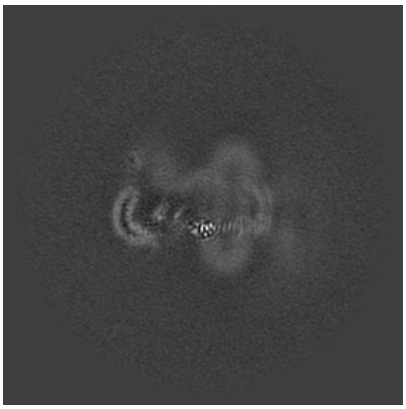
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

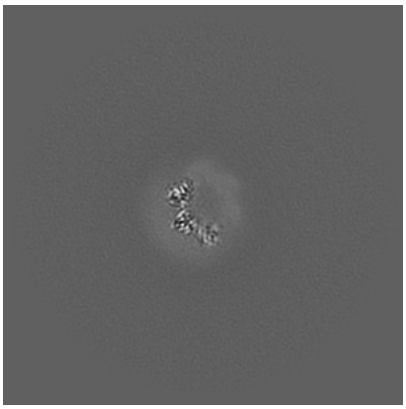
6.2.1 Primary map



X Index: 208

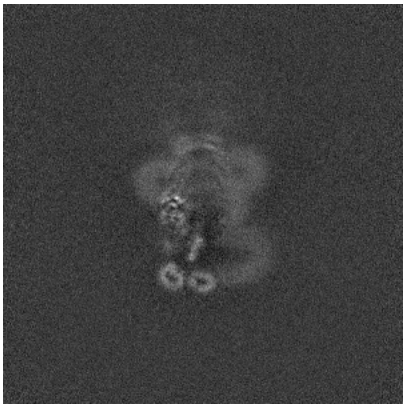


Y Index: 208



Z Index: 208

6.2.2 Raw map



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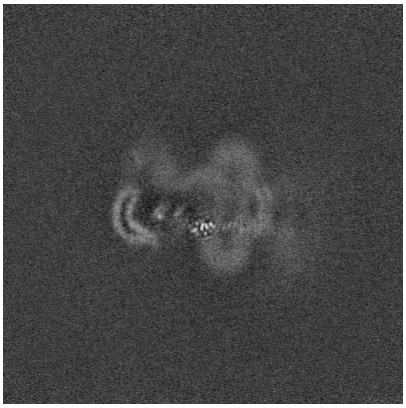
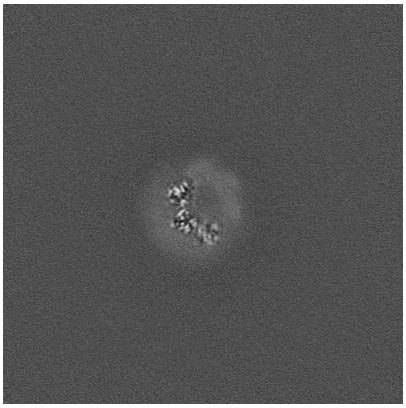


Y Index: 208

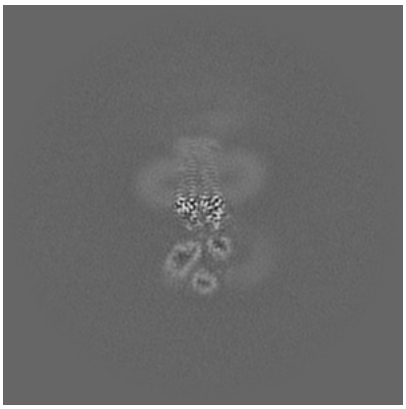


Z Index: 208

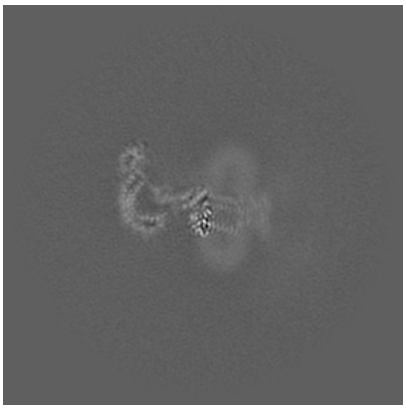
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

