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wwPDB EM Validation Summary Report ⓘ

Nov 23, 2022 – 05:29 PM JST

PDB ID	:	7VBI
EMDB ID	:	EMD-31880
Title	:	Cryo-EM structure of the non-acylated tirzepatide (LY3298176)-bound human GLP-1R-Gs complex
Authors	:	Zhao, F.H.; Zhou, Q.T.; Cong, Z.T.; Hang, K.N.; Zou, X.Y.; Zhang, C.; Chen, Y.; Dai, A.T.; Liang, A.Y.; Ming, Q.Q.; Wang, M.; Chen, L.N.; Xu, P.Y.; Chang, R.L.; Feng, W.B.; Xia, T.; Zhang, Y.; Wu, B.L.; Yang, D.H.; Zhao, L.H.; Xu, H.E.; Wang, M.W.
Deposited on	:	2021-08-31
Resolution	:	3.00 Å(reported)

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

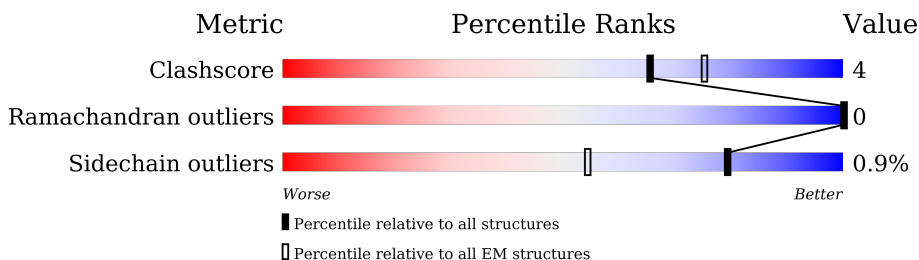|		
EMDB validation analysis	:	0.0.1.dev43
Mogul	:	1.8.5 (274361), CSD as541be (2020)
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.9
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.31.3

1 Overall quality at a glance ⓘ

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

The reported resolution of this entry is 3.00 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	Percentile Ranks	Value
Clashscore		4
Ramachandran outliers		0
Sidechain outliers		0.9%

Worse   *Better*

▮ Percentile relative to all structures
▯ Percentile relative to all EM structures

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	357	54% 9% · 36%
2	N	140	77% 12% · 10%
3	P	29	83% 17%
4	R	440	80% 7% 13%
5	B	345	83% 14% ·
6	G	70	7% 69% 13% 19%

2   Entry composition ⓘ

There are 6 unique types of molecules in this entry. The entry contains 9223 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 3 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	227	Total	C	N	O	S	0	0
			1891	1197	347	341	6		

There are 19 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	54	ASN	SER	conflict	UNP P63092-3
A	226	ALA	GLY	conflict	UNP P63092-3
A	?	-	SER	deletion	UNP P63092-3
A	?	-	TYR	deletion	UNP P63092-3
A	?	-	ASN	deletion	UNP P63092-3
A	?	-	MET	deletion	UNP P63092-3
A	?	-	VAL	deletion	UNP P63092-3
A	?	-	ILE	deletion	UNP P63092-3
A	?	-	ARG	deletion	UNP P63092-3
A	?	-	GLU	deletion	UNP P63092-3
A	?	-	ASP	deletion	UNP P63092-3
A	?	-	ASN	deletion	UNP P63092-3
A	?	-	GLN	deletion	UNP P63092-3
A	268	ALA	GLU	conflict	UNP P63092-3
A	271	LYS	ASN	conflict	UNP P63092-3
A	274	ASP	LYS	conflict	UNP P63092-3
A	280	LYS	ARG	conflict	UNP P63092-3
A	284	ASP	THR	conflict	UNP P63092-3
A	285	THR	ILE	conflict	UNP P63092-3

- Molecule 2 is a protein called Nanobody 35.

Mol	Chain	Residues	Atoms					AltConf	Trace
2	N	126	Total	C	N	O	S	0	0
			961	599	168	188	6		

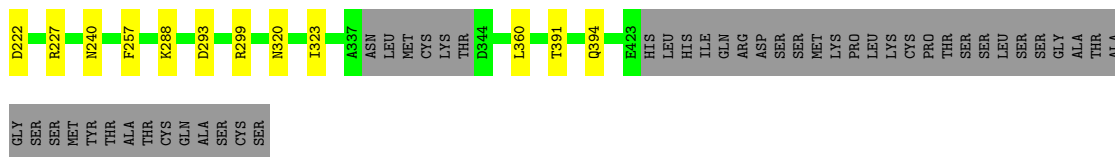- Molecule 3 is a protein called Non-acylated_tirzepatide.

Mol	Chain	Residues	Atoms				AltConf	Trace
3	P	29	Total 229	C 152	N 34	O 43	0	0

- Molecule 4 is a protein called Glucagon-like peptide 1 receptor.

Mol	Chain	Residues	Atoms					AltConf	Trace
4	R	382	Total 3115	C 2062	N 504	O 531	S 18	0	0

- Molecule 5 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1.

