wwPDB EM Validation Summary Report

Feb 13, 2024 – 10:35 PM EST

PDB ID	:	3J7H
EMDB ID	:	EMD-5995
Title	:	Structure of beta-galactosidase at 3.2-A resolution obtained by cryo-electron microscopy
Authors	:	Bartesaghi, A.; Matthies, D.; Banerjee, S.; Merk, A.; Subramaniam, S.
Deposited on	:	2014-06-30
Resolution	:	3.20 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:

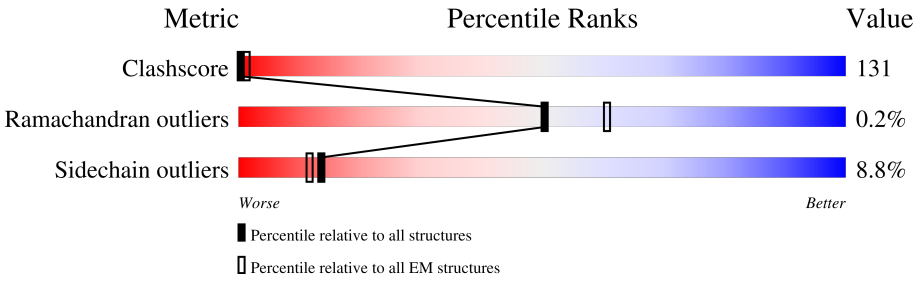
EMDB validation analysis	:	0.0.1.dev70
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

The reported resolution of this entry is 3.20 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	1024	8% 22% 71% 7%
1	B	1024	8% 22% 71% 7%
1	C	1024	8% 22% 71% 7%
1	D	1024	8% 22% 71% 7%

2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 32828 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 Beta-galactosidase.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	1022	Total	C	N	O	S	0	0
			8206	5190	1452	1525	39		
1	B	1022	Total	C	N	O	S	0	0
			8206	5190	1452	1525	39		
1	C	1022	Total	C	N	O	S	0	0
			8206	5190	1452	1525	39		
1	D	1022	Total	C	N	O	S	0	0
			8206	5190	1452	1525	39		

- Molecule 2 is MAGNESIUM ION (three-letter code: MG) (formula: Mg).

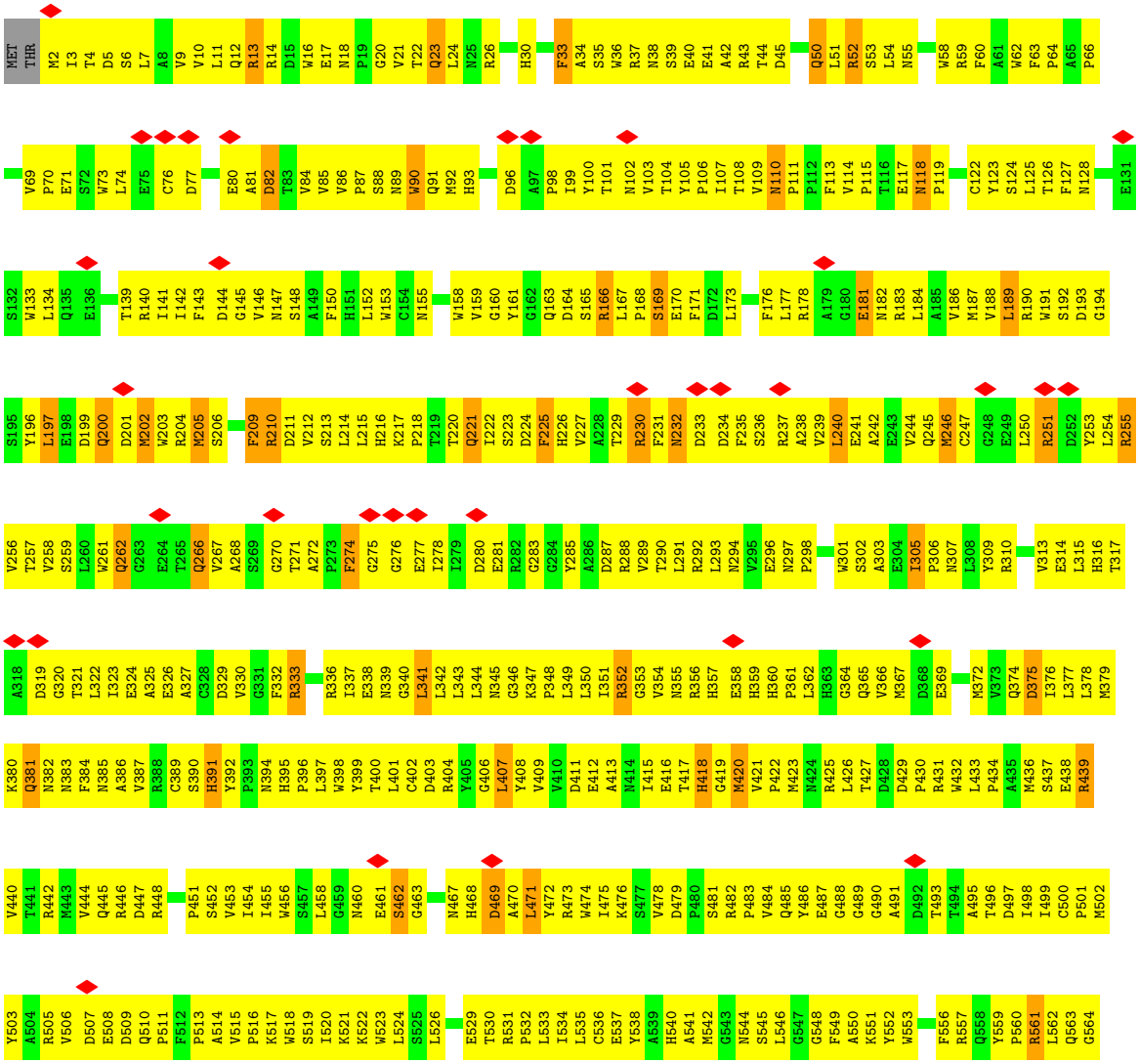
Mol	Chain	Residues	Atoms		AltConf
2	A	1	Total	Mg	0
			1	1	
2	B	1	Total	Mg	0
			1	1	
2	C	1	Total	Mg	0
			1	1	
2	D	1	Total	Mg	0
			1	1	

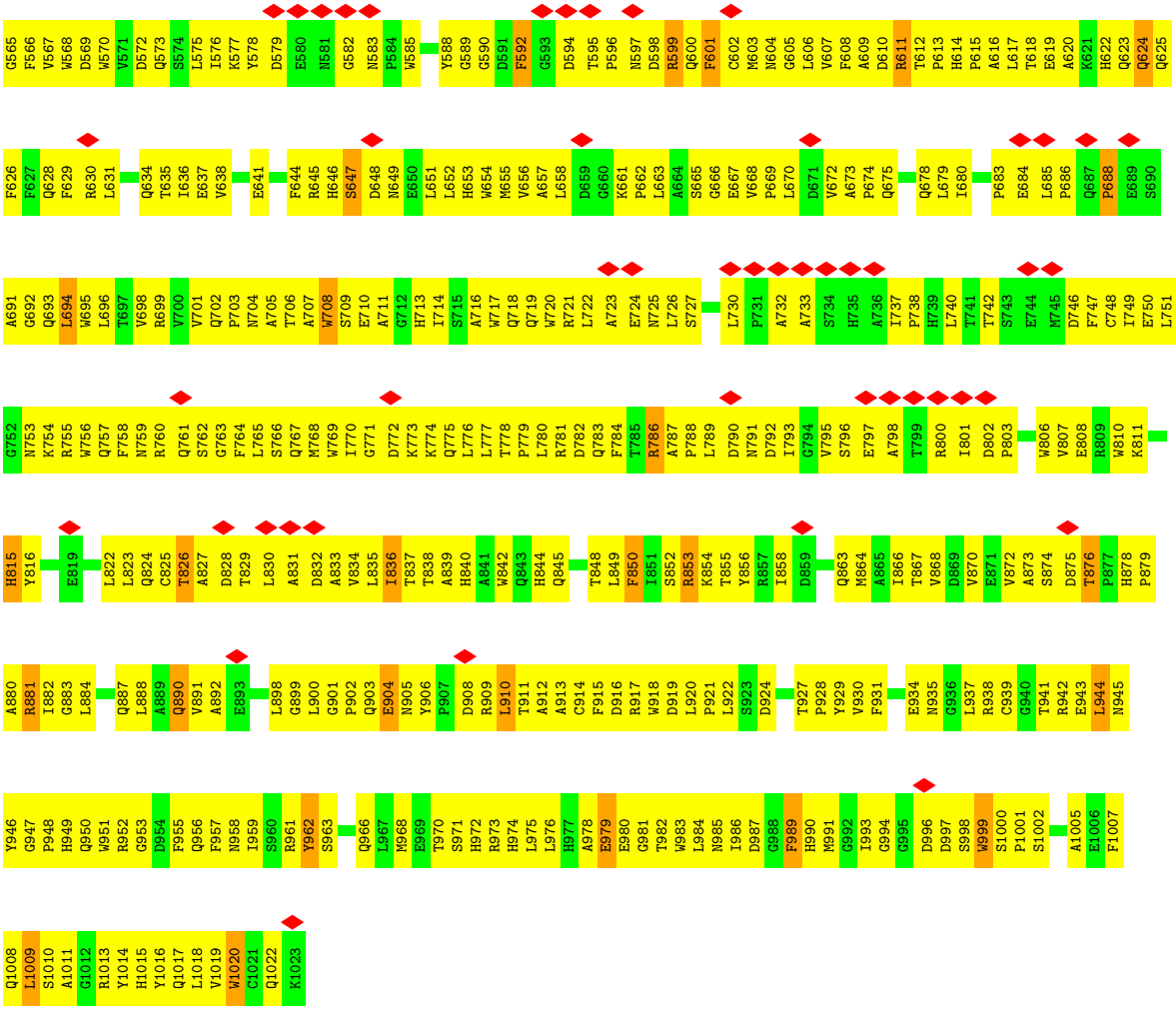
3 Residue-property plots (i)

