wwPDB EM Validation Summary Report (i)

Nov 1, 2022 – 08:49 PM EDT

PDB ID	:	5KUF
EMDB ID	:	EMD-8289
Title	:	GluK2EM with 2S,4R-4-methylglutamate
Authors	:	Meyerson, J.R.; Chittori, S.; Merk, A.; Rao, P.; Han, T.H.; Serpe, M.; Mayer, M.L.; Subramaniam, S.
Deposited on	:	2016-07-13
Resolution	:	3.80 Å(reported)

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

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

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

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

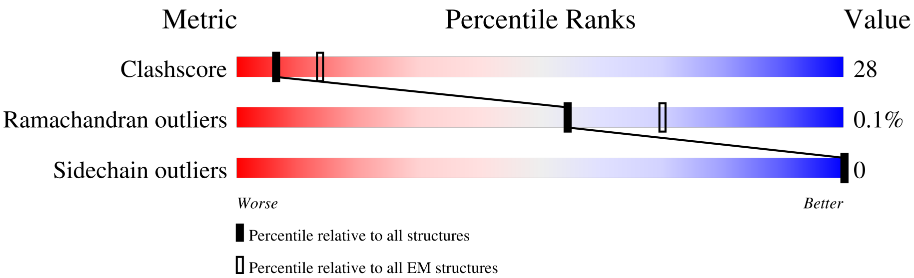
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.31.2

1 Overall quality at a glance (i)

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

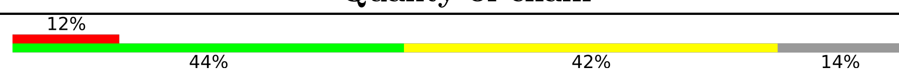
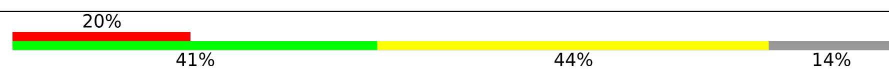
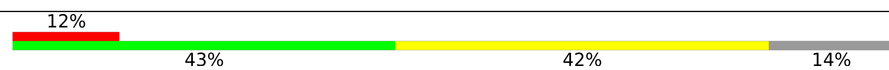
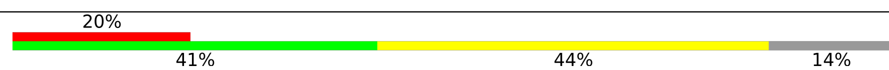
The reported resolution of this entry is 3.80 Å.

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	877	
1	B	877	
1	C	877	
1	D	877	

The following table lists non-polymeric compounds, carbohydrate monomers and non-standard residues in protein, DNA, RNA chains that are outliers for geometric or electron-density-fit criteria:

Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
2	SYM	B	901	-	-	X	-

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Mol	Type	Chain	Res	Chirality	Geometry	Clashes	Electron density
2	SYM	D	901	-	-	X	-

2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 23728 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 Glutamate receptor ionotropic, kainate 2.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	750	Total 5921	C 3802	N 985	O 1103	S 31	0	0
1	B	750	Total 5921	C 3802	N 985	O 1103	S 31	0	0
1	C	750	Total 5921	C 3802	N 985	O 1103	S 31	0	0
1	D	750	Total 5921	C 3802	N 985	O 1103	S 31	0	0

There are 24 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	487	THR	ALA	engineered mutation	UNP P42260
A	536	VAL	ILE	variant	UNP P42260
A	545	VAL	CYS	variant	UNP P42260
A	658	SER	ALA	engineered mutation	UNP P42260
A	690	SER	ASN	engineered mutation	UNP P42260
A	704	LEU	PHE	engineered mutation	UNP P42260
B	487	THR	ALA	engineered mutation	UNP P42260
B	536	VAL	ILE	variant	UNP P42260
B	545	VAL	CYS	variant	UNP P42260
B	658	SER	ALA	engineered mutation	UNP P42260
B	690	SER	ASN	engineered mutation	UNP P42260
B	704	LEU	PHE	engineered mutation	UNP P42260
C	487	THR	ALA	engineered mutation	UNP P42260
C	536	VAL	ILE	variant	UNP P42260
C	545	VAL	CYS	variant	UNP P42260
C	658	SER	ALA	engineered mutation	UNP P42260
C	690	SER	ASN	engineered mutation	UNP P42260
C	704	LEU	PHE	engineered mutation	UNP P42260
D	487	THR	ALA	engineered mutation	UNP P42260
D	536	VAL	ILE	variant	UNP P42260
D	545	VAL	CYS	variant	UNP P42260
D	658	SER	ALA	engineered mutation	UNP P42260

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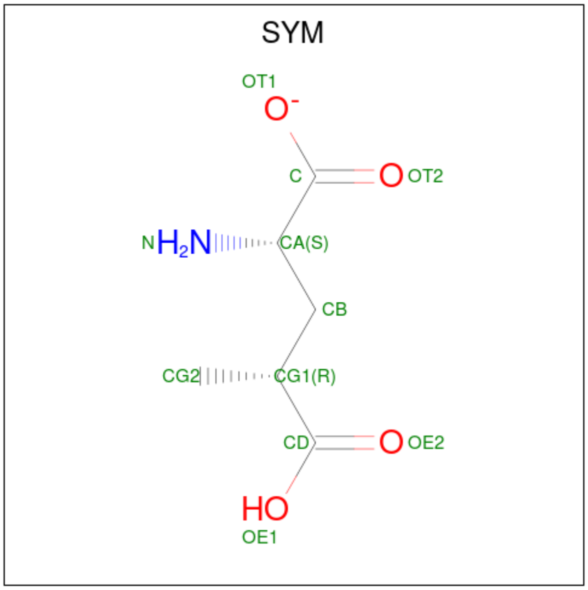
Chain	Residue	Modelled	Actual	Comment	Reference
D	690	SER	ASN	engineered mutation	UNP P42260
D	704	LEU	PHE	engineered mutation	UNP P42260

- Molecule 2 is 2S,4R-4-METHYLGLUTAMATE (three-letter code: SYM) (formula: $C_6H_{10}NO_4$).