Mol	Chain	Residues	Atoms					AltConf	Trace
5	B	337	Total 2591	C 1599	N 466	O 505	S 21	0	0

There are 6 discrepancies between the modelled and reference sequences:
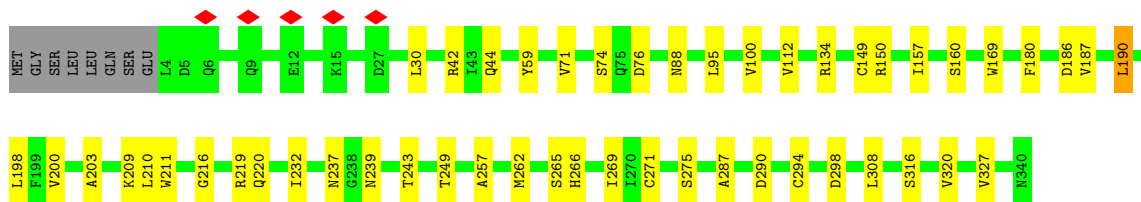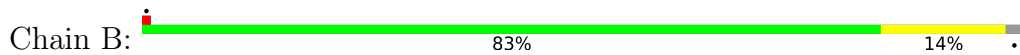
Chain	Residue	Modelled	Actual	Comment	Reference
B	-4	MET	-	initiating methionine	UNP P54311
B	-3	GLY	-	expression tag	UNP P54311
B	-2	SER	-	expression tag	UNP P54311
B	-1	LEU	-	expression tag	UNP P54311
B	0	LEU	-	expression tag	UNP P54311
B	1	GLN	-	expression tag	UNP P54311

- Molecule 6 is a protein called Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2.

Mol	Chain	Residues	Atoms					AltConf	Trace
6	G	57	Total 436	C 273	N 77	O 83	S 3	0	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: Isoform 3 of Guanine nucleotide-binding protein G(s) subunit alpha isoforms short

Chain A:

54%   9%   ·   36%

• Molecule 2: Nanobody 35

Chain N:

77%   12%   ·   10%

• Molecule 3: Non-acylated_tirzepatide

Chain P:

83%   17%

• Molecule 4: Glucagon-like peptide 1 receptor

Chain R:

80%   7%   13%

D222 R227 N240 F257 K288 D293 R299 N320 I323 A337 ASN LEU MET CYS LYS THR D344 L360 T391 Q394 E423 HIS LEU HIS ILE GLN ARG ASP SER SER MET LYS PRO LEU LYS CYS PRO THR SER SER LEU SER SER GLY ALA THR ALA

GLY SER SER MET TYR THR ALA THR CYS GLN ALA SER CYS SER

● Molecule 5: Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1

Chain B: 83%   14%

MET GLY SER LEU LEU GLN SER GLU L4 D5 Q6 Q9 E12 K15 D27 L30 R42 I43 Q44 Y59 V71 S74 Q75 D76 N88 L95 V100 V112 R134 C149 R150 I157 S160 W169 F180 D186 V187 L190

L198 F199 V200 A203 K209 L210 W211 G216 R219 Q220 I232 N237 G238 N239 T243 T249 A257 M262 S265 H266 I269 I270 C271 S275 A287 D290 C294 D298 L308 S316 V320 V327 N340

● Molecule 6: Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2

7%

Chain G: 69%   13%   19%

ALA SER ASN ASN T6 A7 S8 I9 A10 Q11 I25 D26 R27 I28 K29 V30 S31 K46 E47 N59 P60 F61 R62 GLU LYS LYS PHE PHE CYS ALA ILE LEU

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	1320686	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 $(e^-/\text{Å}^2)$	80	Depositor
Minimum defocus (nm)	Not provided	
Maximum defocus (nm)	Not provided	
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	6.863	Depositor
Minimum map value	-4.566	Depositor
Average map value	-0.001	Depositor
Map value standard deviation	0.108	Depositor
Recommended contour level	0.232	Depositor
Map size (Å)	274.176, 274.176, 274.176	wwPDB
Map dimensions	256, 256, 256	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	1.071, 1.071, 1.071	Depositor

5 Model quality ⓘ

5.1 Standard geometry ⓘ

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

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	0.55	0/1927	0.61	2/2588 (0.1%)
2	N	0.61	0/981	0.64	0/1329
3	P	0.41	0/222	0.49	0/300
4	R	0.44	0/3209	0.59	2/4376 (0.0%)
5	B	0.63	0/2638	0.70	1/3577 (0.0%)
6	G	0.32	0/442	0.54	0/597
All	All	0.53	0/9419	0.63	5/12767 (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	1
2	N	0	1
4	R	0	2
All	All	0	4

There are no bond length outliers.

All (5) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
4	R	217	LEU	CA-CB-CG	6.00	129.11	115.30
1	A	270	LEU	CA-CB-CG	5.38	127.67	115.30
4	R	192	LEU	CA-CB-CG	5.26	127.39	115.30
5	B	190	LEU	CA-CB-CG	5.25	127.37	115.30
1	A	302	LEU	CA-CB-CG	5.04	126.89	115.30

There are no chirality outliers.

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All (4) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	251	SER	Peptide
2	N	99	CYS	Peptide
4	R	217	LEU	Peptide
4	R	55	PRO	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	1891	0	1888	20	0
2	N	961	0	928	10	0
3	P	229	0	227	4	0
4	R	3115	0	3041	15	0
5	B	2591	0	2499	27	0
6	G	436	0	448	5	0
All	All	9223	0	9031	74	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 4.

