wwPDB EM Validation Summary Report (i)

Apr 8, 2024 – 04:34 PM JST

PDB ID	:	8WYA
EMDB ID	:	EMD-37921
Title	:	Cryo-EM structure of DSR2-tube complex
Authors	:	Zhang, J.T.; Jia, N.; Liu, X.Y.
Deposited on	:	2023-10-30
Resolution	:	3.62 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:

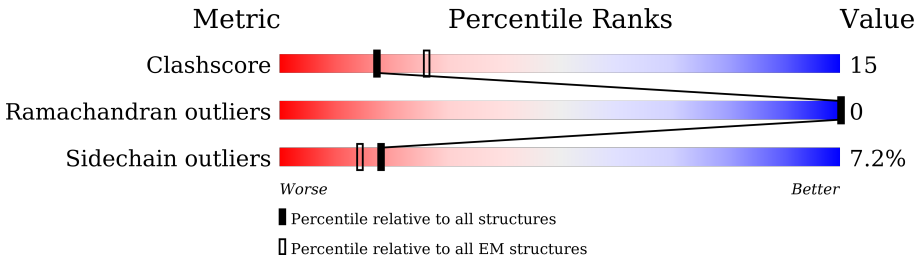
EMDB validation analysis	:	0.0.1.dev70
MolProbity	:	4.02b-467
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
MapQ	:	1.9.13
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

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 3.62 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	1005	47% 56% 32% • 9%
1	B	1005	44% 57% 32% • 8%
1	D	1005	59% 58% 32% • 8%
1	E	1005	60% 60% 30% • 9%
2	C	264	31% 21% 19% 5% 56%
2	F	264	44% 31% 12% • 56%

2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 32621 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 SIR2 family protein.

Mol	Chain	Residues	Atoms					AltConf	Trace
1	A	915	Total	C	N	O	S	0	0
			7655	4965	1230	1430	30		
1	B	923	Total	C	N	O	S	0	0
			7706	4999	1241	1437	29		
1	D	921	Total	C	N	O	S	0	0
			7705	4995	1241	1439	30		
1	E	919	Total	C	N	O	S	0	0
			7677	4982	1236	1430	29		

- Molecule 2 is a protein called Bacillus phage SPbeta tube protein.

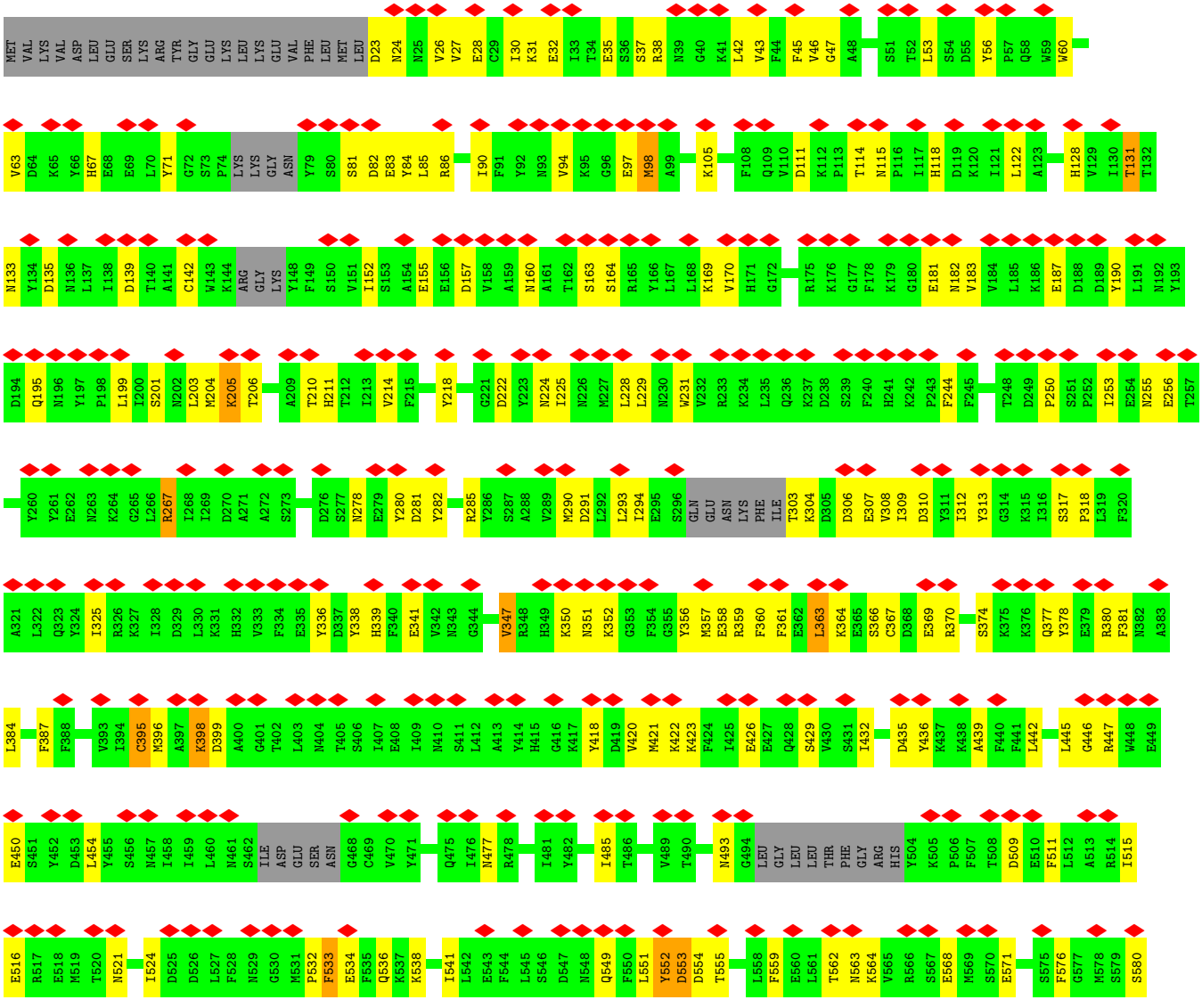
Mol	Chain	Residues	Atoms					AltConf	Trace
2	C	116	Total	C	N	O	S	0	0
			939	604	146	186	3		
2	F	116	Total	C	N	O	S	0	0
			939	604	146	186	3		