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: Beta-galactosidase

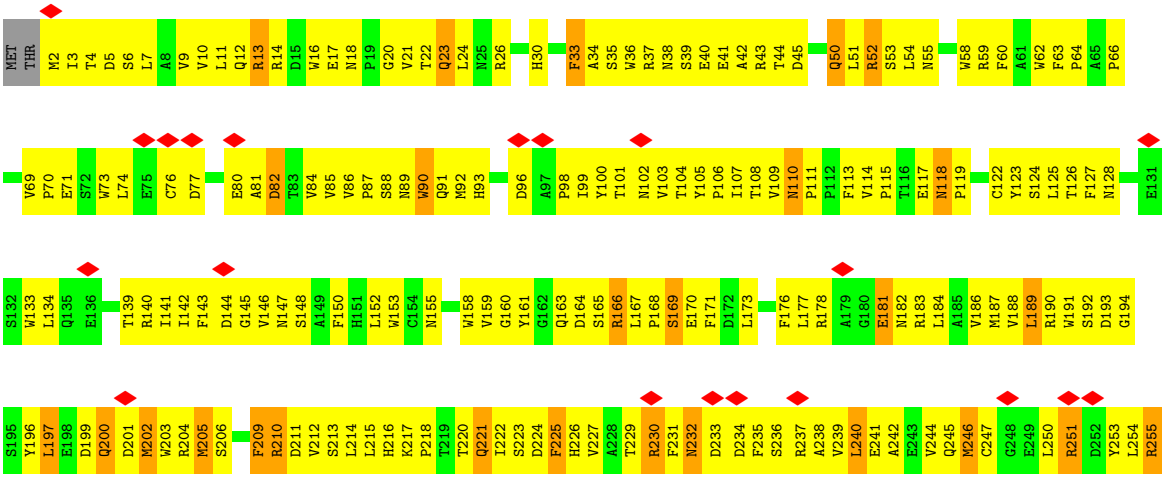
Chain A:

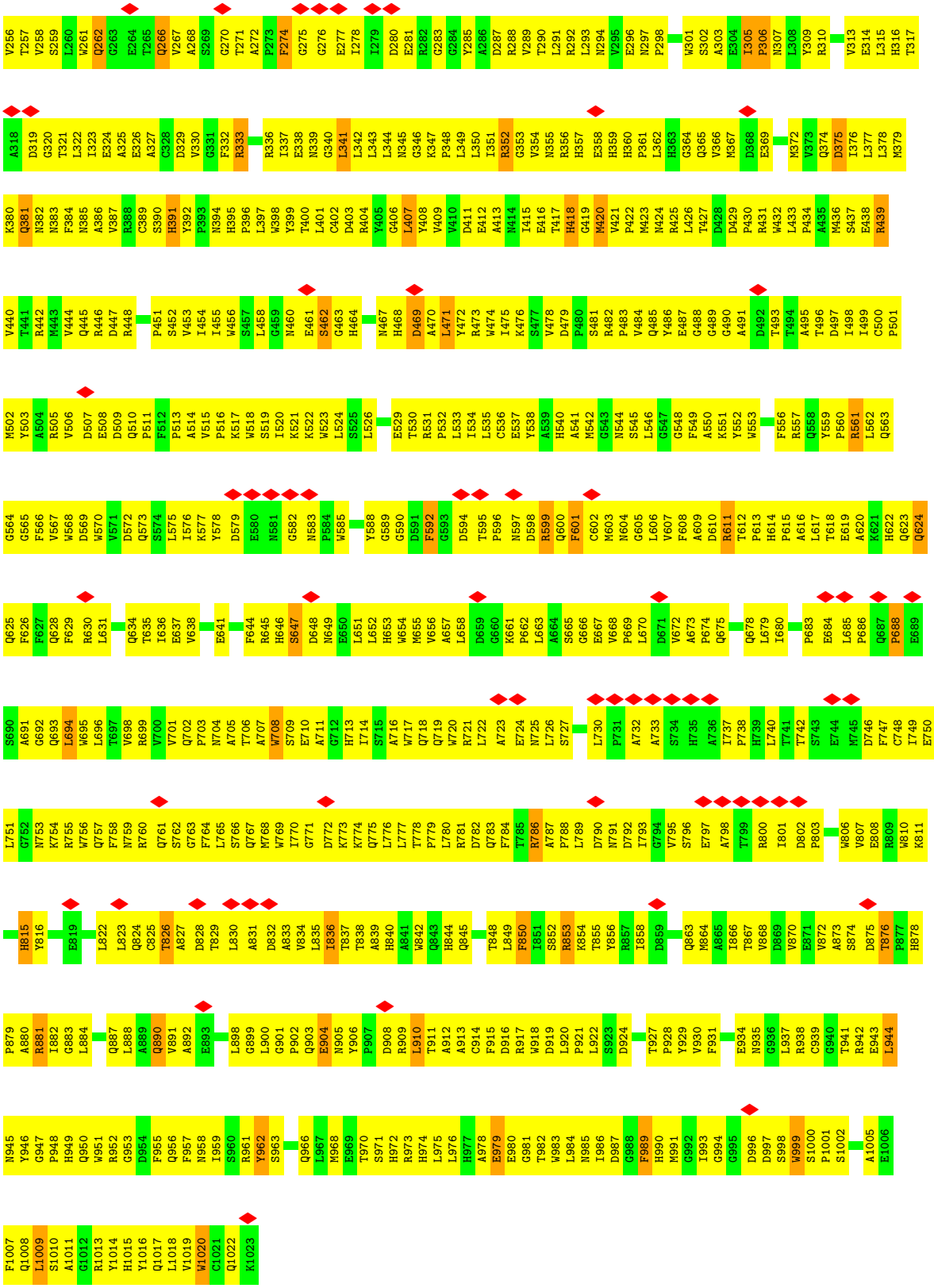




• Molecule 1: Beta-galactosidase

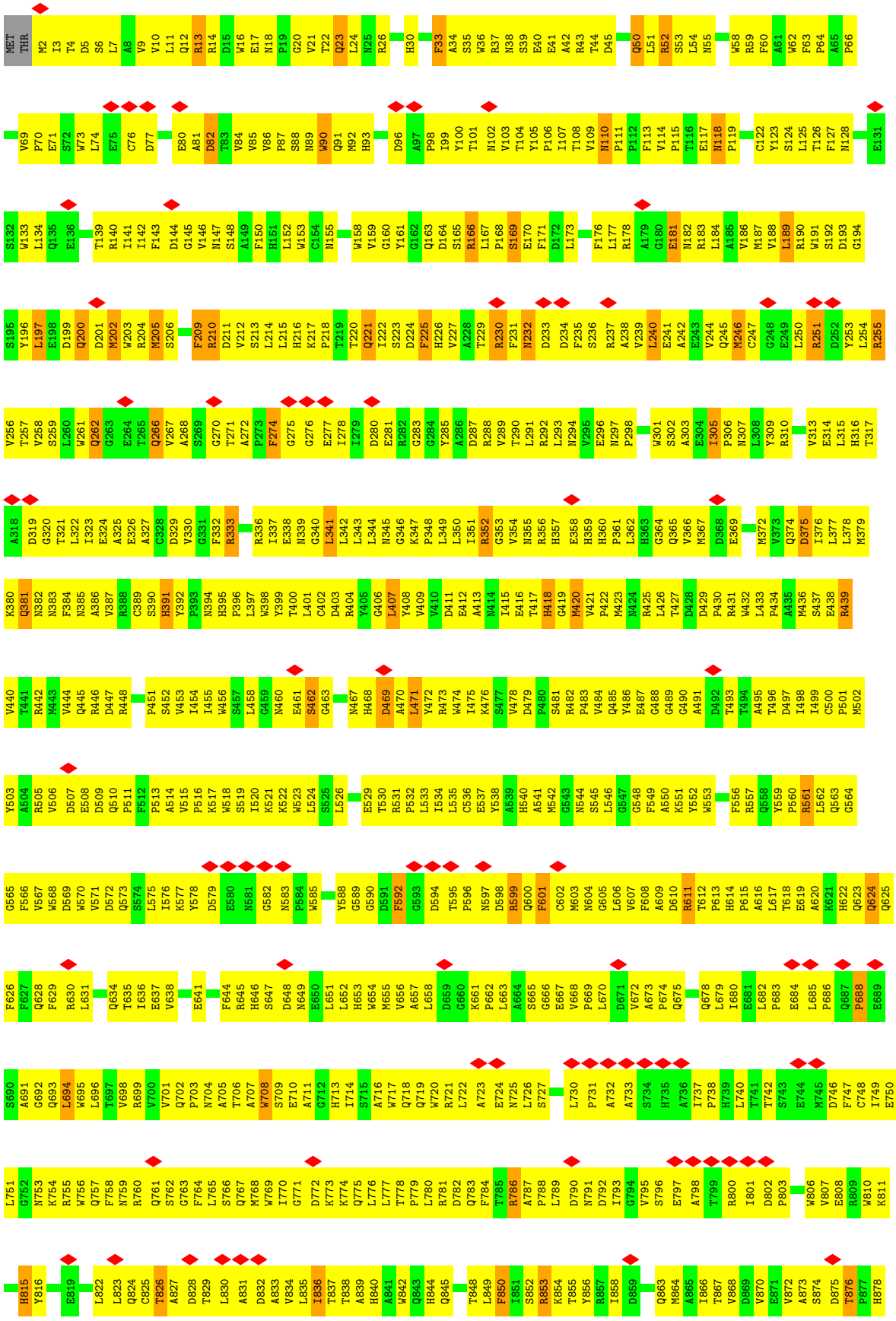
Chain B:





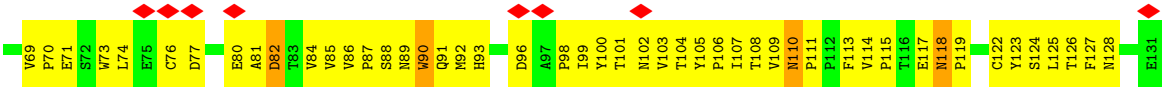
• Molecule 1: Beta-galactosidase

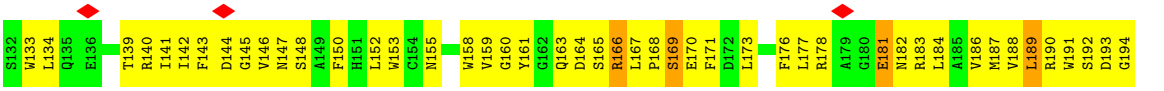
Chain C:

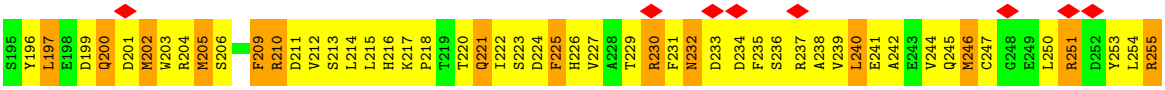


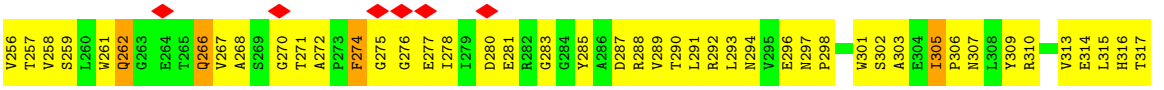
• Molecule 1: Beta-galactosidase

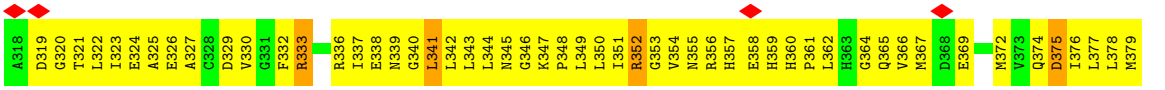
Chain D:

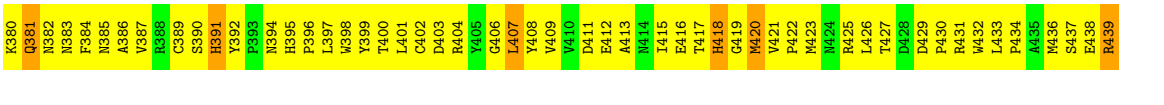


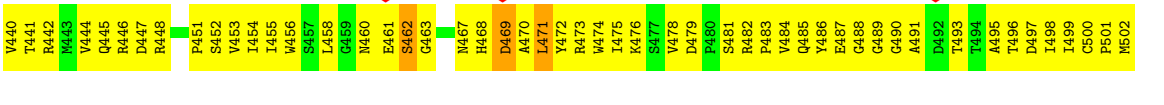


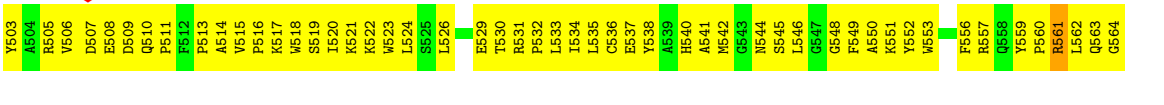


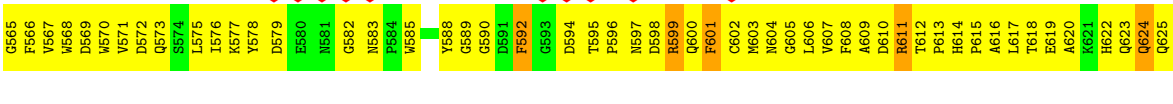


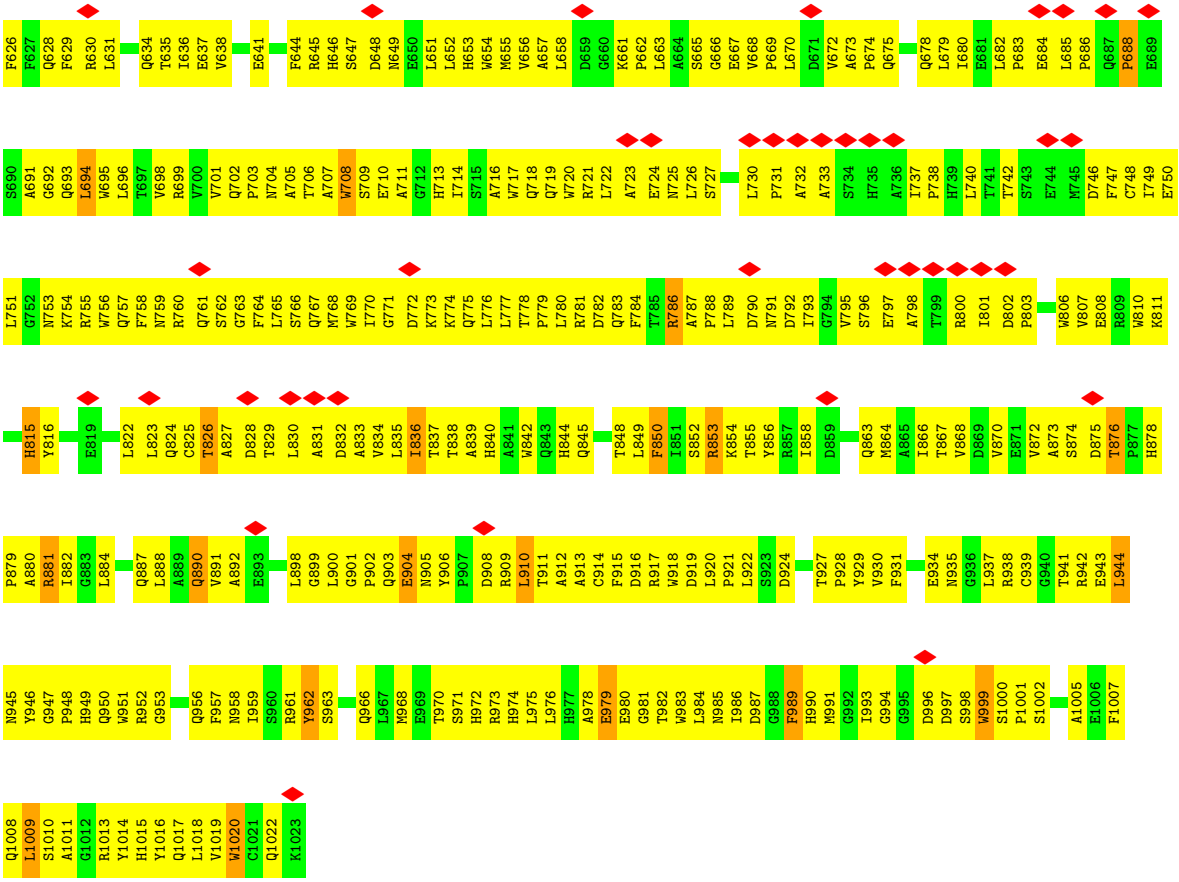











F626 F627 Q628 F629 R630 L631 Q634 T635 I636 E637 V638 E641 F644 R645 H646 S647 D648 N649 E650 L651 L652 H653 W654 M655 V656 A657 L658 D659 G660 K661 P662 L663 A664 S665 G666 E667 V668 P669 L670 D671 V672 A673 P674 Q675 Q678 L679 I680 E681 L682 P683 E684 L685 P686 Q687 P688 E689
S690 A691 G692 Q693 L694 W695 L696 T697 V698 R699 V700 V701 Q702 P703 N704 A705 T706 A707 W708 S709 E710 A711 G712 H713 I714 S715 A716 W717 Q718 Q719 W720 R721 L722 A723 E724 N725 L726 S727 L730 P731 A732 A733 S734 H735 A736 I737 P738 H739 L740 T741 T742 S743 E744 M745 D746 F747 C748 I749 E750
L751 G752 N753 K754 R755 W756 Q757 F758 N759 R760 Q761 S762 G763 F764 L765 S766 Q767 M768 W769 I770 G771 D772 K773 K774 Q775 L776 L777 T778 P779 L780 R781 D782 Q783 F784 T785 R786 A787 P788 L789 D790 N791 D792 I793 G794 V795 S796 E797 A798 T799 R800 I801 D802 P803 W806 V807 E808 R809 W810 K811
H815 Y816 E819 L822 L823 Q824 C825 T826 A827 D828 T829 L830 A831 D832 A833 V834 L835 I836 T837 T838 A839 H840 A841 W842 Q843 H844 Q845 T848 L849 F850 I851 S852 R853 K854 T855 Y856 R857 I858 D859 Q863 M864 A865 I866 T867 V868 D869 V870 E871 V872 A873 S874 D875 T876 P877 H878
P879 A880 R881 I882 G883 L884 Q887 L888 A889 Q890 V891 A892 E893 L898 G899 L900 G901 P902 Q903 E904 N905 Y906 P907 D908 R909 L910 T911 A912 A913 C914 F915 D916 R917 W918 D919 L920 P921 L922 S923 D924 T927 P928 Y929 V930 F931 E934 N935 G936 L937 R938 C939 G940 T941 R942 E943 L944
N945 Y946 G947 P948 H949 Q950 W951 R952 G953 Q956 F957 N958 I959 S960 R961 Y962 S963 Q966 L967 M968 E969 T970 S971 H972 R973 H974 L975 L976 H977 A978 E979 E980 G981 T982 W983 L984 N985 I986 D987 G988 F989 H990 M991 G992 I993 G994 G995 D996 D997 S998 W999 S1000 P1001 S1002 A1005 E1006 F1007
Q1008 L1009 S1010 A1011 G1012 R1013 Y1014 H1015 Y1016 Q1017 L1018 V1019 W1020 C1021 Q1022 K1023

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, D2	Depositor
Number of particles used	11726	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	Individual frames of each movie were aligned by cross-correlation using the cumulative average of previously aligned frames as a reference to align the remaining frames. Parameters of the contrast transfer function for each micrograph were estimated from power spectra obtained using periodogram averaging with tiles of size 512x512 pixels extracted from all frames of each movie. These power spectra were then radially averaged and used to estimate the defocus for each image using frequencies in the 15.0-3.0 Angstrom range. CTF correction was done for each particle as implemented in FREALIGN's reconstruction protocol.	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	45	Depositor
Minimum defocus (nm)	1000	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	105000	Depositor
Image detector	GATAN K2 (4k x 4k)	Depositor
Maximum map value	0.088	Depositor
Minimum map value	-0.051	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.006	Depositor
Recommended contour level	0.0224	Depositor
Map size (Å)	216.75, 216.75, 216.75	wwPDB
Map dimensions	340, 340, 340	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.6375, 0.6375, 0.6375	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	0.26	0/8448	0.47	2/11526 (0.0%)
1	B	0.26	1/8448 (0.0%)	0.47	2/11526 (0.0%)
1	C	0.26	0/8448	0.47	2/11526 (0.0%)
1	D	0.26	0/8448	0.47	2/11526 (0.0%)
All	All	0.26	1/33792 (0.0%)	0.47	8/46104 (0.0%)

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	306	PRO	N-CD	5.03	1.54	1.47

The worst 5 of 8 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	305	ILE	C-N-CD	5.72	140.41	128.40
1	C	305	ILE	C-N-CD	5.72	140.41	128.40
1	D	305	ILE	C-N-CD	5.71	140.39	128.40
1	B	305	ILE	C-N-CD	5.71	140.39	128.40
1	B	110	ASN	C-N-CD	5.11	139.14	128.40

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	8206	0	7791	2164	0
1	B	8206	0	7791	2165	0
1	C	8206	0	7791	2156	0
1	D	8206	0	7791	2161	0
2	A	1	0	0	0	0
2	B	1	0	0	0	0
2	C	1	0	0	0	0
2	D	1	0	0	0	0
All	All	32828	0	31164	8379	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 131.

The worst 5 of 8379 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:C:427:THR:HA	1:C:436:MET:CE	1.21	1.67
1:D:427:THR:HA	1:D:436:MET:CE	1.21	1.67
1:C:159:VAL:HG22	1:C:176:PHE:CE1	1.25	1.64
1:D:159:VAL:HG22	1:D:176:PHE:CE1	1.25	1.64
1:A:159:VAL:HG22	1:A:176:PHE:CE1	1.25	1.63

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	1020/1024 (100%)	993 (97%)	25 (2%)	2 (0%)	47 79
1	B	1020/1024 (100%)	993 (97%)	25 (2%)	2 (0%)	47 79
1	C	1020/1024 (100%)	993 (97%)	25 (2%)	2 (0%)	47 79
1	D	1020/1024 (100%)	993 (97%)	25 (2%)	2 (0%)	47 79

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
All	All	4080/4096 (100%)	3972 (97%)	100 (2%)	8 (0%)	50 79

5 of 8 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	511	PRO
1	B	511	PRO
1	C	511	PRO
1	D	511	PRO
1	A	688	PRO

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	874/876 (100%)	797 (91%)	77 (9%)	10 36
1	B	874/876 (100%)	797 (91%)	77 (9%)	10 36
1	C	874/876 (100%)	797 (91%)	77 (9%)	10 36
1	D	874/876 (100%)	797 (91%)	77 (9%)	10 36
All	All	3496/3504 (100%)	3188 (91%)	308 (9%)	13 36

5 of 308 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	D	13	ARG
1	D	667	GLU
1	D	118	ASN
1	D	274	PHE
1	D	910	LEU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 160 such sidechains are listed below:

Mol	Chain	Res	Type
1	C	905	ASN
1	D	646	HIS
1	C	1022	GLN
1	D	266	GLN
1	D	815	HIS

5.3.3 RNA

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains

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

5.5 Carbohydrates

There are no monosaccharides in this entry.