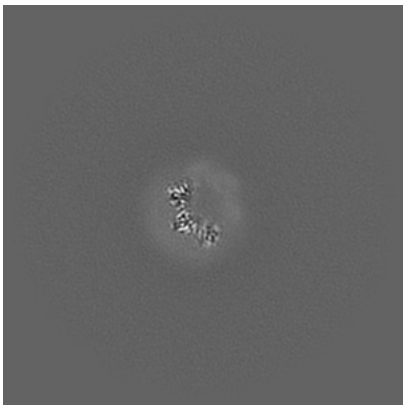
6.3.1 Primary map



X Index: 187

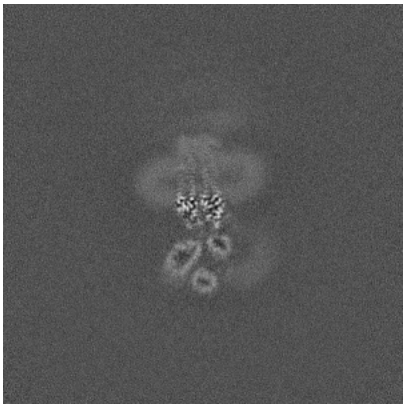


Y Index: 184

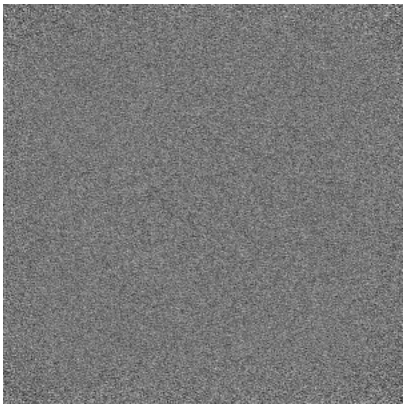


Z Index: 209

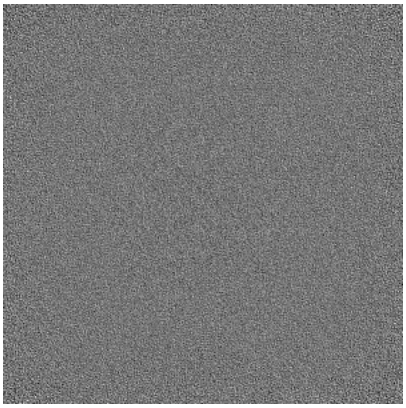
6.3.2 Raw map



X Index: 187



Y Index: 0

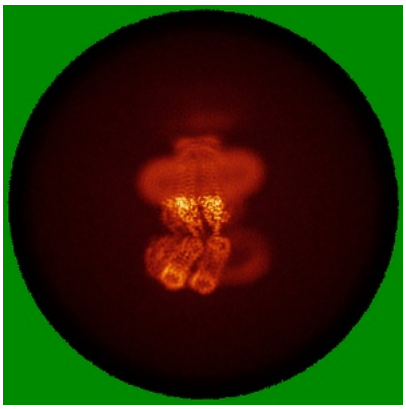


Z Index: 0

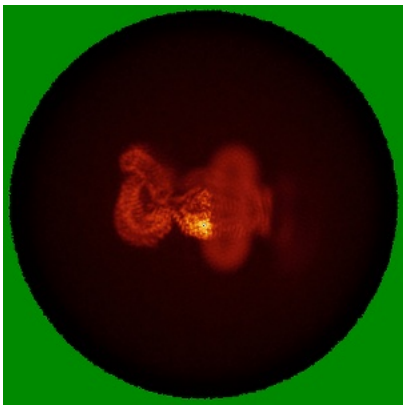
The images above show the largest variance slices of the map in three orthogonal directions.