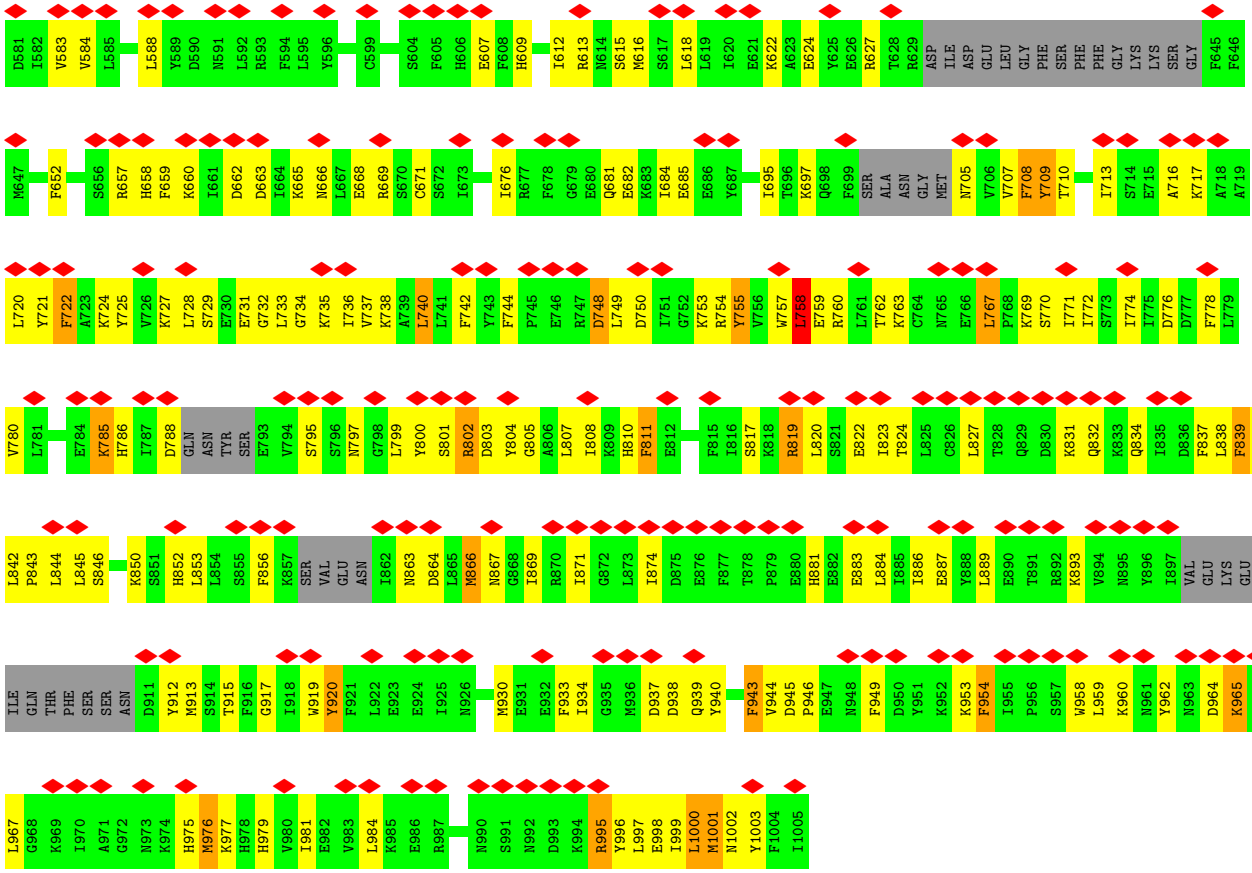
3 Residue-property plots (i)

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: SIR2 family protein

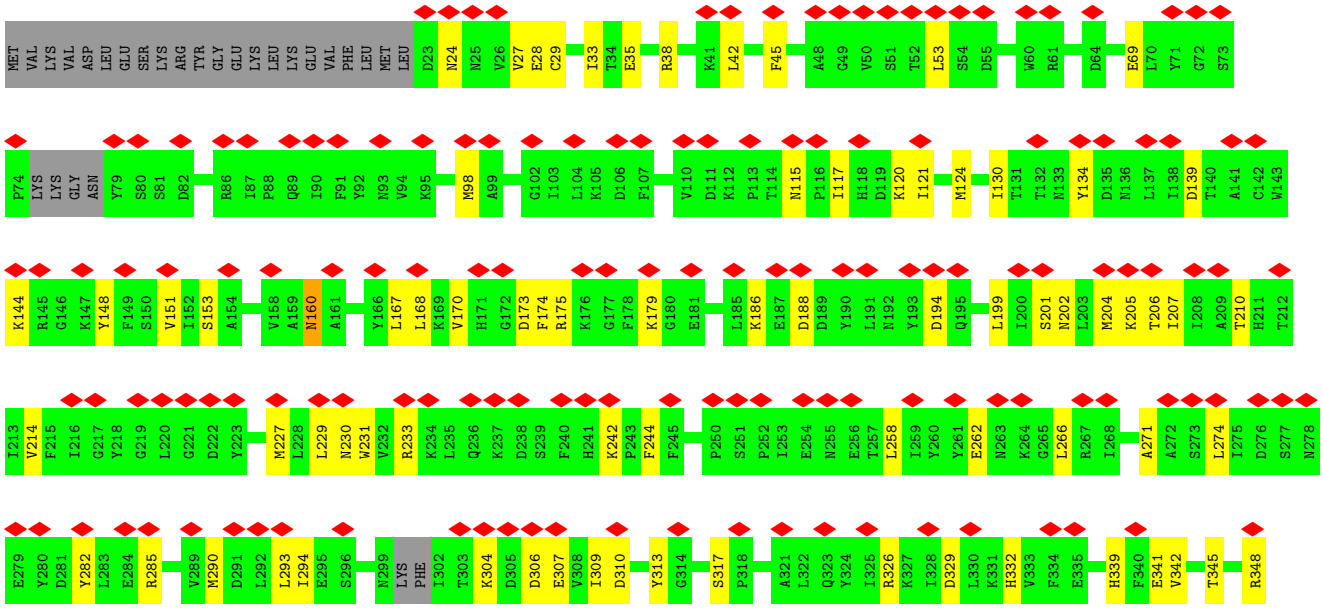
Chain A:

