



wwPDB EM Validation Summary Report ⓘ

Jan 30, 2024 – 04:56 PM EST

PDB ID : 7T96
EMDB ID : EMD-25752
Title : Cryo-EM structure of S2 state ACh-bound M2R-Go signaling complex with a PAM
Authors : Xu, J.; Wang, Q.; Du, Y.; Kobilka, B.K.
Deposited on : 2021-12-18
Resolution : 3.22 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
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) : 2.36

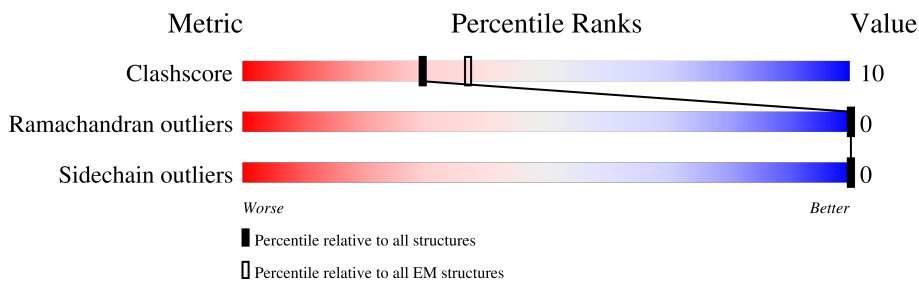
1 Overall quality at a glance i

The following experimental techniques were used to determine the structure:

ELECTRON MICROSCOPY

The reported resolution of this entry is 3.22 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 $\leq 5\%$. 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	353	
2	B	354	
3	C	345	
4	D	71	
5	E	256	

2 Entry composition

There are 7 unique types of molecules in this entry. The entry contains 8450 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 Muscarinic acetylcholine receptor M2.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
1	A	274	2015	1332	324	344	15	0	0

There are 27 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	-4	ASP	-	expression tag	UNP P08172
A	-3	TYR	-	expression tag	UNP P08172
A	-2	LYS	-	expression tag	UNP P08172
A	-1	ASP	-	expression tag	UNP P08172
A	0	ASP	-	expression tag	UNP P08172
A	1	ASP	-	expression tag	UNP P08172
A	2	ASP	-	expression tag	UNP P08172
A	3	ALA	-	expression tag	UNP P08172
A	6	ASP	ASN	conflict	UNP P08172
A	9	ASP	ASN	conflict	UNP P08172
A	360	ILE	-	linker	UNP P08172
A	361	VAL	-	linker	UNP P08172
A	362	ALA	-	linker	UNP P08172
A	363	ARG	-	linker	UNP P08172
A	364	LYS	-	linker	UNP P08172
A	365	ILE	-	linker	UNP P08172
A	366	VAL	-	linker	UNP P08172
A	367	LYS	-	linker	UNP P08172
A	467	PRO	-	expression tag	UNP P08172
A	468	ALA	-	expression tag	UNP P08172
A	469	GLY	-	expression tag	UNP P08172
A	470	LEU	-	expression tag	UNP P08172
A	471	GLU	-	expression tag	UNP P08172
A	472	VAL	-	expression tag	UNP P08172
A	473	LEU	-	expression tag	UNP P08172
A	474	PHE	-	expression tag	UNP P08172
A	475	GLN	-	expression tag	UNP P08172

- Molecule 2 is a protein called Guanine nucleotide-binding protein G(o) subunit alpha.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
2	B	218	1705	1088	288	318	11	0	0

There are 4 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
B	9	ASP	GLU	conflict	UNP P09471
B	10	LYS	ARG	conflict	UNP P09471
B	13	VAL	LEU	conflict	UNP P09471
B	18	MET	ALA	conflict	UNP P09471

- Molecule 3 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
3	C	338	2541	1573	456	493	19	0	0

There are 6 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
C	-4	GLY	-	expression tag	UNP P62873
C	-3	PRO	-	expression tag	UNP P62873
C	-2	GLY	-	expression tag	UNP P62873
C	-1	SER	-	expression tag	UNP P62873
C	0	SER	-	expression tag	UNP P62873
C	1	GLY	-	expression tag	UNP P62873

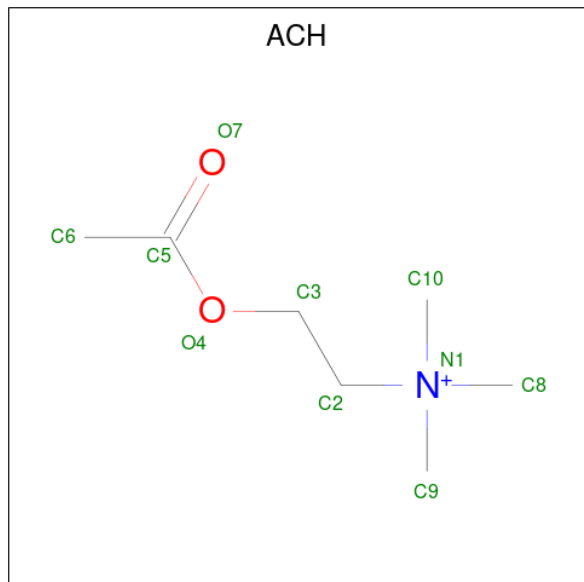
- Molecule 4 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2.

Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
4	D	56	403	252	69	79	3	0	0

- Molecule 5 is a protein called scFV16.

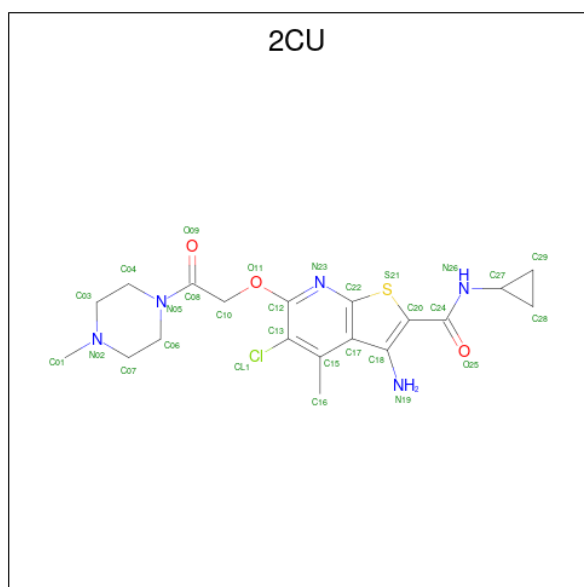
Mol	Chain	Residues	Atoms					AltConf	Trace
			Total	C	N	O	S		
5	E	236	1747	1111	288	338	10	0	0

- Molecule 6 is ACETYLCHOLINE (three-letter code: ACH) (formula: $C_7H_{16}NO_2$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms				AltConf
			Total	C	N	O	
6	A	1	10	7	1	2	0

- Molecule 7 is 3-amino-5-chloro-N-cyclopropyl-4-methyl-6-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]thieno[2,3-b]pyridine-2-carboxamide (three-letter code: 2CU) (formula: $C_{19}H_{24}ClN_5O_3S$) (labeled as "Ligand of Interest" by depositor).

