wwPDB EM Validation Summary Report

Nov 25, 2024 – 10:22 AM EST

PDB ID	:	9CE3
EMDB ID	:	EMD-45492
Title	:	Structure of the TSC:WIPI3 lysosomal recruitment complex
Authors	:	Bayly-Jones, C.; Lupton, C.J.; D'Andrea, L.; Ellisdon, A.M.
Deposited on	:	2024-06-26
Resolution	:	2.90 Å(reported)
Based on initial models	:	5EJC, ., 9C9I

This is a wwPDB EM Validation Summary Report for a publicly released PDB entry.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/EMValidationReportHelp
with specific help available everywhere you see the ⓘ symbol.

The types of validation reports are described at
http://www.wwpdb.org/validation/2017/FAQs#types.

The following versions of software and data (see references ⓘ) were used in the production of this report:

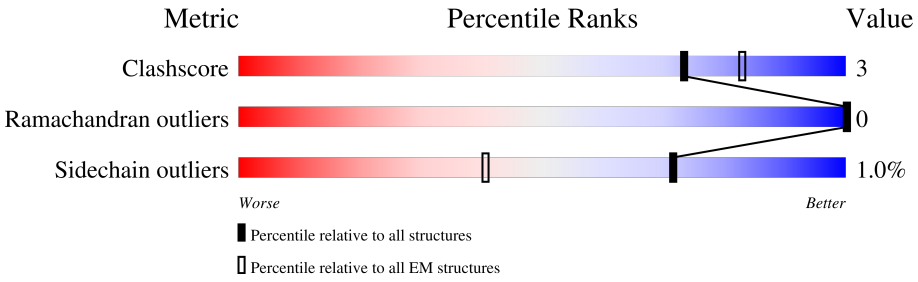
EMDB validation analysis	:	0.0.1.dev113
MolProbity	:	4.02b-467
Percentile statistics	:	20231227.v01 (using entries in the PDB archive December 27th 2023)
MapQ	:	1.9.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.40

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 2.90 Å.

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



Metric	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	210492	15764
Ramachandran outliers	207382	16835
Sidechain outliers	206894	16415

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	1792	65% 7% 28%
1	B	1792	65% 7% 28%
2	C	1191	47% 5% 48%
2	D	1191	45% 51%
3	E	301	82% 9% 8%
4	F	316	54% 94%
5	G	12	42% 100%
5	H	12	100%

2 Entry composition (i)

There are 5 unique types of molecules in this entry. The entry contains 35086 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 Isoform 4 of Tuberin.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	1295	Total	C	N	O	S	0	0
			10341	6610	1780	1886	65		
1	B	1282	Total	C	N	O	S	0	0
			10200	6523	1751	1861	65		

There are 18 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-7	MET	-	initiating methionine	UNP P49815
A	-6	ASP	-	expression tag	UNP P49815
A	-5	TYR	-	expression tag	UNP P49815
A	-4	LYS	-	expression tag	UNP P49815
A	-3	ASP	-	expression tag	UNP P49815
A	-2	ASP	-	expression tag	UNP P49815
A	-1	ASP	-	expression tag	UNP P49815
A	0	ASP	-	expression tag	UNP P49815
A	1	LYS	-	expression tag	UNP P49815
B	-7	MET	-	initiating methionine	UNP P49815
B	-6	ASP	-	expression tag	UNP P49815
B	-5	TYR	-	expression tag	UNP P49815
B	-4	LYS	-	expression tag	UNP P49815
B	-3	ASP	-	expression tag	UNP P49815
B	-2	ASP	-	expression tag	UNP P49815
B	-1	ASP	-	expression tag	UNP P49815
B	0	ASP	-	expression tag	UNP P49815
B	1	LYS	-	expression tag	UNP P49815

- Molecule 2 is a protein called Hamartin.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	C	616	Total	C	N	O	S	0	0
			5042	3188	901	930	23		

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Mol	Chain	Residues	Atoms					AltConf	Trace
2	D	581	Total	C	N	O	S	0	0
			4773	3020	861	868	24		

There are 54 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
C	1165	GLY	-	expression tag	UNP Q92574
C	1166	THR	-	expression tag	UNP Q92574
C	1167	LYS	-	expression tag	UNP Q92574
C	1168	LEU	-	expression tag	UNP Q92574
C	1169	GLY	-	expression tag	UNP Q92574
C	1170	PRO	-	expression tag	UNP Q92574
C	1171	GLU	-	expression tag	UNP Q92574
C	1172	GLN	-	expression tag	UNP Q92574
C	1173	LYS	-	expression tag	UNP Q92574
C	1174	LEU	-	expression tag	UNP Q92574
C	1175	ILE	-	expression tag	UNP Q92574
C	1176	SER	-	expression tag	UNP Q92574
C	1177	GLU	-	expression tag	UNP Q92574
C	1178	GLU	-	expression tag	UNP Q92574
C	1179	ASP	-	expression tag	UNP Q92574
C	1180	LEU	-	expression tag	UNP Q92574
C	1181	ASN	-	expression tag	UNP Q92574
C	1182	SER	-	expression tag	UNP Q92574
C	1183	ALA	-	expression tag	UNP Q92574
C	1184	VAL	-	expression tag	UNP Q92574
C	1185	ASP	-	expression tag	UNP Q92574
C	1186	HIS	-	expression tag	UNP Q92574
C	1187	HIS	-	expression tag	UNP Q92574
C	1188	HIS	-	expression tag	UNP Q92574
C	1189	HIS	-	expression tag	UNP Q92574
C	1190	HIS	-	expression tag	UNP Q92574
C	1191	HIS	-	expression tag	UNP Q92574
D	1165	GLY	-	expression tag	UNP Q92574
D	1166	THR	-	expression tag	UNP Q92574
D	1167	LYS	-	expression tag	UNP Q92574
D	1168	LEU	-	expression tag	UNP Q92574
D	1169	GLY	-	expression tag	UNP Q92574
D	1170	PRO	-	expression tag	UNP Q92574
D	1171	GLU	-	expression tag	UNP Q92574
D	1172	GLN	-	expression tag	UNP Q92574
D	1173	LYS	-	expression tag	UNP Q92574

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Chain	Residue	Modelled	Actual	Comment	Reference
D	1174	LEU	-	expression tag	UNP Q92574
D	1175	ILE	-	expression tag	UNP Q92574
D	1176	SER	-	expression tag	UNP Q92574
D	1177	GLU	-	expression tag	UNP Q92574
D	1178	GLU	-	expression tag	UNP Q92574
D	1179	ASP	-	expression tag	UNP Q92574
D	1180	LEU	-	expression tag	UNP Q92574
D	1181	ASN	-	expression tag	UNP Q92574
D	1182	SER	-	expression tag	UNP Q92574
D	1183	ALA	-	expression tag	UNP Q92574
D	1184	VAL	-	expression tag	UNP Q92574
D	1185	ASP	-	expression tag	UNP Q92574
D	1186	HIS	-	expression tag	UNP Q92574
D	1187	HIS	-	expression tag	UNP Q92574
D	1188	HIS	-	expression tag	UNP Q92574
D	1189	HIS	-	expression tag	UNP Q92574
D	1190	HIS	-	expression tag	UNP Q92574
D	1191	HIS	-	expression tag	UNP Q92574

- Molecule 3 is a protein called TBC1 domain family member 7.