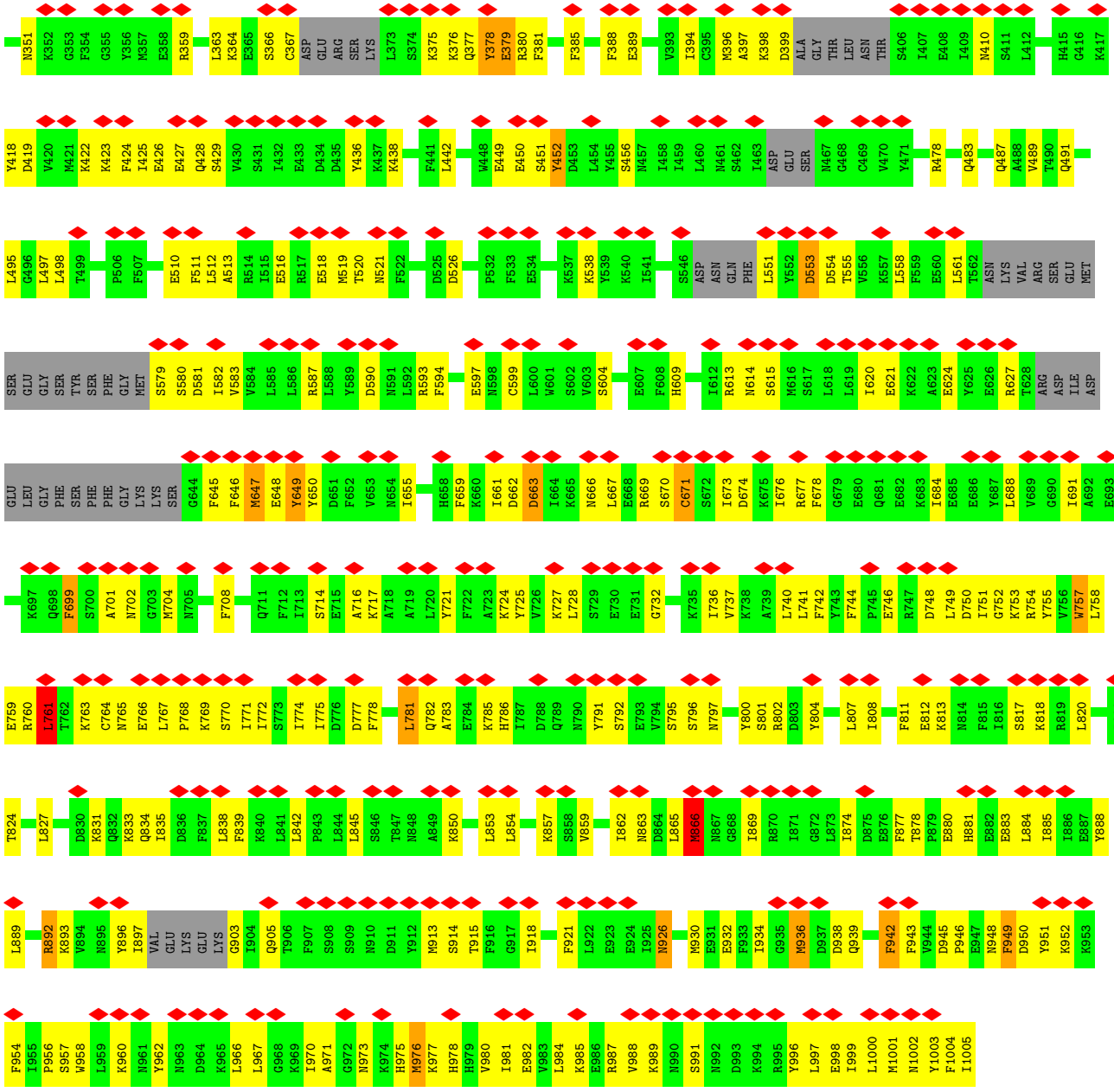




• Molecule 1: SIR2 family protein

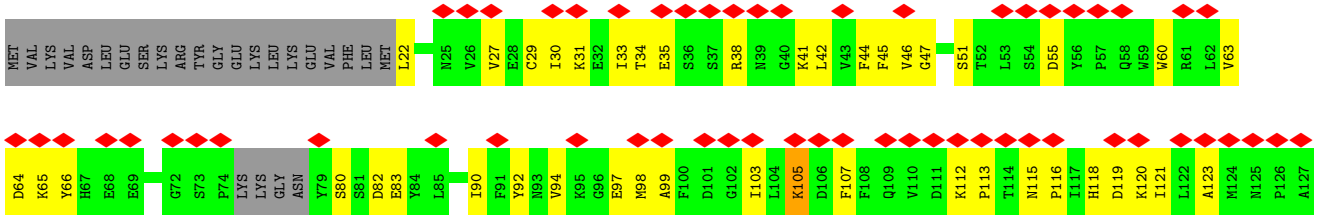
Chain B:

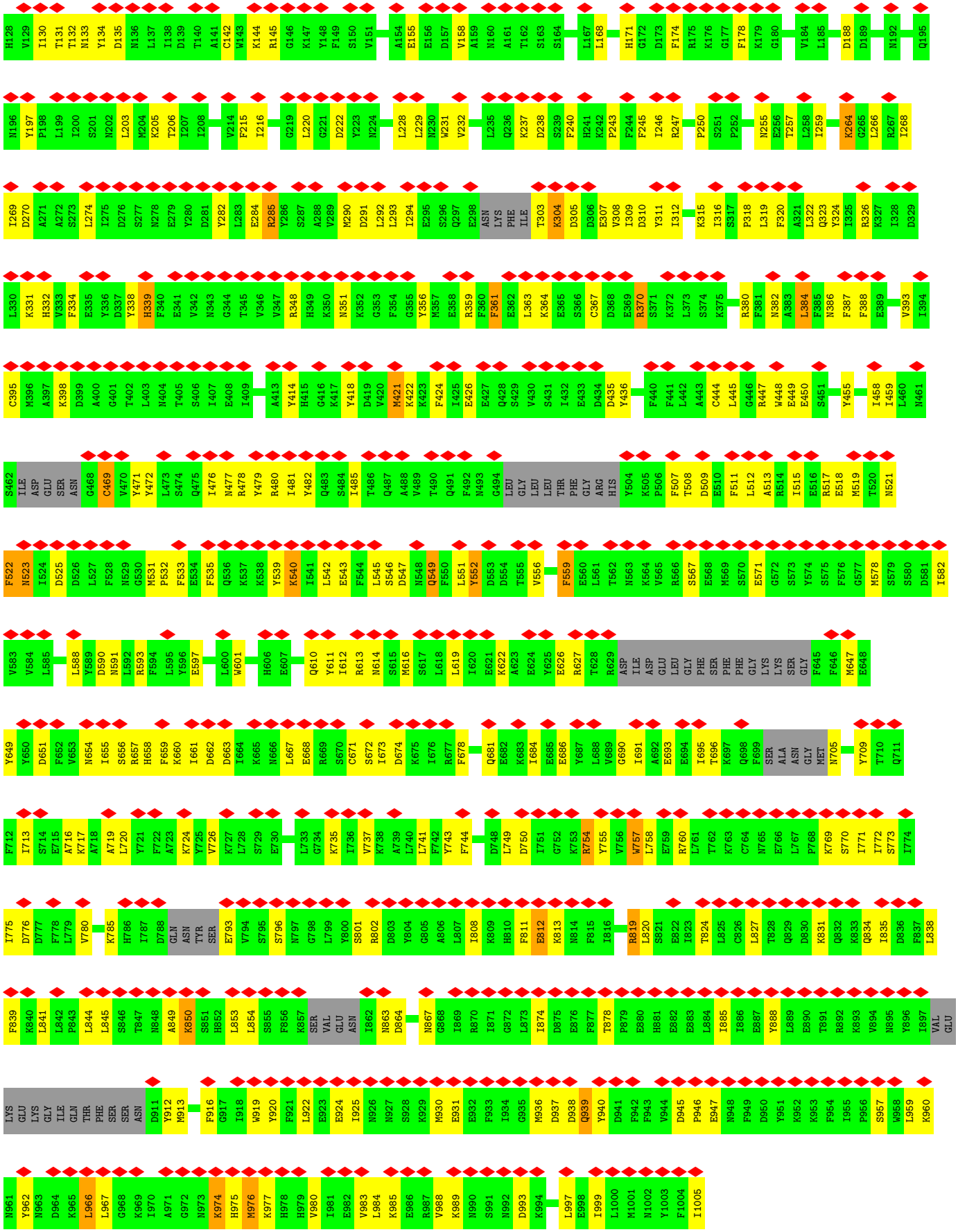




• Molecule 1: SIR2 family protein

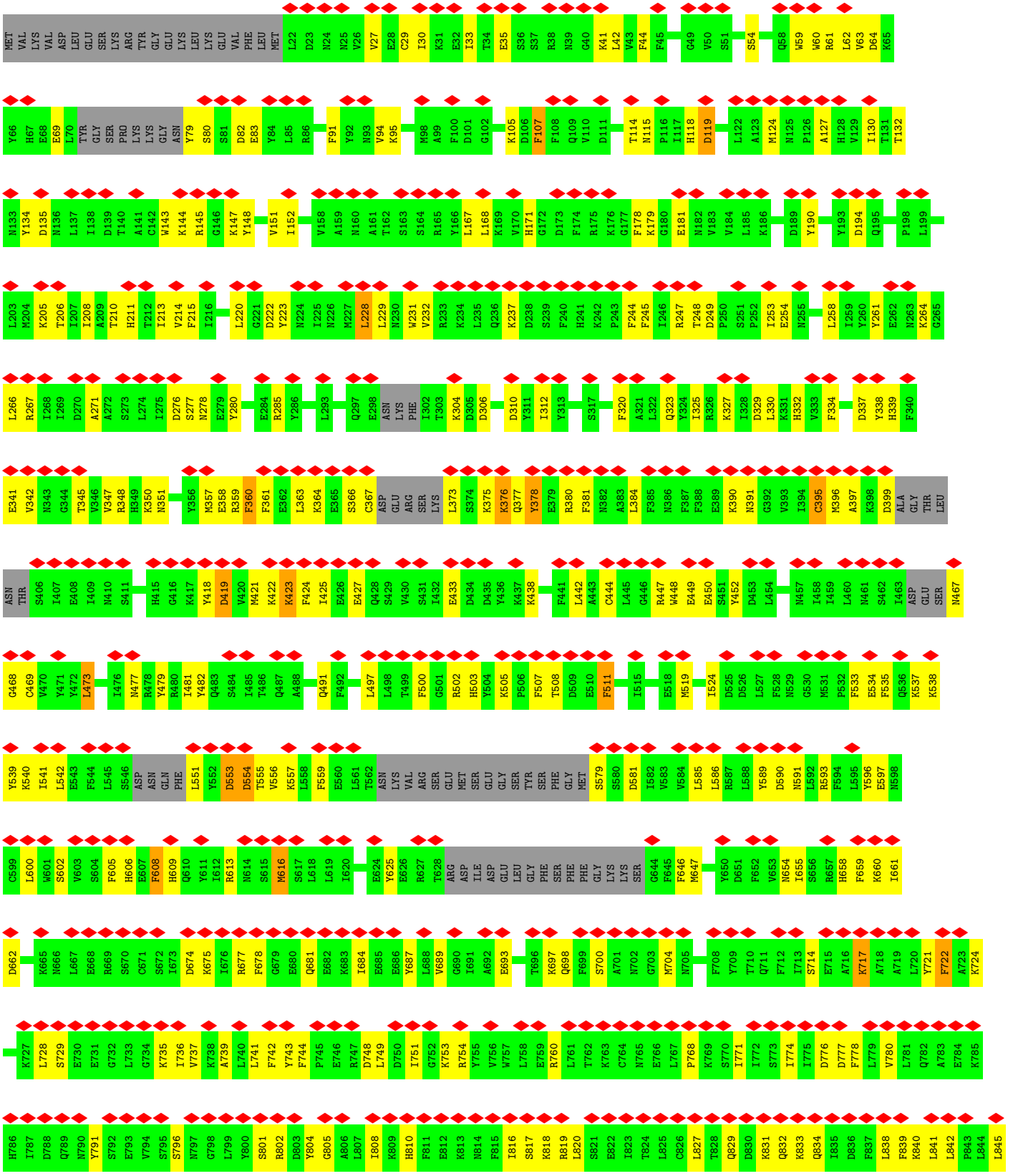
Chain D:

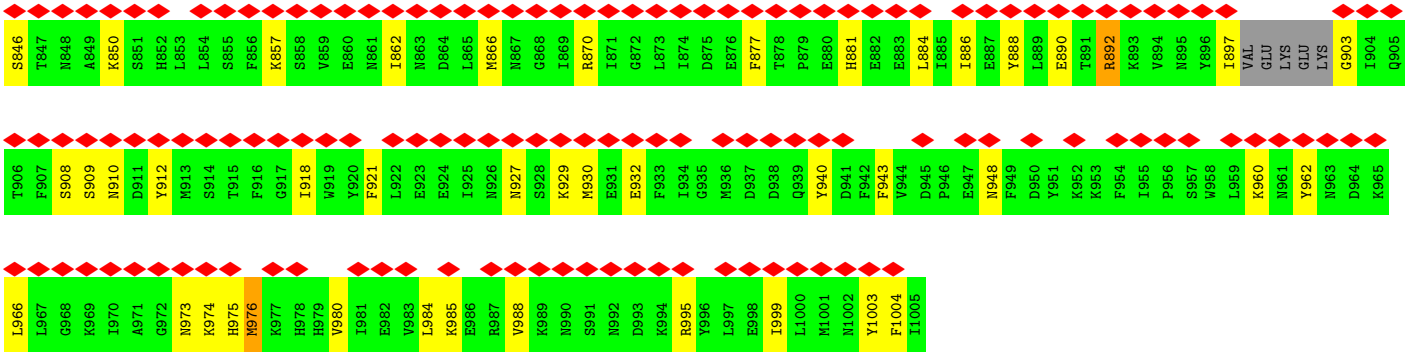



H128 V129 I130 T131 T132 N133 Y134 D135 N136 L137 I138 D139 T140 A141 C142 W143 K144 R145 G146 K147 Y148 F149 S150 V151 A154 E155 E156 D157 V158 A159 N160 A161 T162 S163 S164 L167 L168 H171 G172 D173 F174 R175 K176 G177 F178 K179 G180 V184 L185 D188 D189 N192 Q195
N196 Y197 P198 L199 I200 S201 N202 L203 M204 K205 T206 I207 I208 V214 F215 I216 G219 L220 G221 D222 Y223 N224 L228 L229 N230 W231 V232 L235 Q236 K237 D238 S239 F240 H241 K242 P243 F244 F245 I246 R247 P250 S251 P252 N255 E256 T257 L258 I259 K264 G265 L266 R267 I268
I269 D270 A271 A272 S273 L274 I275 D276 S277 N278 E279 Y280 D281 Y282 L283 E284 R285 Y286 S287 A288 V289 M290 D291 L292 L293 I294 E295 S296 Q297 E298 ASN LYS PHE ILE T303 K304 D305 D306 E307 V308 I309 D310 Y311 I312 K315 I316 S317 P318 L319 F320 A321 L322 Q323 Y324 I325 R326 K327 I328 D329
L330 K331 H332 V333 F334 E335 Y336 D337 Y338 H339 F340 E341 V342 N343 G344 T345 V346 V347 R348 H349 K350 N351 K352 G353 F354 G355 Y356 M357 E358 R359 F360 F361 E362 L363 K364 E365 S366 C367 D368 E369 R370 S371 K372 L373 S374 K375 R380 F381 N382 A383 L384 F385 N386 F387 F388 E389 V393 I394
C395 M396 A397 K398 D399 A400 G401 T402 L403 N404 T405 S406 I407 E408 I409 A413 Y414 H415 G416 K417 Y418 D419 V420 M421 K422 K423 F424 I425 E426 E427 Q428 S429 V430 S431 I432 E433 D434 D435 Y436 F440 F441 L442 A443 C444 L445 G446 R447 W448 E449 E450 S451 Y455 I458 I459 L460 N461
S462 ILE ASP GLU SER ASN G468 C469 V470 Y471 Y472 L473 S474 Q475 I476 N477 R478 Y479 R480 I481 Y482 Q483 S484 I485 T486 Q487 A488 V489 T490 Q491 F492 N493 G494 LEU GLY LEU LEU THR PHE GLY ARG HIS Y504 K505 P506 F507 T508 D509 E510 F511 L512 A513 R514 I515 E516 R517 E518 M519 T520 N521
F522 N523 I524 D525 D526 L527 F528 N529 G530 M531 P532 F533 E534 F535 Q536 K537 K538 Y539 K540 I541 L542 E543 F544 L545 S546 D547 N548 Q549 F550 L551 Y552 D553 D554 T555 V556 F559 E560 L561 T562 N563 K564 V565 R566 S567 E568 M569 S570 E571 G572 S573 Y574 S575 F576 G577 M578 S579 S580 D581 I582
V583 V584 L585 L588 Y589 D590 N591 L592 R593 F594 L595 Y596 E597 L600 W601 H606 E607 Q610 Y611 I612 R613 N614 S615 M616 S617 L618 L619 I620 E621 K622 A623 E624 Y625 E626 R627 T628 R629 ASP ILE ASP GLU LEU GLY PHE SER PHE PHE GLY LYS LYS SER GLY F645 F646 M647 E648
Y649 Y650 D651 F652 V653 N654 I655 S656 R657 H658 F659 K660 I661 D662 D663 I664 K665 N666 L667 E668 R669 S670 C671 S672 I673 D674 K675 I676 R677 F678 Q681 E682 K683 I684 E685 E686 Y687 L688 V689 G690 I691 A692 E693 E694 I695 T696 K697 Q698 F699 SER ALA ASN GLY MET N705 Y709 T710 Q711
F712 I713 S714 E715 A716 K717 A718 A719 L720 Y721 F722 A723 K724 Y725 V726 K727 L728 S729 E730 L733 G734 K735 I736 V737 K738 A739 L740 L741 F742 Y743 F744 D748 L749 D750 I751 G752 K753 R754 Y755 V756 W757 L758 E759 R760 L761 T762 K763 C764 N765 E766 L767 P768 K769 S770 I771 I772 S773 I774
I775 D776 D777 F778 L779 V780 K785 H786 I787 D788 GLN ASN TYR SER E793 V794 S795 S796 N797 G798 L799 Y800 S801 R802 D803 Y804 G805 A806 L807 I808 K809 H810 F811 E812 K813 N814 F815 I816 R819 L820 S821 E822 I823 T824 L825 C826 L827 T828 Q829 D830 K831 Q832 K833 Q834 I835 D836 F837 L838
F839 K840 L841 L842 P843 L844 L845 S846 T847 N848 A849 K850 S851 H852 L853 L854 S855 F856 K857 SER VAL GLU ASN I862 N863 D864 N867 G868 I869 R870 I871 G872 L873 I874 D875 E876 F877 T878 P879 E880 H881 E882 E883 L884 I885 I886 E887 Y888 L889 E890 T891 R892 K893 V894 N895 Y896 I897 VAL GLU
LYS GLU LYS GLY ILE GLN THR PHE SER SER ASN D911 Y912 M913 F916 G917 I918 W919 Y920 F921 L922 E923 E924 I925 N926 N927 S928 K929 M930 E931 E932 F933 I934 G935 M936 D937 D938 Q939 Y940 D941 F942 F943 V944 D945 P946 E947 N948 F949 D950 Y951 K952 K953 F954 I955 P956 S957 W958 L959 K960
N961 Y962 N963 D964 K965 L966 L967 G968 K969 I970 A971 G972 N973 K974 H975 M976 K977 H978 H979 V980 I981 E982 V983 L984 K985 E986 R987 V988 K989 N990 S991 N992 D993 K994 L997 E998 I999 L1000 M1001 N1002 Y1003 F1004 I1005