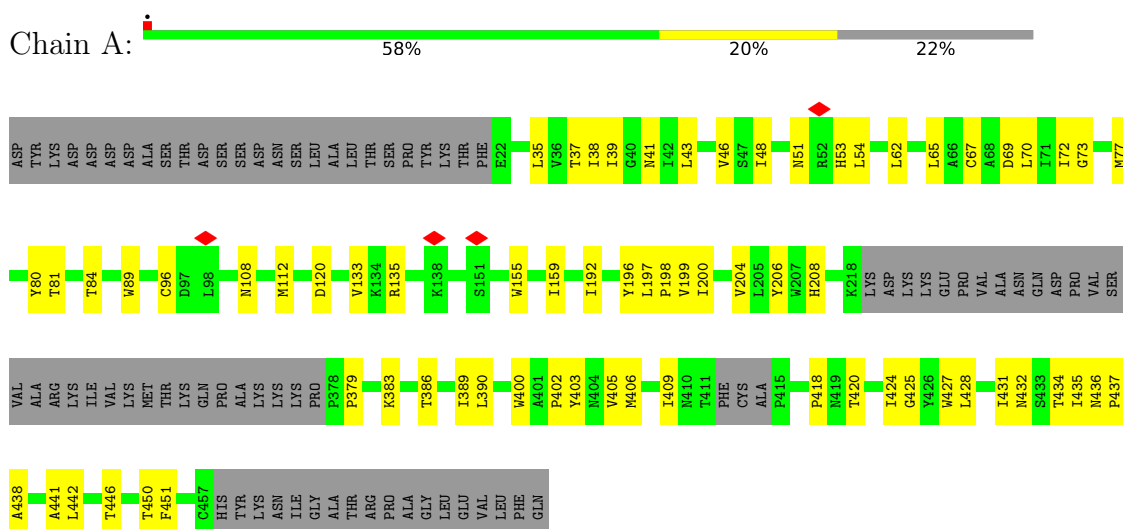


Mol	Chain	Residues	Atoms					AltConf	
			Total	C	Cl	N	O		S
7	A	1	29	19	1	5	3	1	0

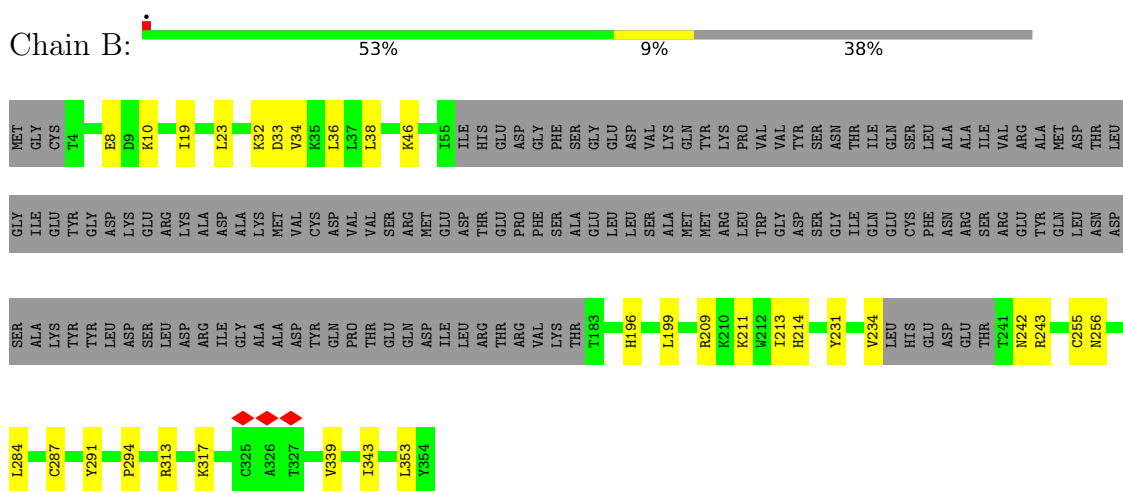
3 Residue-property plots [i](#)

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: Muscarinic acetylcholine receptor M2

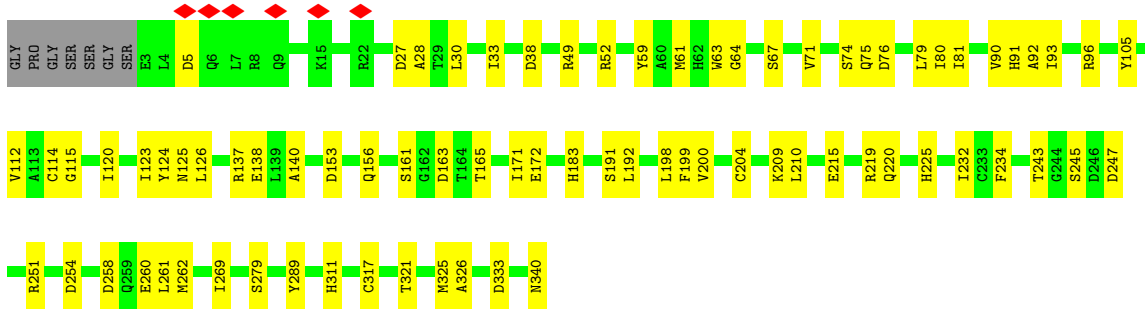


- Molecule 2: Guanine nucleotide-binding protein G(o) subunit alpha

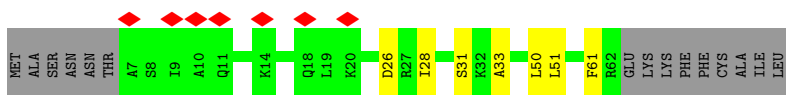


- Molecule 3: Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1

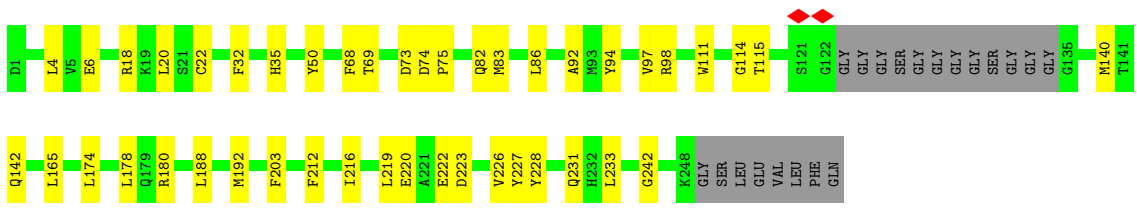
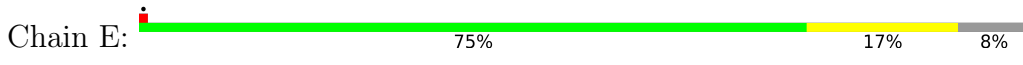