5.6 Ligand geometry

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

There are no such residues in this entry.

5.8 Polymer linkage issues

There are no chain breaks in this entry.

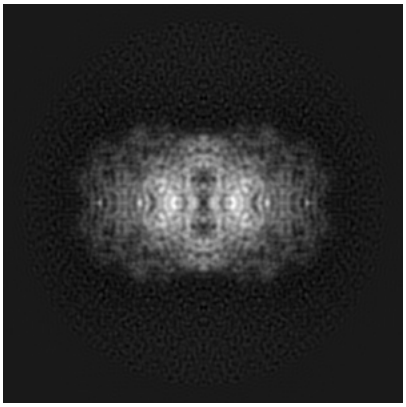
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-5995. These allow visual inspection of the internal detail of the map and identification of artifacts.

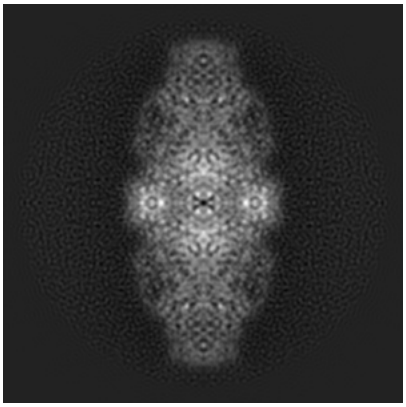
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections

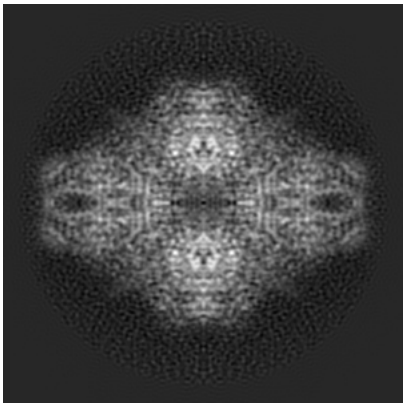
6.1.1 Primary map



X



Y

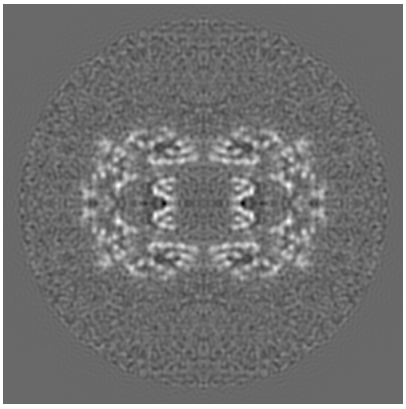


Z

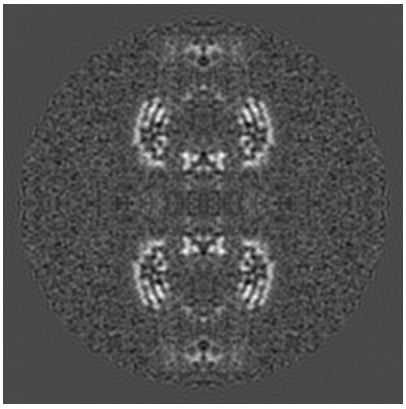
The images above show the map projected in three orthogonal directions.

6.2 Central slices

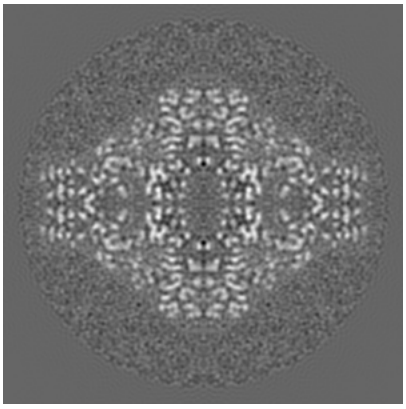
6.2.1 Primary map



X Index: 170



Y Index: 170

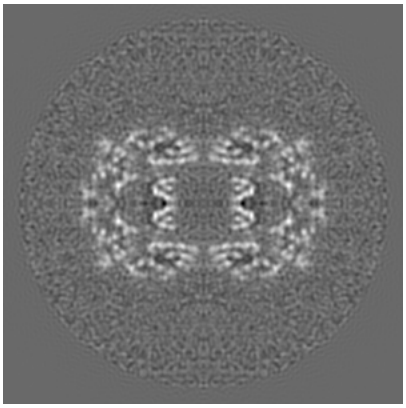


Z Index: 170

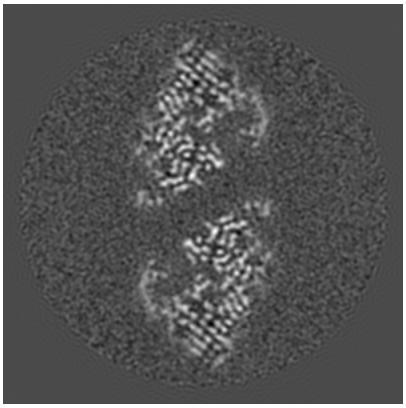
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

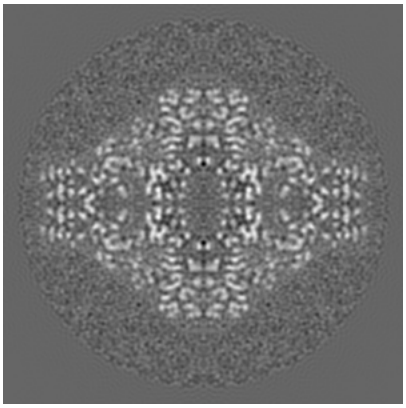
6.3.1 Primary map



X Index: 170



Y Index: 183

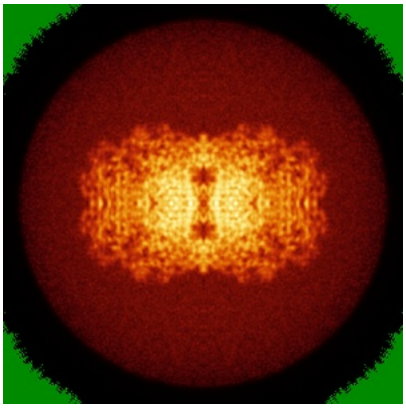


Z Index: 170

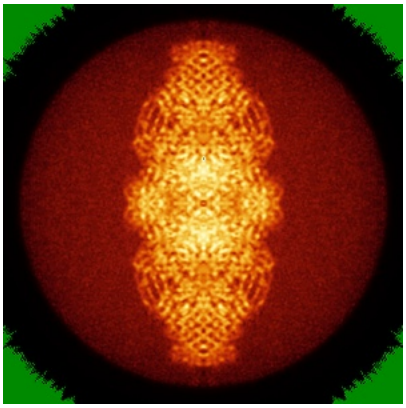
The images above show the largest variance slices of the map in three orthogonal directions.