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

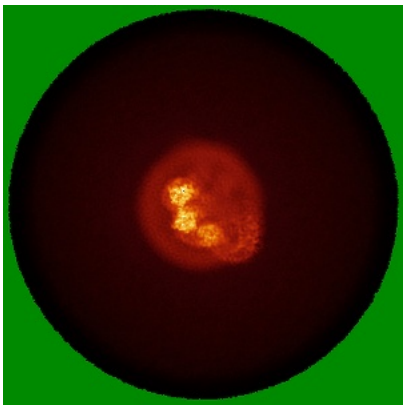
6.4.1 Primary map



X

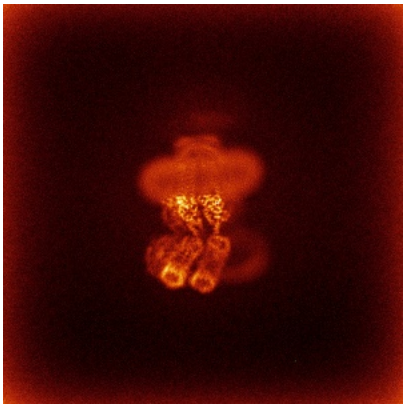


Y

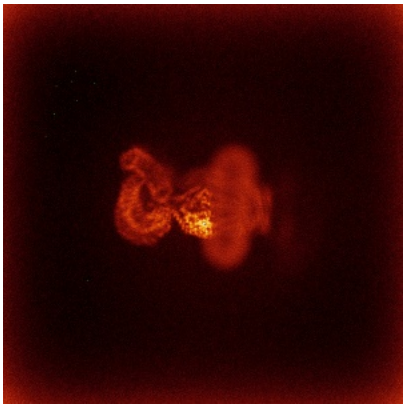


Z

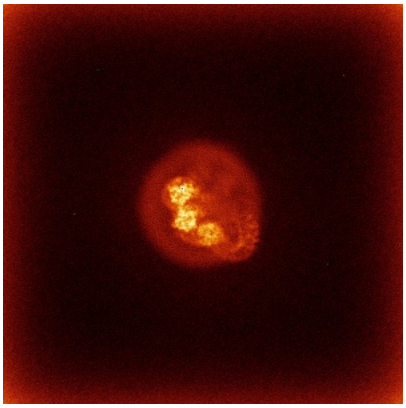
6.4.2 Raw map



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.

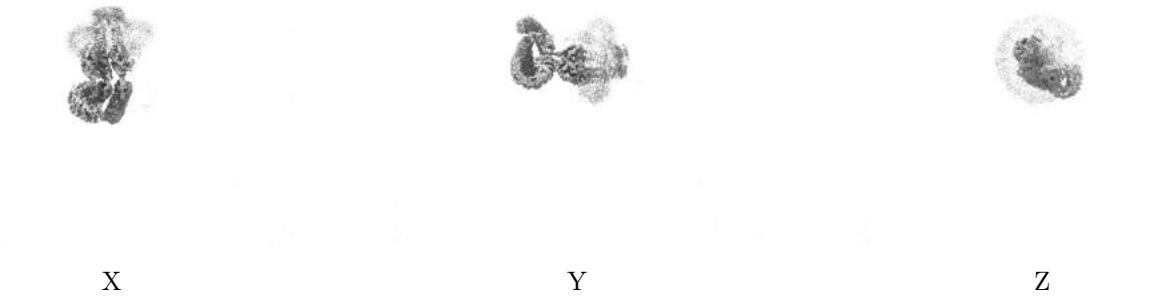
6.5 Orthogonal surface views (i)

6.5.1 Primary map

X Y Z

The images above show the 3D surface view of the map at the recommended contour level 0.45. 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.

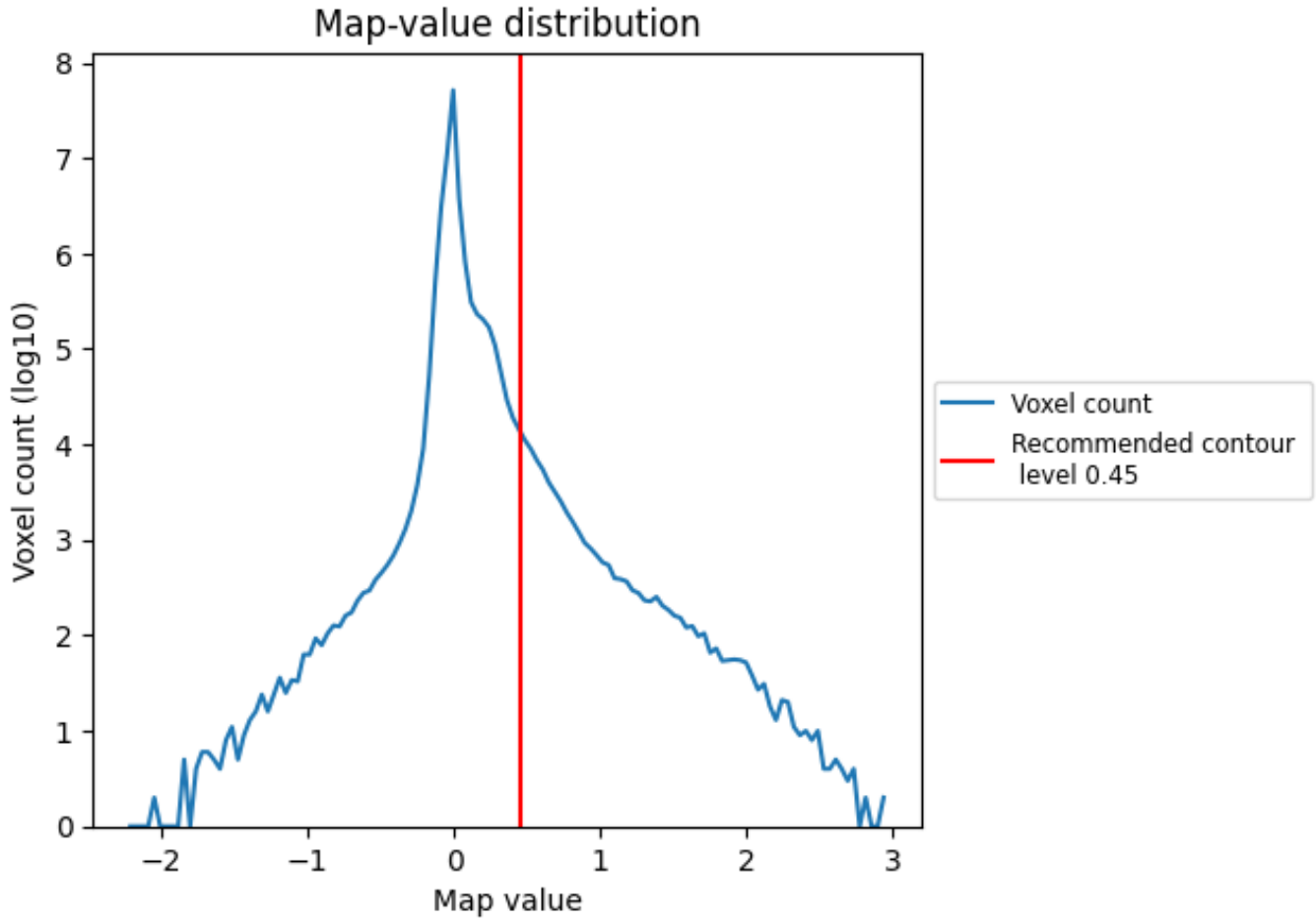
6.6 Mask visualisation (i)