- Molecule 4: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2



- Molecule 5: scFV16



4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	80017	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	NONE	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/\text{\AA}^2$)	60	Depositor
Minimum defocus (nm)	1000	Depositor
Maximum defocus (nm)	2000	Depositor
Magnification	Not provided	
Image detector	GATAN K3 BIOQUANTUM (6k x 4k)	Depositor
Maximum map value	2.058	Depositor
Minimum map value	-0.002	Depositor
Average map value	0.001	Depositor
Map value standard deviation	0.025	Depositor
Recommended contour level	0.06	Depositor
Map size (\AA)	272.0, 272.0, 272.0	wwPDB
Map dimensions	320, 320, 320	wwPDB
Map angles ($^\circ$)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (\AA)	0.85, 0.85, 0.85	Depositor

5 Model quality [i](#)

5.1 Standard geometry [i](#)

Bond lengths and bond angles in the following residue types are not validated in this section: 2CU, ACH

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	0.45	0/2065	0.62	0/2841
2	B	0.37	0/1733	0.51	0/2333
3	C	0.36	0/2588	0.51	0/3516
4	D	0.29	0/409	0.43	0/556
5	E	0.41	0/1791	0.51	0/2439
All	All	0.39	0/8586	0.54	0/11685

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 [i](#)

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	2015	0	1929	65	0
2	B	1705	0	1699	19	0
3	C	2541	0	2411	50	0
4	D	403	0	386	6	0
5	E	1747	0	1632	29	0
6	A	10	0	16	3	0
7	A	29	0	24	3	0
All	All	8450	0	8097	167	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 10.

The worst 5 of 167 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:54:LEU:HD21	1:A:446:THR:HG22	1.37	1.03
1:A:81:THR:O	1:A:84:THR:HG22	1.65	0.96
1:A:72:ILE:O	1:A:77:MET:HG2	1.64	0.96
5:E:94:TYR:O	5:E:114:GLY:HA2	1.75	0.87
7:A:502:2CU:H1	7:A:502:2CU:H19	1.38	0.85

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	268/353 (76%)	246 (92%)	22 (8%)	0	100	100
2	B	212/354 (60%)	201 (95%)	11 (5%)	0	100	100
3	C	336/345 (97%)	310 (92%)	26 (8%)	0	100	100
4	D	54/71 (76%)	51 (94%)	3 (6%)	0	100	100
5	E	232/256 (91%)	214 (92%)	18 (8%)	0	100	100
All	All	1102/1379 (80%)	1022 (93%)	80 (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	196/310 (63%)	196 (100%)	0	100	100
2	B	185/309 (60%)	185 (100%)	0	100	100
3	C	265/285 (93%)	265 (100%)	0	100	100
4	D	39/58 (67%)	39 (100%)	0	100	100
5	E	183/208 (88%)	183 (100%)	0	100	100
All	All	868/1170 (74%)	868 (100%)	0	100	100

There are no protein residues with a non-rotameric sidechain to report.

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (4) such sidechains are listed below:

Mol	Chain	Res	Type
1	A	51	ASN
2	B	242	ASN
3	C	75	GLN
3	C	183	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
6	ACH	A	501	-	9,9,9	1.63	2 (22%)	12,12,12	0.99	0
7	2CU	A	502	-	28,32,32	3.31	14 (50%)	33,47,47	1.97	9 (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. '2' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
6	ACH	A	501	-	-	3/7/7/7	-
7	2CU	A	502	-	-	5/13/29/29	0/4/4/4

The worst 5 of 16 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
7	A	502	2CU	C01-N02	-8.89	1.26	1.46
7	A	502	2CU	C24-N26	6.62	1.48	1.34
7	A	502	2CU	C18-C17	6.51	1.51	1.41
7	A	502	2CU	C08-N05	6.22	1.47	1.35
7	A	502	2CU	O11-C12	4.16	1.41	1.35

The worst 5 of 9 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(°)	Ideal(°)
7	A	502	2CU	C20-C24-N26	5.17	128.70	115.43
7	A	502	2CU	C18-C17-C22	4.50	114.67	107.94
7	A	502	2CU	C27-N26-C24	4.40	128.63	122.55
7	A	502	2CU	C12-C13-C15	-3.18	118.53	121.98
7	A	502	2CU	C29-C27-N26	-2.78	114.62	118.61

There are no chirality outliers.

5 of 8 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
7	A	502	2CU	C20-C24-N26-C27
7	A	502	2CU	O25-C24-N26-C27