The worst 5 of 74 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:362:HIS:NE2	1:A:378:ASP:OD1	2.29	0.62
1:A:223:ASP:N	1:A:223:ASP:OD1	2.33	0.62
1:A:318:TYR:O	1:A:336:ARG:NH2	2.33	0.62
5:B:160:SER:HB2	5:B:190:LEU:HD23	1.82	0.61
5:B:74:SER:OG	5:B:76:ASP:OD1	2.18	0.60

There are no symmetry-related clashes.

5.3   Torsion angles ⓘ

5.3.1   Protein backbone ⓘ

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	221/357 (62%)	210 (95%)	11 (5%)	0	100	100
2	N	124/140 (89%)	117 (94%)	7 (6%)	0	100	100
3	P	25/29 (86%)	24 (96%)	1 (4%)	0	100	100
4	R	376/440 (86%)	352 (94%)	24 (6%)	0	100	100
5	B	335/345 (97%)	317 (95%)	18 (5%)	0	100	100
6	G	55/70 (79%)	53 (96%)	2 (4%)	0	100	100
All	All	1136/1381 (82%)	1073 (94%)	63 (6%)	0	100	100

There are no Ramachandran outliers to report.

5.3.2   Protein sidechains ⓘ

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	203/319 (64%)	200 (98%)	3 (2%)	65	87
2	N	104/116 (90%)	103 (99%)	1 (1%)	76	91
3	P	22/22 (100%)	22 (100%)	0	100	100
4	R	329/392 (84%)	326 (99%)	3 (1%)	78	92
5	B	280/287 (98%)	278 (99%)	2 (1%)	84	94
6	G	46/57 (81%)	46 (100%)	0	100	100
All	All	984/1193 (82%)	975 (99%)	9 (1%)	79	92

5 of 9 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
5	B	59	TYR
5	B	220	GLN
2	N	99	CYS
4	R	257	PHE
4	R	299	ARG

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 16 such sidechains are listed below:

Mol	Chain	Res	Type
5	B	237	ASN
5	B	155	ASN
4	R	212	HIS
5	B	132	ASN
4	R	140	GLN

5.3.3　RNA ⓘ

There are no RNA molecules in this entry.

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

2 non-standard protein/DNA/RNA residues 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	$\#|Z| > 2$
3	AIB	P	2	3	1,5,6	0.82	0	2,7,9	1.50	0
3	AIB	P	13	3	1,5,6	1.06	0	2,7,9	1.20	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
3	AIB	P	2	3	-	0/2/3/6	-
3	AIB	P	13	3	-	0/2/3/6	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no torsion outliers.

There are no ring outliers.

No monomer is involved in short contacts.

5.5 Carbohydrates ⓘ

There are no monosaccharides in this entry.

5.6 Ligand geometry ⓘ

There are no ligands in this entry.

5.7 Other polymers ⓘ

There are no such residues in this entry.

5.8 Polymer linkage issues ⓘ

There are no chain breaks in this entry.

6　Map visualisation (i)

This section contains visualisations of the EMDB entry EMD-31880. 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 |
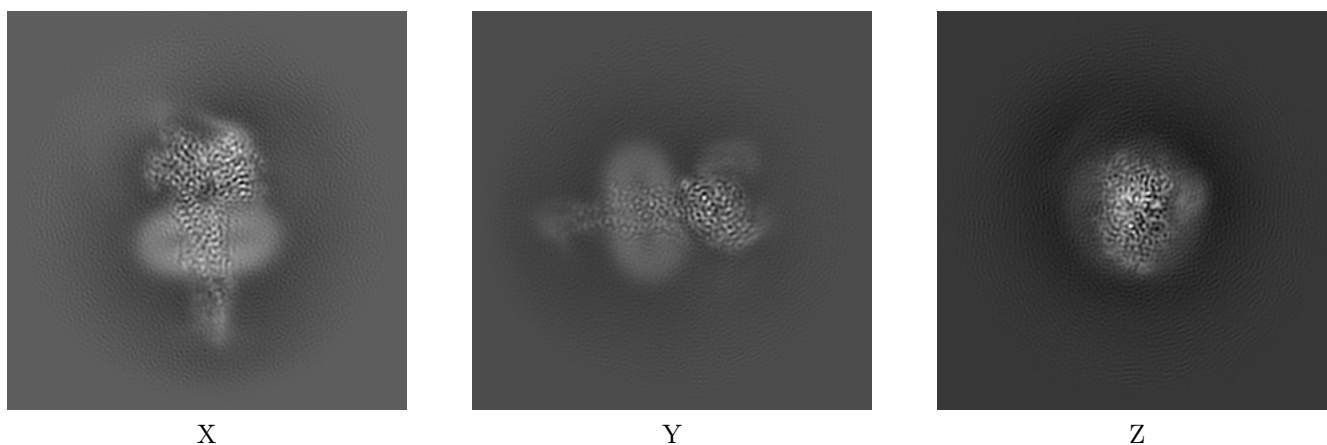
The images above show the map projected in three orthogonal directions.