• Molecule 1: SIR2 family protein

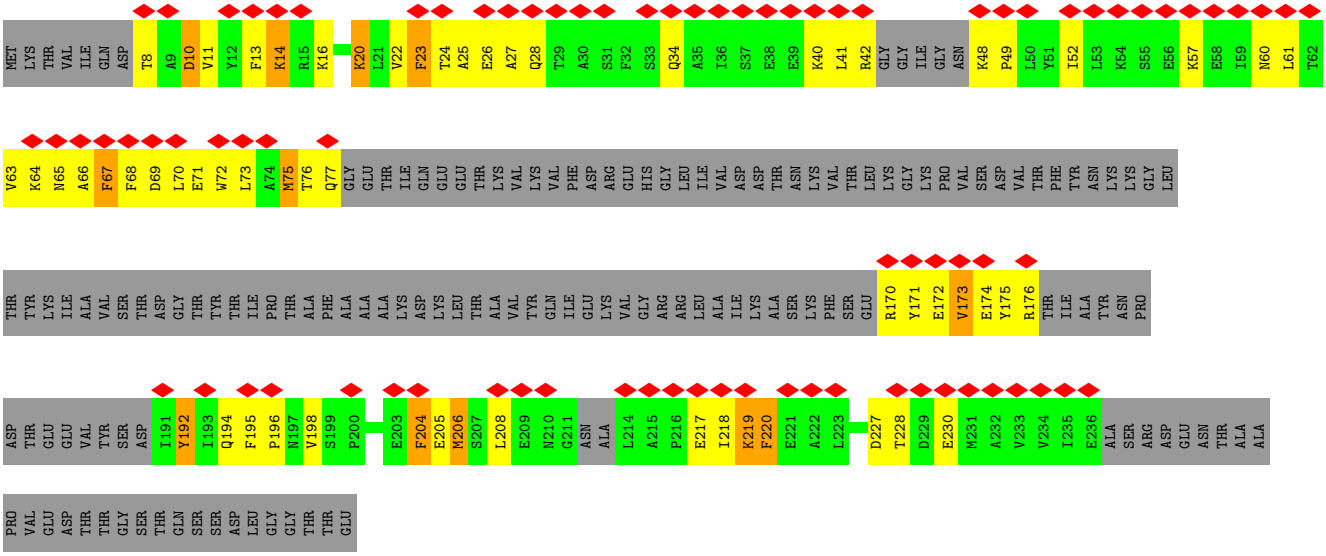
Chain E:





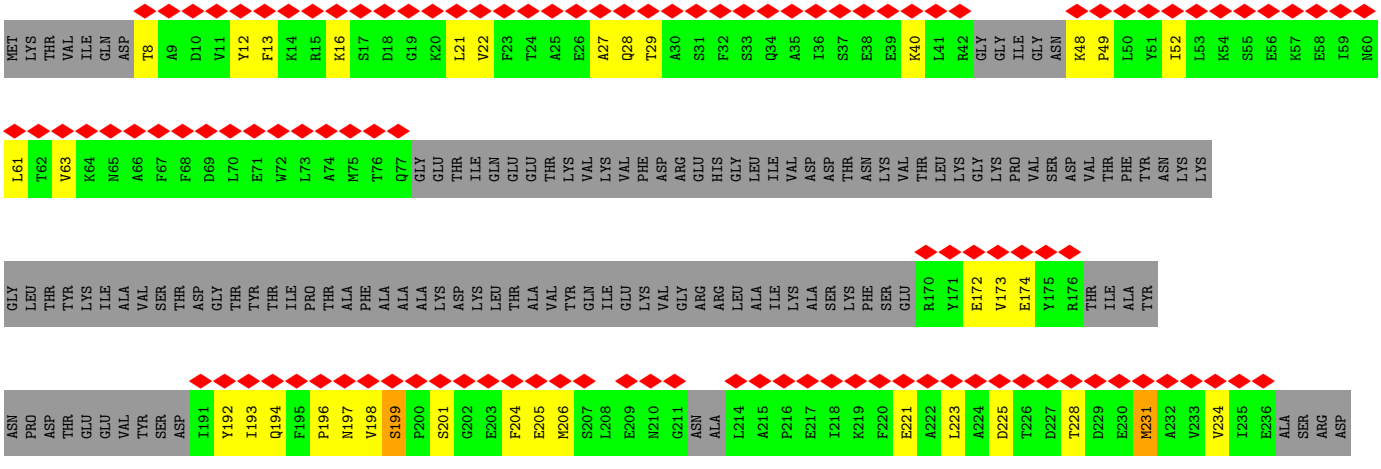
• Molecule 2: Bacillus phage SPbeta tube protein

Chain C:



• Molecule 2: Bacillus phage SPbeta tube protein

Chain F:



GLU
ASN
THR
ALA
ALA
PRO
VAL
GLU
ASP
THR
THR
GLY
SER
THR
GLN
SER
SER
ASP
LEU
GLY
GLY
THR
THR
GLU

4 Experimental information (i)

Property	Value	Source
EM reconstruction method	SINGLE PARTICLE	Depositor
Imposed symmetry	POINT, Not provided	
Number of particles used	16411	Depositor
Resolution determination method	FSC 0.143 CUT-OFF	Depositor
CTF correction method	PHASE FLIPPING ONLY	Depositor
Microscope	FEI TITAN KRIOS	Depositor
Voltage (kV)	300	Depositor
Electron dose ($e^-/Å^2$)	50	Depositor
Minimum defocus (nm)	1500	Depositor
Maximum defocus (nm)	2500	Depositor
Magnification	Not provided	
Image detector	GATAN K3 (6k x 4k)	Depositor
Maximum map value	1.732	Depositor
Minimum map value	-0.956	Depositor
Average map value	-0.000	Depositor
Map value standard deviation	0.044	Depositor
Recommended contour level	0.25	Depositor
Map size (Å)	454.47998, 454.47998, 454.47998	wwPDB
Map dimensions	460, 460, 460	wwPDB
Map angles (°)	90.0, 90.0, 90.0	wwPDB
Pixel spacing (Å)	0.988, 0.988, 0.988	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	0.27	0/7829	0.52	3/10541 (0.0%)
1	B	0.28	0/7882	0.53	4/10617 (0.0%)
1	D	0.26	0/7880	0.48	0/10609
1	E	0.26	0/7851	0.48	0/10574
2	C	0.30	0/953	0.64	1/1280 (0.1%)
2	F	0.25	0/953	0.47	0/1280
All	All	0.27	0/33348	0.51	8/44901 (0.0%)

There are no bond length outliers.

The worst 5 of 8 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	B	956	PRO	CA-N-CD	-9.41	98.32	111.50
1	A	758	LEU	CA-CB-CG	6.46	130.15	115.30
1	B	781	LEU	CA-CB-CG	5.78	128.60	115.30
1	B	761	LEU	CA-CB-CG	5.47	127.87	115.30
1	B	866	MET	CA-CB-CG	5.45	122.56	113.30

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	7655	0	7487	253	0
1	B	7706	0	7544	243	0

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Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	D	7705	0	7542	227	0
1	E	7677	0	7525	206	0
2	C	939	0	919	54	0
2	F	939	0	919	22	0
All	All	32621	0	31936	980	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 15.

The worst 5 of 980 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:E:724:LYS:HB2	1:E:760:ARG:HB3	1.57	0.87
1:A:309:ILE:HD11	1:A:377:GLN:HG3	1.58	0.85
1:E:395:CYS:SG	1:E:396:MET:N	2.52	0.81
1:D:247:ARG:HD2	1:D:268:ILE:HD11	1.66	0.78
1:D:250:PRO:HA	1:D:285:ARG:HH22	1.48	0.78

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	893/1005 (89%)	861 (96%)	32 (4%)	0	100	100
1	B	903/1005 (90%)	874 (97%)	29 (3%)	0	100	100
1	D	901/1005 (90%)	878 (97%)	23 (3%)	0	100	100
1	E	899/1005 (90%)	864 (96%)	35 (4%)	0	100	100
2	C	106/264 (40%)	96 (91%)	10 (9%)	0	100	100
2	F	106/264 (40%)	104 (98%)	2 (2%)	0	100	100

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Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles
All	All	3808/4548 (84%)	3677 (97%)	131 (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	844/923 (91%)	785 (93%)	59 (7%)	15 48
1	B	849/923 (92%)	794 (94%)	55 (6%)	17 51
1	D	849/923 (92%)	786 (93%)	63 (7%)	13 45
1	E	846/923 (92%)	784 (93%)	62 (7%)	14 46
2	C	102/225 (45%)	86 (84%)	16 (16%)	2 17
2	F	102/225 (45%)	98 (96%)	4 (4%)	32 65
All	All	3592/4142 (87%)	3333 (93%)	259 (7%)	18 47

5 of 259 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	E	616	MET
1	E	698	GLN
1	B	866	MET
1	B	813	LYS
1	E	810	HIS

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 22 such sidechains are listed below:

Mol	Chain	Res	Type
1	D	765	ASN
1	E	339	HIS
1	D	973	ASN
1	E	377	GLN
1	B	786	HIS

5.3.3 RNA

There are no RNA molecules in this entry.

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

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

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.

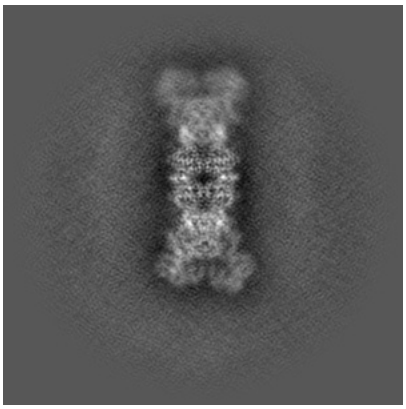
6 Map visualisation

This section contains visualisations of the EMDB entry EMD-37921. These allow visual inspection of the internal detail of the map and identification of artifacts.

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

6.1 Orthogonal projections

6.1.1 Primary map



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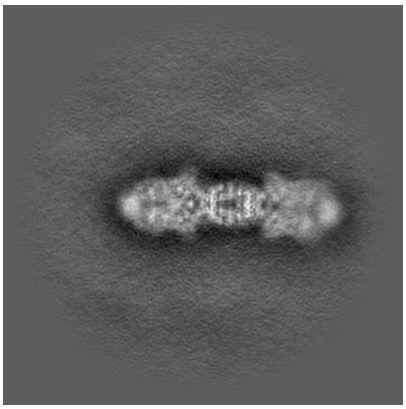
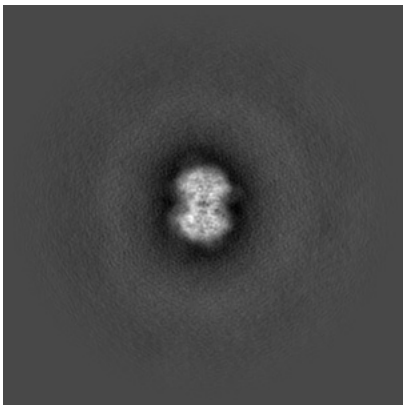
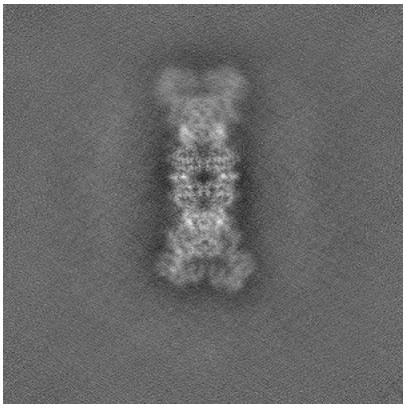


Y



Z

6.1.2 Raw map



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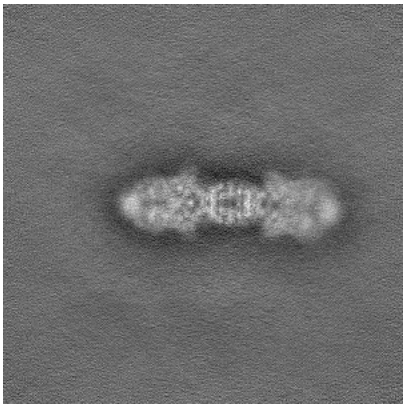
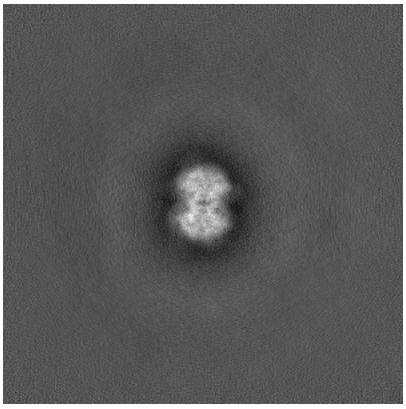