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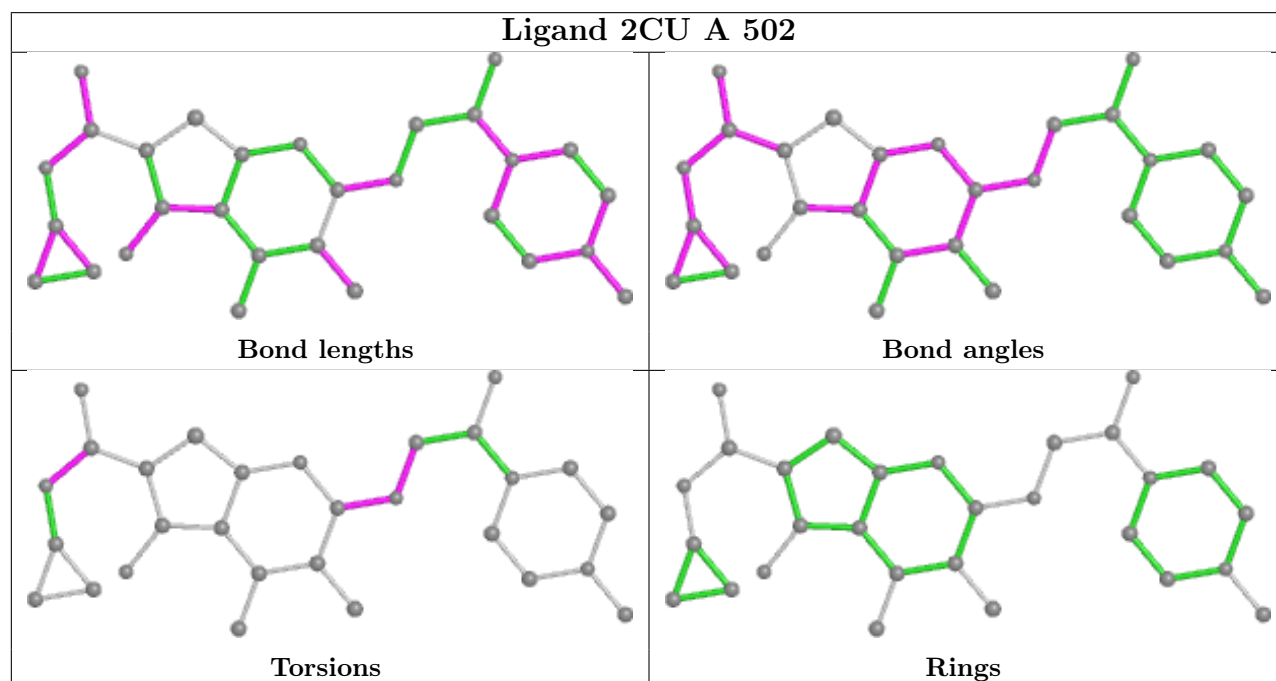
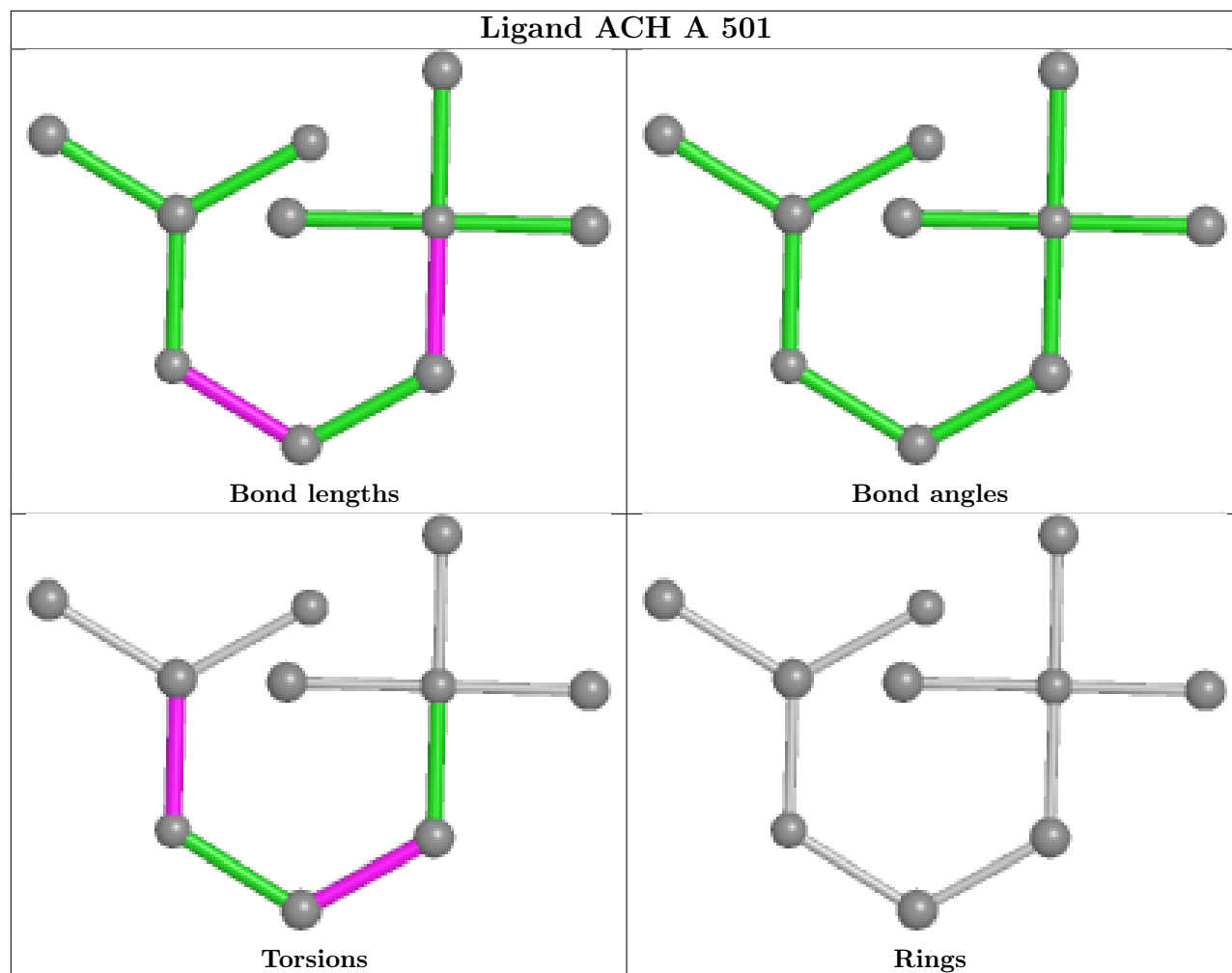
Mol	Chain	Res	Type	Atoms
7	A	502	2CU	C13-C12-O11-C10
7	A	502	2CU	N23-C12-O11-C10
6	A	501	ACH	O7-C5-O4-C3

There are no ring outliers.

2 monomers are involved in 6 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
6	A	501	ACH	3	0
7	A	502	2CU	3	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 than 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.



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.

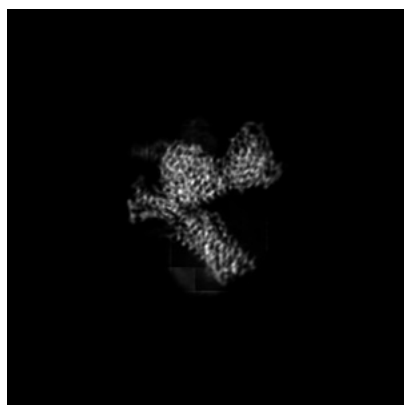
6 Map visualisation [i](#)

This section contains visualisations of the EMDB entry EMD-25752. These allow visual inspection of the internal detail of the map and identification of artifacts.