6.2　Central slices (i)

6.2.1　Primary map

| X Index: 128 | Y Index: 128 | Z Index: 128 |
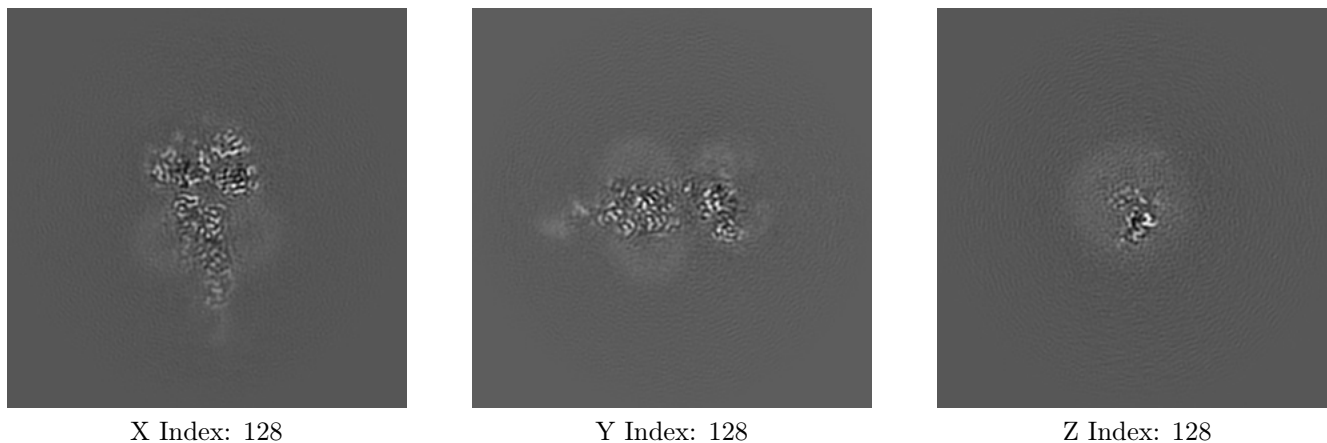
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices ⓘ
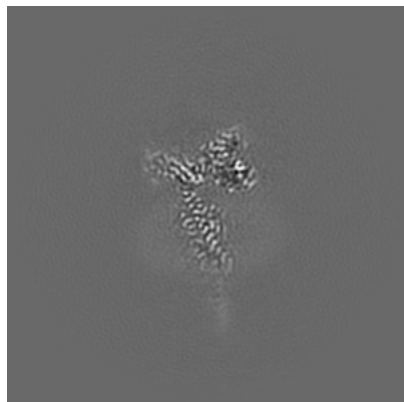
6.3.1 Primary map
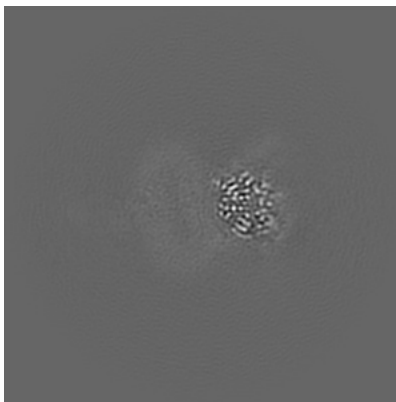
| X Index: 133 | Y Index: 148 | Z Index: 151 |
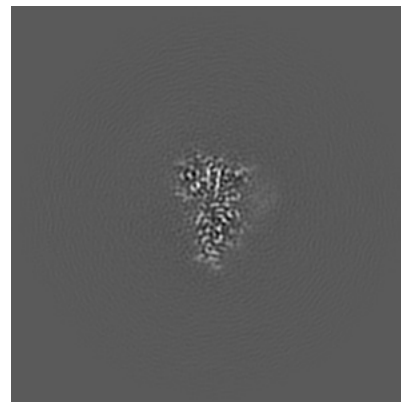
The images above show the largest variance slices of the map in three orthogonal directions.

6.4 Orthogonal surface views ⓘ

6.4.1 Primary map

| X | Y | Z |

The images above show the 3D surface view of the map at the recommended contour level 0.232. 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 ⓘ