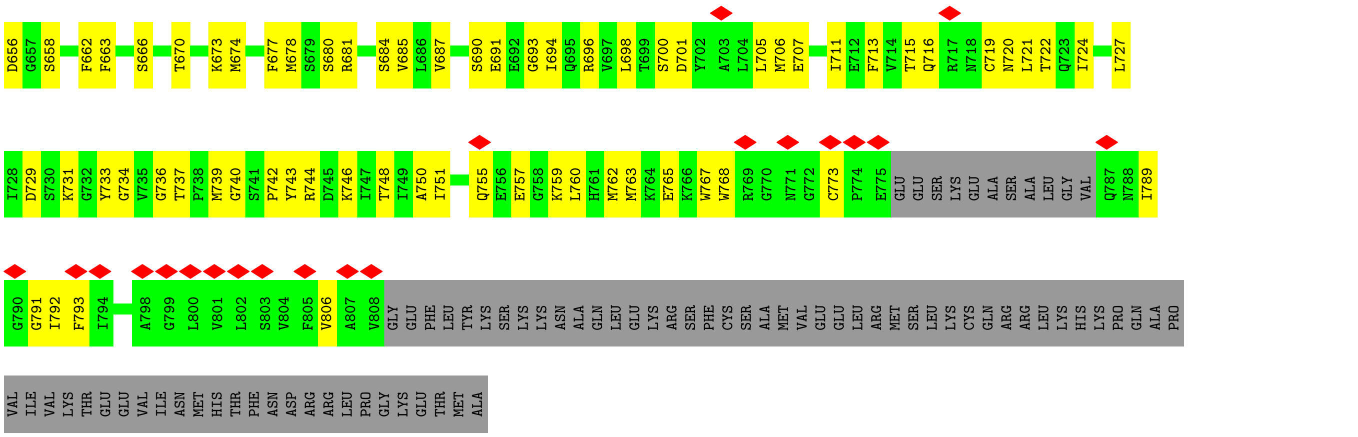


Mol	Chain	Residues	Atoms	AltConf
2	A	1	Total C N O 11 6 1 4	0
2	B	1	Total C N O 11 6 1 4	0
2	C	1	Total C N O 11 6 1 4	0
2	D	1	Total C N O 11 6 1 4	0

3 Residue-property plots

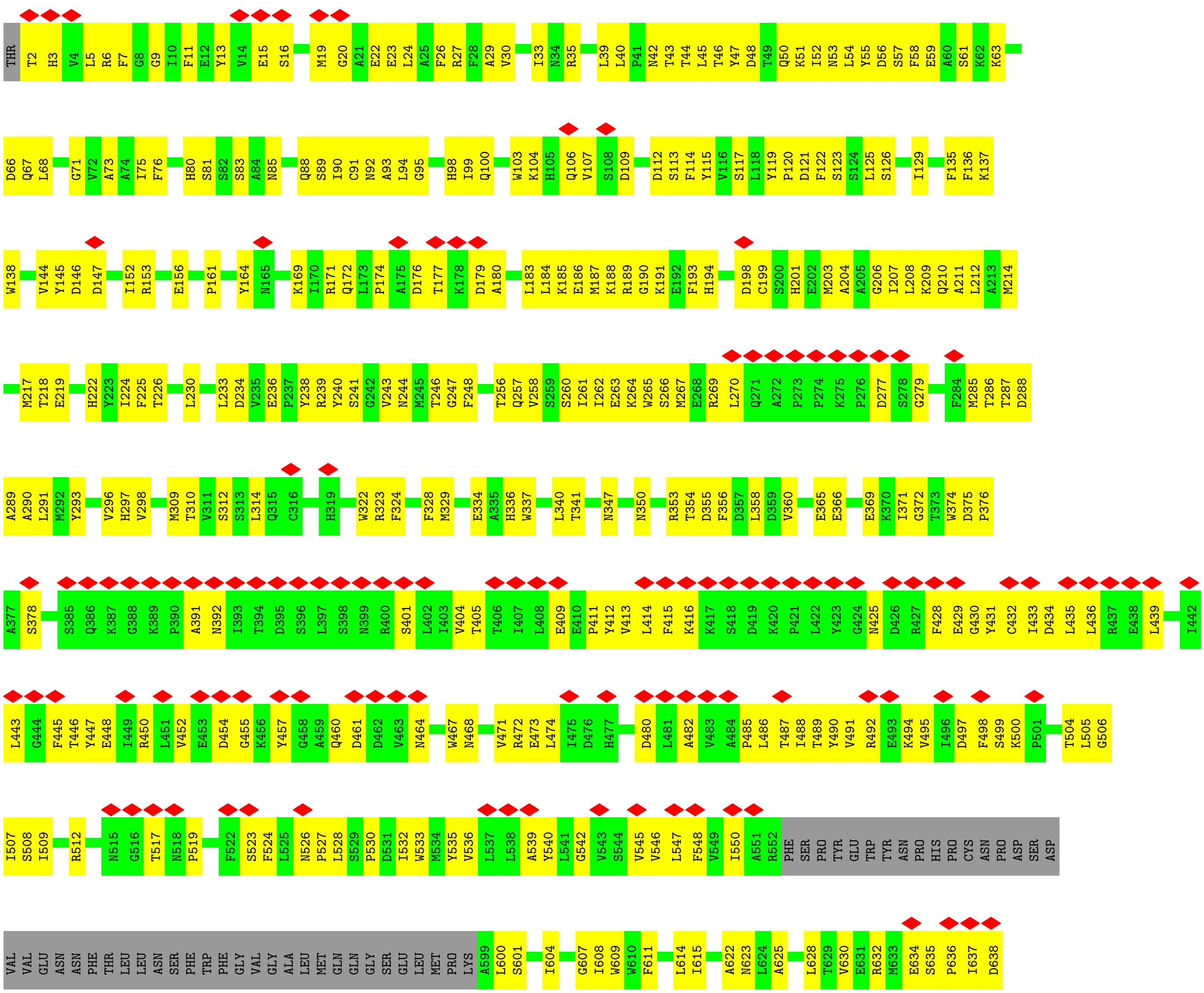
These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and atom inclusion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

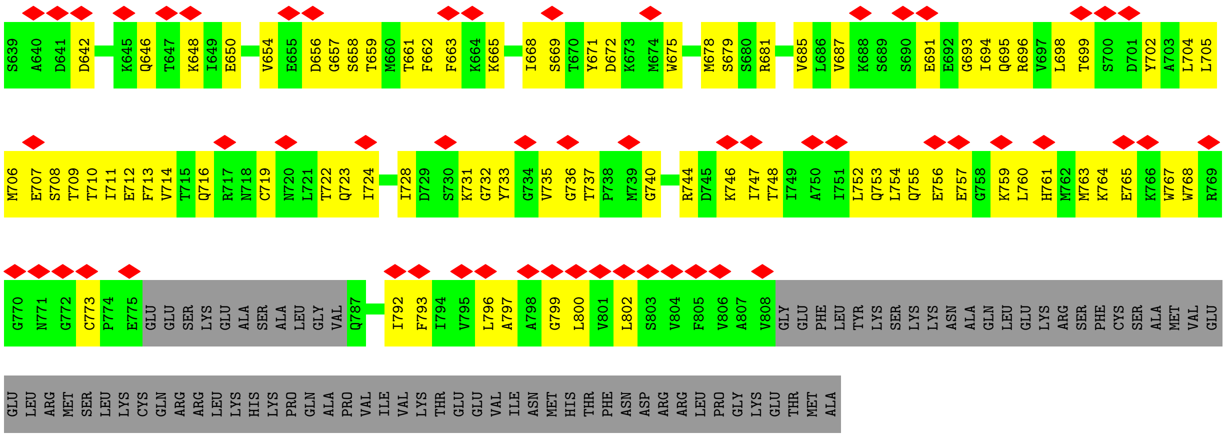
- Molecule 1: Glutamate receptor ionotropic, kainate 2