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

7 Map analysis

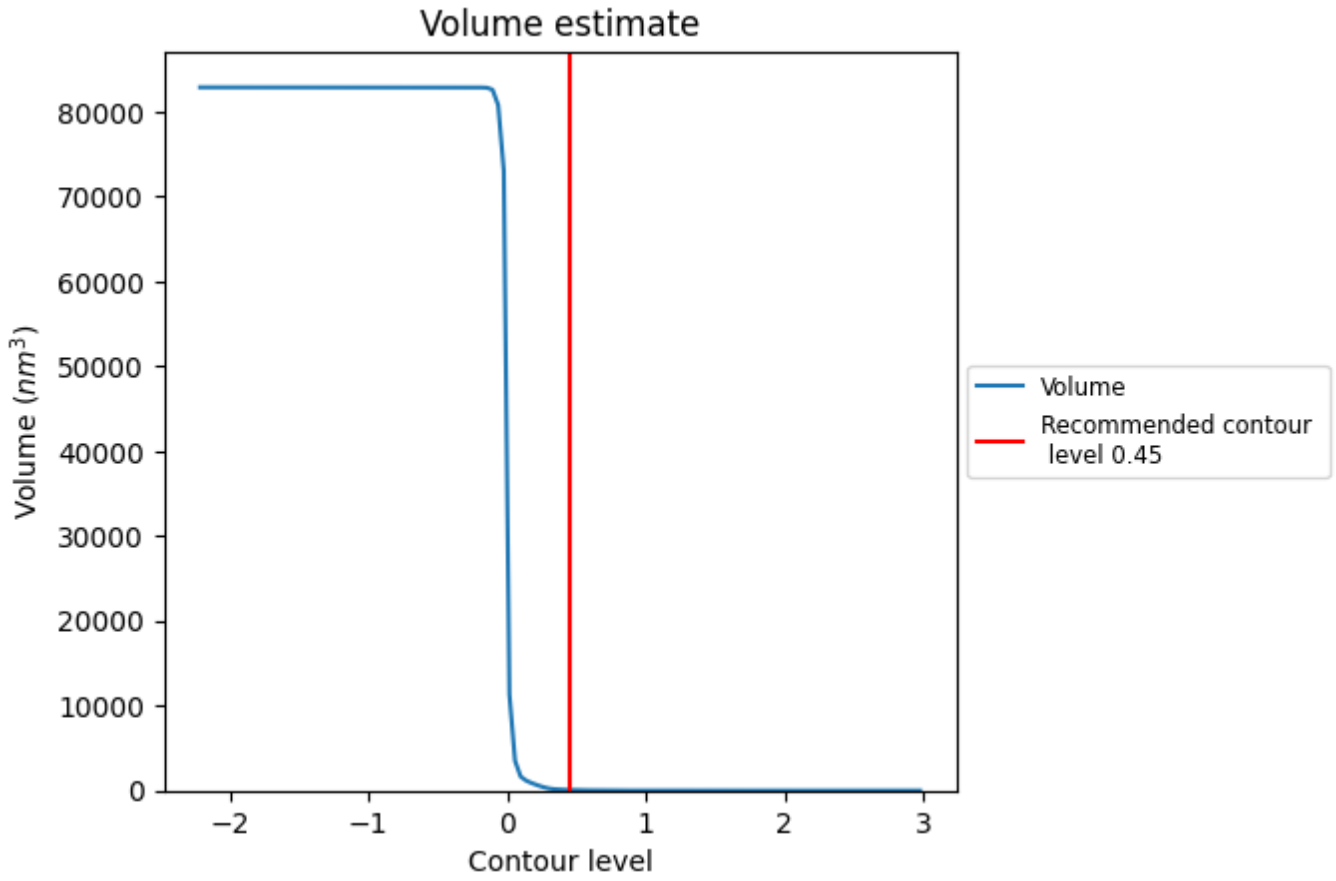
This section contains the results of statistical analysis of the map.