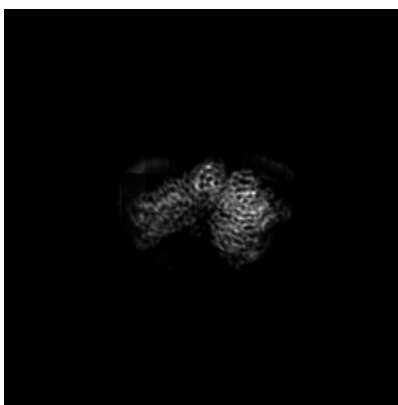
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections [i](#)

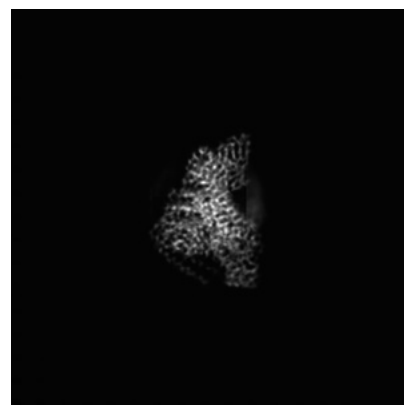
6.1.1 Primary map



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: 160



Y Index: 160



Z Index: 160

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: 172



Y Index: 159

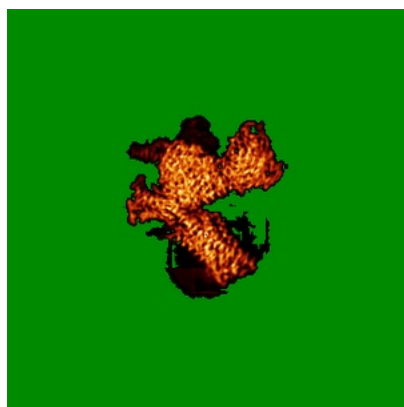


Z Index: 190

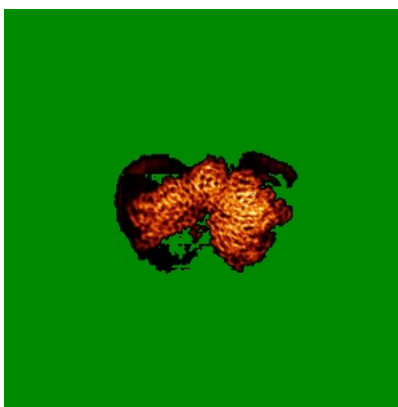
The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal standard-deviation projections (False-color) [i](#)

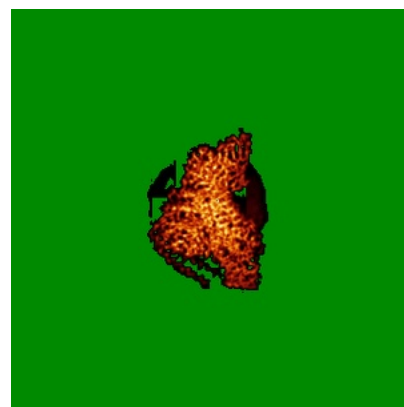
6.4.1 Primary map



X



Y

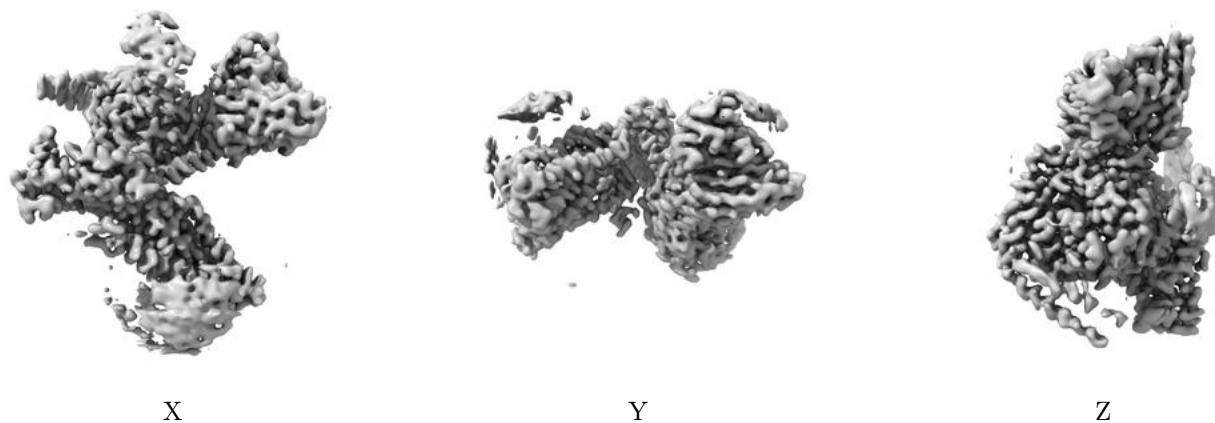


Z

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



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

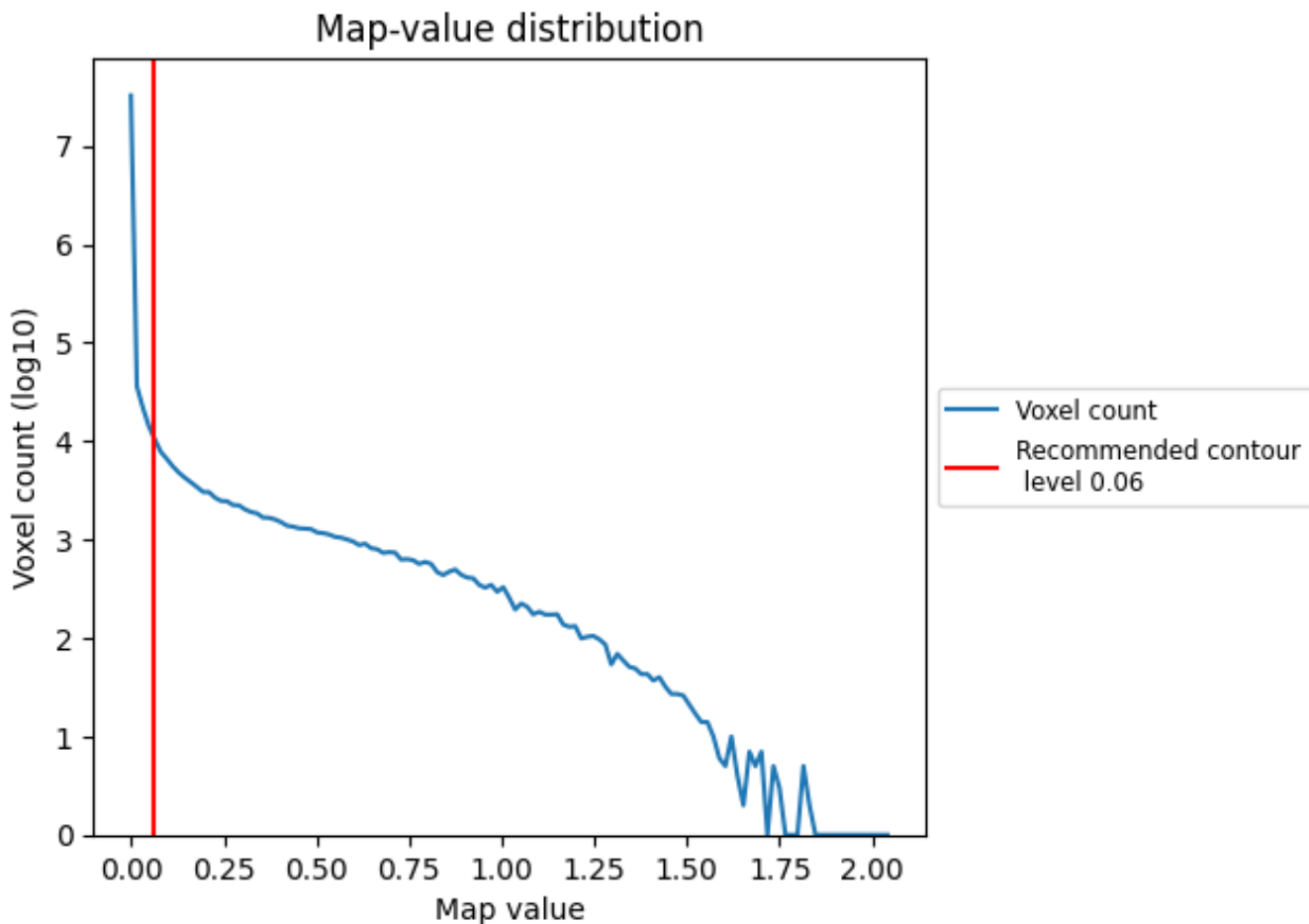
6.6 Mask visualisation [i](#)