• Molecule 1: Glutamate receptor ionotropic, kainate 2

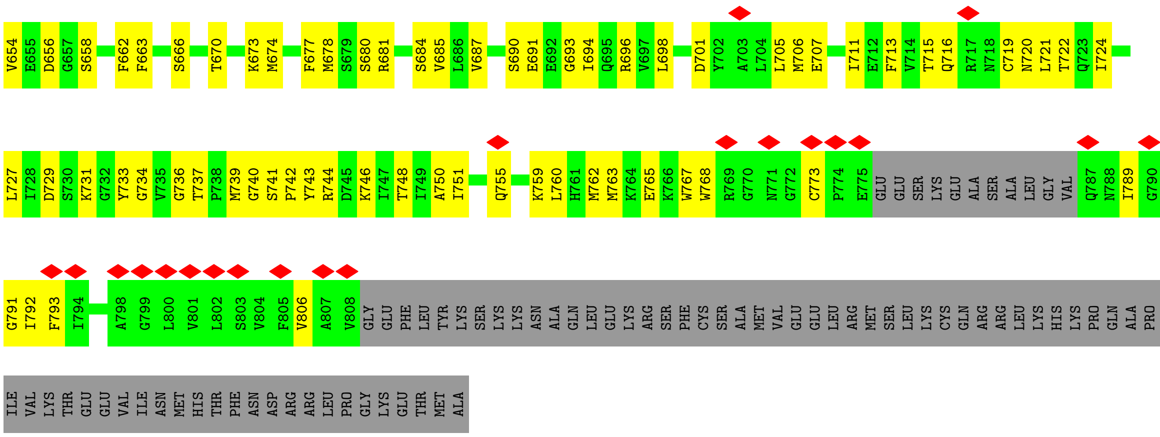
Chain B:





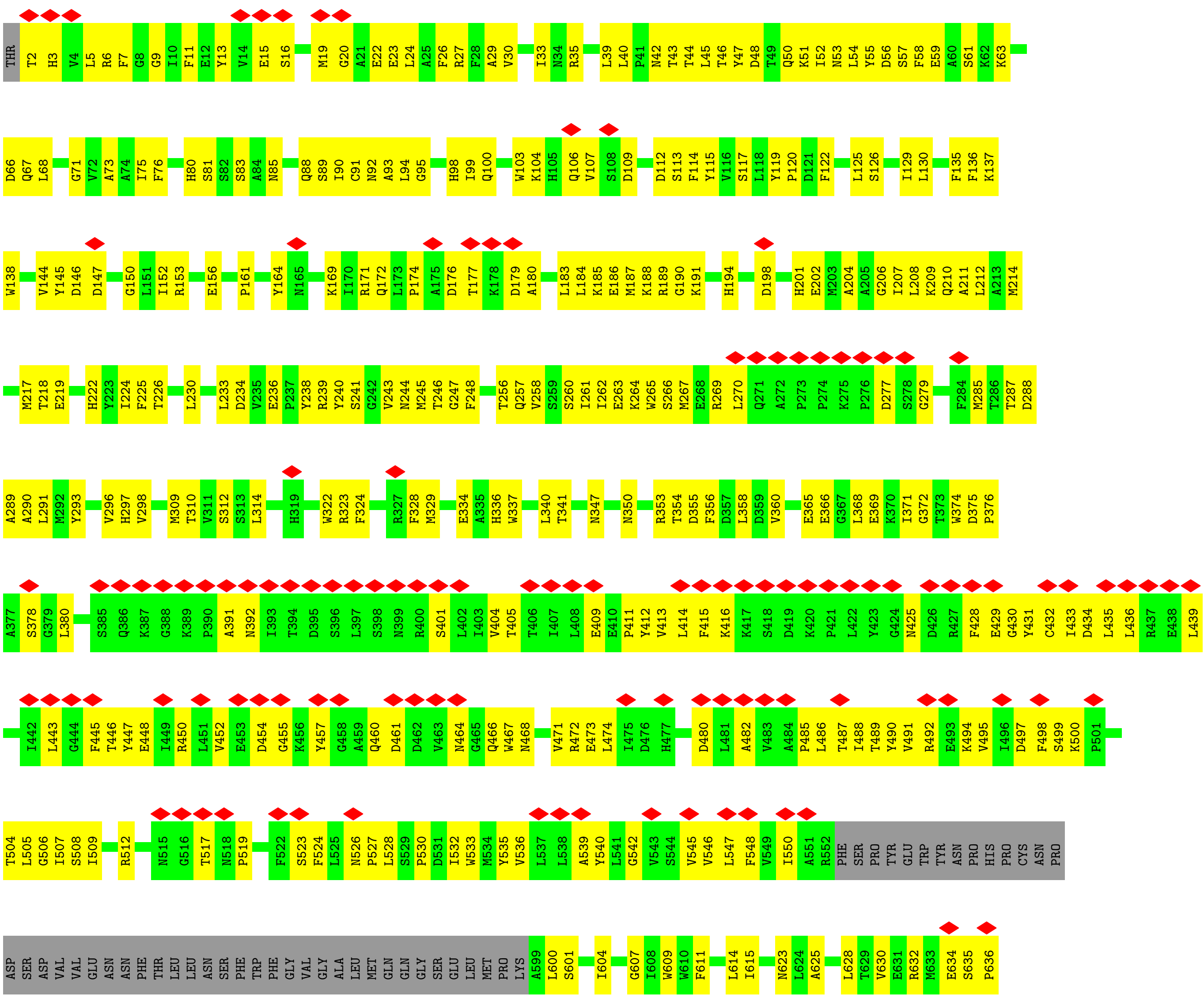
• Molecule 1: Glutamate receptor ionotropic, kainate 2