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.

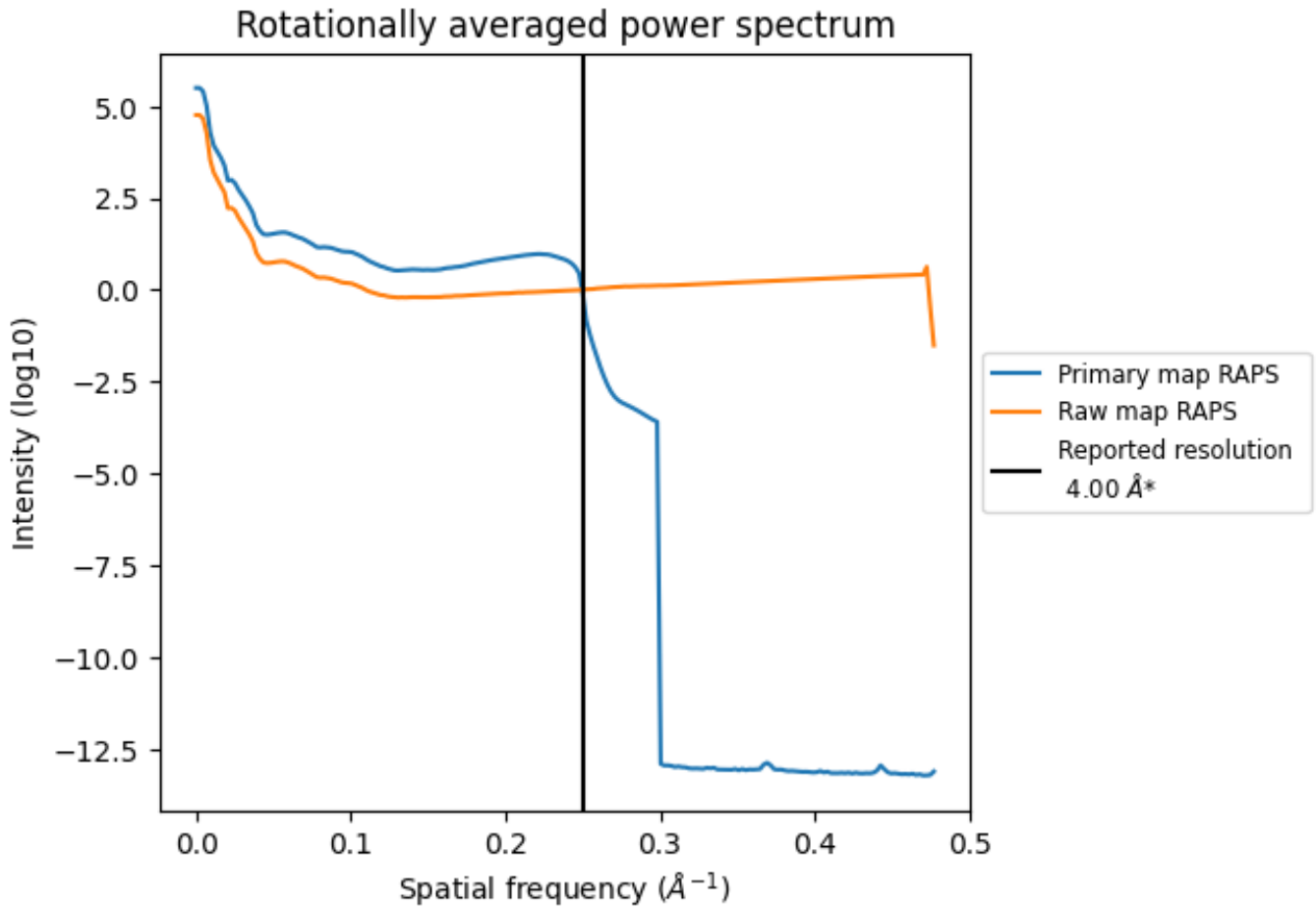
7.2 Volume estimate (i)



