wwPDB EM Validation Summary Report ⓘ

Oct 2, 2023 – 06:22 PM JST

PDB ID	:	8HW2
EMDB ID	:	EMD-35049
Title	:	Cryo-EM structure of beta-estradiol 17-(beta-D-glucuronide)-bound human ABC transporter ABCC3 in nanodiscs
Authors	:	Wang, J.; Wang, F.F.; Chen, Y.X.; Zhou, C.Z.
Deposited on	:	2022-12-28
Resolution	:	3.65 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:

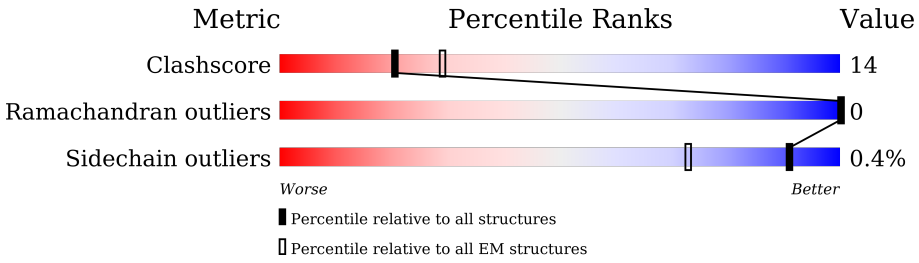
EMDB validation analysis	:	0.0.1.dev50
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.35.1

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 3.65 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	1527	24% 64% 26% 10%

2 Entry composition

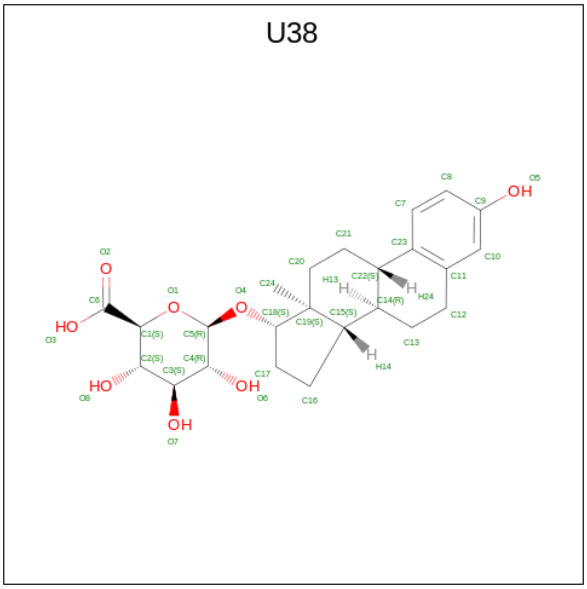
There are 2 unique types of molecules in this entry. The entry contains 10853 atoms, of which 0 are hydrogens and 0 are deuteriums.

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 ATP-binding cassette sub-family C member 3.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	1375	10789	7015	1812	1905	57	0	0

- Molecule 2 is Estradiol-17beta-glucuronide (three-letter code: U38) (formula: $C_{24}H_{32}O_8$) (labeled as "Ligand of Interest" by depositor).



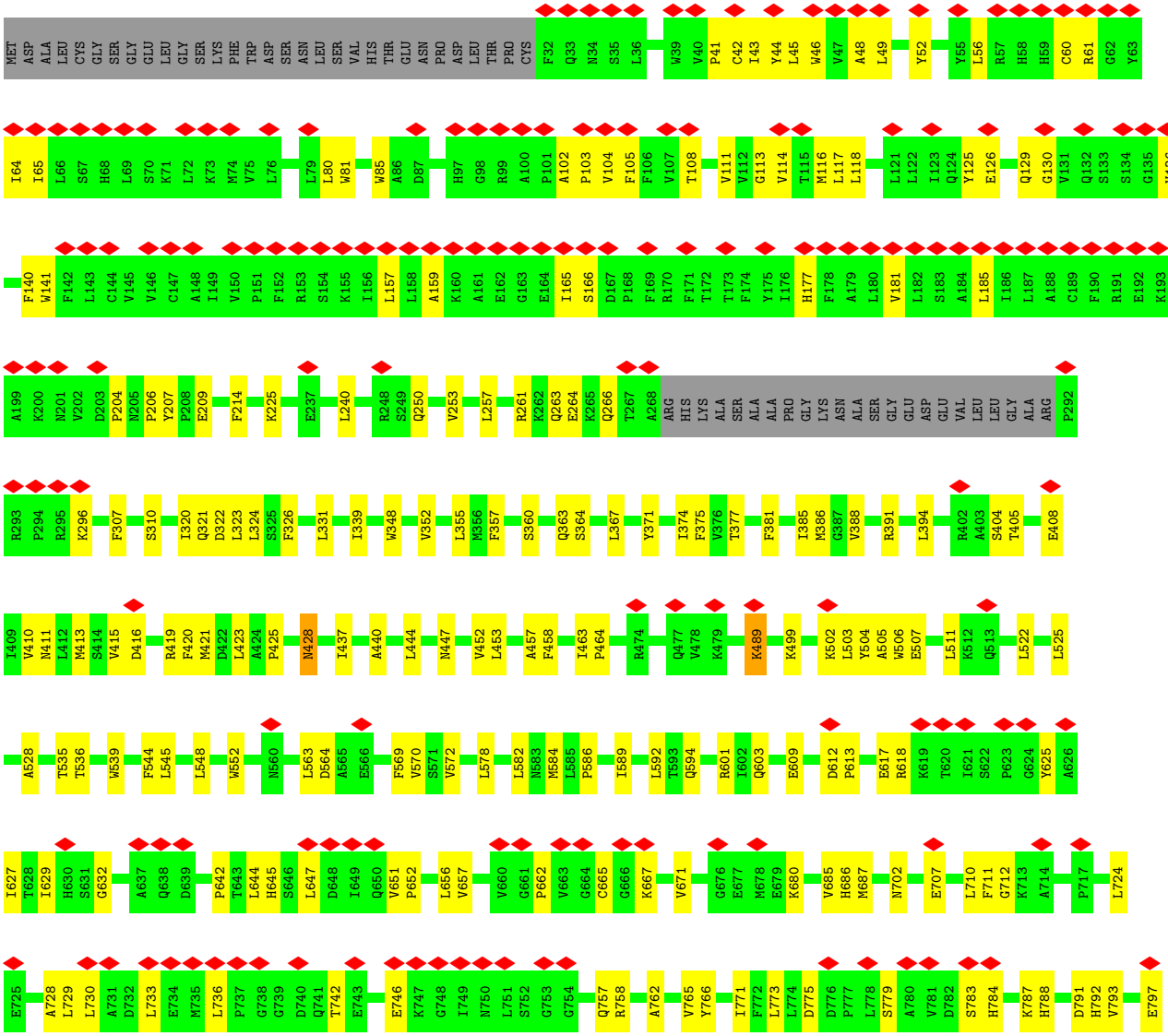
Mol	Chain	Residues	Atoms			AltConf
			Total	C	O	
2	A	1	32	24	8	0
2	A	1	32	24	8	0