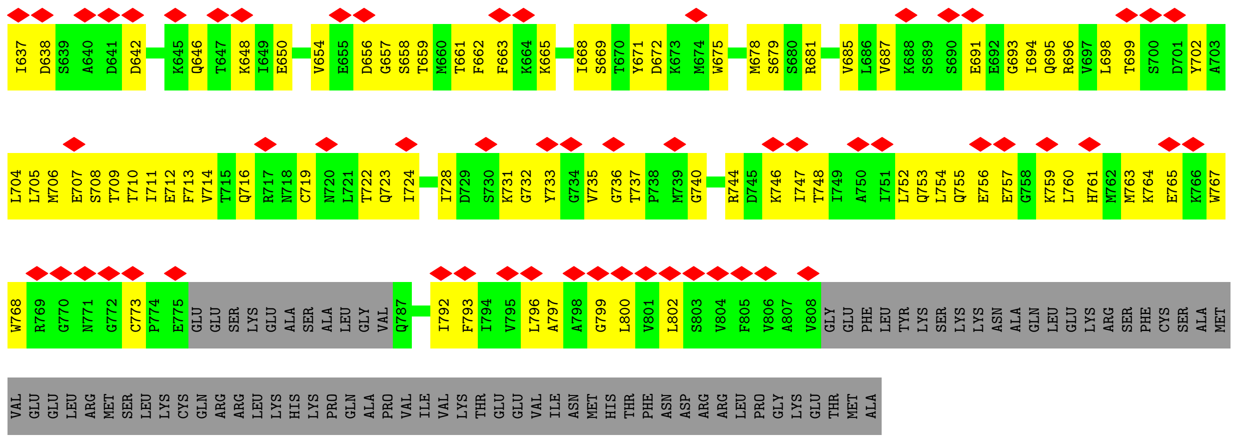
Chain C:



- Molecule 1: Glutamate receptor ionotropic, kainate 2

Chain D:




I637 D638 S639 A640 D641 D642 K645 Q646 T647 K648 I649 E650 V654 E655 D656 G657 S658 T659 M660 T661 F662 F663 K664 K665 I668 S669 T670 Y671 D672 K673 M674 W675 M678 S679 S680 R681 V685 L686 V687 K688 S689 S690 E691 E692 G693 I694 Q695 R696 V697 L698 T699 S700 D701 Y702 A703
L704 L705 M706 E707 S708 T709 T710 I711 E712 F713 V714 I715 Q716 R717 N718 C719 N720 L721 T722 Q723 I724 I728 D729 S730 K731 G732 Y733 G734 V735 G736 T737 P738 M739 G740 R744 D745 K746 I747 T748 I749 A750 I751 L752 Q753 L754 Q755 E756 E757 G758 K759 L760 H761 M762 M763 K764 E765 K766 W767
W768 R769 G770 N771 G772 C773 P774 E775 GLU GLU SER LYS GLU ALA SER ALA LEU GLY VAL Q787 I792 F793 I794 V795 L796 A797 A798 G799 L800 V801 L802 S803 V804 F805 V806 A807 V808 GLY GLU PHE LEU TYR LYS SER LYS LYS ASN ALA GLN LEU GLU LYS ARG SER PHE CYS SER ALA MET
VAL GLU GLU LEU ARG MET SER LEU LYS CYS GLN ARG ARG LEU LYS HIS LYS PRO GLN ALA PRO VAL ILE VAL LYS THR GLU GLU VAL ILE ASN MET HIS THR PHE ASN ASP ARG ARG LEU PRO GLY LYS GLU THR MET ALA

4 Experimental information

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, C2	Depositor
Number of particles used	62244	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 $(e^-/\text{Å}^2)$	45	Depositor
Minimum defocus (nm)	Not provided	
Maximum defocus (nm)	Not provided	
Magnification	Not provided	
Image detector	GATAN K2 SUMMIT (4k x 4k)	Depositor
Maximum map value	0.161	Depositor
Minimum map value	-0.080	Depositor
Average map value	-0.000	Depositor
Map value standard deviation	0.007	Depositor
Recommended contour level	0.037	Depositor
Map size (Å)	397.2, 397.2, 397.2	wwPDB
Map dimensions	300, 300, 300	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.324, 1.324, 1.324	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: SYM

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	0.48	0/6050	0.58	1/8194 (0.0%)
1	B	0.48	0/6050	0.59	0/8194
1	C	0.48	0/6050	0.58	1/8194 (0.0%)
1	D	0.48	0/6050	0.59	0/8194
All	All	0.48	0/24200	0.58	2/32776 (0.0%)

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	3
1	B	0	1
1	C	0	3
1	D	0	1
All	All	0	8

There are no bond length outliers.

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	513	LYS	C-N-CD	-5.10	109.37	120.60
1	C	513	LYS	C-N-CD	-5.09	109.40	120.60

There are no chirality outliers.

5 of 8 planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	14	VAL	Peptide
1	A	380	LEU	Peptide
1	A	80	HIS	Peptide
1	B	35	ARG	Peptide
1	C	14	VAL	Peptide

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	5921	0	5917	324	0
1	B	5921	0	5917	360	0
1	C	5921	0	5917	332	0
1	D	5921	0	5917	364	0
2	A	11	0	9	5	0
2	B	11	0	9	6	0
2	C	11	0	9	5	0
2	D	11	0	9	7	0
All	All	23728	0	23704	1337	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 28.

The worst 5 of 1337 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:5:LEU:HB3	1:C:45:LEU:HD22	1.44	0.98
1:A:5:LEU:HB3	1:A:45:LEU:HD22	1.44	0.97
1:B:92:ASN:HD21	1:B:113:SER:H	0.98	0.96
1:D:92:ASN:HD21	1:D:113:SER:H	0.98	0.91
1:D:88:GLN:HE22	1:D:106:GLN:HE22	1.21	0.88

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	744/877 (85%)	627 (84%)	116 (16%)	1 (0%)	51	83
1	B	744/877 (85%)	630 (85%)	114 (15%)	0	100	100
1	C	744/877 (85%)	625 (84%)	118 (16%)	1 (0%)	51	83
1	D	744/877 (85%)	630 (85%)	114 (15%)	0	100	100
All	All	2976/3508 (85%)	2512 (84%)	462 (16%)	2 (0%)	54	83

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	15	GLU
1	C	15	GLU

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	649/767 (85%)	649 (100%)	0	100	100
1	B	649/767 (85%)	649 (100%)	0	100	100
1	C	649/767 (85%)	649 (100%)	0	100	100
1	D	648/767 (84%)	648 (100%)	0	100	100
All	All	2595/3068 (85%)	2595 (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. 5 of 34 such sidechains are listed below:

Mol	Chain	Res	Type
1	D	297	HIS
1	D	319	HIS
1	D	623	ASN
1	B	297	HIS
1	B	92	ASN

5.3.3 RNA (i)

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains (i)

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

5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

5.6 Ligand geometry (i)

4 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	SYM	B	901	-	9,10,10	1.00	0	10,13,13	0.77	0
2	SYM	D	901	-	9,10,10	1.00	0	10,13,13	0.78	0
2	SYM	C	901	-	9,10,10	1.01	0	10,13,13	0.86	0
2	SYM	A	901	-	9,10,10	1.02	0	10,13,13	0.87	0

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	SYM	B	901	-	-	4/12/12/12	-
2	SYM	D	901	-	-	4/12/12/12	-
2	SYM	C	901	-	-	6/12/12/12	-
2	SYM	A	901	-	-	6/12/12/12	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

5 of 20 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	A	901	SYM	CA-CB-CG1-CG2
2	A	901	SYM	CA-CB-CG1-CD
2	A	901	SYM	OE2-CD-CG1-CB
2	C	901	SYM	CA-CB-CG1-CG2
2	C	901	SYM	CA-CB-CG1-CD

There are no ring outliers.