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

7 Map analysis [i](#)

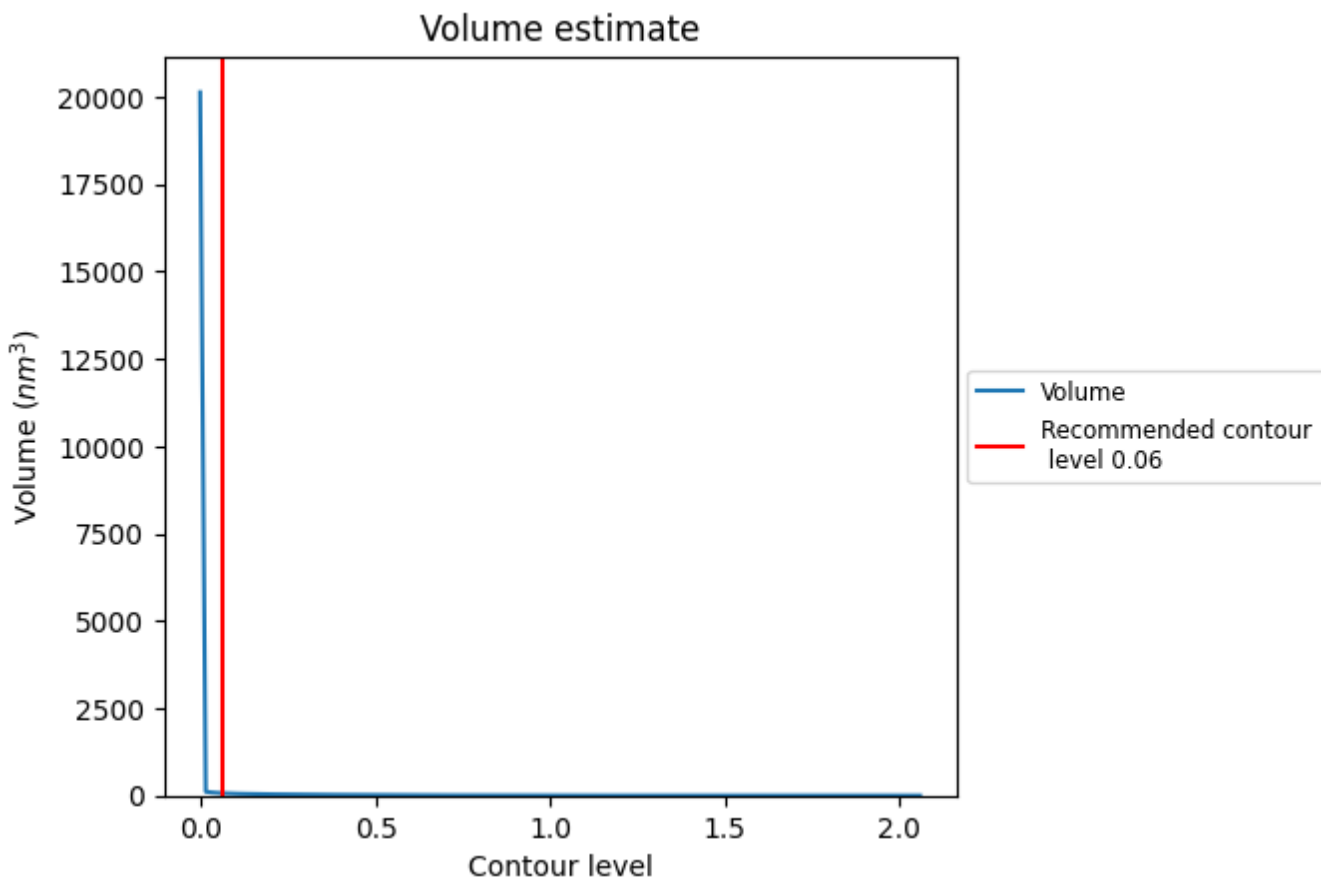
This section contains the results of statistical analysis of the map.

7.1 Map-value distribution [i](#)



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.