Mol	Chain	Residues	Atoms					AltConf	Trace
3	E	276	Total	C	N	O	S	0	0
			2238	1446	378	399	15		

There are 8 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
E	294	ASP	-	expression tag	UNP Q9P0N9
E	295	TYR	-	expression tag	UNP Q9P0N9
E	296	LYS	-	expression tag	UNP Q9P0N9
E	297	ASP	-	expression tag	UNP Q9P0N9
E	298	ASP	-	expression tag	UNP Q9P0N9
E	299	ASP	-	expression tag	UNP Q9P0N9
E	300	ASP	-	expression tag	UNP Q9P0N9
E	301	LYS	-	expression tag	UNP Q9P0N9

- Molecule 4 is a protein called WD repeat domain phosphoinositide-interacting protein 3.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	F	305	Total	C	N	O	S	0	0
			2372	1512	406	436	18		

There are 31 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
F	5	MET	-	initiating methionine	UNP Q5MNZ6
F	6	HIS	-	expression tag	UNP Q5MNZ6
F	7	HIS	-	expression tag	UNP Q5MNZ6
F	8	HIS	-	expression tag	UNP Q5MNZ6
F	9	HIS	-	expression tag	UNP Q5MNZ6
F	10	HIS	-	expression tag	UNP Q5MNZ6
F	11	HIS	-	expression tag	UNP Q5MNZ6
F	?	-	PRO	deletion	UNP Q5MNZ6
F	?	-	LYS	deletion	UNP Q5MNZ6
F	?	-	TYR	deletion	UNP Q5MNZ6
F	?	-	PRO	deletion	UNP Q5MNZ6
F	?	-	PRO	deletion	UNP Q5MNZ6
F	?	-	ASN	deletion	UNP Q5MNZ6
F	?	-	ARG	deletion	UNP Q5MNZ6
F	?	-	ASN	deletion	UNP Q5MNZ6
F	?	-	LYS	deletion	UNP Q5MNZ6
F	?	-	GLN	deletion	UNP Q5MNZ6
F	?	-	SER	deletion	UNP Q5MNZ6
F	?	-	SER	deletion	UNP Q5MNZ6
F	?	-	LEU	deletion	UNP Q5MNZ6
F	?	-	ALA	deletion	UNP Q5MNZ6
F	?	-	SER	deletion	UNP Q5MNZ6
F	?	-	ALA	deletion	UNP Q5MNZ6
F	?	-	SER	deletion	UNP Q5MNZ6
F	?	-	PHE	deletion	UNP Q5MNZ6
F	?	-	LEU	deletion	UNP Q5MNZ6
F	?	-	PRO	deletion	UNP Q5MNZ6
F	?	-	LYS	deletion	UNP Q5MNZ6
F	?	-	TYR	deletion	UNP Q5MNZ6
F	?	-	PHE	deletion	UNP Q5MNZ6
F	?	-	SER	deletion	UNP Q5MNZ6

- Molecule 5 is a protein called Unknown fragment.

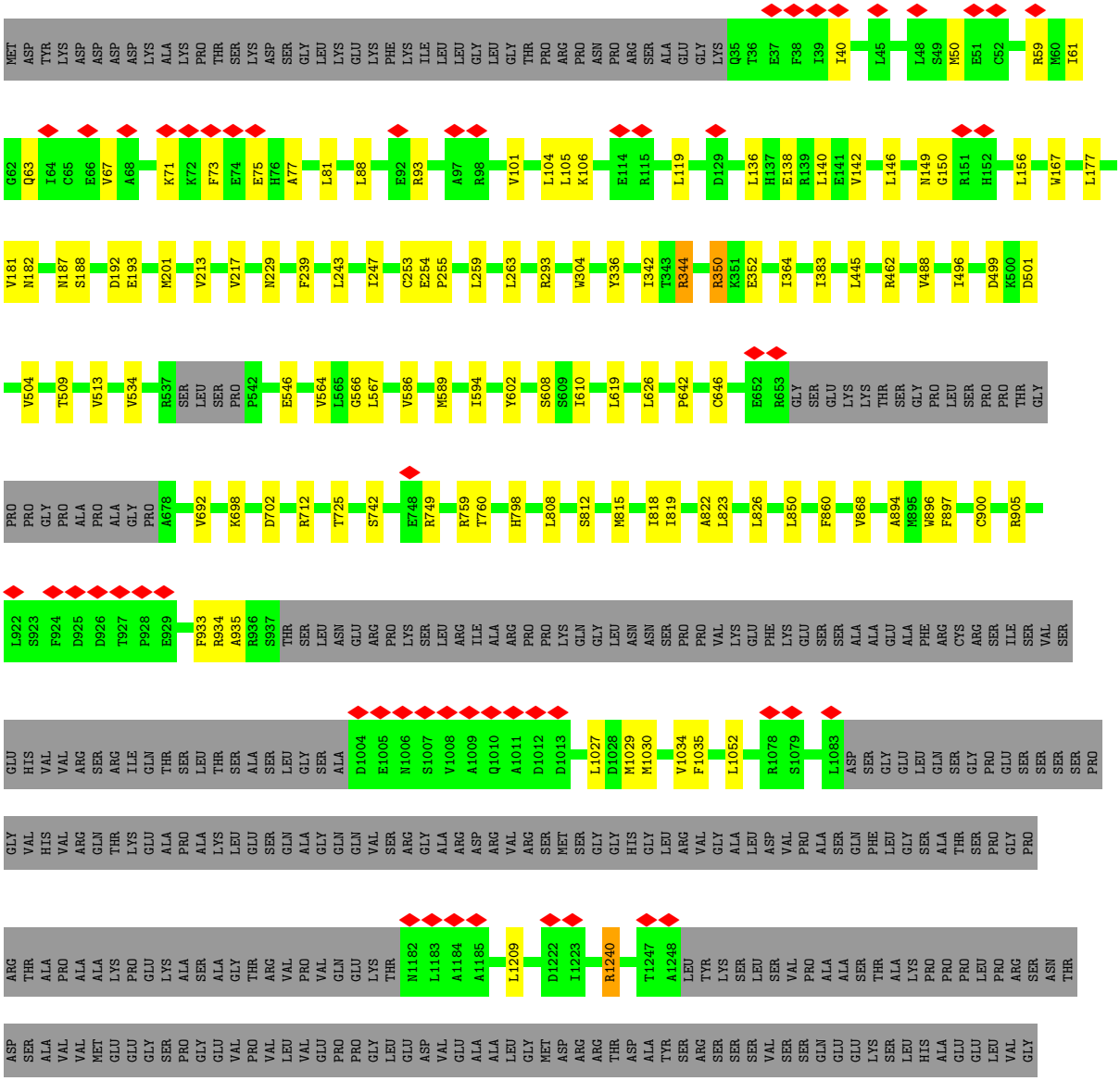
Mol	Chain	Residues	Atoms				AltConf	Trace
5	G	12	Total	C	N	O	0	0
			60	36	12	12		
5	H	12	Total	C	N	O	0	0
			60	36	12	12		

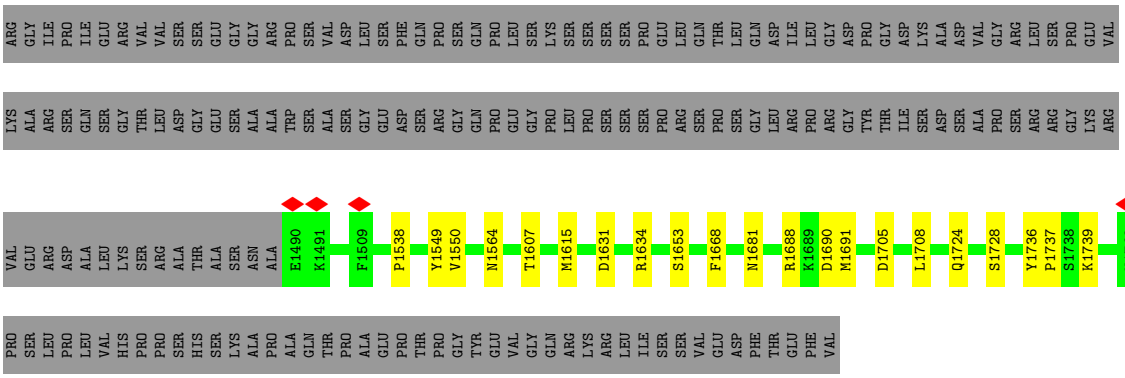
3 Residue-property plots (i)