4 monomers are involved in 23 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	B	901	SYM	6	0
2	D	901	SYM	7	0
2	C	901	SYM	5	0
2	A	901	SYM	5	0

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.

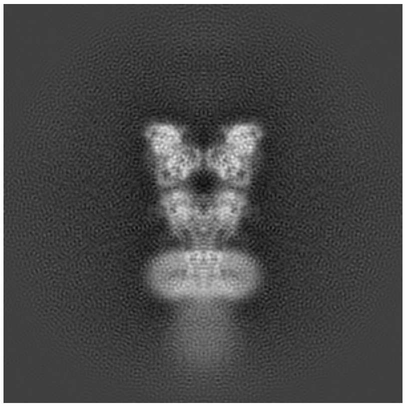
6 Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-8289. These allow visual inspection of the internal detail of the map and identification of artifacts.

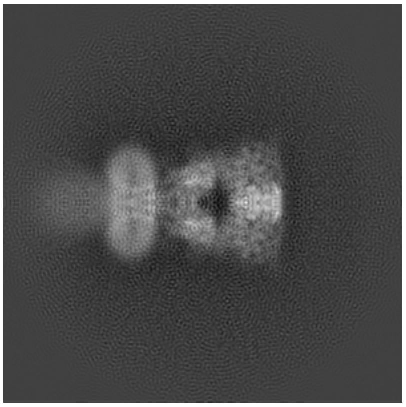
No raw map or half-maps were deposited for this entry and therefore no images, graphs, etc. pertaining to the raw map can be shown.

6.1 Orthogonal projections (i)

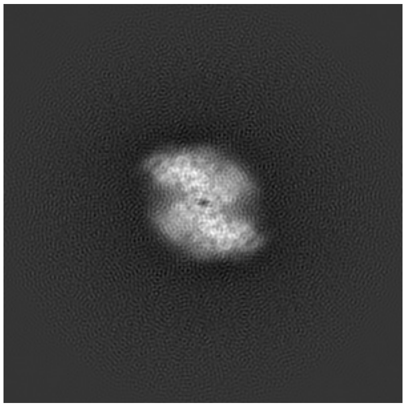
6.1.1 Primary map



X



Y

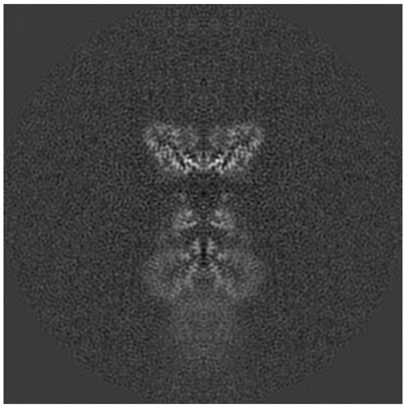


Z

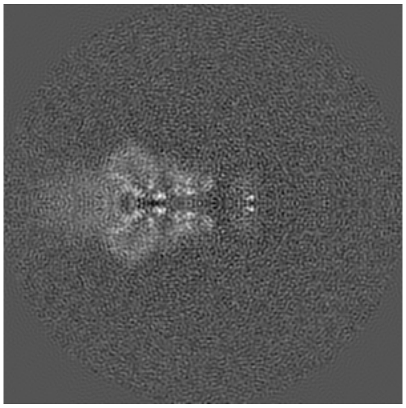
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

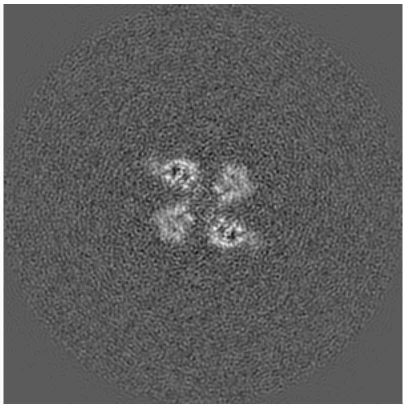
6.2.1 Primary map



X Index: 150



Y Index: 150

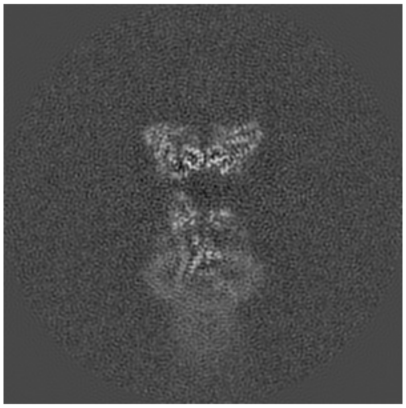


Z Index: 150

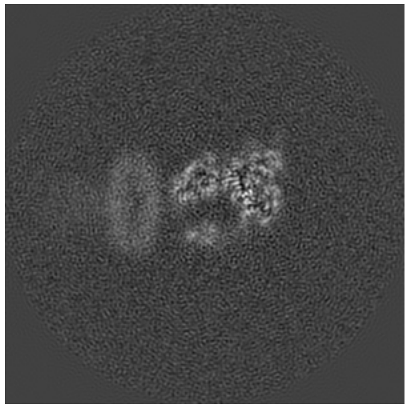
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

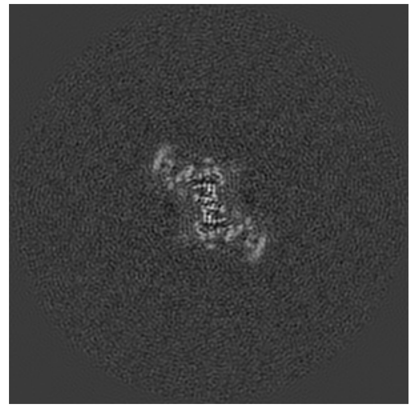
6.3.1 Primary map



X Index: 153



Y Index: 128



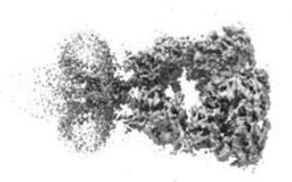
Z Index: 182

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

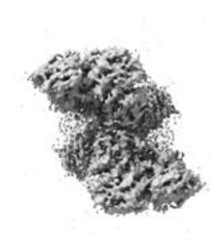
6.4 Orthogonal surface views (i)

6.4.1 Primary map

X



Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.037. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