The volume at the recommended contour level is 77 nm^3; this corresponds to an approximate mass of 70 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)

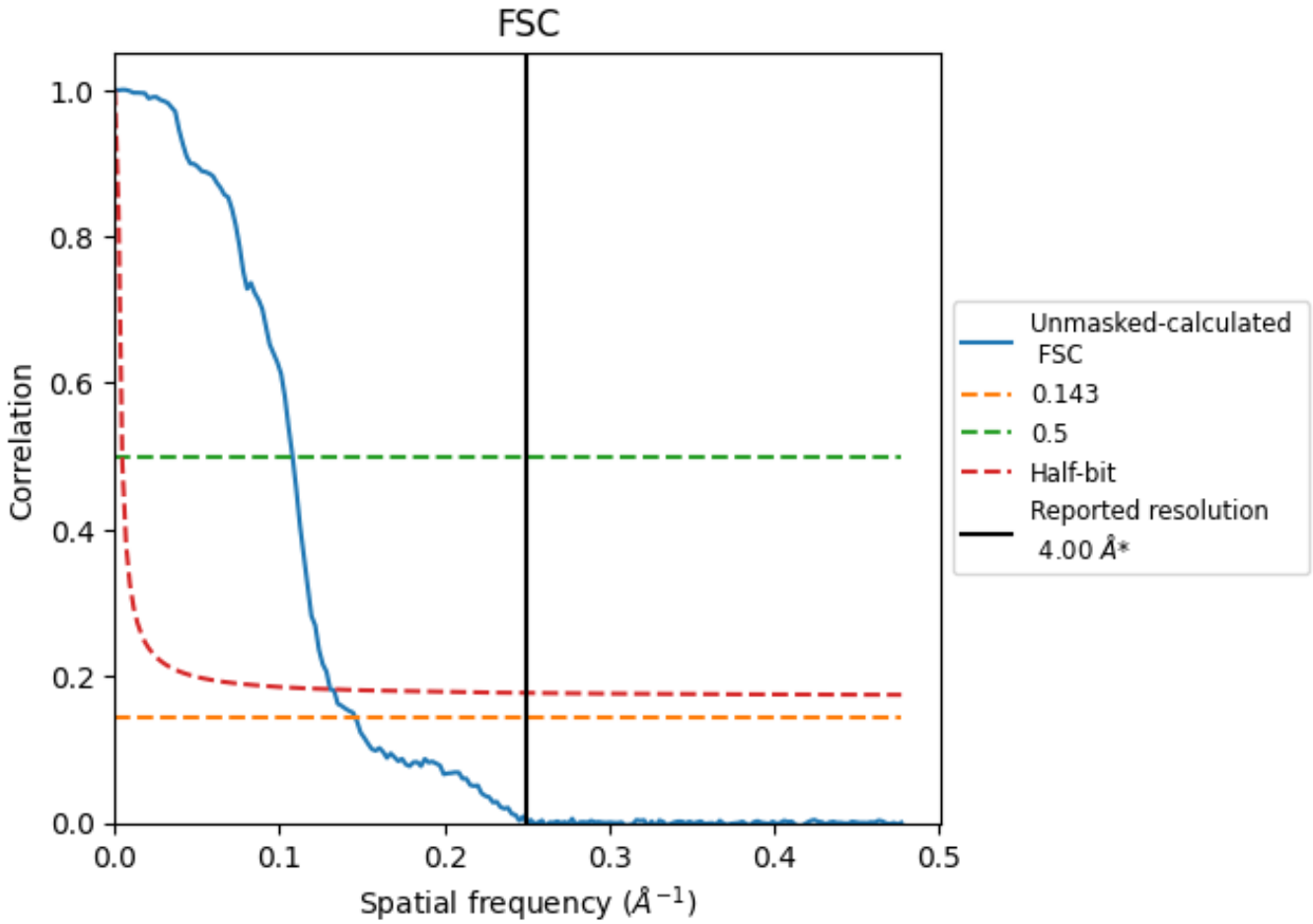


*Reported resolution corresponds to spatial frequency of 0.250 $Å^{-1}$

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 0.250 Å^{-1}

8.2 Resolution estimates (i)

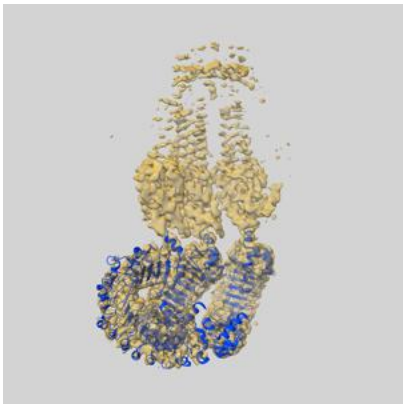
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	4.00	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	6.85	9.28	7.65