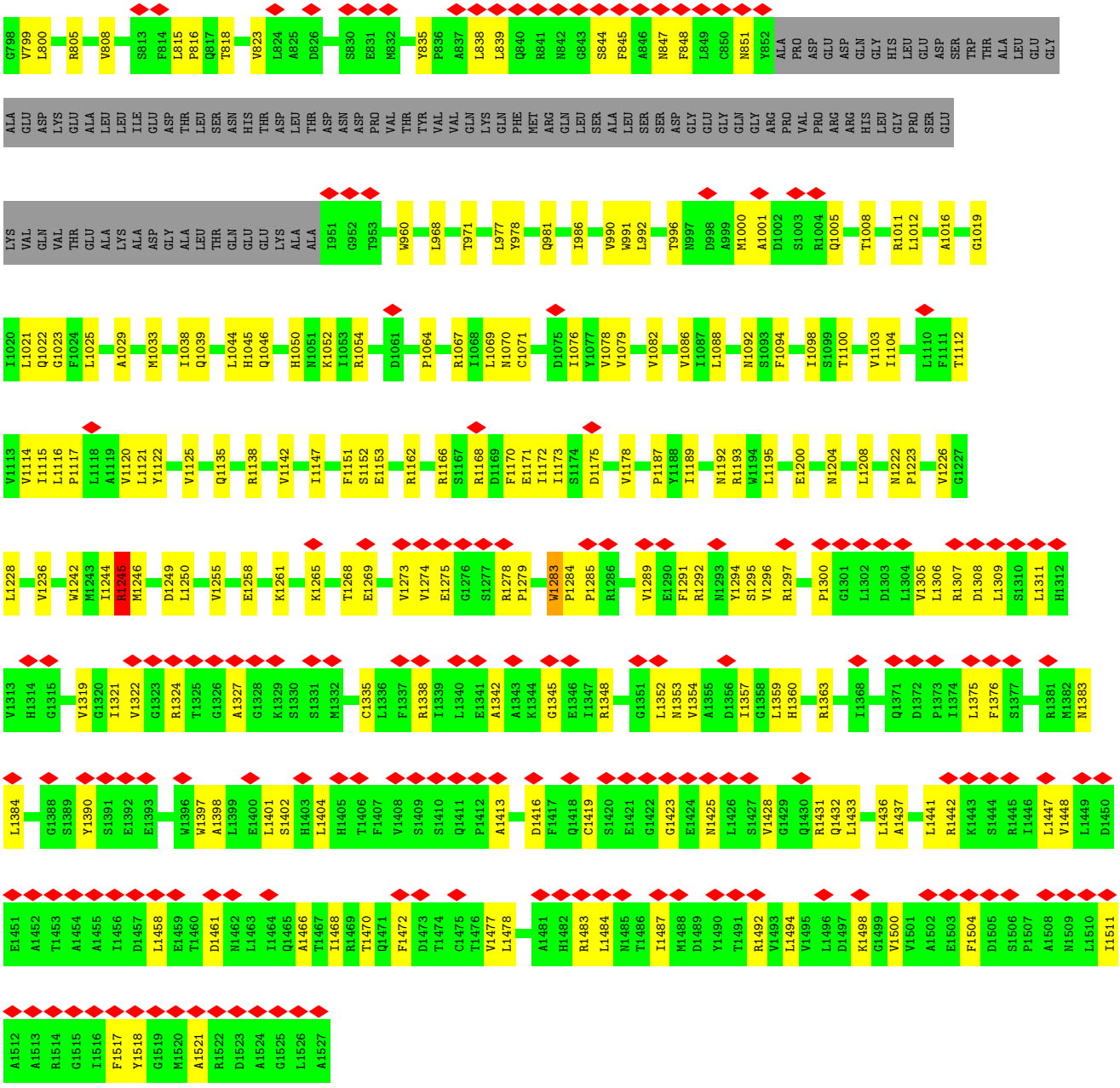
3 Residue-property plots (i)

These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and atom inclusion in map density. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. A red diamond above a residue indicates a poor fit to the EM map for this residue (all-atom inclusion < 40%). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

- Molecule 1: ATP-binding cassette sub-family C member 3

Chain A:




G798 V799 L800 R805 V808 S813 F814 L815 P816 Q817 T818 V823 L824 A825 D826 S830 E831 M832 Y835 P836 A837 L838 L839 Q840 R841 N842 G843 S844 F845 A846 N847 F848 L849 C850 N851 Y852 ALA PRO ASP GLU ASP GLN GLY HIS LEU GLU ASP SER TRP THR ALA LEU GLU GLY
ALA GLU ASP LYS GLU ALA LEU LEU ILE GLU ASP THR LEU SER ASN HIS THR ASP LEU THR ASP ASN ASP PRO VAL THR TYR VAL VAL GLN LYS GLN PHE MET ARG GLN LEU SER ALA LEU SER SER ASP GLY GLU GLY GLN GLY ARG PRO VAL PRO ARG ARG HIS LEU GLY PRO SER GLU
LYS VAL GLN VAL THR GLU ALA LYS ALA ASP GLY ALA LEU THR GLN GLU GLU LYS ALA ALA I951 G952 T953 W960 L968 T971 L977 Y978 Q981 I986 V990 W991 L992 T996 N997 D998 A999 M1000 A1001 D1002 S1003 R1004 Q1005 T1008 R1011 L1012 A1016 G1019
I1020 L1021 Q1022 G1023 F1024 L1025 A1029 M1033 I1038 Q1039 L1044 H1045 Q1046 H1050 N1051 K1052 I1053 R1054 D1061 P1064 R1067 I1068 L1069 N1070 C1071 D1075 I1076 Y1077 V1078 V1079 V1082 V1086 I1087 L1088 N1092 S1093 F1094 I1098 S1099 T1100 V1103 I1104 L1110 F1111 T1112
V1113 V1114 I1115 L1116 P1117 L1118 A1119 V1120 L1121 Y1122 V1125 Q1135 R1138 V1142 I1147 F1151 S1152 E1153 R1162 R1166 S1167 R1168 D1169 F1170 E1171 I1172 I1173 S1174 D1175 V1178 P1187 Y1188 I1189 N1192 R1193 W1194 L1195 E1200 N1204 L1208 N1222 P1223 V1226 G1227
L1228 V1236 W1242 M1243 I1244 R1245 M1246 D1249 L1250 V1255 E1258 K1261 K1265 T1268 E1269 V1273 V1274 E1275 G1276 S1277 R1278 P1279 W1283 P1284 P1285 R1286 V1289 E1290 F1291 R1292 N1293 Y1294 S1295 V1296 R1297 P1300 G1301 L1302 D1303 L1304 V1305 L1306 R1307 D1308 L1309 S1310 L1311 H1312
V1313 H1314 G1315 V1319 G1320 T1321 V1322 G1323 R1324 T1325 G1326 A1327 G1328 K1329 S1330 S1331 M1332 C1335 L1336 F1337 R1338 I1339 L1340 E1341 A1342 A1343 K1344 G1345 E1346 I1347 R1348 G1351 L1352 N1353 V1354 A1355 D1356 I1357 G1358 L1359 H1360 R1363 I1368 Q1371 D1372 P1373 I1374 L1375 F1376 S1377 R1381 M1382 N1383
L1384 G1388 S1389 Y1390 S1391 E1392 E1393 W1396 W1397 A1398 L1399 E1400 L1401 S1402 H1403 L1404 H1405 T1406 F1407 V1408 S1409 S1410 Q1411 P1412 A1413 D1416 F1417 Q1418 C1419 S1420 E1421 G1422 G1423 E1424 N1425 L1426 S1427 V1428 G1429 Q1430 R1431 Q1432 L1433 L1436 A1437 L1441 R1442 K1443 S1444 R1445 I1446 L1447 V1448 L1449 D1450
E1451 A1452 T1453 A1454 A1455 I1456 D1457 L1458 E1459 T1460 D1461 N1462 L1463 I1464 Q1465 A1466 T1467 I1468 R1469 T1470 Q1471 F1472 D1473 T1474 C1475 T1476 V1477 L1478 A1481 H1482 R1483 L1484 N1485 T1486 I1487 M1488 D1489 Y1490 T1491 R1492 V1493 L1494 V1495 L1496 D1497 K1498 G1499 V1500 V1501 A1502 E1503 F1504 D1505 S1506 P1507 A1508 N1509 L1510 I1511
A1512 A1513 R1514 G1515 I1516 F1517 Y1518 G1519 M1520 A1521 R1522 D1523 A1524 G1525 L1526 A1527

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	177500	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING AND AMPLITUDE CORRECTION	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	54	Depositor
Minimum defocus (nm)	1500	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	3.165	Depositor
Minimum map value	-2.215	Depositor
Average map value	0.003	Depositor
Map value standard deviation	0.059	Depositor
Recommended contour level	0.447	Depositor
Map size (Å)	273.92, 273.92, 273.92	wwPDB
Map dimensions	256, 256, 256	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.07, 1.07, 1.07	Depositor

5 Model quality

5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: U38

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	0.27	0/11041	0.48	0/15014

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand.A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
1	A	0	2

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	1245	ARG	Sidechain
1	A	1283	TRP	Peptide

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	10789	0	11029	296	0

Continued on next page...