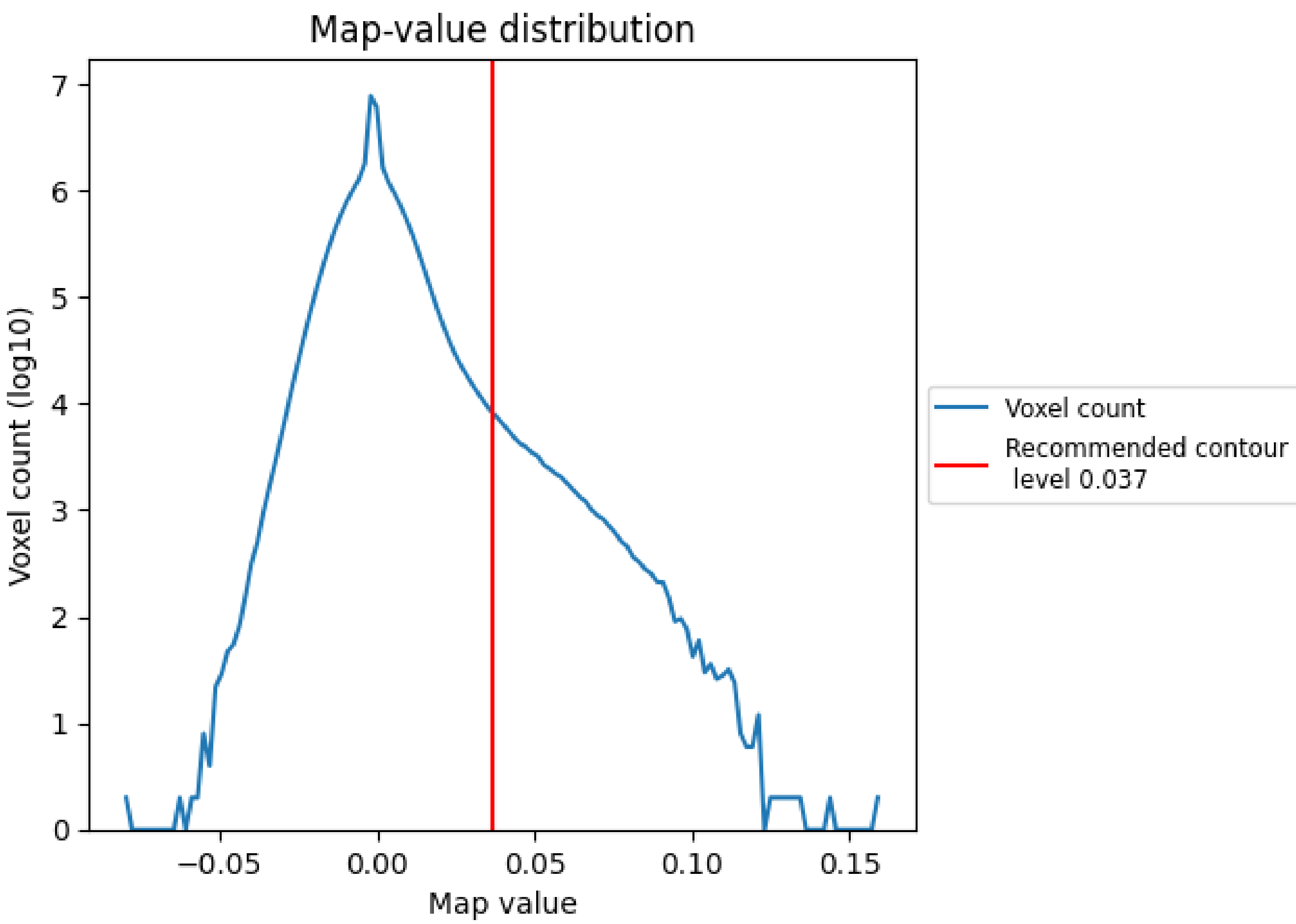
6.5 Mask visualisation (i)

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

7 Map analysis (i)

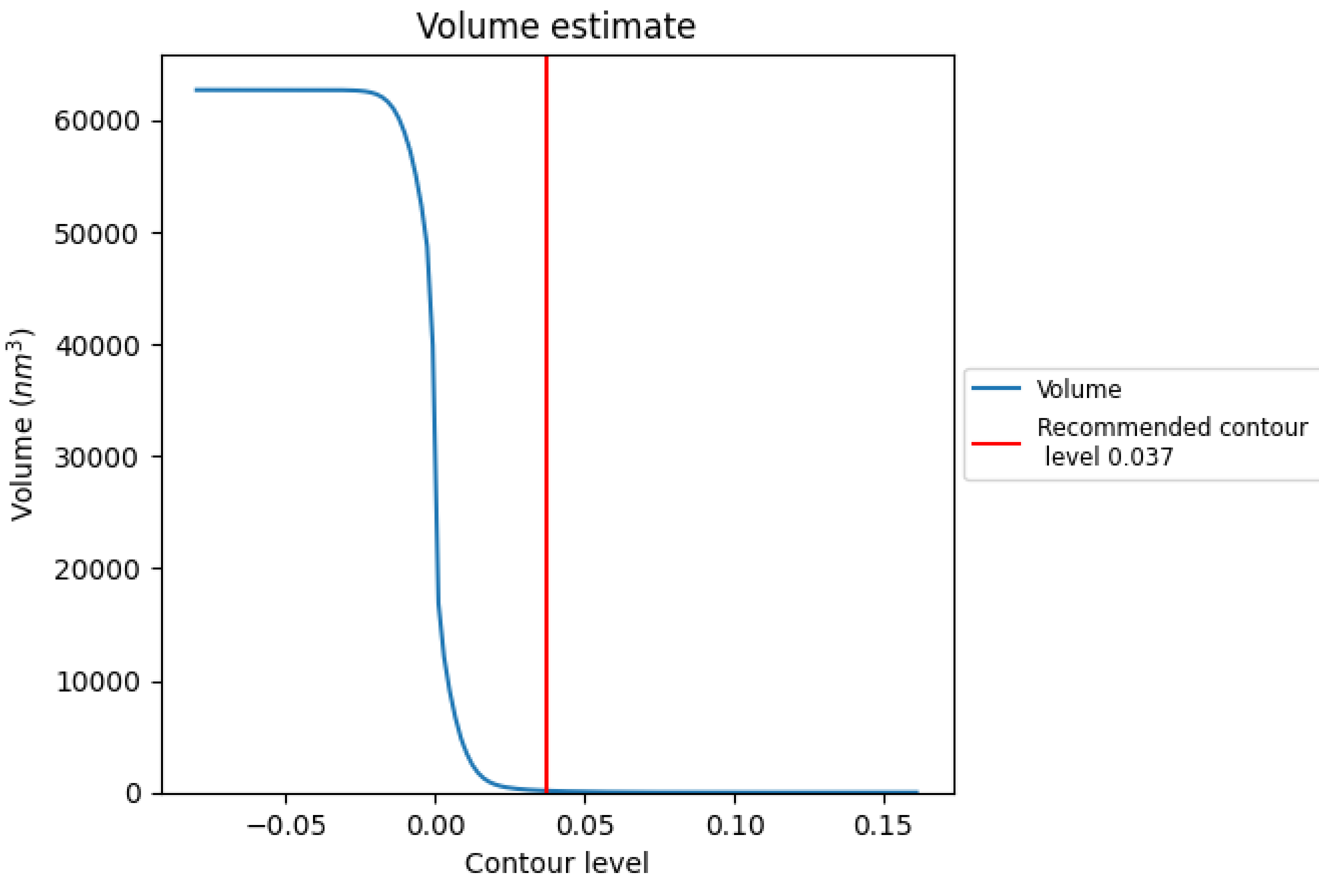
This section contains the results of statistical analysis of the map.