*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 6.85 differs from the reported value 4.0 by more than 10 %

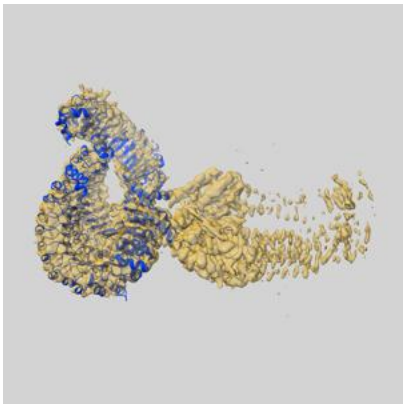
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-28895 and PDB model 8F75. Per-residue inclusion information can be found in section 3 on page 5.

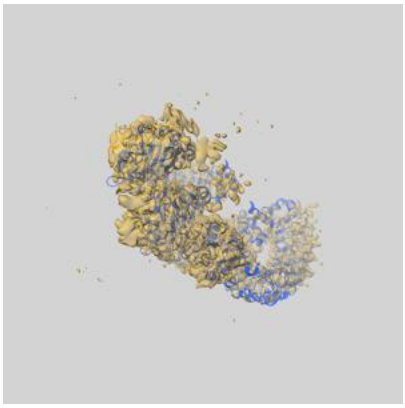
9.1 Map-model overlay



X



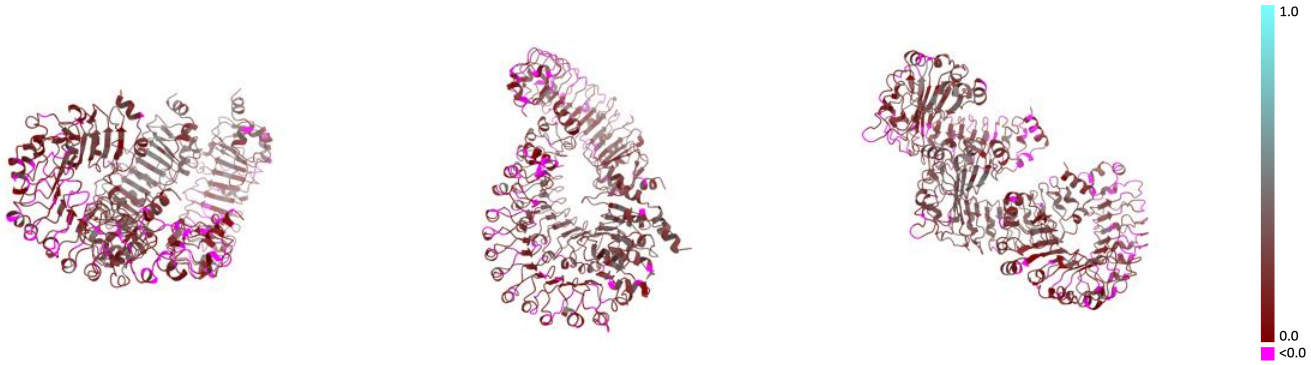
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.45 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.

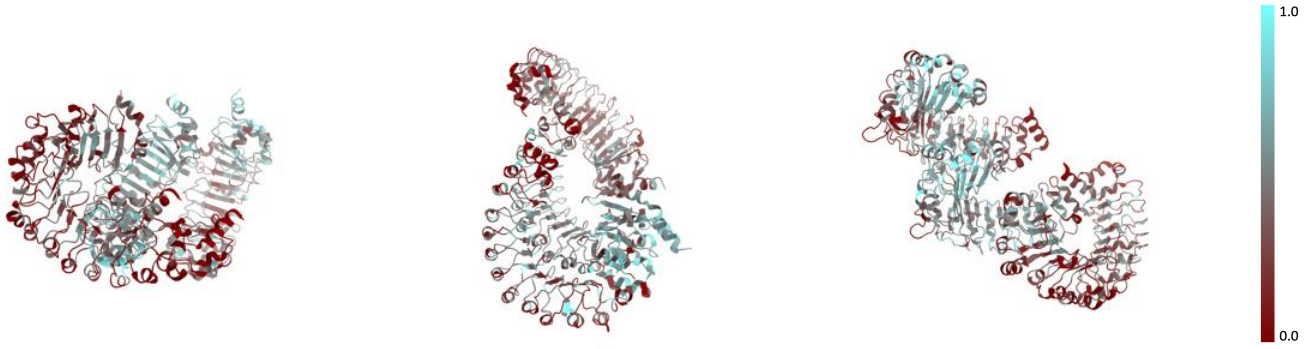
9.2 Q-score mapped to coordinate model



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.