Continued from previous page...

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
2	A	64	0	0	24	0
All	All	10853	0	11029	296	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 14.

The worst 5 of 296 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:584:MET:CE	2:A:1601:U38:C12	1.97	1.42
1:A:584:MET:HE2	2:A:1601:U38:C12	1.57	1.21
1:A:584:MET:HE3	2:A:1601:U38:C12	1.66	1.15
1:A:584:MET:CE	2:A:1601:U38:C13	2.28	1.09
1:A:584:MET:HE2	2:A:1601:U38:C13	1.90	1.00

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	1369/1527 (90%)	1271 (93%)	98 (7%)	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	1176/1299 (90%)	1171 (100%)	5 (0%)	91 95

All (5) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	177	HIS
1	A	428	ASN
1	A	489	LYS
1	A	680	LYS
1	A	1245	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (5) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	263	GLN
1	A	368	GLN
1	A	1092	ASN
1	A	1312	HIS
1	A	1314	HIS

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)

2 ligands are modelled in this entry.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and

the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. 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 $|Z| > 2$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Type	Chain	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\#\|Z\| > 2$	Counts	RMSZ	$\#\|Z\| > 2$
2	U38	A	1601	-	36,36,36	5.37	14 (38%)	55,56,56	2.80	21 (38%)
2	U38	A	1602	-	36,36,36	5.38	14 (38%)	55,56,56	2.52	15 (27%)

In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	U38	A	1601	-	-	2/8/68/68	0/5/5/5
2	U38	A	1602	-	-	2/8/68/68	0/5/5/5

The worst 5 of 28 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	A	1602	U38	C7-C23	16.11	1.60	1.39
2	A	1601	U38	C7-C23	16.03	1.60	1.39
2	A	1602	U38	C10-C11	12.47	1.60	1.39
2	A	1601	U38	C10-C11	12.39	1.60	1.39
2	A	1601	U38	C22-C14	11.78	1.68	1.54

The worst 5 of 36 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	A	1601	U38	C20-C21-C22	9.35	124.91	112.33
2	A	1602	U38	C20-C21-C22	9.16	124.67	112.33
2	A	1601	U38	C10-C11-C23	-6.50	111.08	119.50
2	A	1602	U38	C10-C11-C23	-6.38	111.22	119.50
2	A	1601	U38	C23-C22-C14	-5.85	104.46	111.58

There are no chirality outliers.

All (4) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	1602	U38	O1-C5-O4-C18

Continued on next page...

Continued from previous page...

Mol	Chain	Res	Type	Atoms
2	A	1602	U38	C4-C5-O4-C18
2	A	1601	U38	O1-C5-O4-C18
2	A	1601	U38	C17-C18-O4-C5

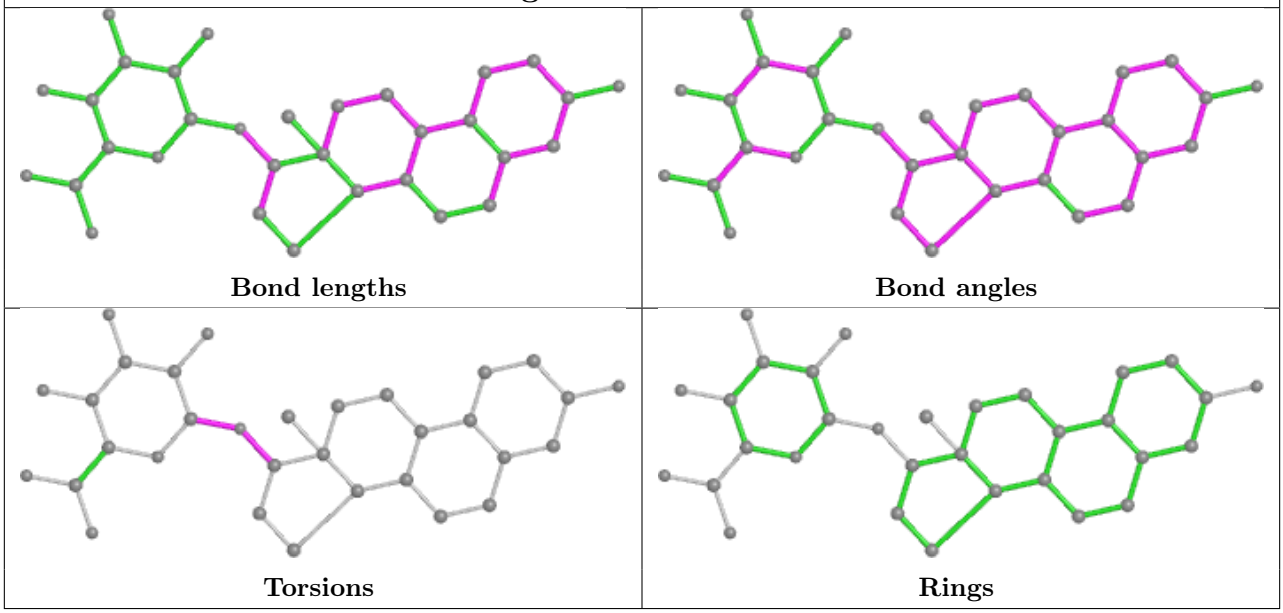
There are no ring outliers.

2 monomers are involved in 24 short contacts:

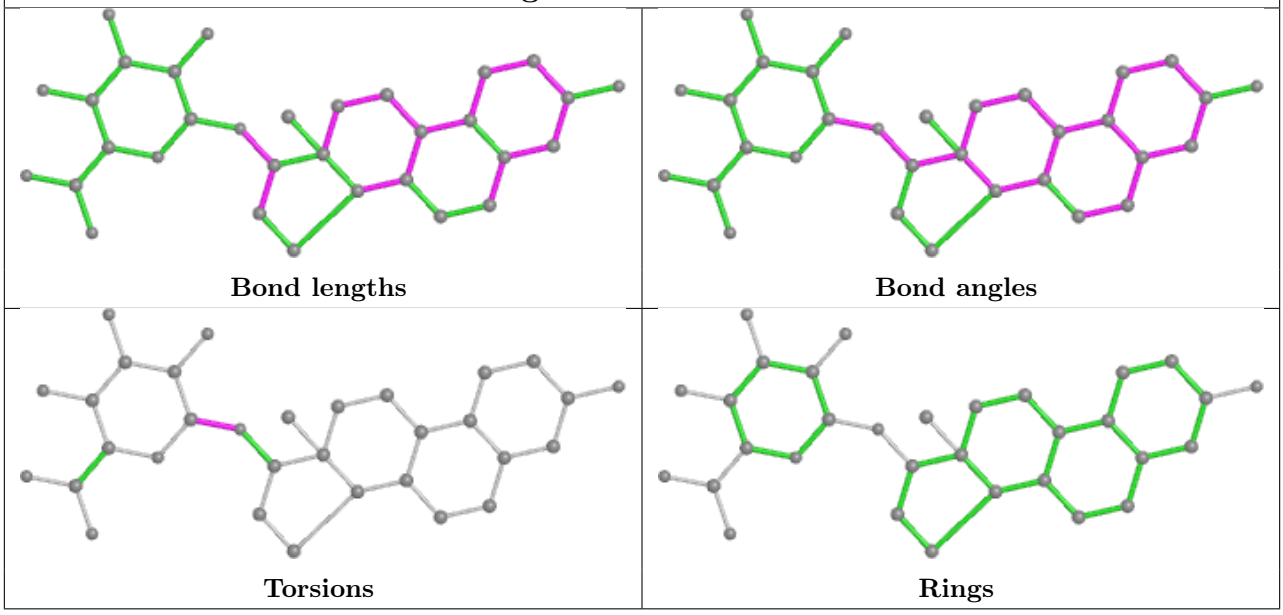
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	1601	U38	12	0
2	A	1602	U38	12	0

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.