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.

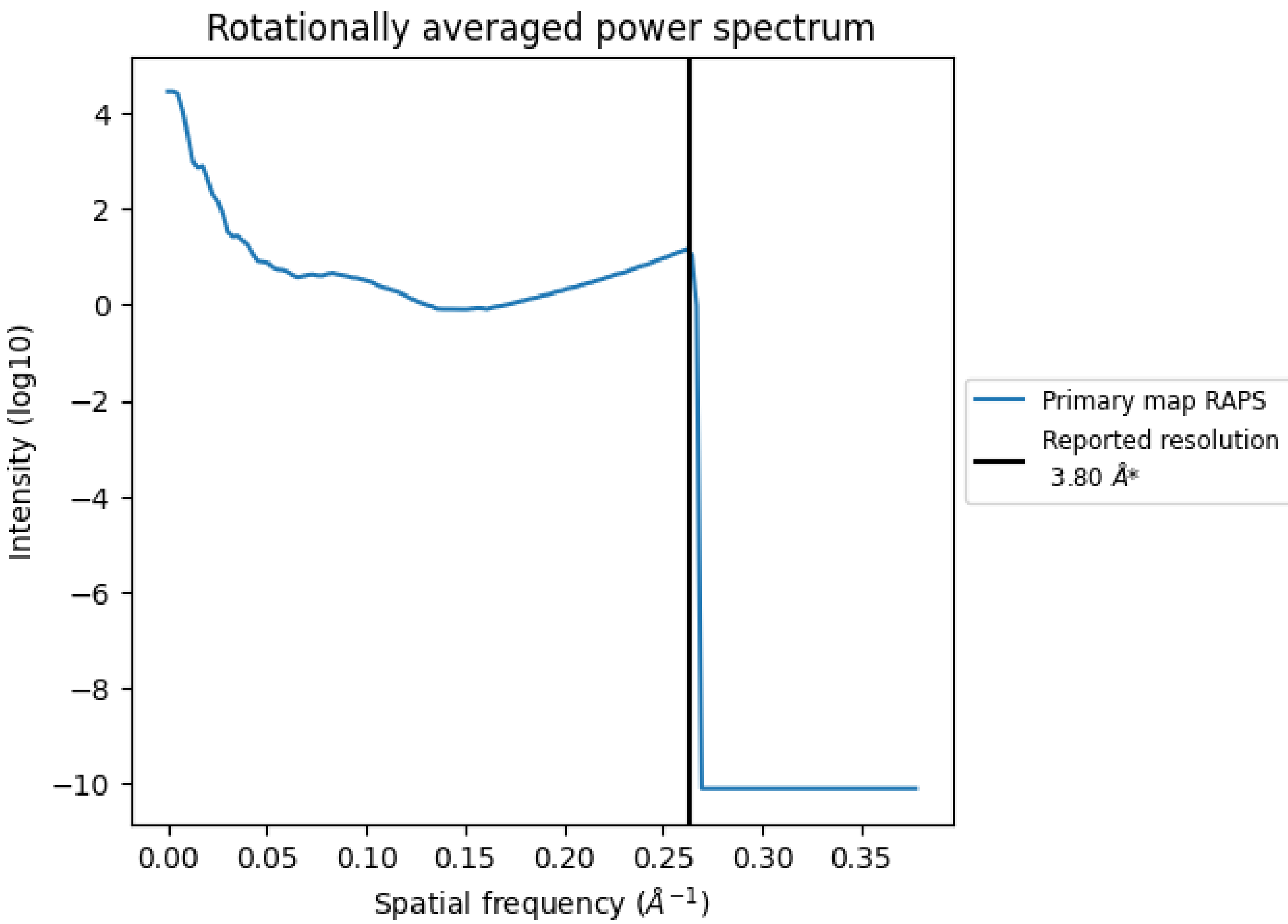
7.2 Volume estimate (i)



The volume at the recommended contour level is 153 nm^3; this corresponds to an approximate mass of 139 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 0.263 $Å^{-1}$

8 Fourier-Shell correlation (i)

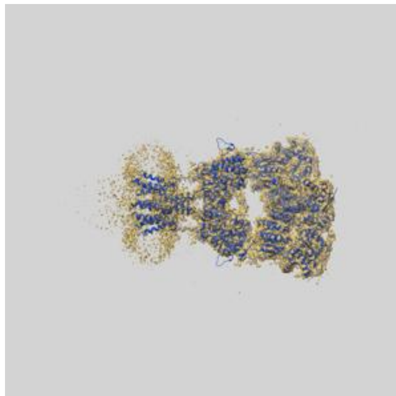
This section was not generated. No FSC curve or half-maps provided.

9 Map-model fit (i)

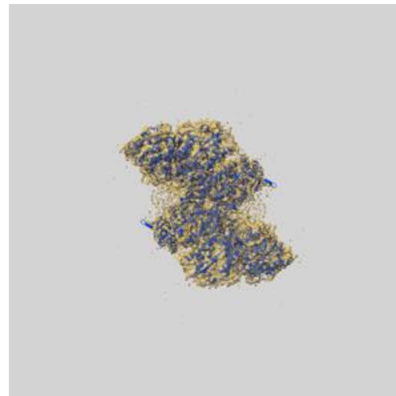
This section contains information regarding the fit between EMDB map EMD-8289 and PDB model 5KUF. Per-residue inclusion information can be found in section 3 on page 6.