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

7.1 Map-value distribution ⓘ
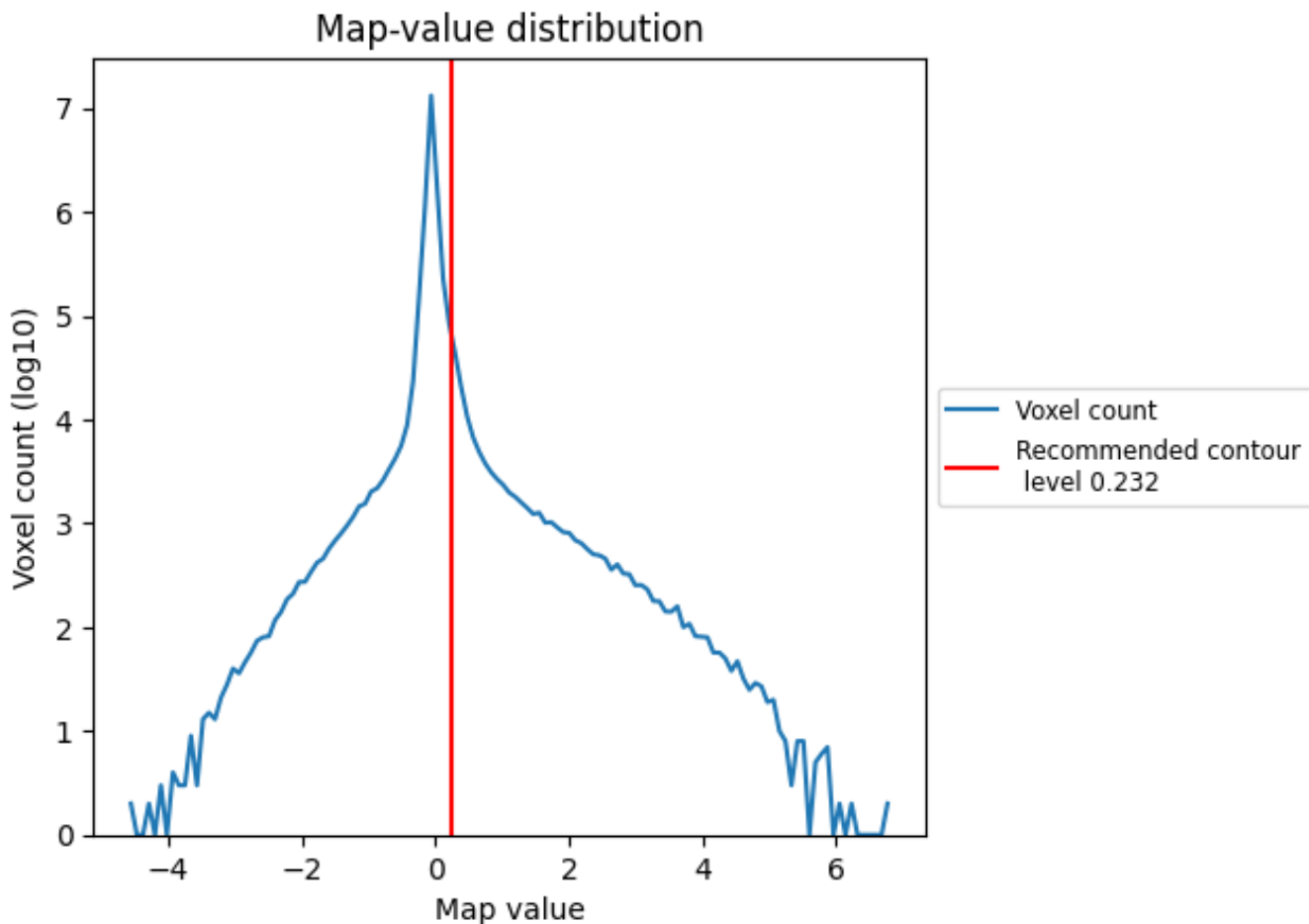
The map-value distribution is plotted in 128 intervals along the x-axis. The y-axis is logarithmic. A spike in this graph at zero usually indicates that the volume has been masked.

7.2   Volume estimate ⓘ
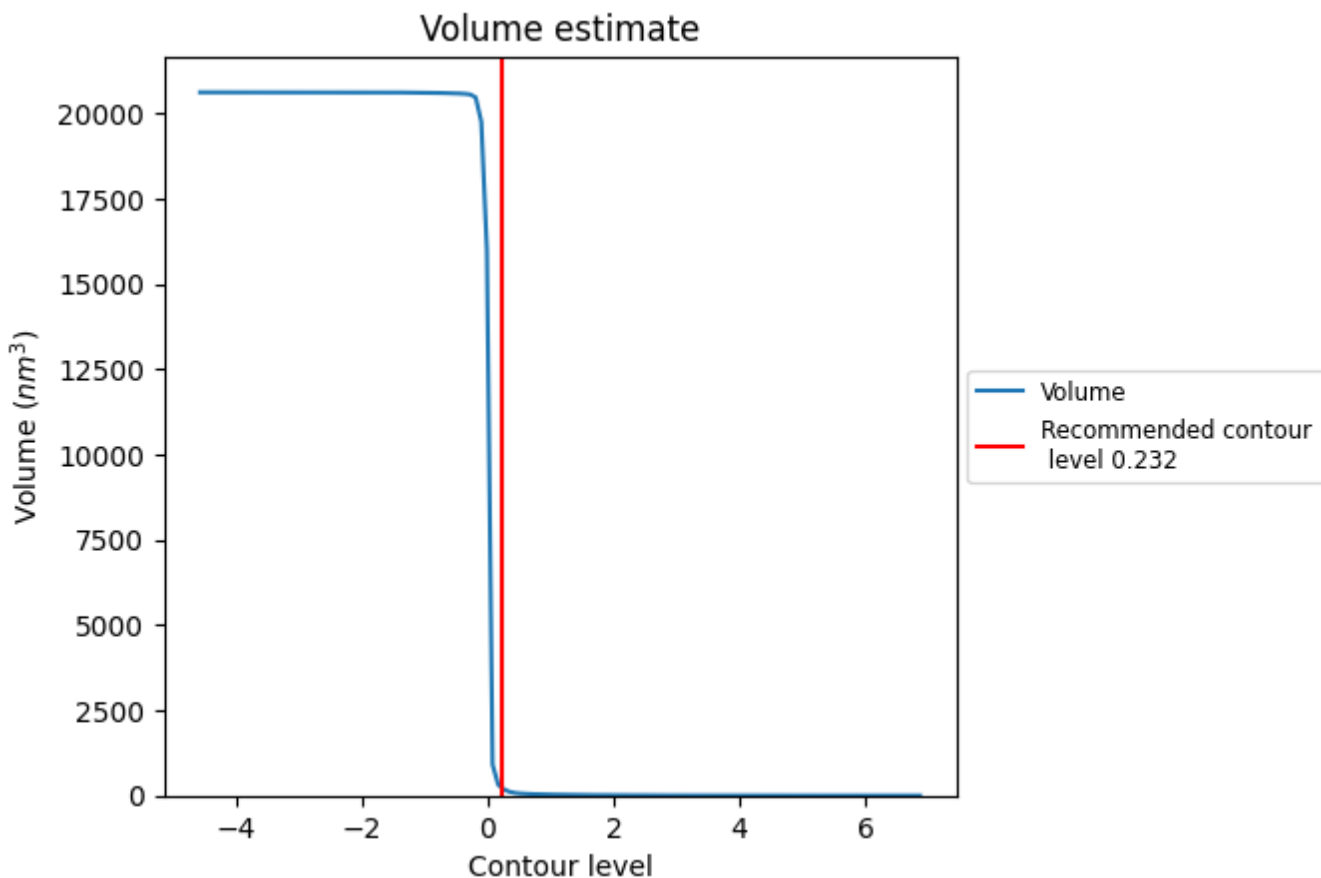
The volume at the recommended contour level is 218 nm^3; this corresponds to an approximate mass of 197 kDa.