Ligand U38 A 1601



Ligand U38 A 1602



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.

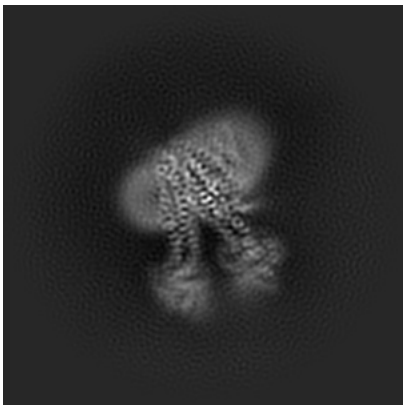
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-35049. These allow visual inspection of the internal detail of the map and identification of artifacts.

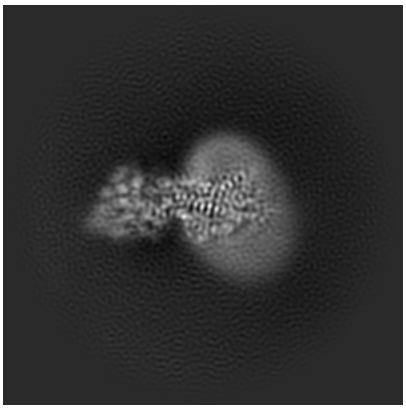
Images derived from a raw map, generated by summing the deposited half-maps, are presented below the corresponding image components of the primary map to allow further visual inspection and comparison with those of the primary map.

6.1 Orthogonal projections

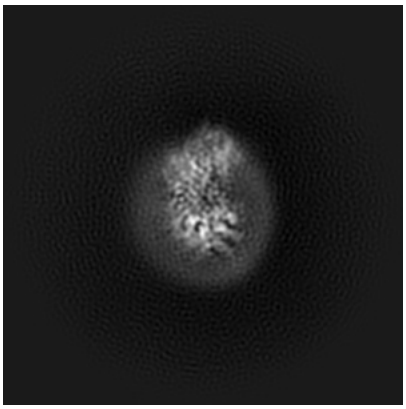
6.1.1 Primary map



X

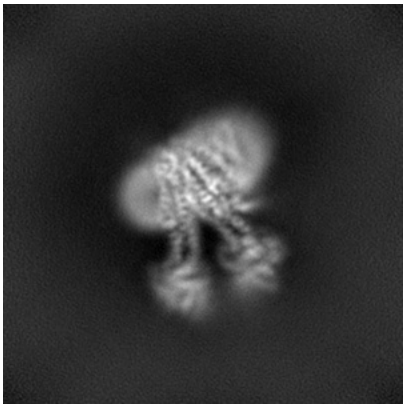


Y

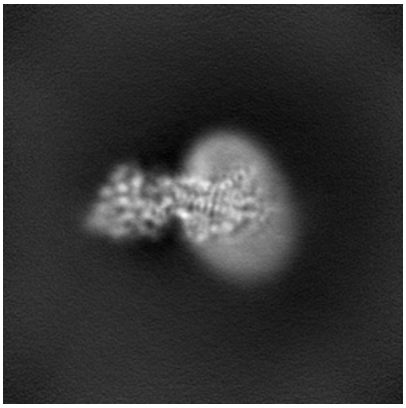


Z

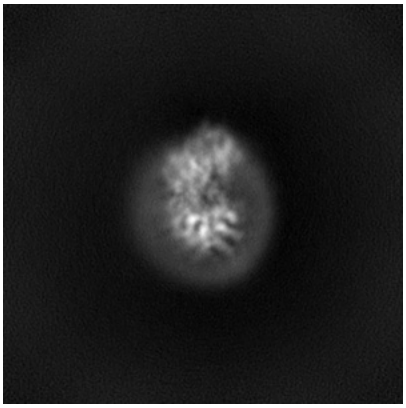
6.1.2 Raw map



X



Y

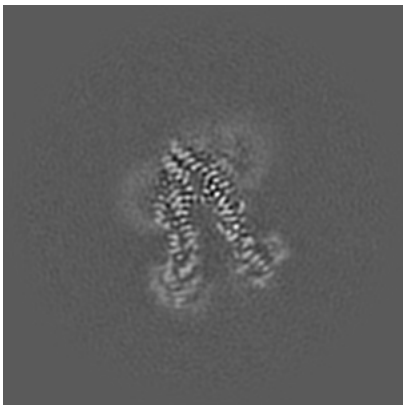


Z

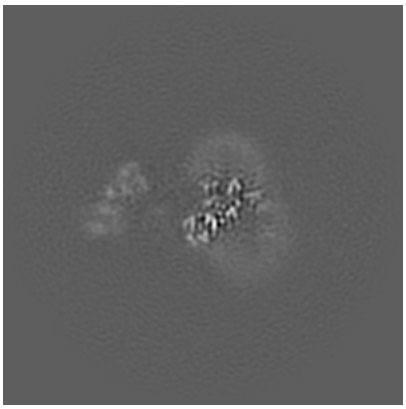
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