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: Isoform 4 of Tuberin

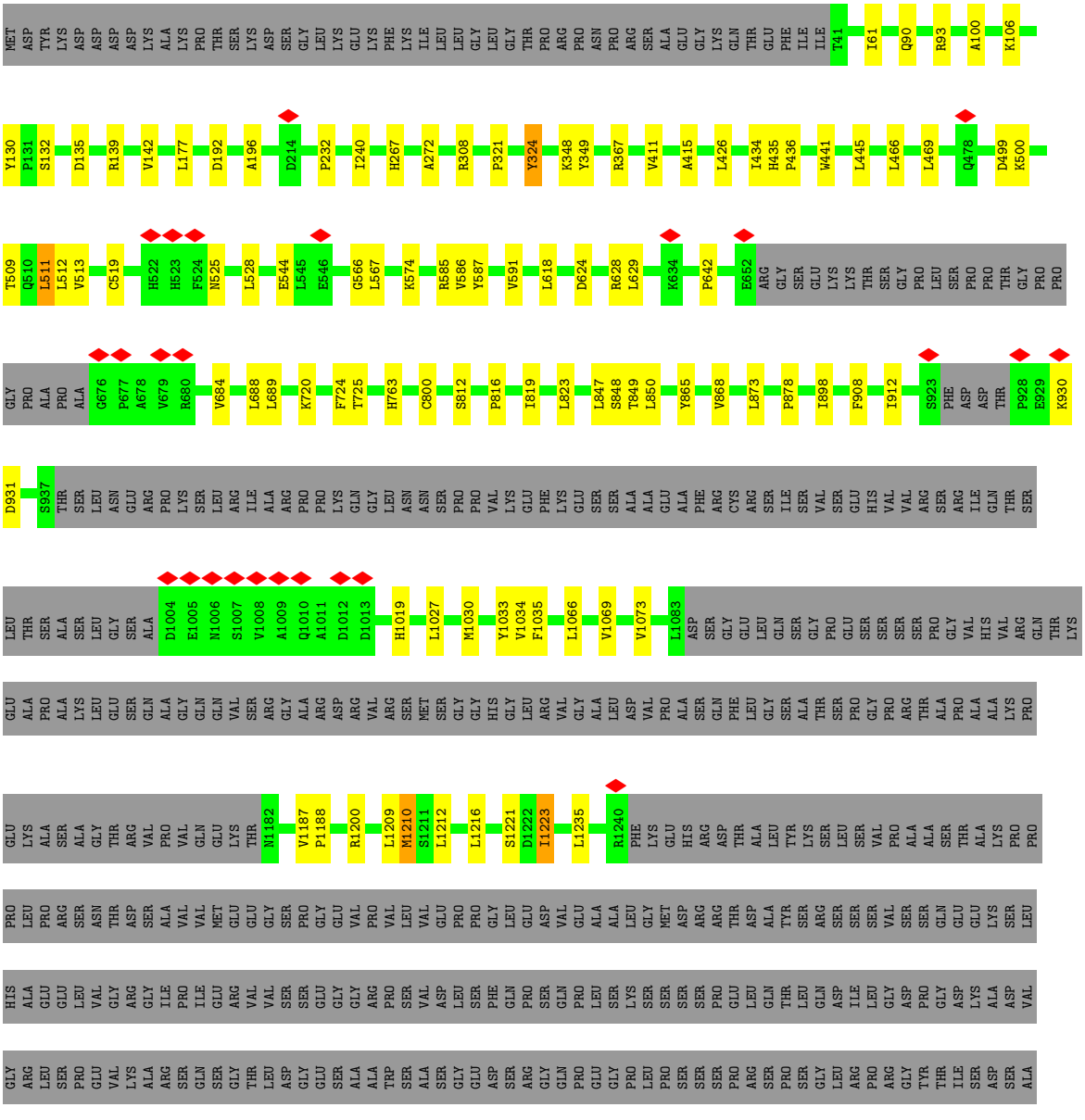
Chain A:

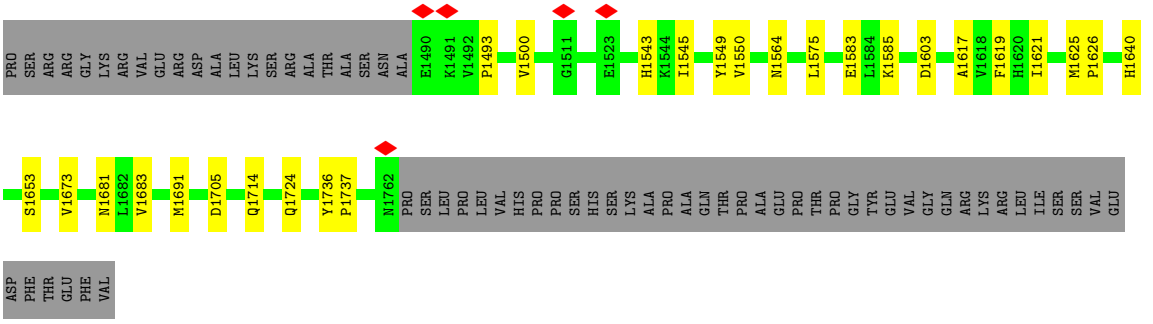




• Molecule 1: Isoform 4 of Tuberin

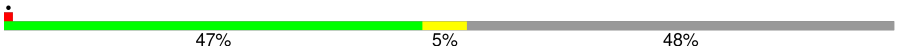
Chain B:

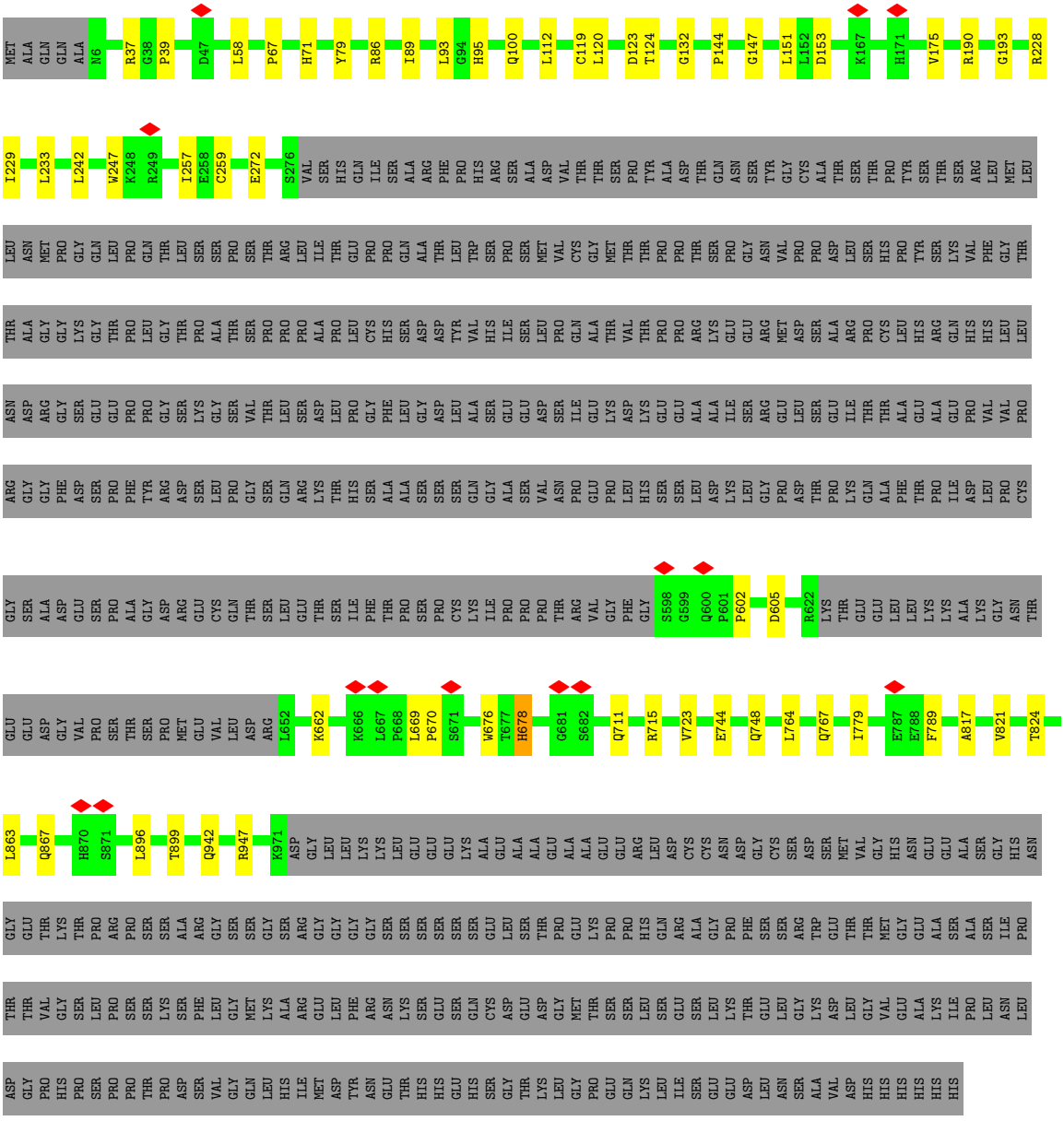




• Molecule 2: Hamartin

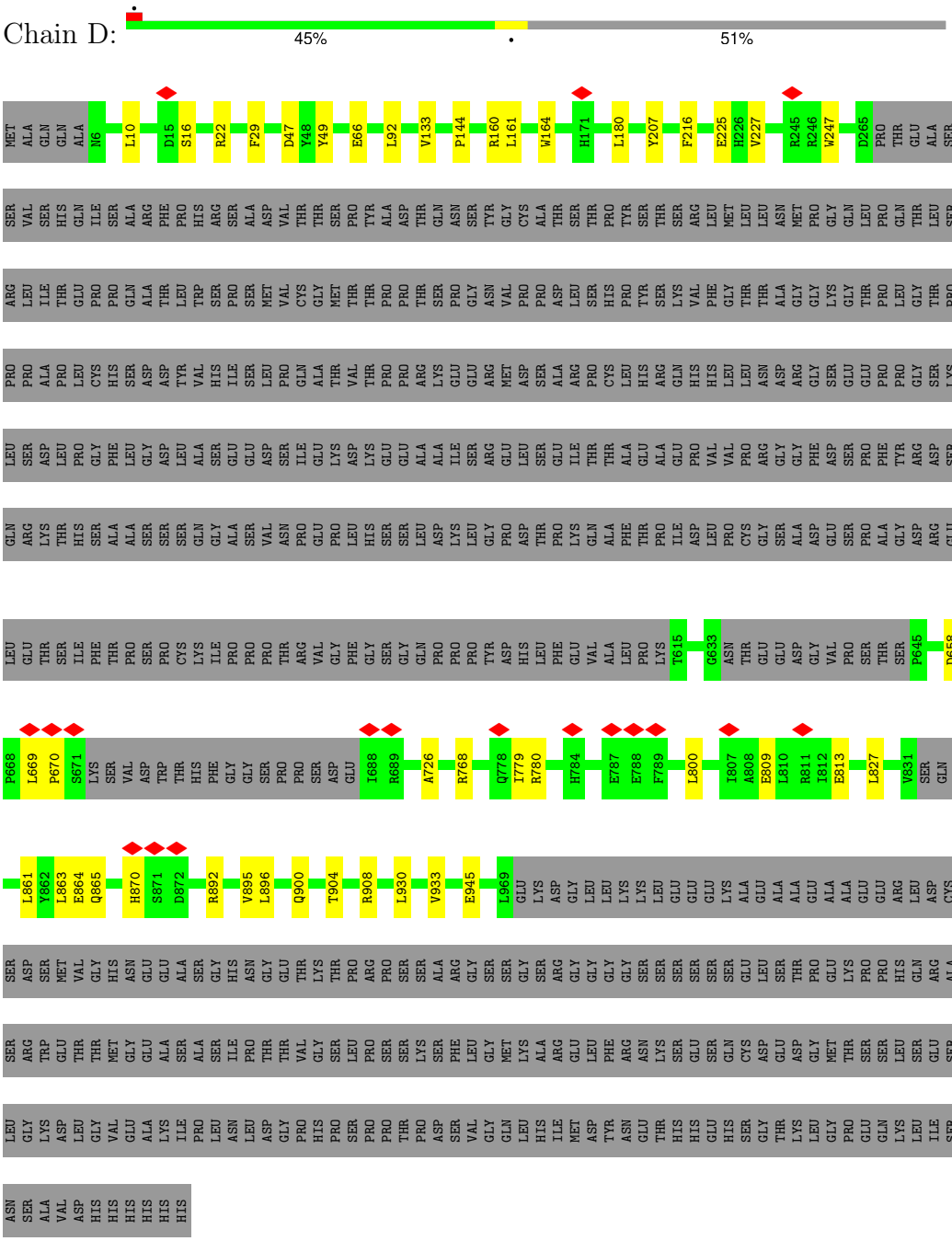
Chain C:





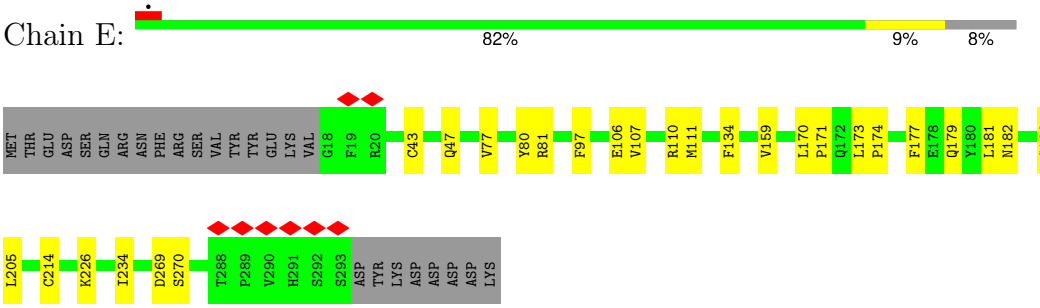
• Molecule 2: Hamartin

Chain D:



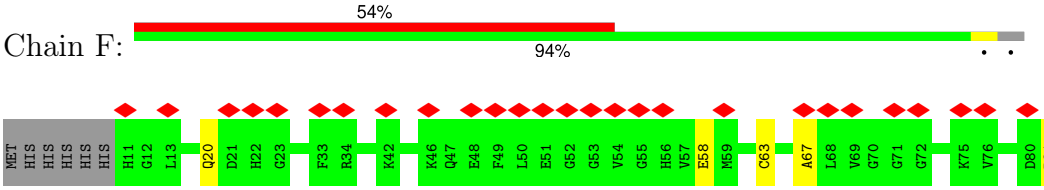
• Molecule 3: TBC1 domain family member 7

Chain E:



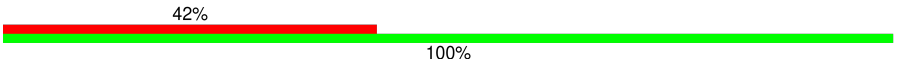
• Molecule 4: WD repeat domain phosphoinositide-interacting protein 3

Chain F:



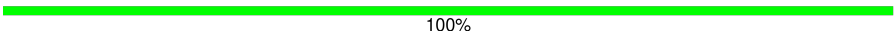
• Molecule 5: Unknown fragment

Chain G:



• Molecule 5: Unknown fragment

Chain H:



There are no outlier residues recorded for this chain.