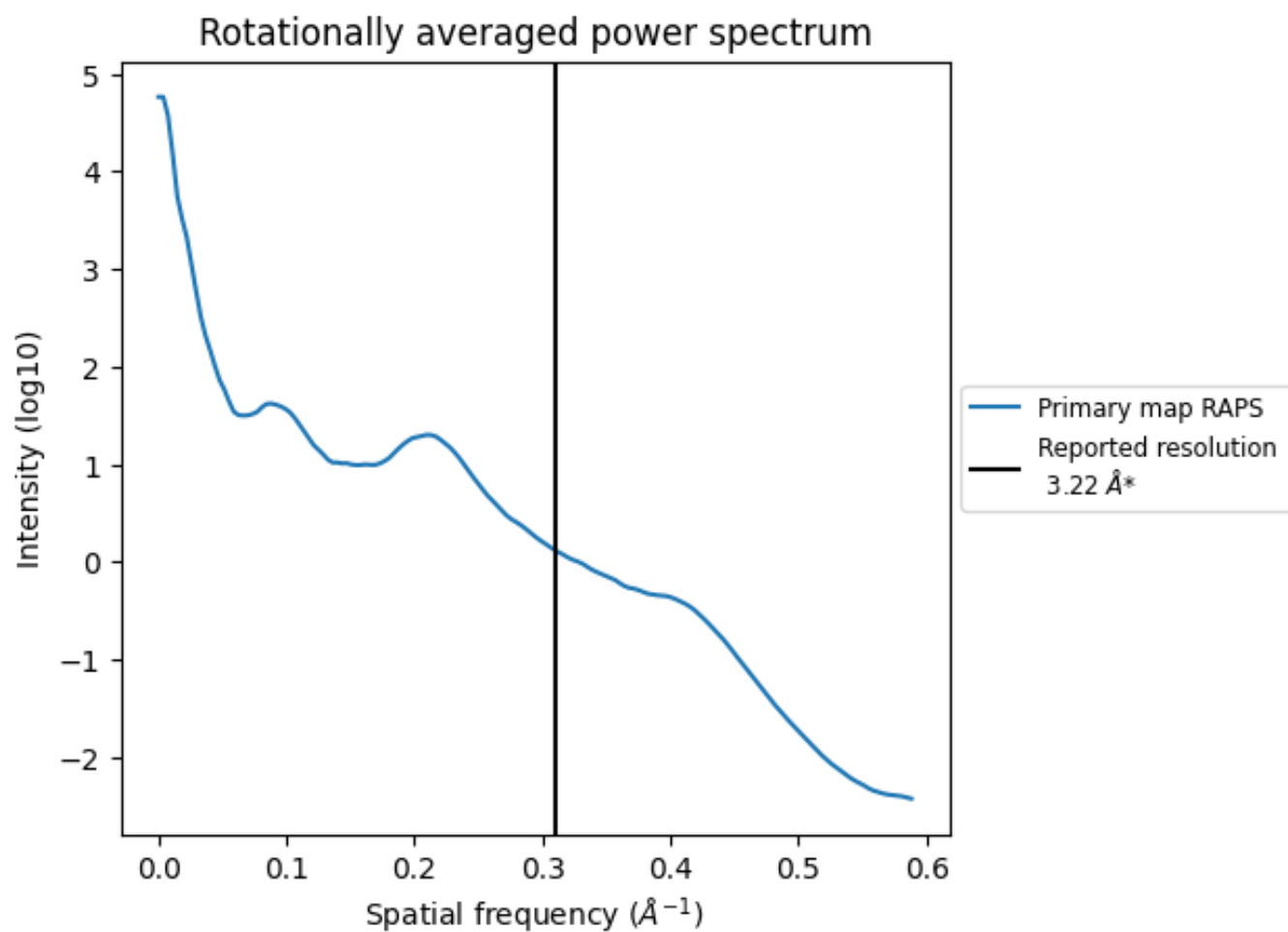
7.2 Volume estimate [i](#)



The volume at the recommended contour level is 68 nm³; this corresponds to an approximate mass of 62 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 0.311 Å⁻¹

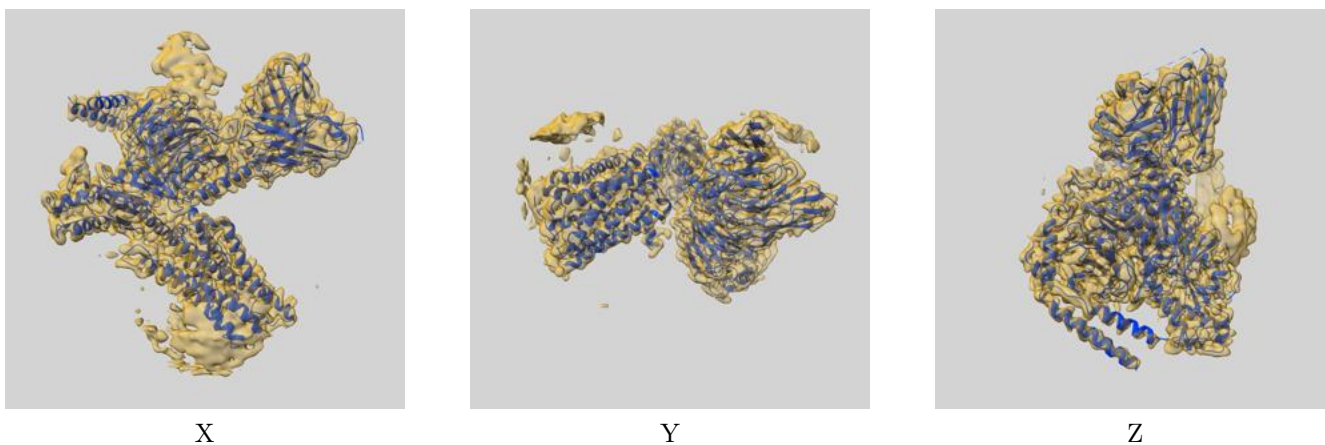
8 Fourier-Shell correlation

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

9 Map-model fit [i](#)

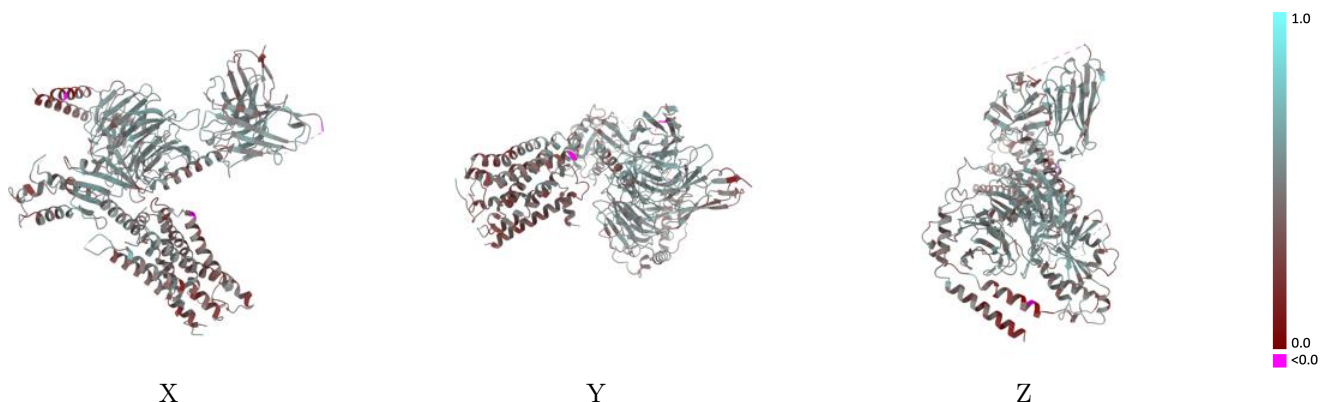
This section contains information regarding the fit between EMDB map EMD-25752 and PDB model 7T96. Per-residue inclusion information can be found in section 3 on page 7.