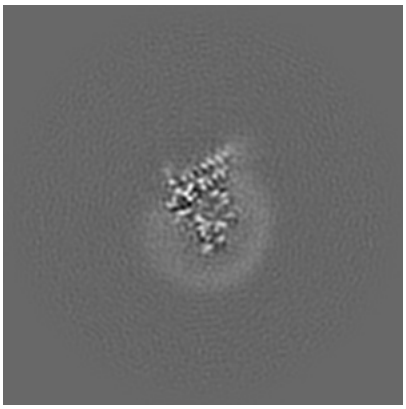
6.2.1 Primary map



X Index: 128

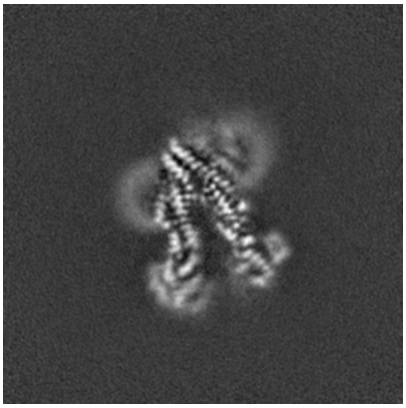


Y Index: 128

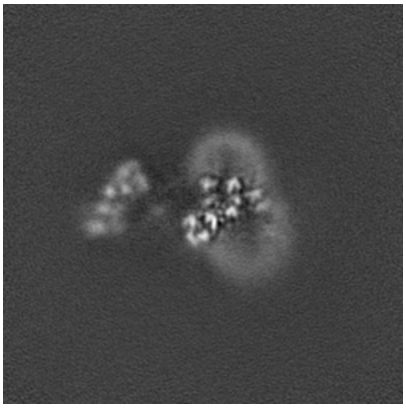


Z Index: 128

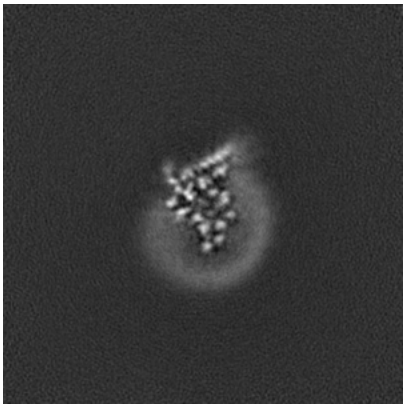
6.2.2 Raw map



X Index: 128



Y Index: 128

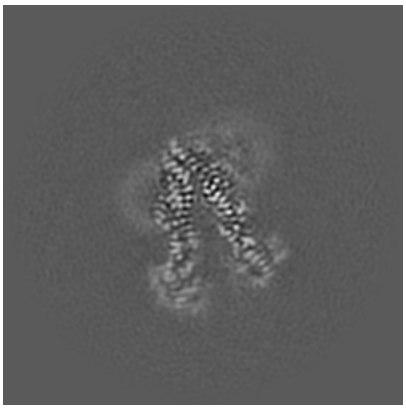


Z Index: 128

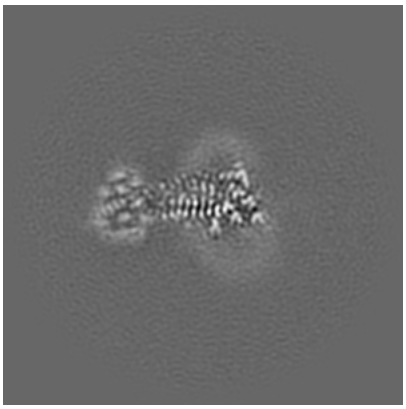
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

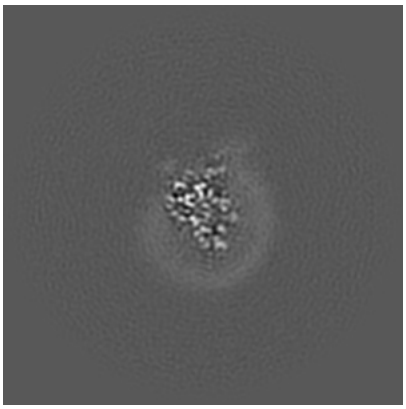
6.3.1 Primary map



X Index: 127

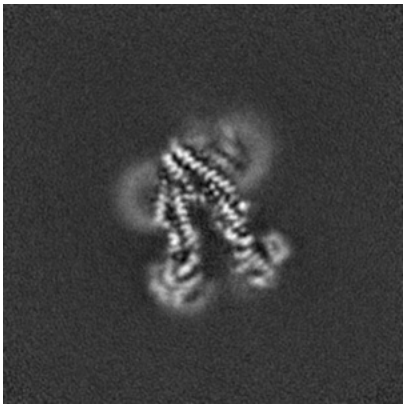


Y Index: 116

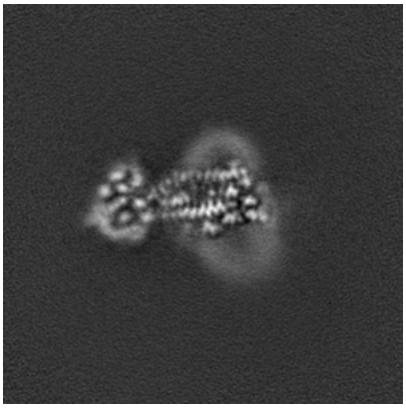


Z Index: 131

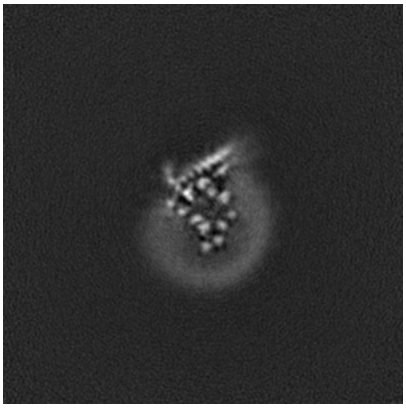
6.3.2 Raw map



X Index: 129



Y Index: 118

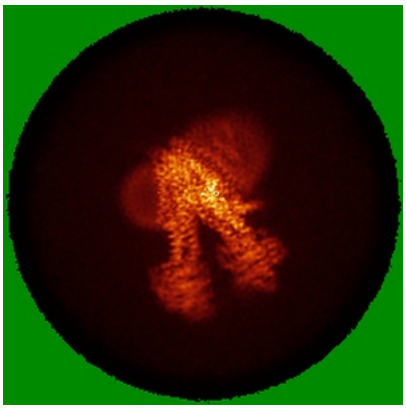


Z Index: 127

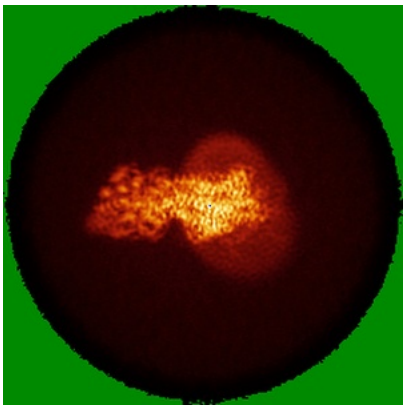
The images above show the largest variance slices of the map in three orthogonal directions.

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

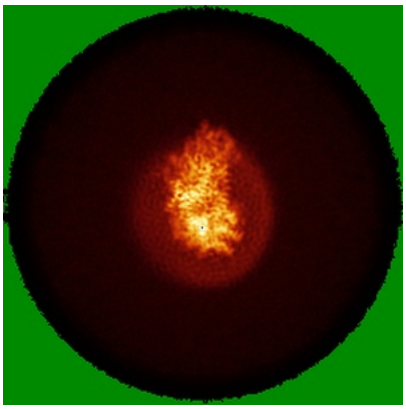
6.4.1 Primary map



X

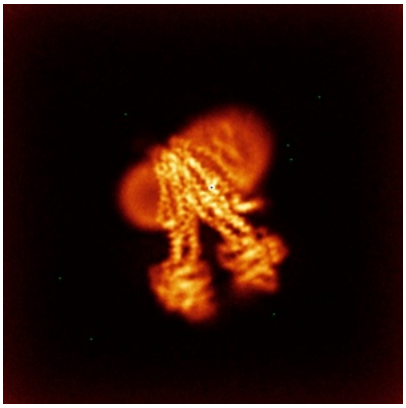


Y

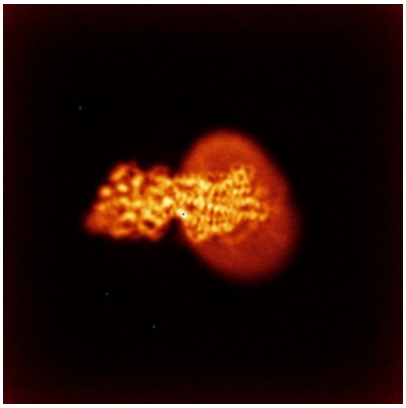


Z

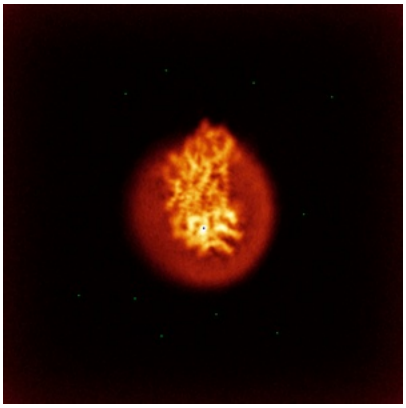
6.4.2 Raw map



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 0.447. 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.