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C1	Depositor
Number of particles used	200000	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 ($e^-/Å^2$)	46.54	Depositor
Minimum defocus (nm)	500	Depositor
Maximum defocus (nm)	3000	Depositor
Magnification	105000	Depositor
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor
Maximum map value	1.624	Depositor
Minimum map value	0.000	Depositor
Average map value	0.000	Depositor
Map value standard deviation	0.010	Depositor
Recommended contour level	0.05	Depositor
Map size (Å)	655.19995, 655.19995, 655.19995	wwPDB
Map dimensions	560, 560, 560	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.17, 1.17, 1.17	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	0.33	0/10554	0.51	0/14310
1	B	0.33	0/10411	0.53	0/14122
2	C	0.30	0/5136	0.50	0/6927
2	D	0.28	0/4850	0.49	0/6526
3	E	0.34	0/2293	0.49	0/3103
4	F	0.27	0/2427	0.50	0/3283
All	All	0.32	0/35671	0.51	0/48271

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	10341	0	10454	78	0
1	B	10200	0	10317	69	0
2	C	5042	0	5116	35	0
2	D	4773	0	4902	32	0
3	E	2238	0	2275	15	0
4	F	2372	0	2345	4	0
5	G	60	0	14	0	0
5	H	60	0	15	0	0
All	All	35086	0	35438	216	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 3.

The worst 5 of 216 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)
3:E:179:GLN:O	3:E:182:ASN:ND2	2.20	0.75
1:B:1603:ASP:OD2	1:B:1640:HIS:NE2	2.24	0.70
1:B:567:LEU:HD22	1:B:586:VAL:HG13	1.75	0.68
1:B:849:THR:HG22	1:B:1714:GLN:HG2	1.74	0.67
1:B:509:THR:HA	1:B:512:LEU:HD12	1.79	0.65

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	1283/1792 (72%)	1254 (98%)	29 (2%)	0	100	100
1	B	1270/1792 (71%)	1228 (97%)	42 (3%)	0	100	100
2	C	610/1191 (51%)	594 (97%)	16 (3%)	0	100	100
2	D	571/1191 (48%)	556 (97%)	15 (3%)	0	100	100
3	E	274/301 (91%)	270 (98%)	4 (2%)	0	100	100
4	F	303/316 (96%)	293 (97%)	10 (3%)	0	100	100
All	All	4311/6583 (66%)	4195 (97%)	116 (3%)	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	1158/1568 (74%)	1147 (99%)	11 (1%)	75 92
1	B	1143/1568 (73%)	1131 (99%)	12 (1%)	73 91
2	C	566/1057 (54%)	559 (99%)	7 (1%)	67 89
2	D	534/1057 (50%)	529 (99%)	5 (1%)	75 92
3	E	251/276 (91%)	250 (100%)	1 (0%)	89 97
4	F	261/272 (96%)	258 (99%)	3 (1%)	70 90
All	All	3913/5798 (68%)	3874 (99%)	39 (1%)	71 91

5 of 39 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
2	C	789	PHE
3	E	97	PHE
2	C	942	GLN
2	D	863	LEU
4	F	144	ASN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 17 such sidechains are listed below:

Mol	Chain	Res	Type
2	D	870	HIS
3	E	182	ASN
2	C	179	HIS
2	C	694	GLN
2	C	711	GLN

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 oligosaccharides 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.

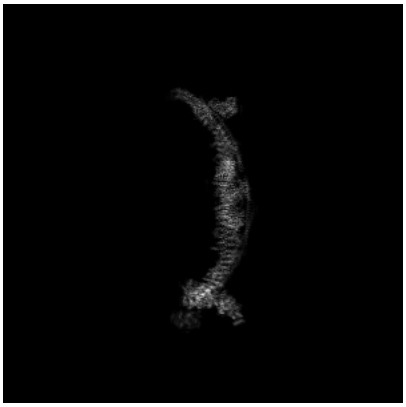
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-45492. These allow visual inspection of the internal detail of the map and identification of artifacts.

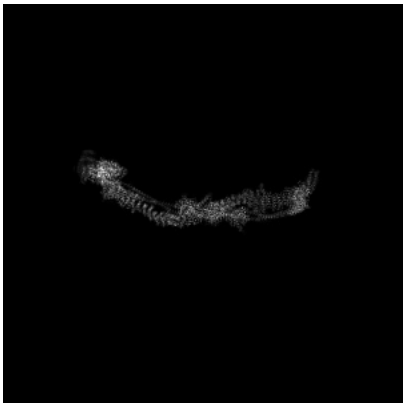
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections

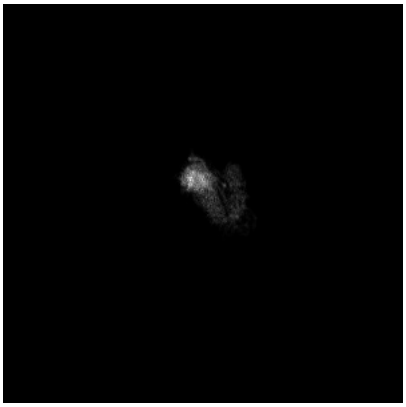
6.1.1 Primary map



X



Y

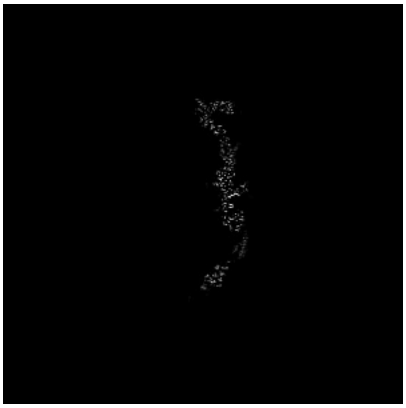


Z

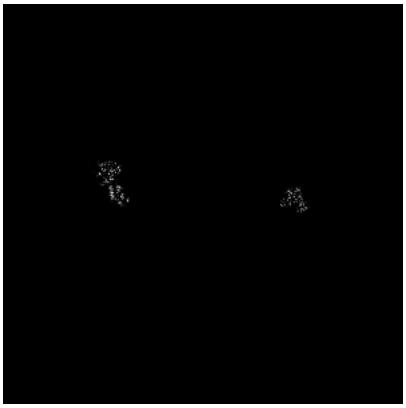
The images above show the map projected in three orthogonal directions.