Y

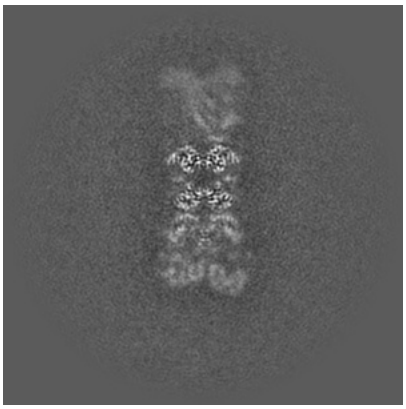


Z

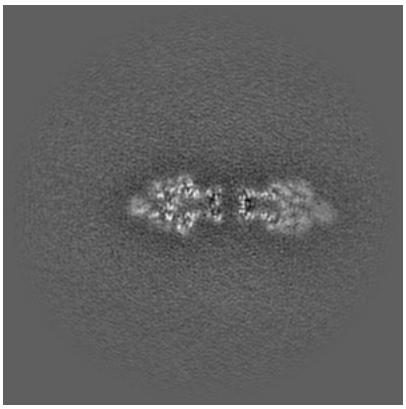
The images above show the map projected in three orthogonal directions.

6.2 Central slices (i)

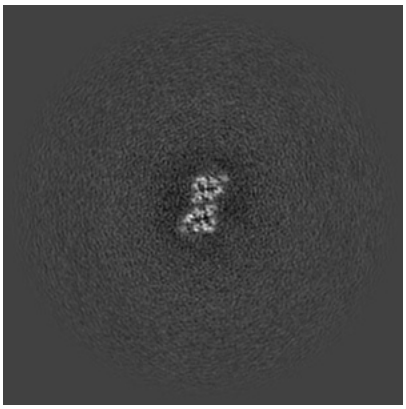
6.2.1 Primary map



X Index: 230

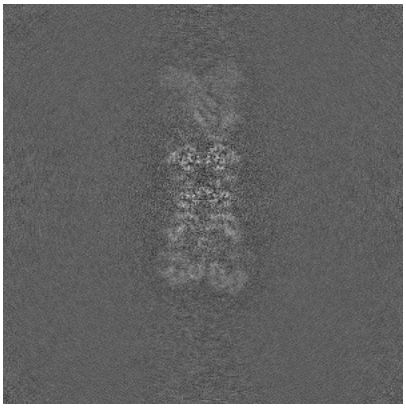


Y Index: 230

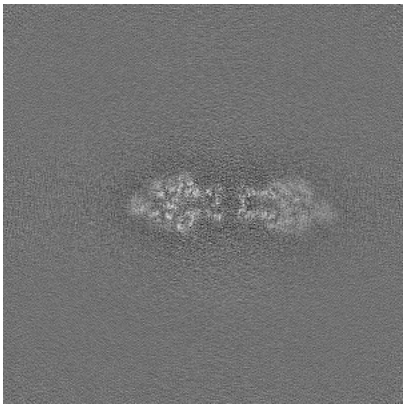


Z Index: 230

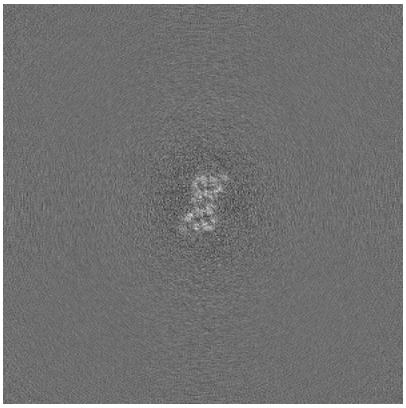
6.2.2 Raw map



X Index: 230



Y Index: 230

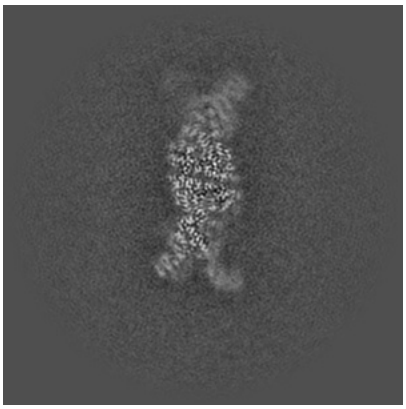


Z Index: 230

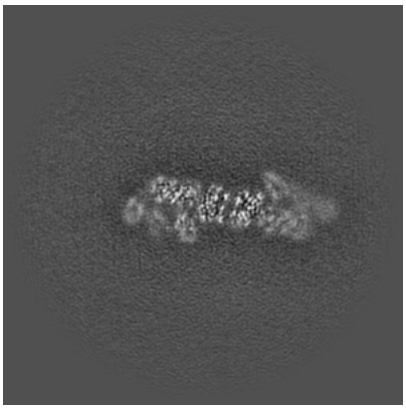
The images above show central slices of the map in three orthogonal directions.