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

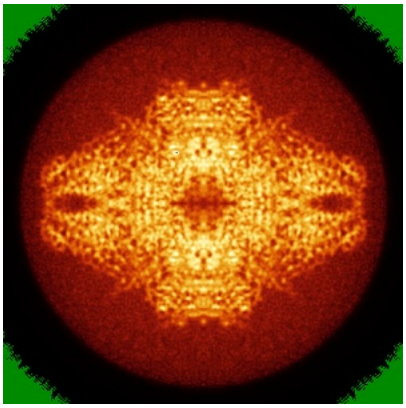
6.4.1 Primary map



X



Y



Z

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 0.0224. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

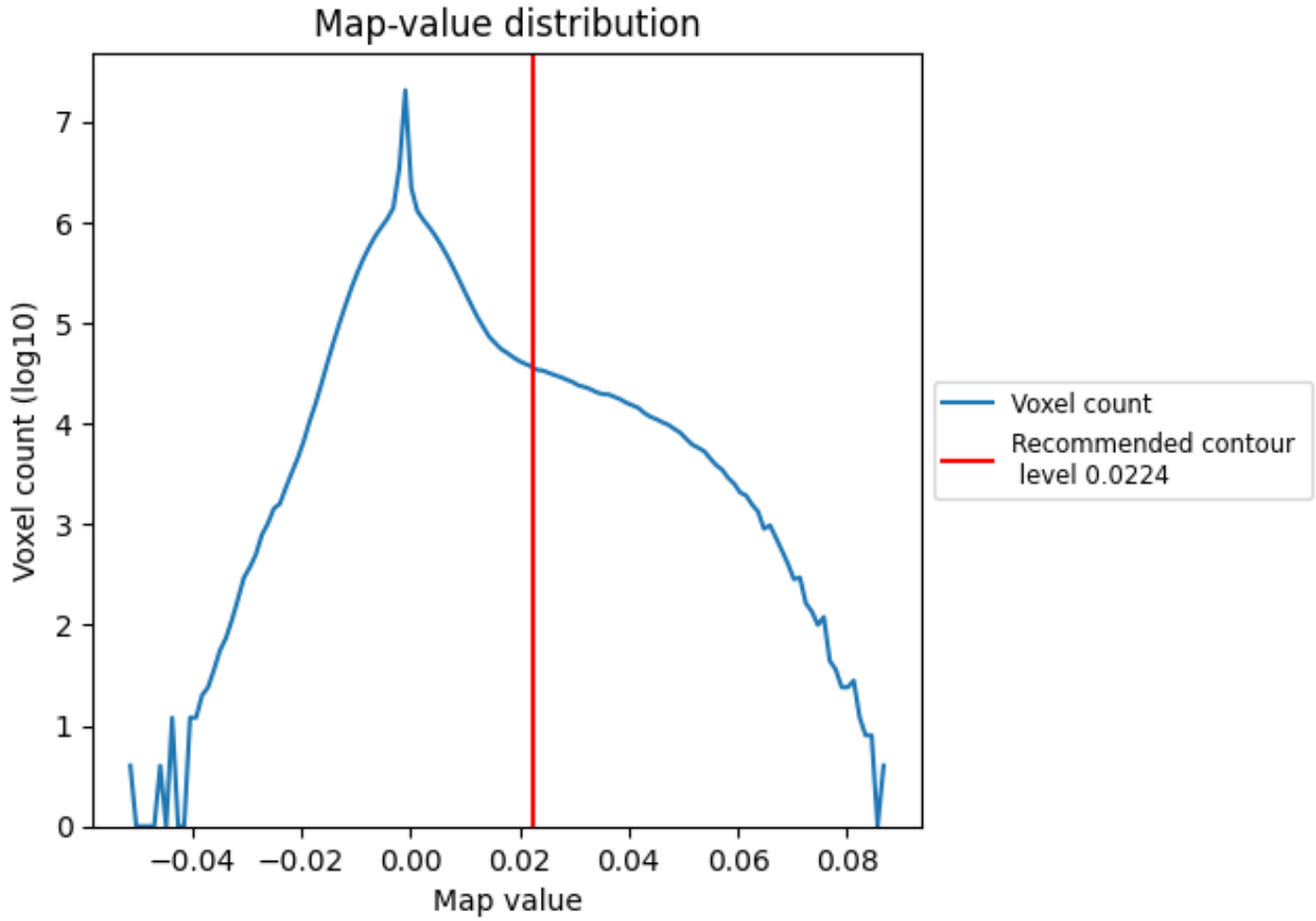
6.6 Mask visualisation (i)

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

7 Map analysis

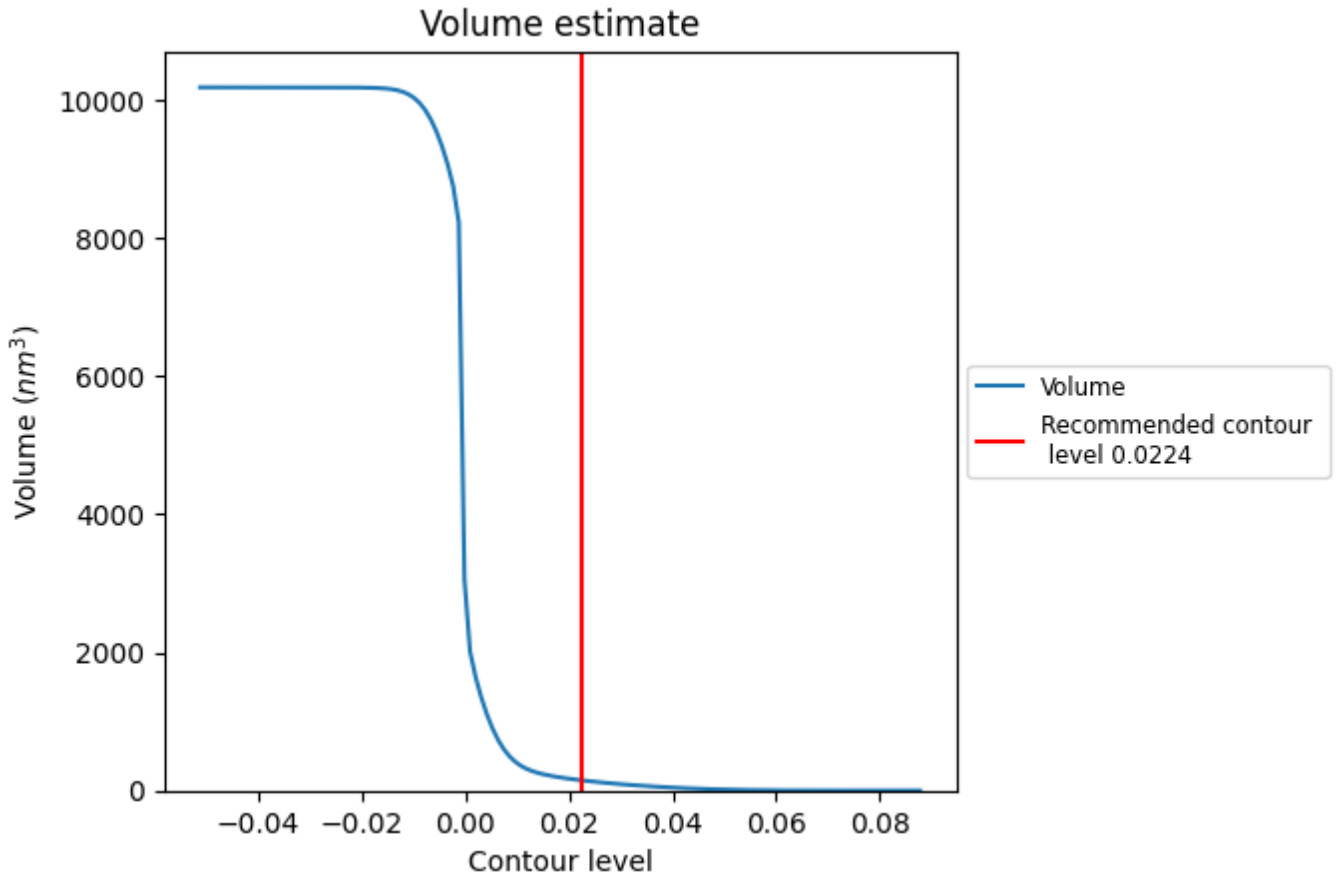
This section contains the results of statistical analysis of the map.

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.