6.2 Central slices

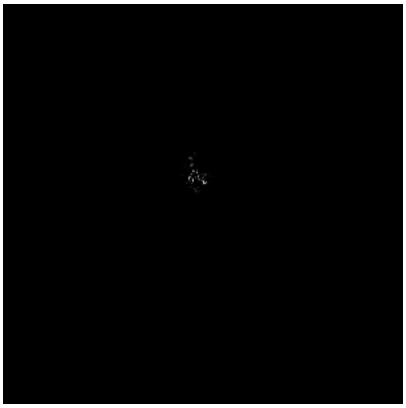
6.2.1 Primary map



X Index: 280



Y Index: 280

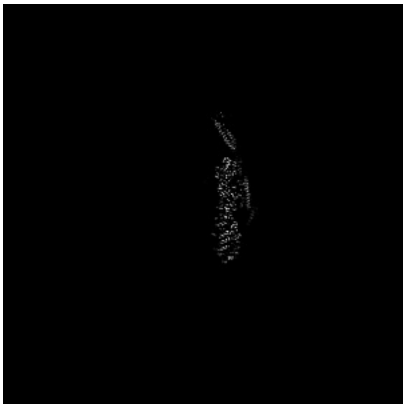


Z Index: 280

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

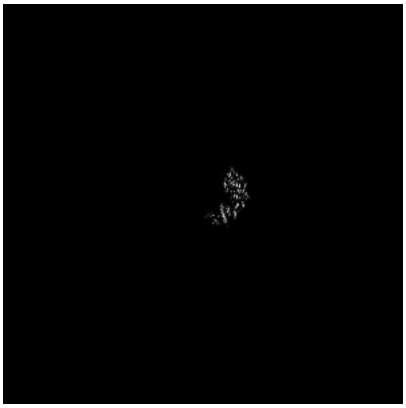
6.3 Largest variance slices (i)

6.3.1 Primary map



X Index: 262

Y Index: 316

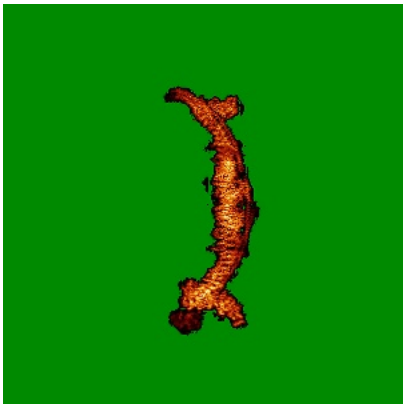


Z Index: 139

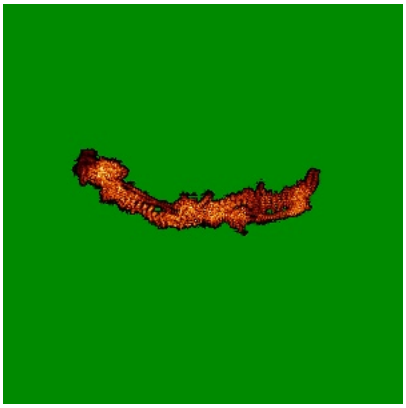
The images above show the largest variance slices of the map in three orthogonal directions.

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

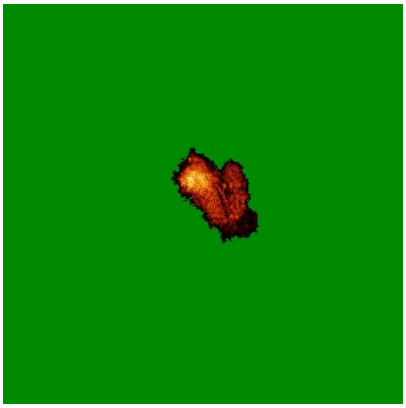
6.4.1 Primary map



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

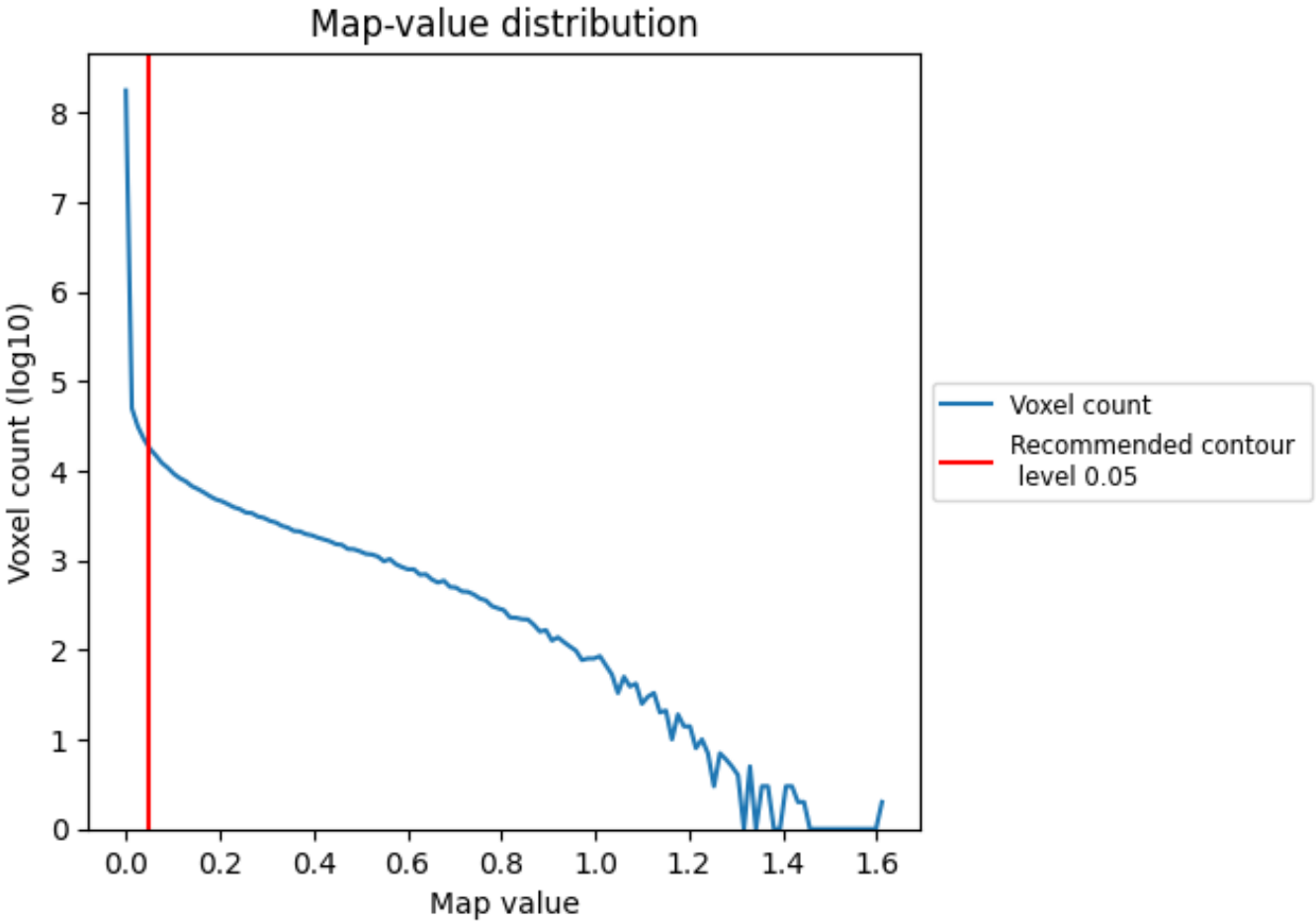
6.6 Mask visualisation (i)