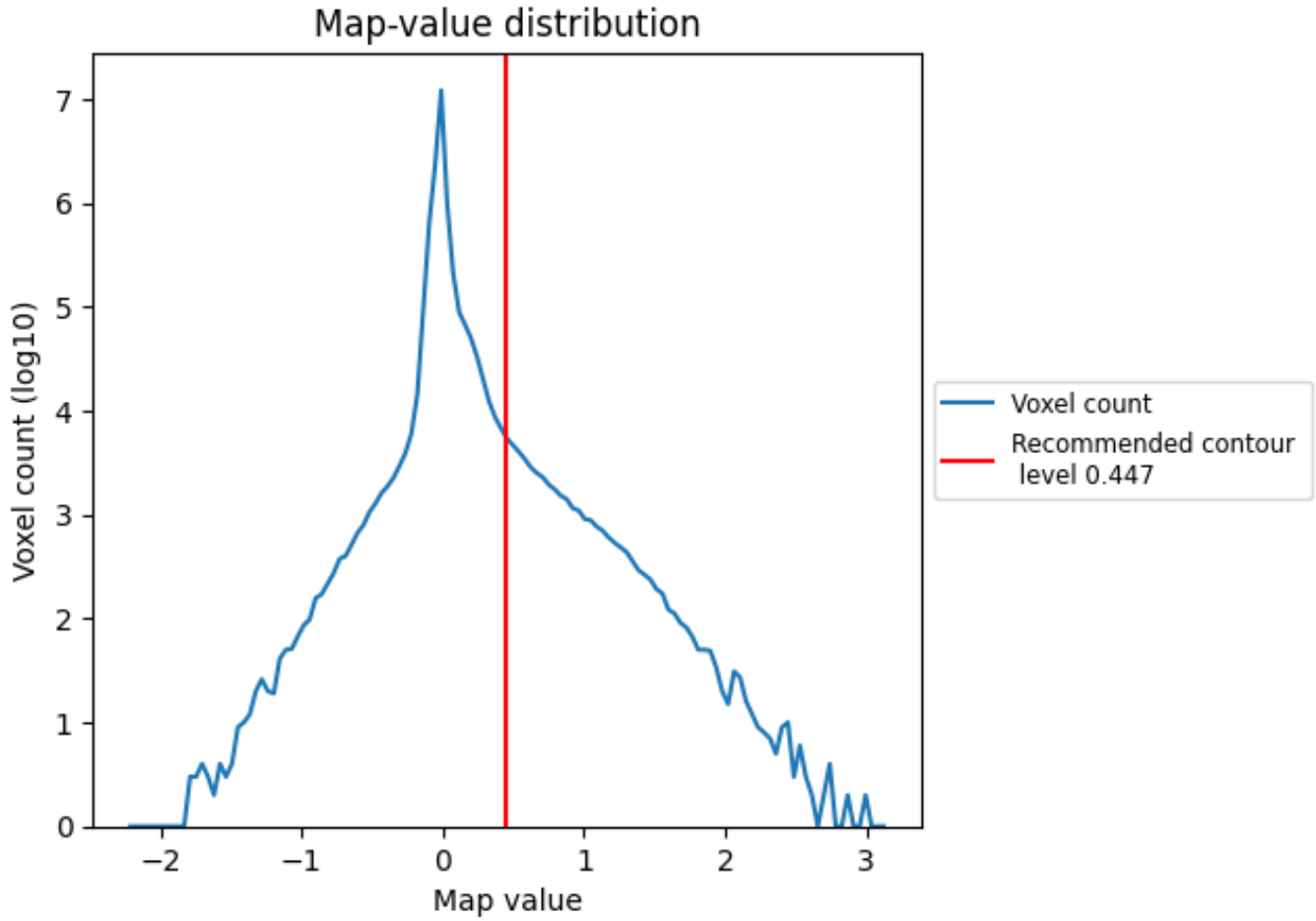
6.6 Mask visualisation (i)

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

7 Map analysis

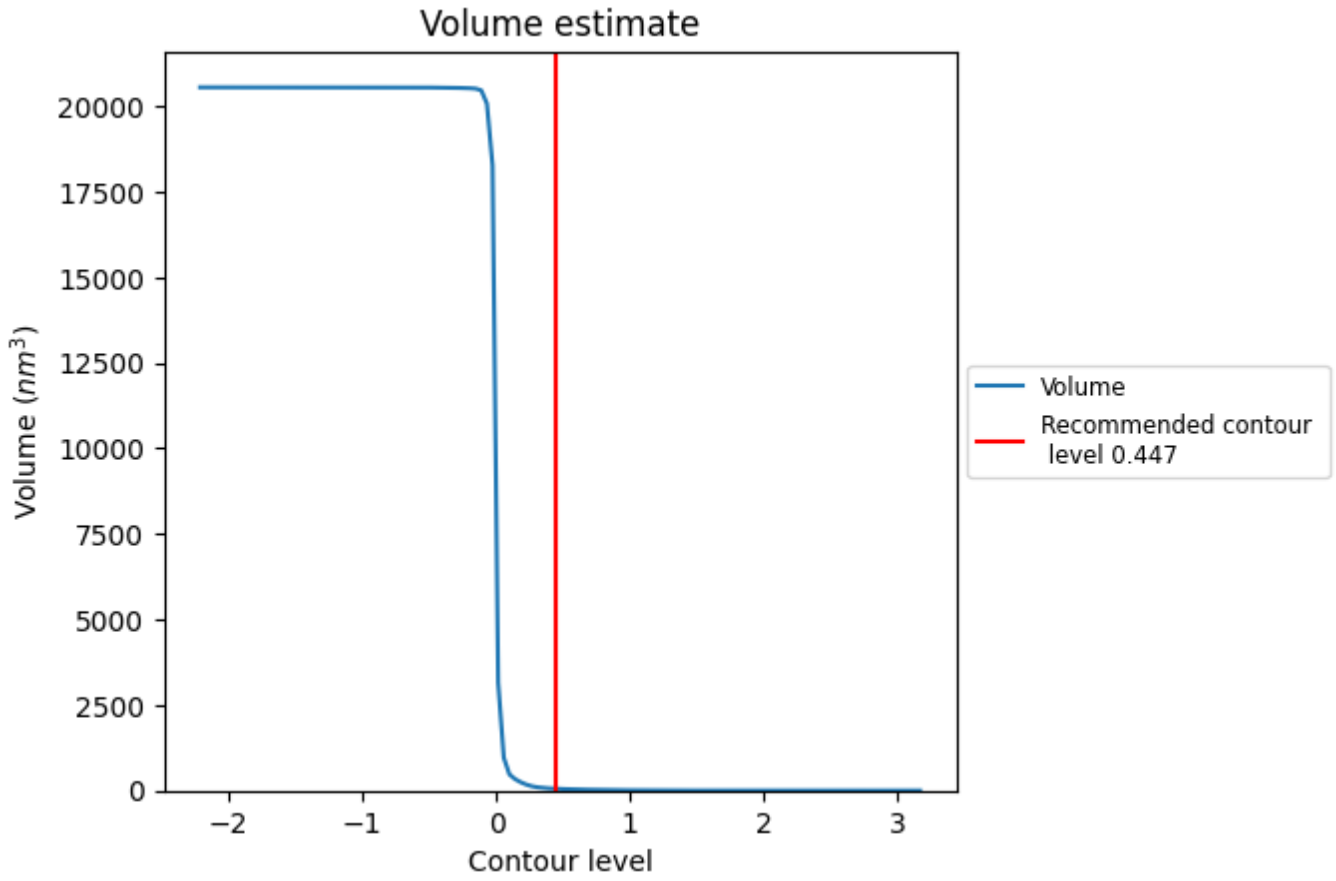
This section contains the results of statistical analysis of the map.

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.