9.1 Map-model overlay (i)

X



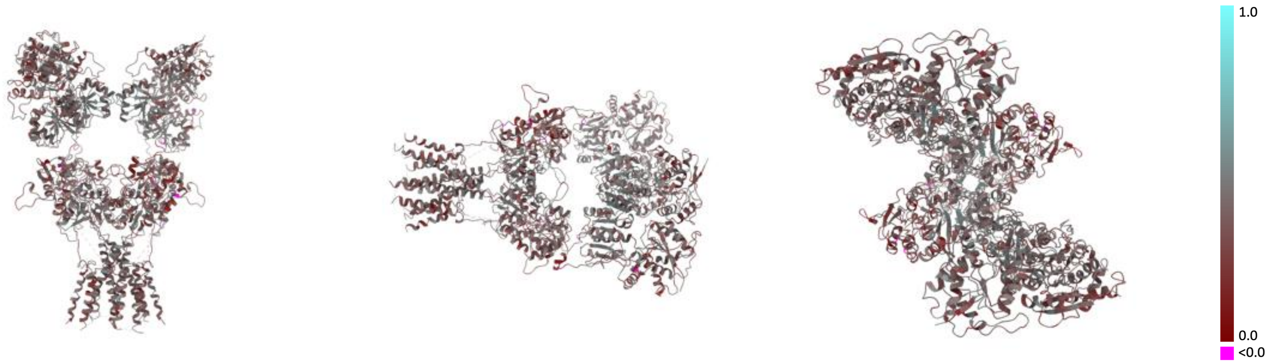
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.037 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.

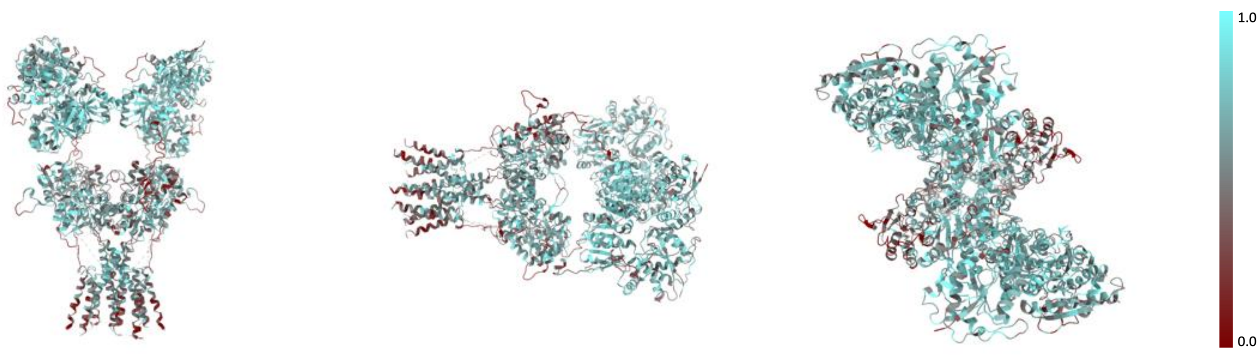
9.2 Q-score mapped to coordinate model (i)



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.

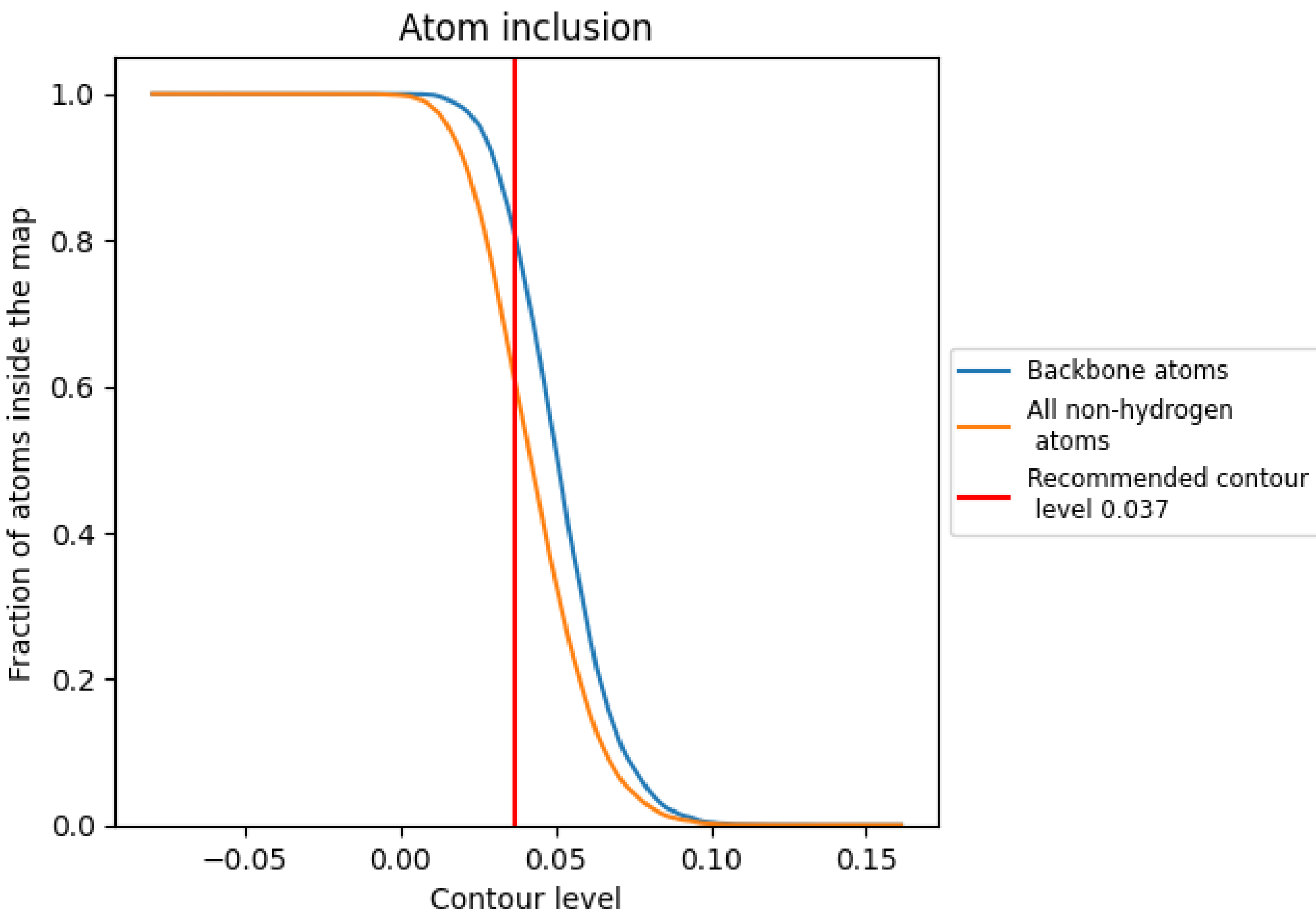
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.037).

9.4 Atom inclusion ⓘ



At the recommended contour level, 81% of all backbone atoms, 61% 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 (0.037) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.6055	0.3780
A	0.6311	0.3880
B	0.5799	0.3680
C	0.6318	0.3870
D	0.5792	0.3700