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

7 Map analysis

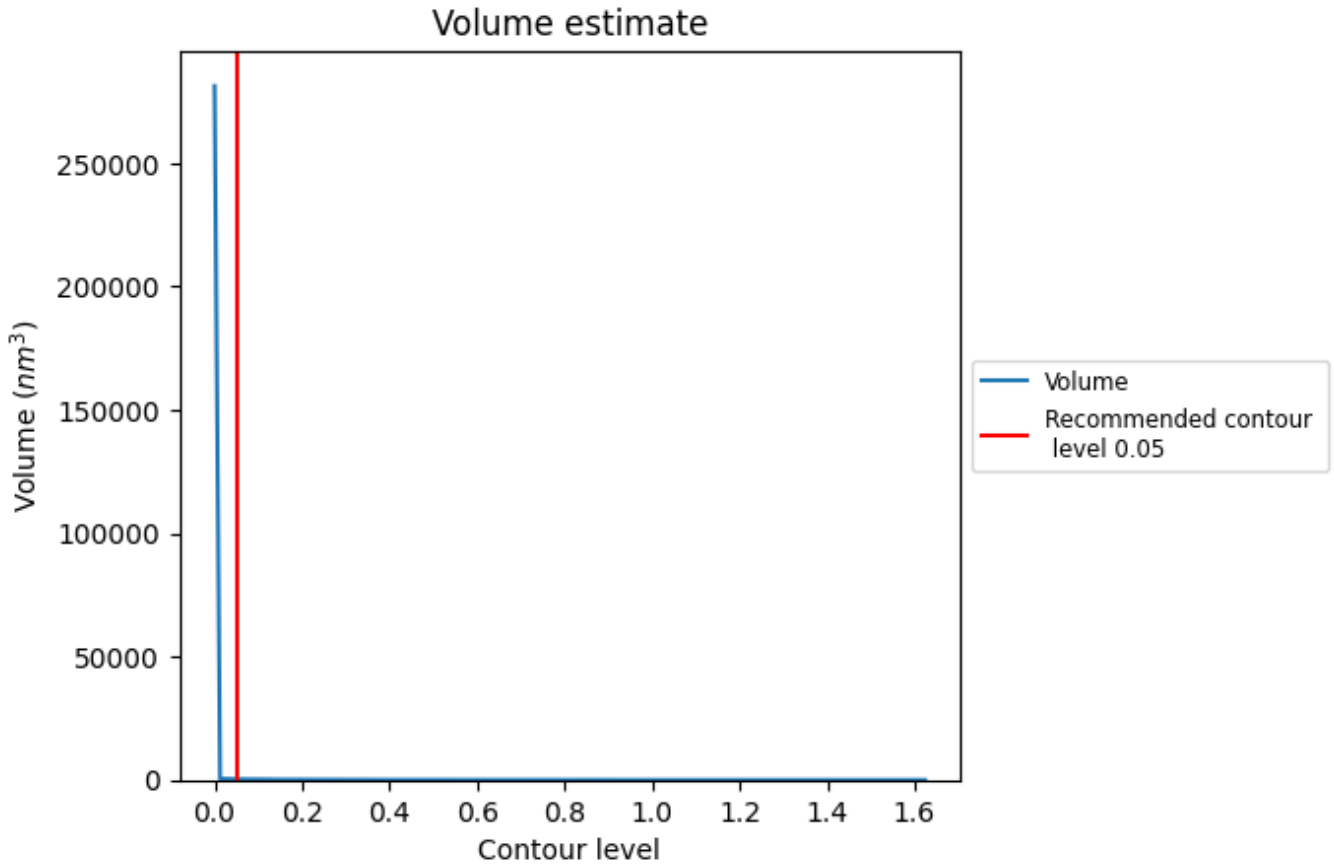
This section contains the results of statistical analysis of the map.

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.

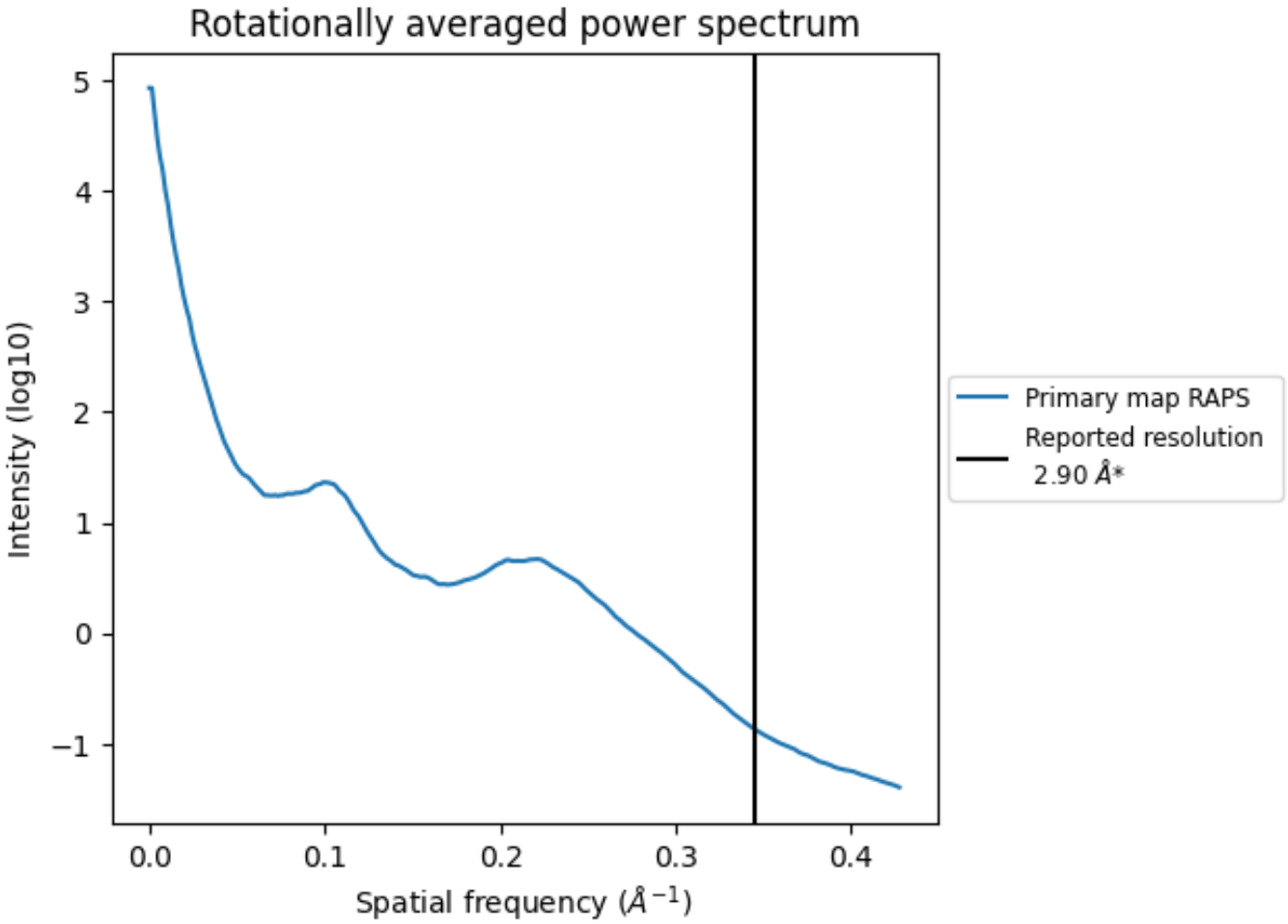
7.2 Volume estimate



The volume at the recommended contour level is 303 nm^3; this corresponds to an approximate mass of 274 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 0.345 Å^{-1}

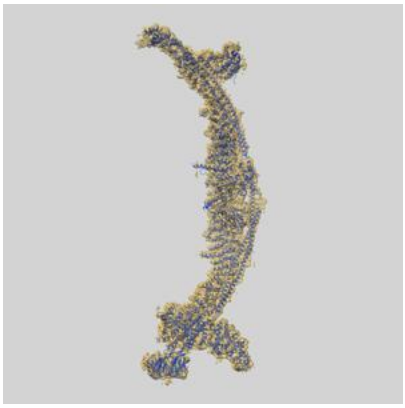
8 Fourier-Shell correlation

This section was not generated. No FSC curve or half-maps provided.

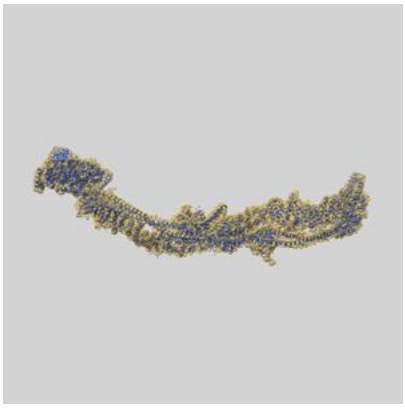
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-45492 and PDB model 9CE3. Per-residue inclusion information can be found in section 3 on page 7.