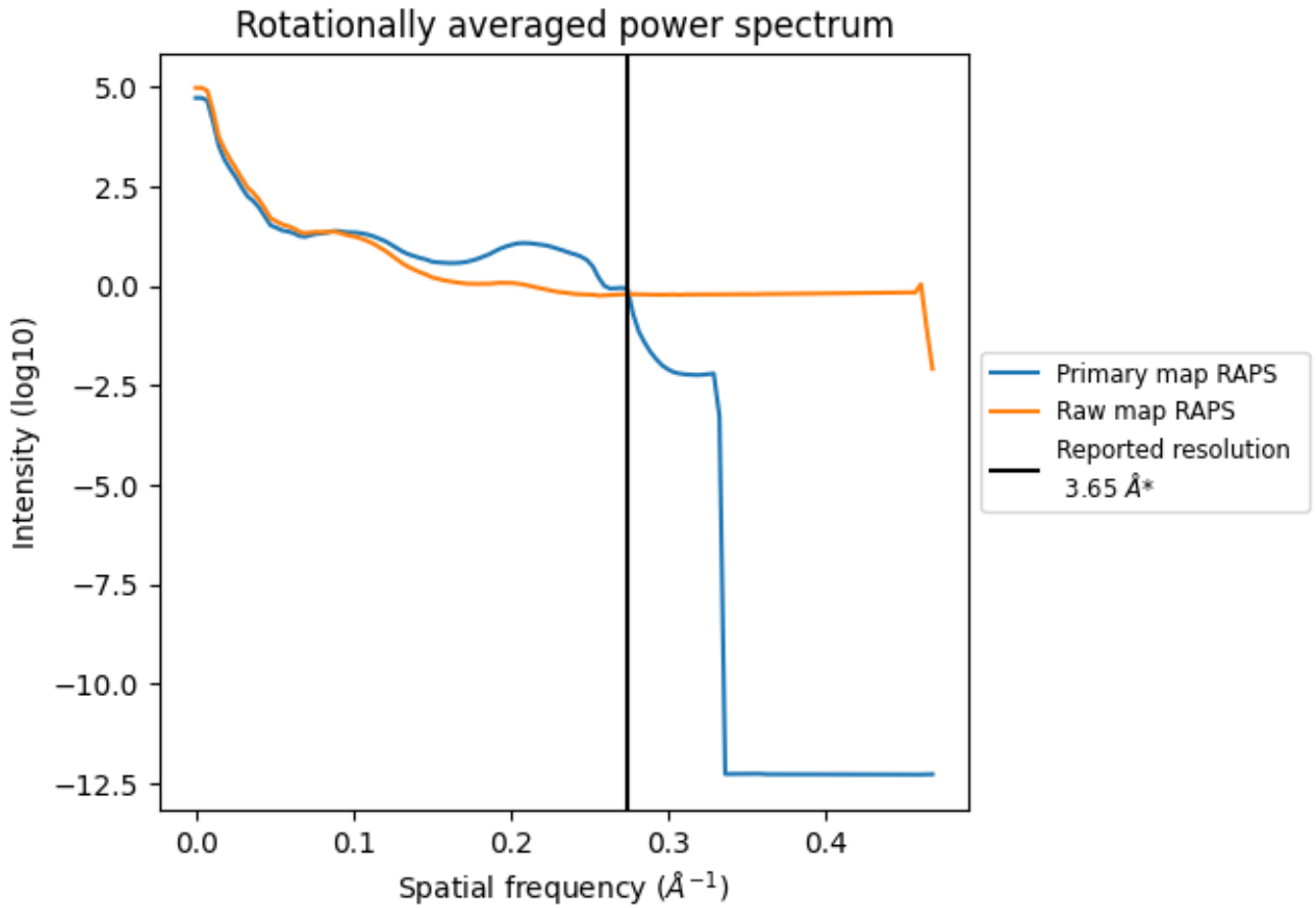
7.2 Volume estimate (i)



The volume at the recommended contour level is 52 nm^3; this corresponds to an approximate mass of 47 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)

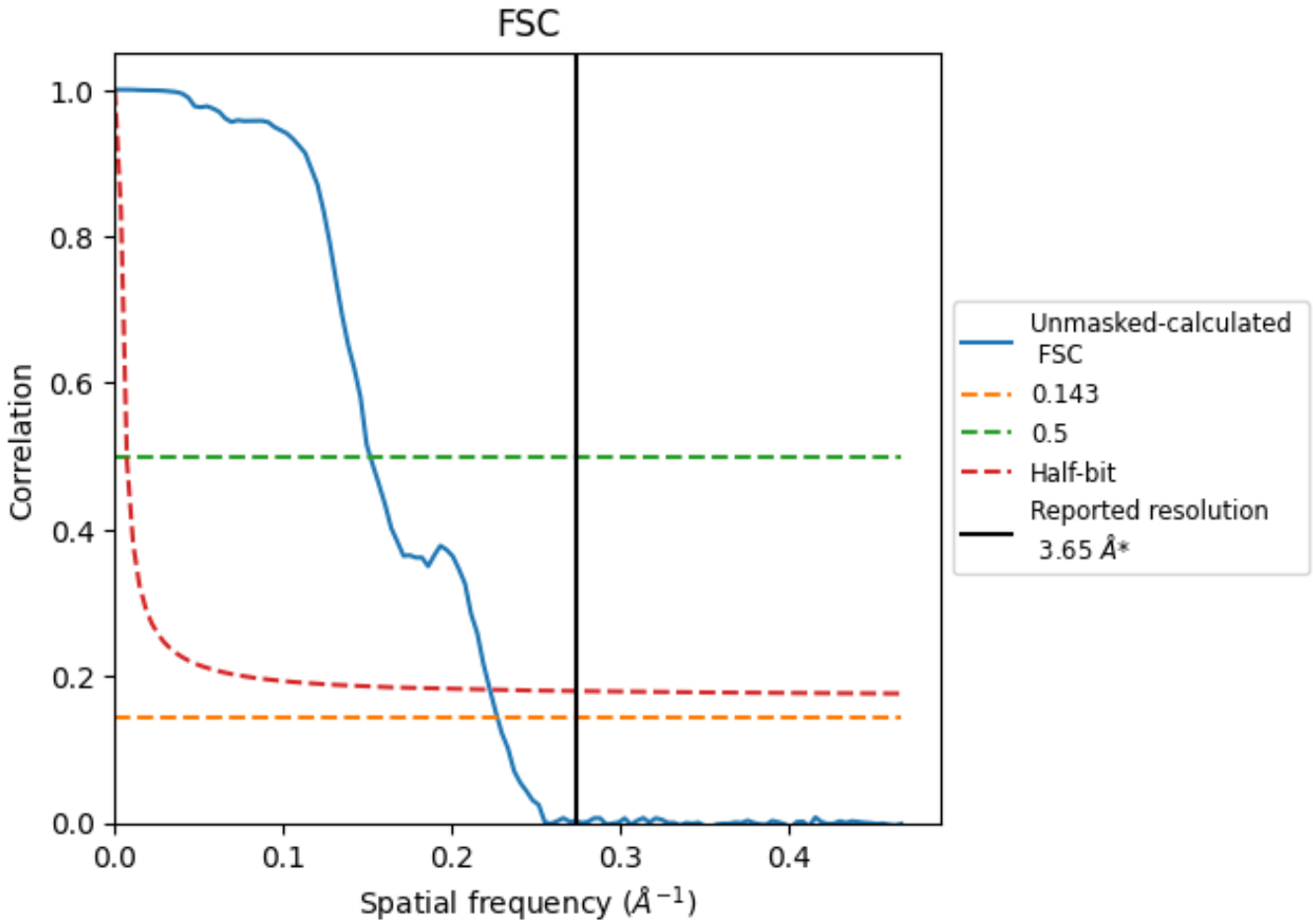


*Reported resolution corresponds to spatial frequency of 0.274 $Å^{-1}$

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 0.274 Å^{-1}

8.2 Resolution estimates (i)

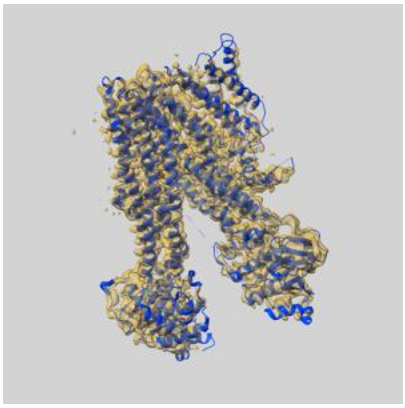
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.65	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	4.40	6.59	4.49