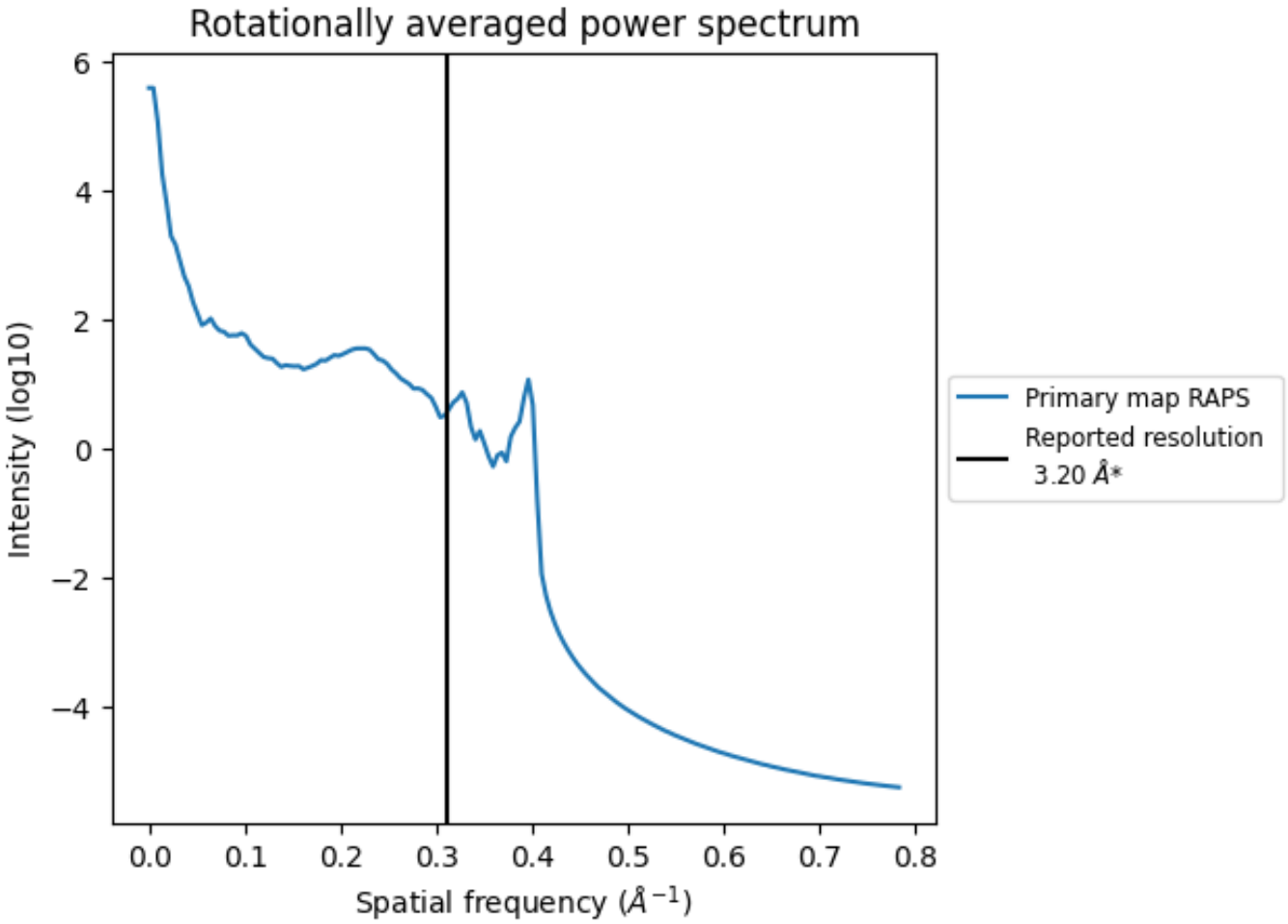
7.2 Volume estimate (i)



The volume at the recommended contour level is 148 nm^3; this corresponds to an approximate mass of 134 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)

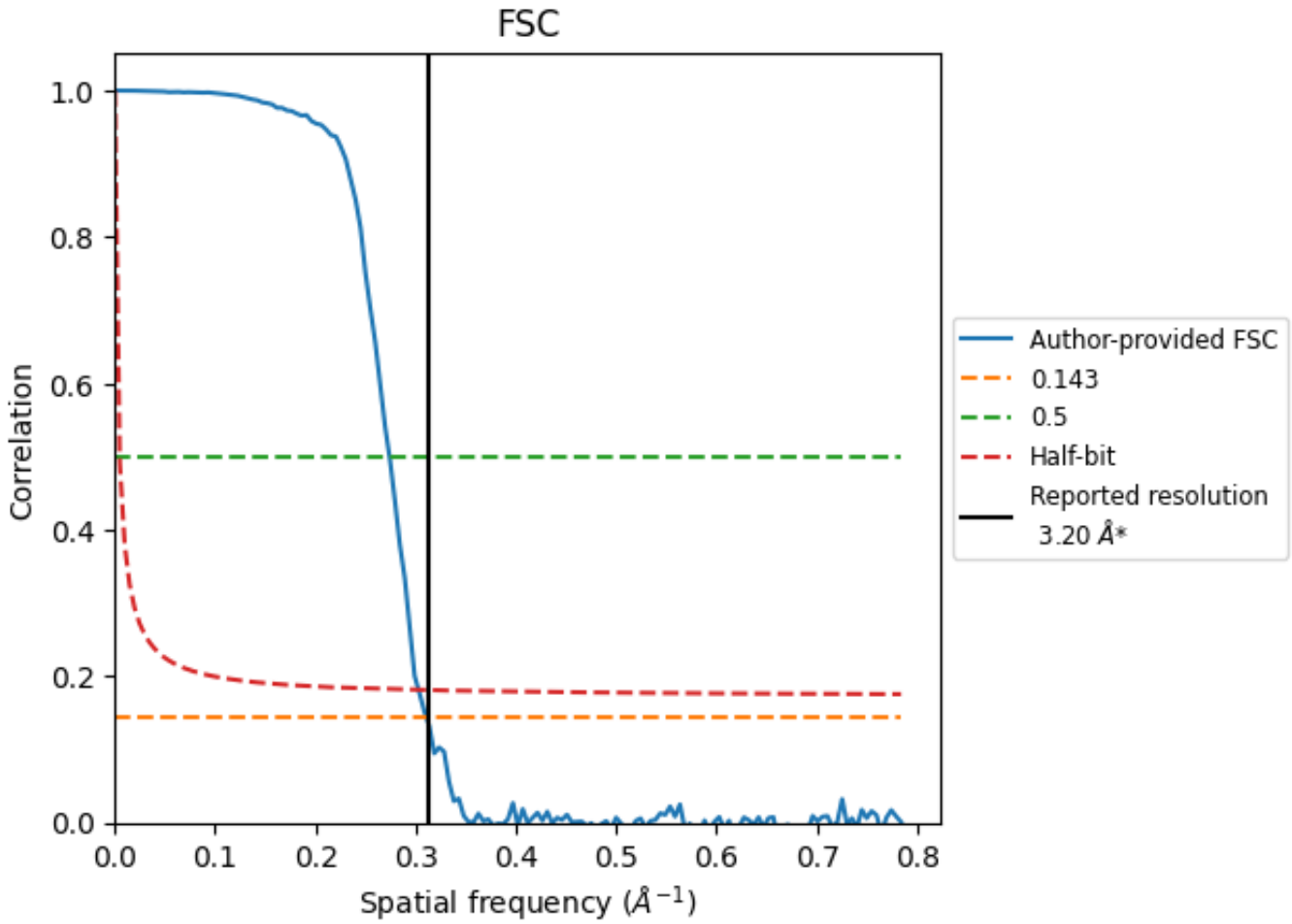


*Reported resolution corresponds to spatial frequency of 0.312 Å^{-1}

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 0.312 Å^{-1}

8.2 Resolution estimates (i)

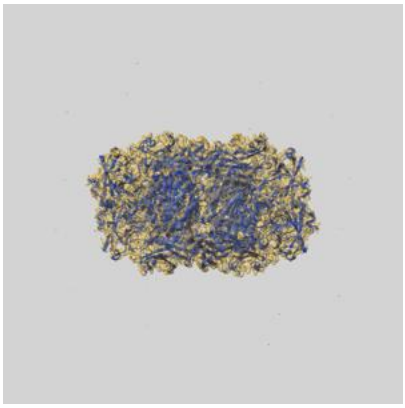
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.20	-	-
Author-provided FSC curve	3.22	3.65	3.30
Unmasked-calculated*	-	-	-

*Resolution estimate based on FSC curve calculated by comparison of deposited half-maps.

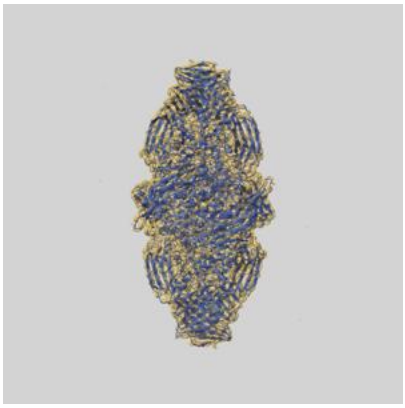
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-5995 and PDB model 3J7H. Per-residue inclusion information can be found in section 3 on page 4.