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 ⓘ
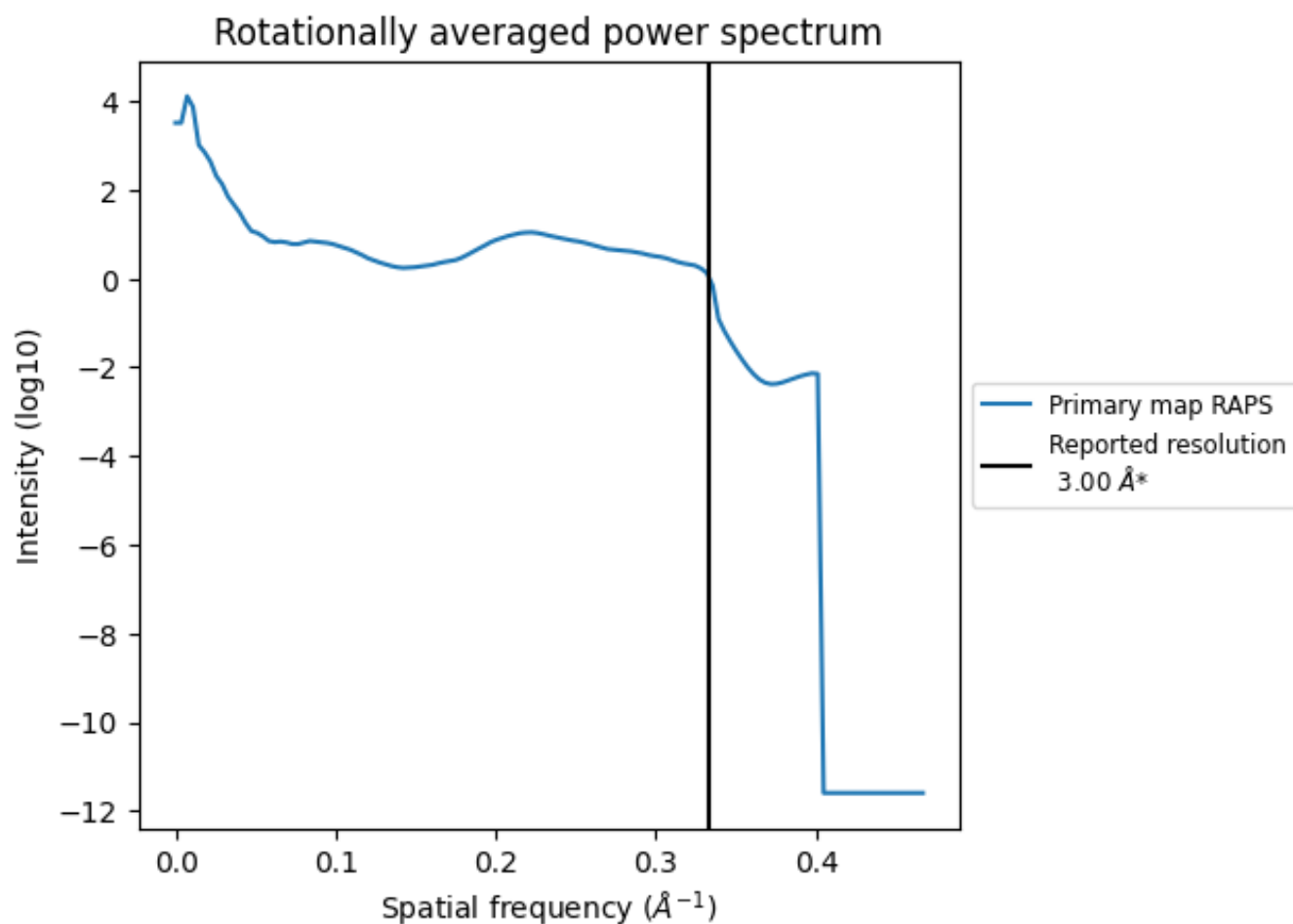
*Reported resolution corresponds to spatial frequency of 0.333 Å^{-1}

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-31880 and PDB model 7VBI. Per-residue inclusion information can be found in section 3 on page 5.