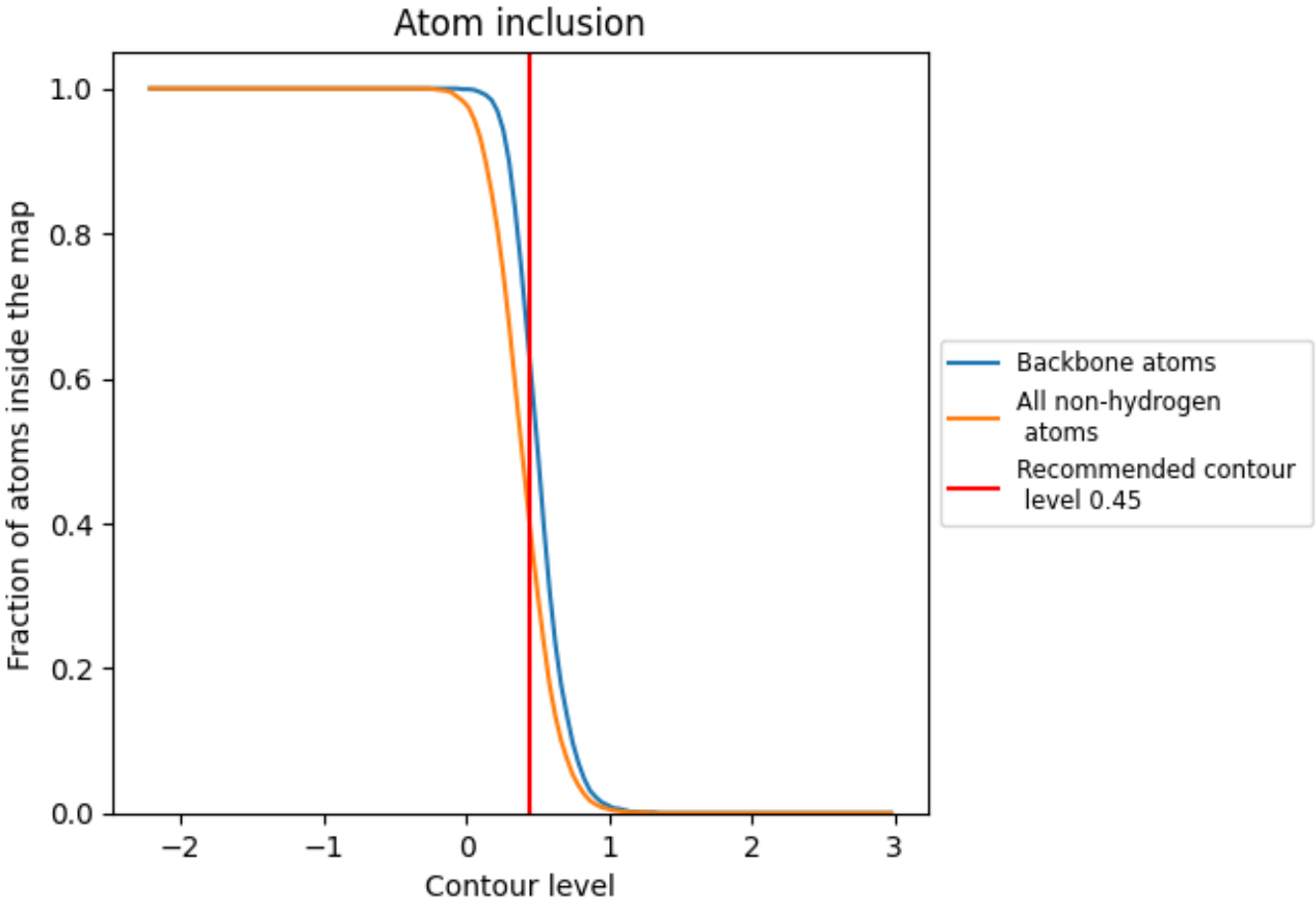
9.3 Atom inclusion mapped to coordinate model



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 (0.45).

9.4 Atom inclusion (i)



At the recommended contour level, 62% of all backbone atoms, 39% 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 (0.45) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.3890	0.1810
A	0.5170	0.2590
B	0.3720	0.1440
F	0.2780	0.1410