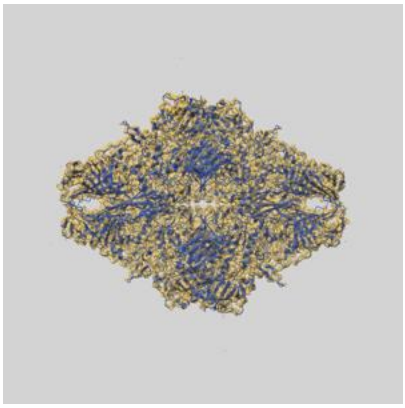
9.1 Map-model overlay



X



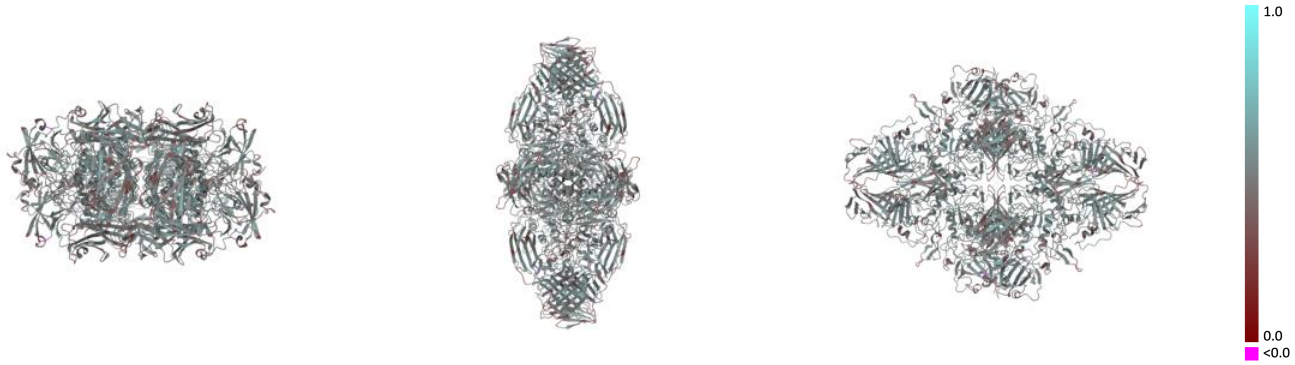
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.0224 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.

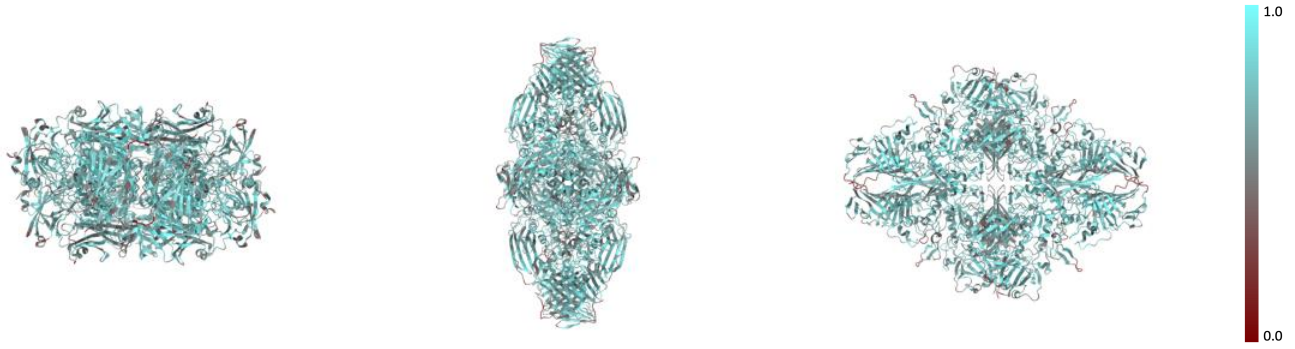
9.2 Q-score mapped to coordinate model



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.

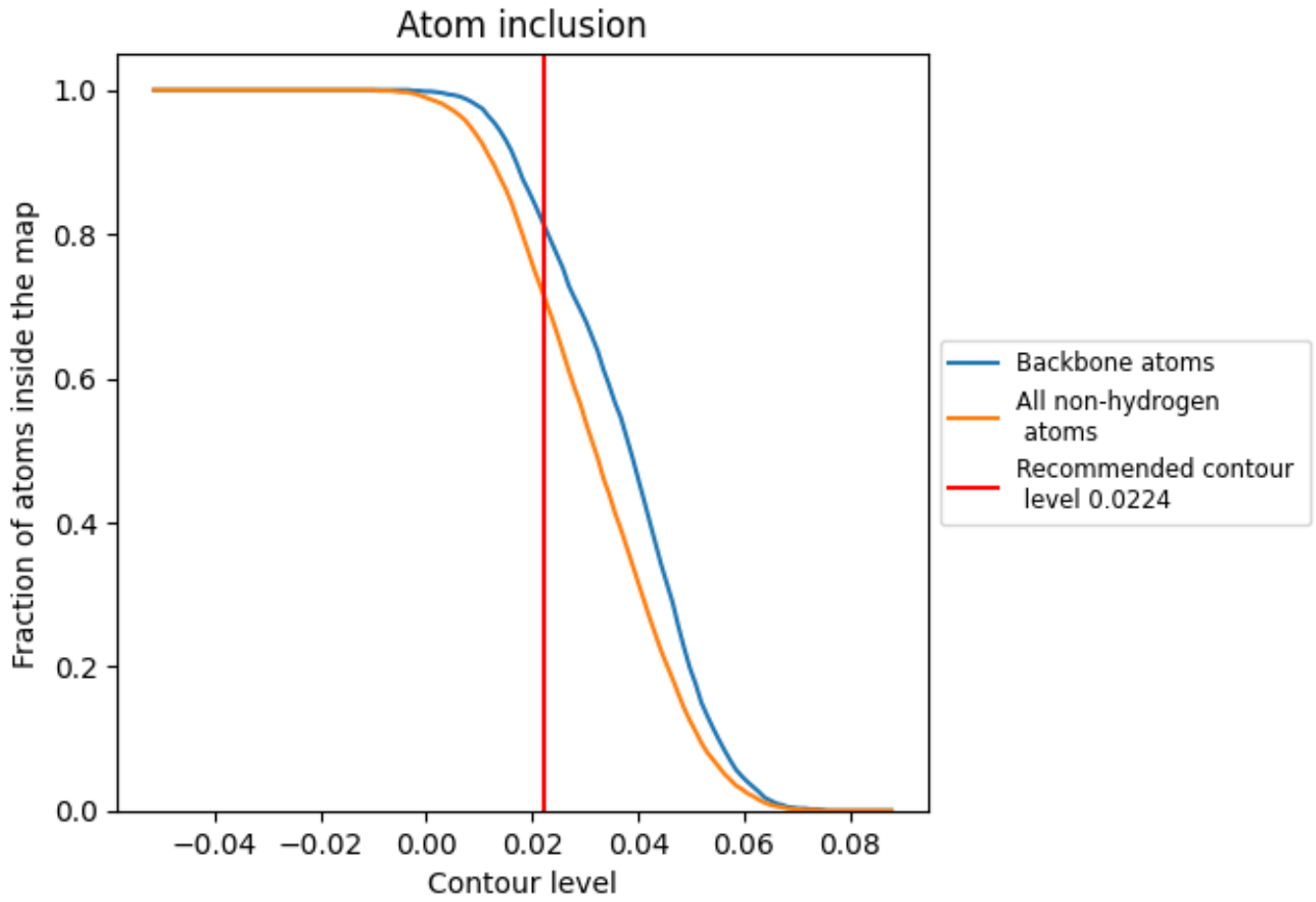
9.3 Atom inclusion mapped to coordinate model



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 (0.0224).

9.4 Atom inclusion (i)



At the recommended contour level, 81% of all backbone atoms, 71% 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 (0.0224) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.7100	0.4920
A	0.7100	0.4930
B	0.7100	0.4920
C	0.7100	0.4920
D	0.7100	0.4920