9.1   Map-model overlay ⓘ

X   Y   Z
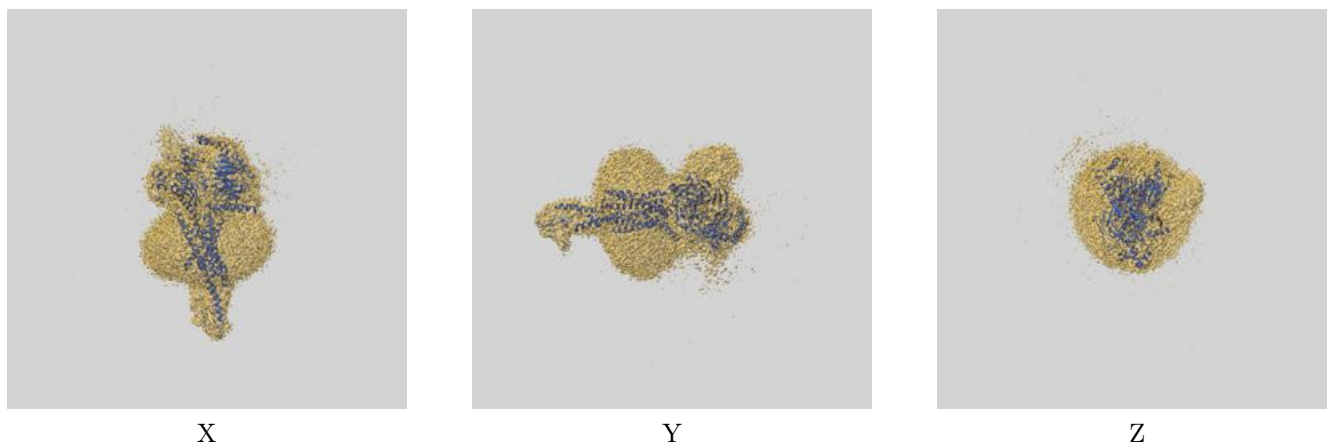
The images above show the 3D surface view of the map at the recommended contour level 0.232 at 50% transparency in yellow overlaid with a ribbon representation of the model coloured in blue. These images allow for the visual assessment of the quality of fit between the atomic model and the map.

9.2   Q-score mapped to coordinate model ⓘ

X   Y   Z
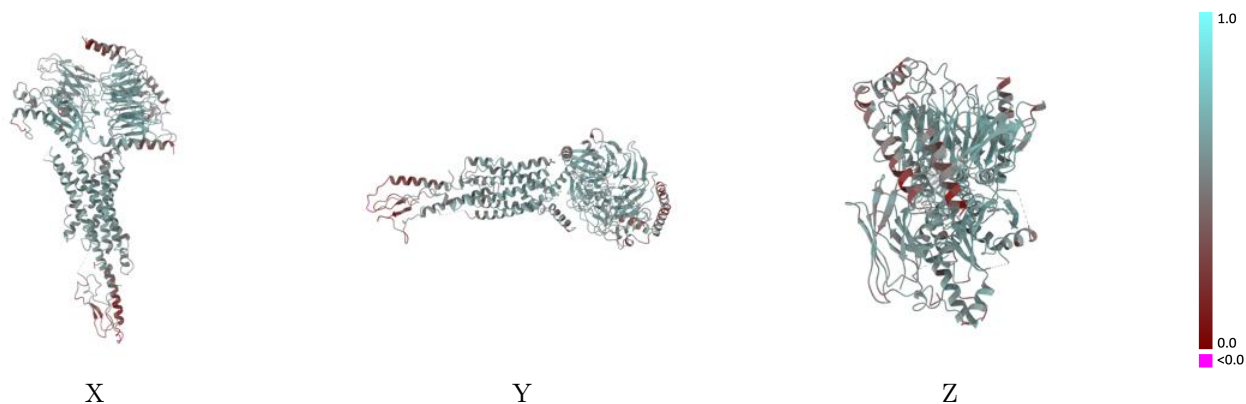
The images above show the model with each residue coloured according its Q-score. This shows their resolvability in the map with higher Q-score values reflecting better resolvability. Please note: Q-score is calculating the resolvability of atoms, and thus high values are only expected at resolutions at which atoms can be resolved. Low Q-score values may therefore be expected for many entries.