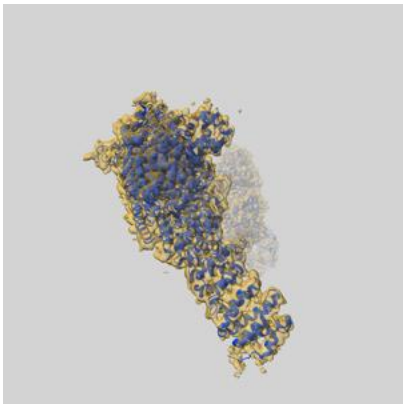
9.1 Map-model overlay



X



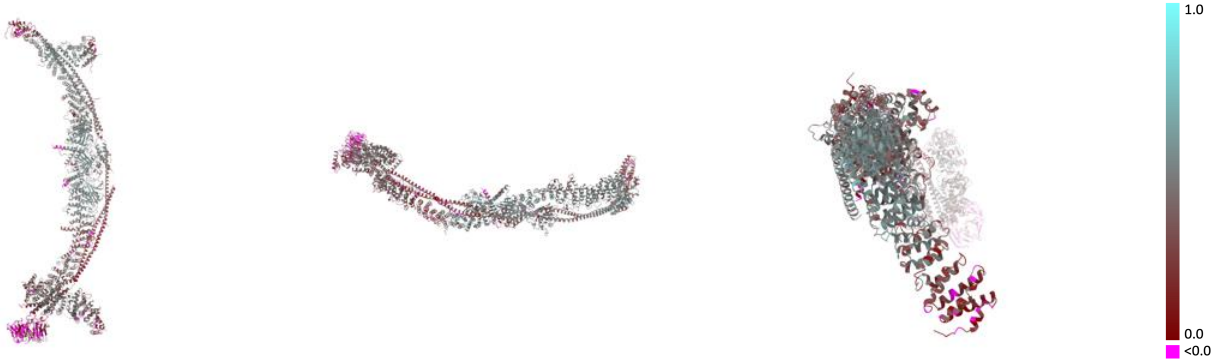
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.05 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.

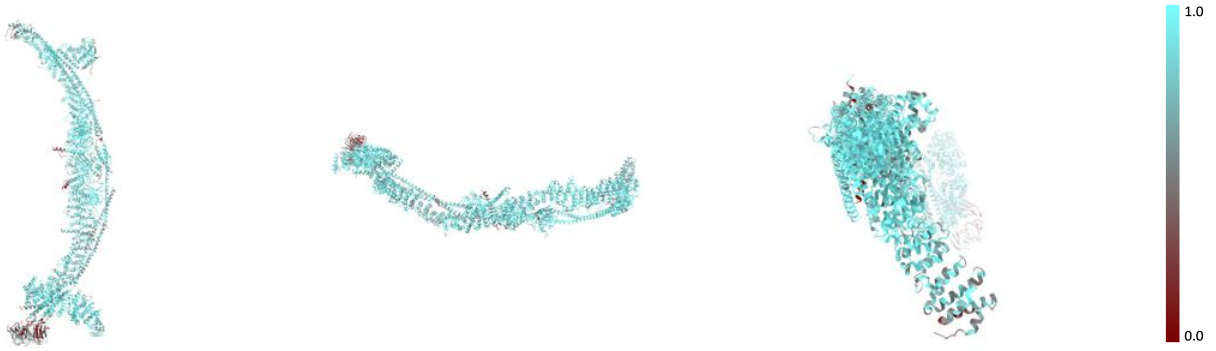
9.2 Q-score mapped to coordinate model (i)



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.

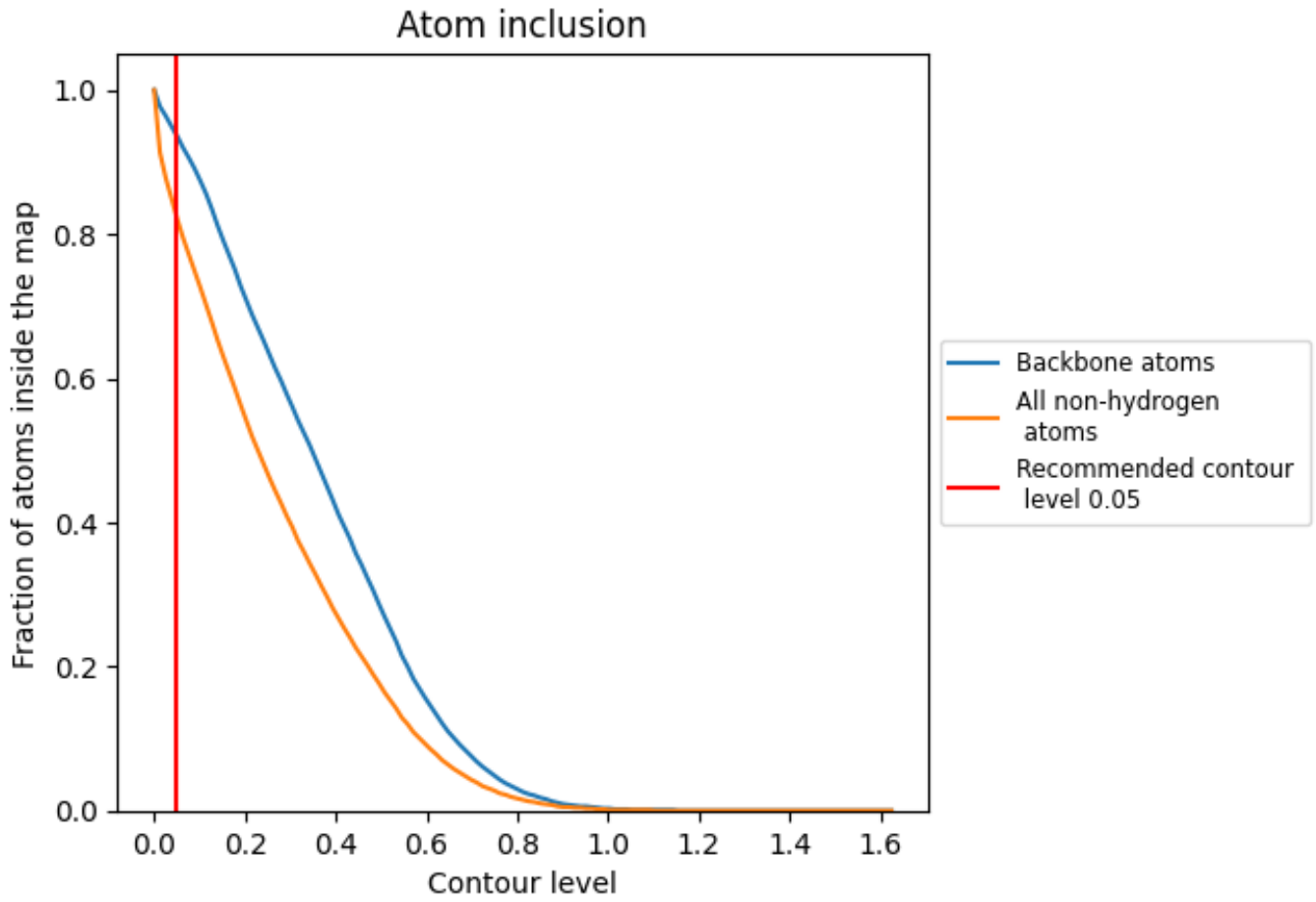
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.05).

9.4 Atom inclusion (i)



At the recommended contour level, 94% of all backbone atoms, 82% 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 (0.05) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.8220	0.3820
A	0.8630	0.4390
B	0.8670	0.4110
C	0.8440	0.3680
D	0.8230	0.3440
E	0.8550	0.4520
F	0.3780	0.0460
G	0.6330	0.3360
H	0.9330	0.4150