9.1 Map-model overlay [i](#)



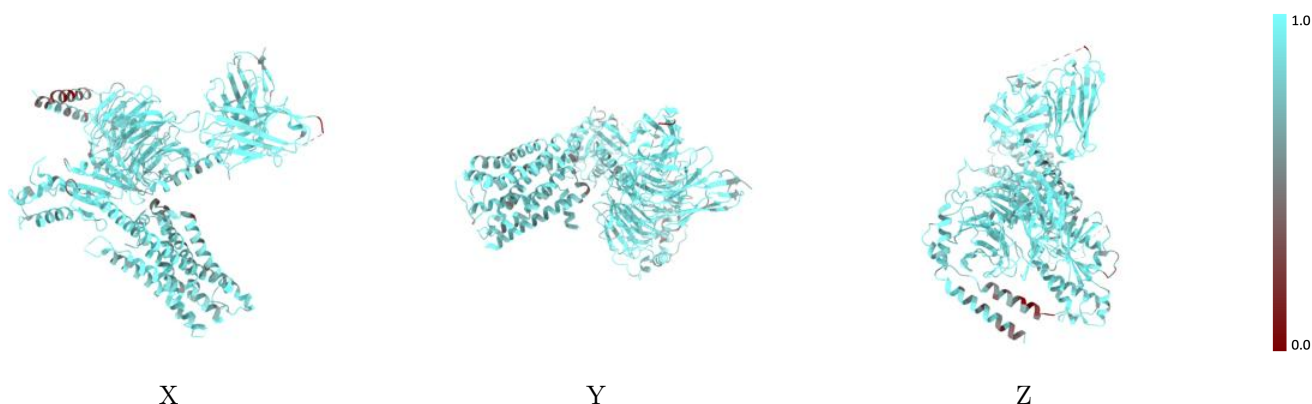
The images above show the 3D surface view of the map at the recommended contour level 0.06 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

9.2 Q-score mapped to coordinate model [\(i\)](#)



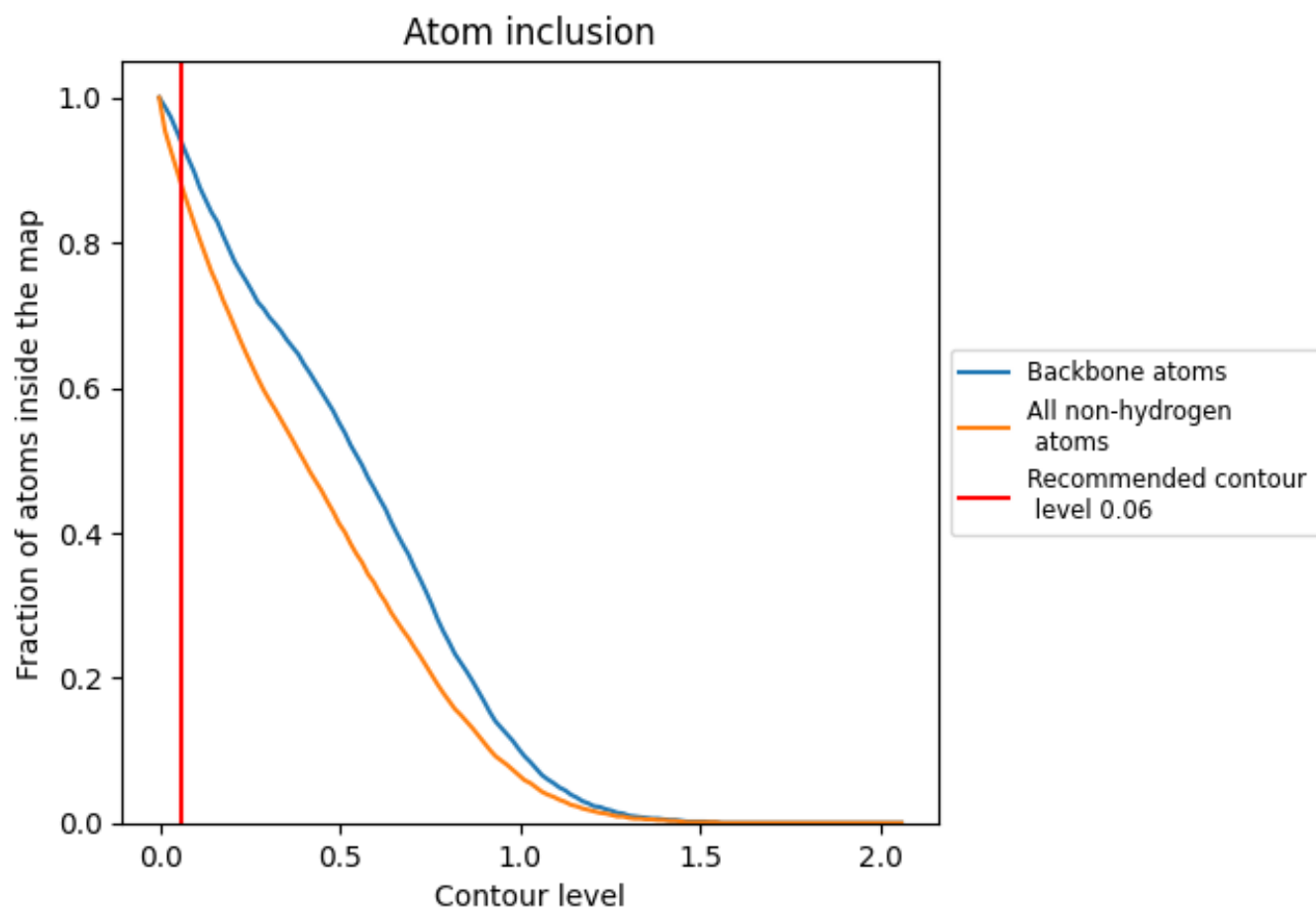
The images above show the model with each residue coloured according to its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions 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 [\(i\)](#)



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













9.4 Atom inclusion [i](#)



At the recommended contour level, 94% of all backbone atoms, 88% of all non-hydrogen atoms, are inside the map.

9.5 Map-model fit summary

The table lists the average atom inclusion at the recommended contour level (0.06) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	 0.8790	 0.4660
A	 0.8390	 0.3950
B	 0.8790	 0.4760
C	 0.9060	 0.5000
D	 0.7400	 0.3930
E	 0.9180	 0.5050