9.3   Atom inclusion mapped to coordinate model ⓘ

X   Y   Z
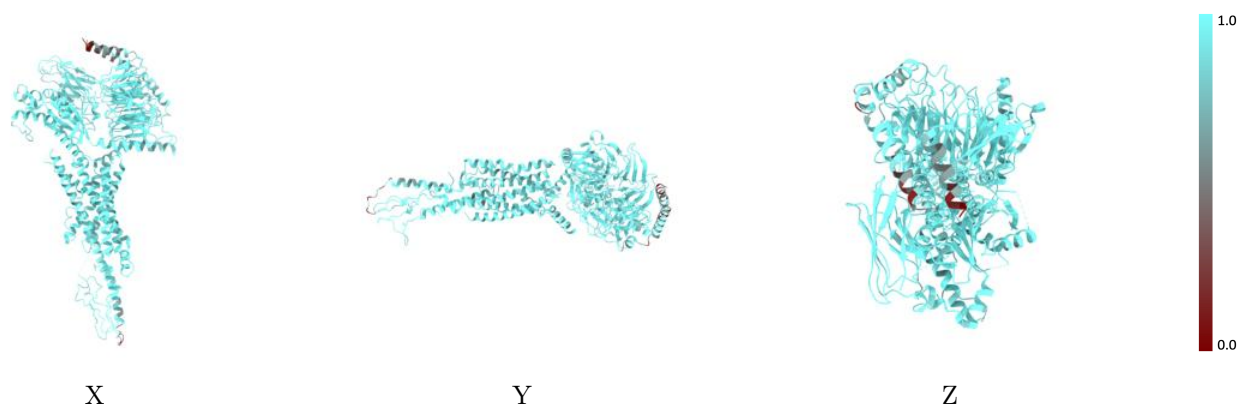
The images above show the model with each residue coloured according to its atom inclusion. This shows to what extent they are inside the map at the recommended contour level (0.232).

9.4 Atom inclusion ⓘ
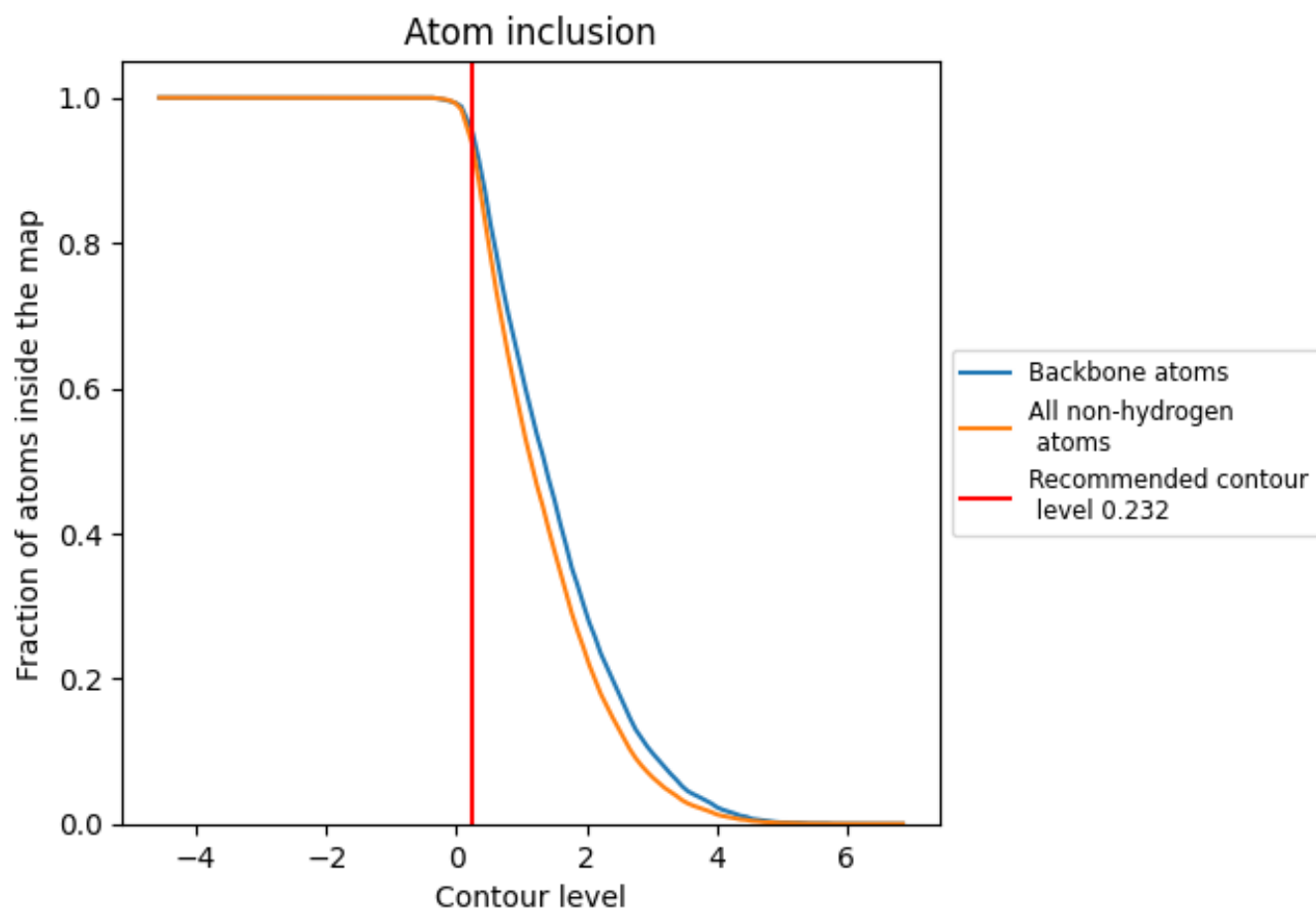
At the recommended contour level, 96% of all backbone atoms, 94% of all non-hydrogen atoms, are inside the map.

9.5   Map-model fit summary ⓘ

The table lists the average atom inclusion at the recommended contour level (0.232) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.9396	0.5330
A	0.9549	0.5590
B	0.9475	0.5650
G	0.7739	0.4430
N	0.9680	0.5470
P	0.9602	0.5390
R	0.9368	0.4980

1.0

0.0
<0.0