*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 4.40 differs from the reported value 3.65 by more than 10 %

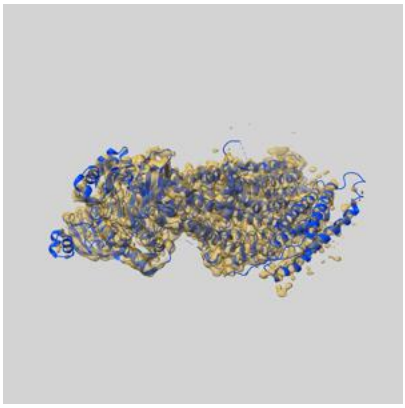
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-35049 and PDB model 8HW2. Per-residue inclusion information can be found in section 3 on page 4.

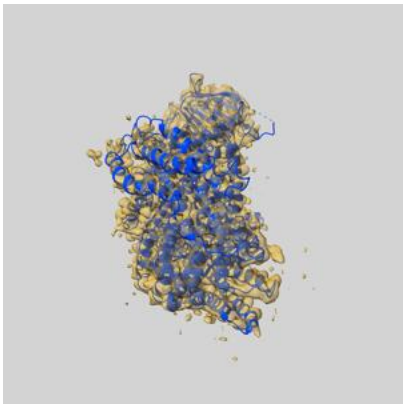
9.1 Map-model overlay



X



Y



Z

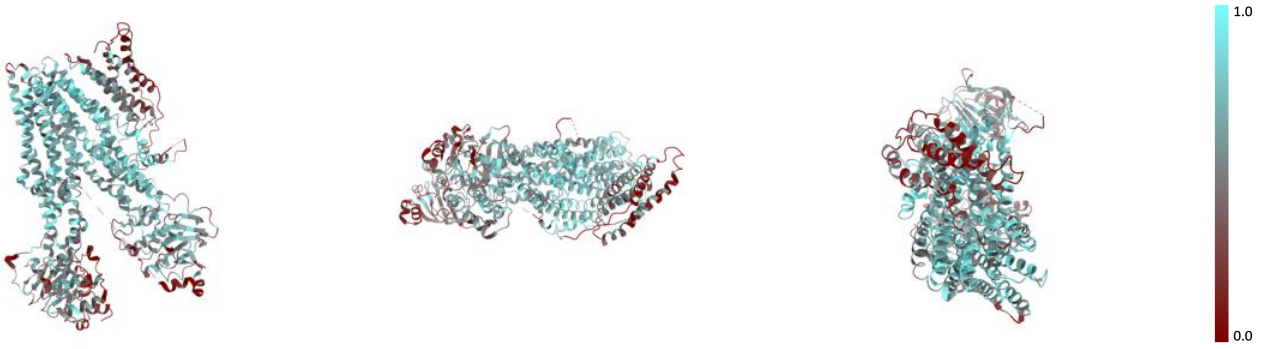
The images above show the 3D surface view of the map at the recommended contour level 0.447 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

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.

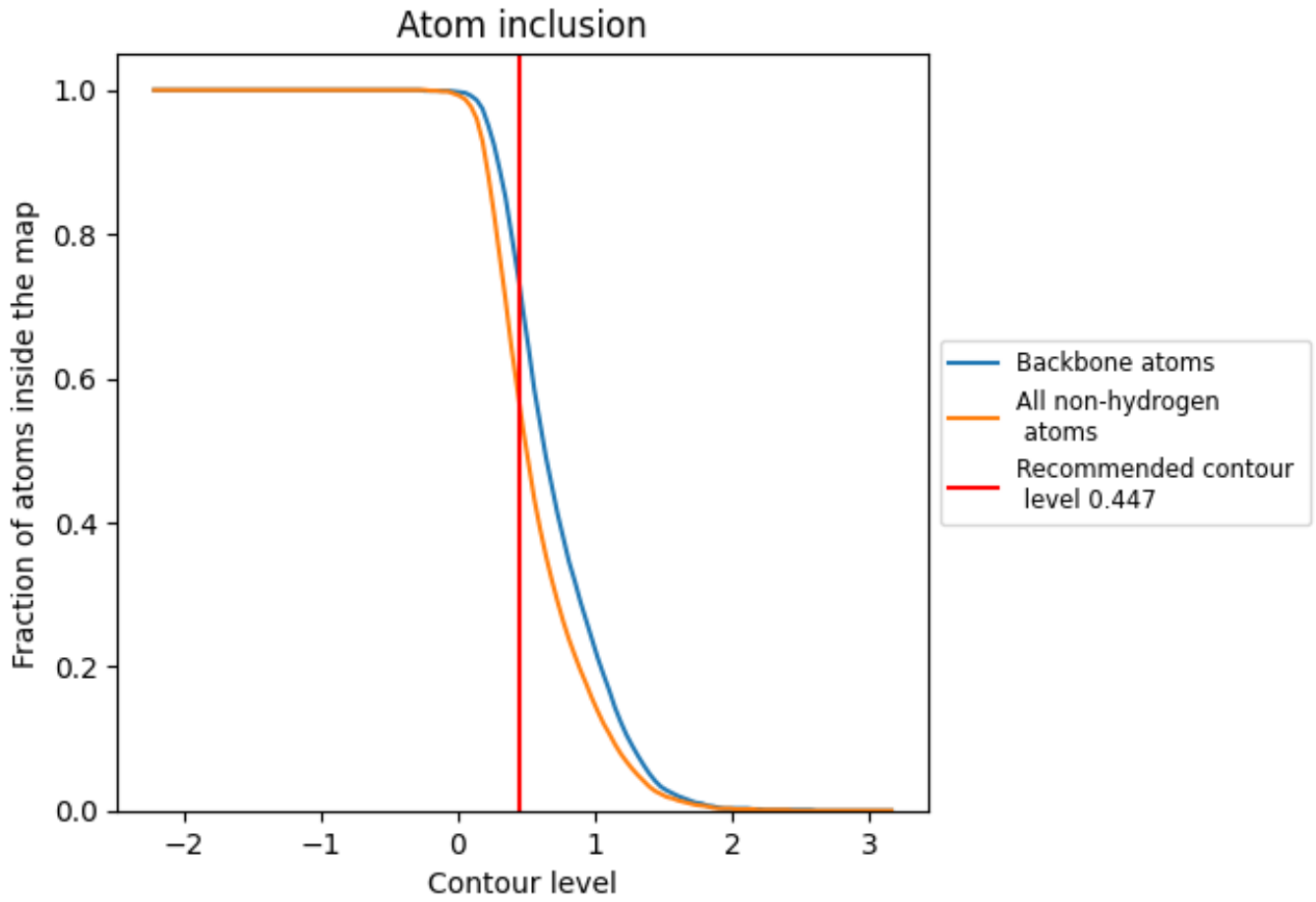
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.447).

9.4 Atom inclusion (i)



At the recommended contour level, 74% of all backbone atoms, 57% of all non-hydrogen atoms, are inside the map.

9.5 Map-model fit summary (i)

The table lists the average atom inclusion at the recommended contour level (0.447) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.5690	0.4040
A	0.5690	0.4040