6.3 Largest variance slices (i)

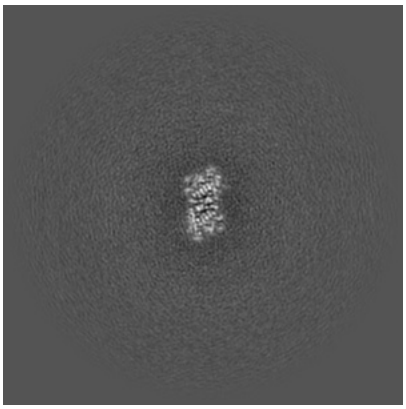
6.3.1 Primary map



X Index: 241

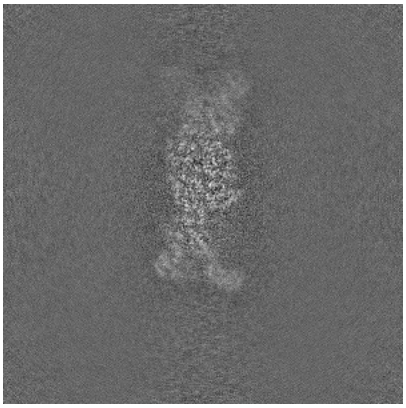


Y Index: 217

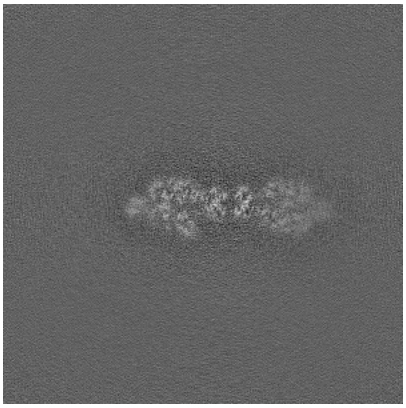


Z Index: 240

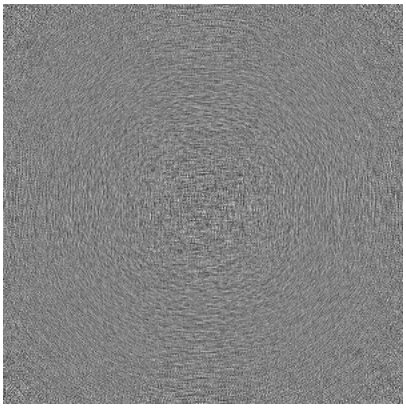
6.3.2 Raw map



X Index: 239



Y Index: 226

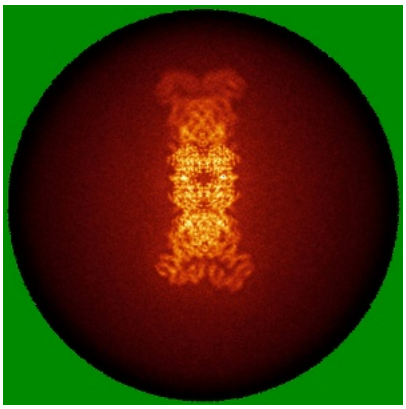


Z Index: 459

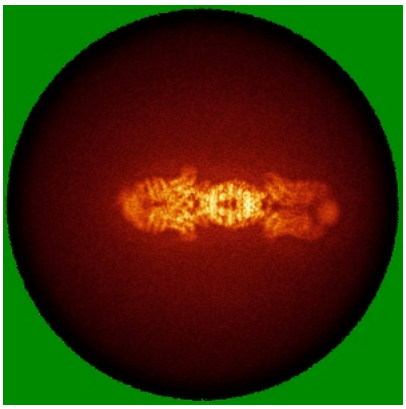
The images above show the largest variance slices of the map in three orthogonal directions.

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

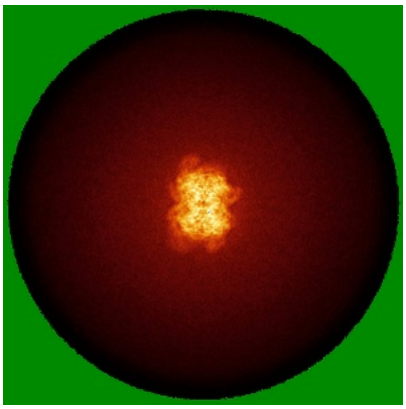
6.4.1 Primary map



X

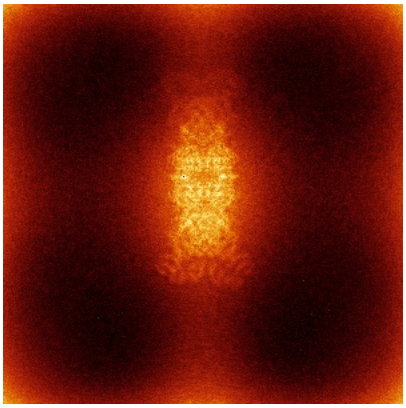


Y

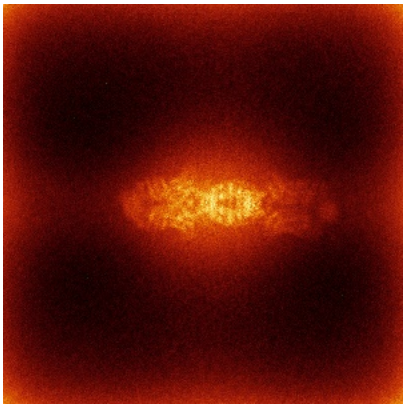


Z

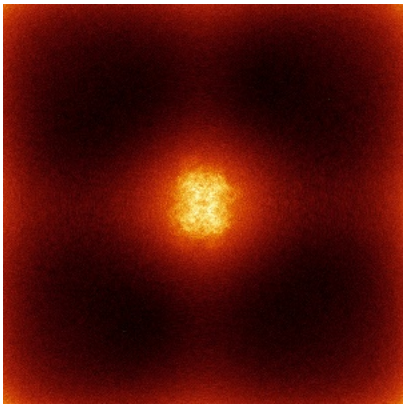
6.4.2 Raw map



X



Y



Z

The images above show the map standard deviation projections with false color in three orthogonal directions. Minimum values are shown in green, max in blue, and dark to light orange shades represent small to large values respectively.

6.5 Orthogonal surface views (i)

6.5.1 Primary map

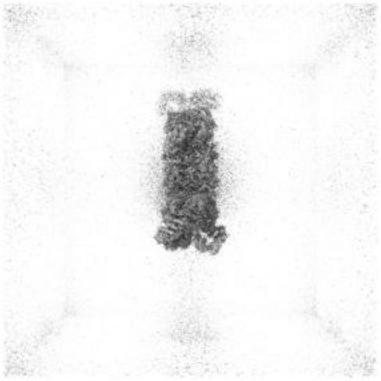
X

Y

Z

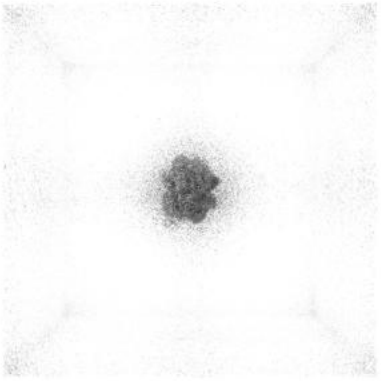
The images above show the 3D surface view of the map at the recommended contour level 0.25. These images, in conjunction with the slice images, may facilitate assessment of whether an appropriate contour level has been provided.

6.5.2 Raw map



X

Y



Z

These images show the 3D surface of the raw map. The raw map's contour level was selected so that its surface encloses the same volume as the primary map does at its recommended contour level.

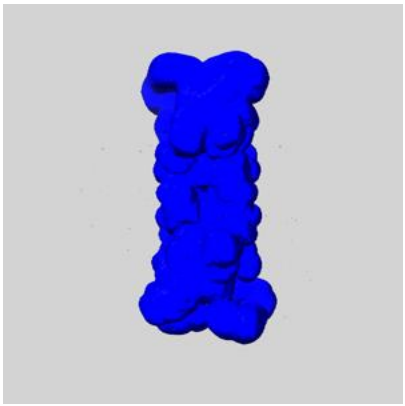
6.6 Mask visualisation (i)

This section shows the 3D surface view of the primary map at 50% transparency overlaid with the specified mask at 0% transparency

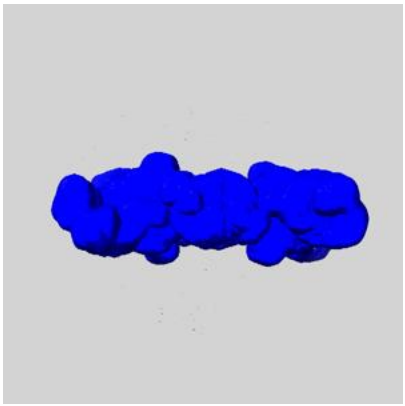
A mask typically either:

- Encompasses the whole structure
- Separates out a domain, a functional unit, a monomer or an area of interest from a larger structure

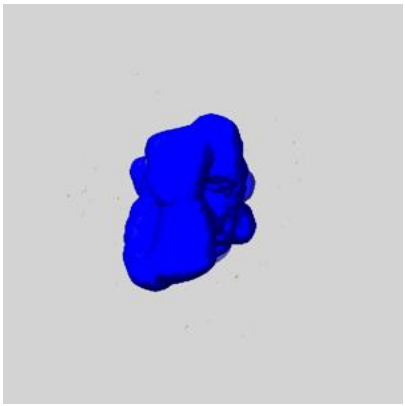
6.6.1 emd_37921_msk_1.map (i)



X



Y

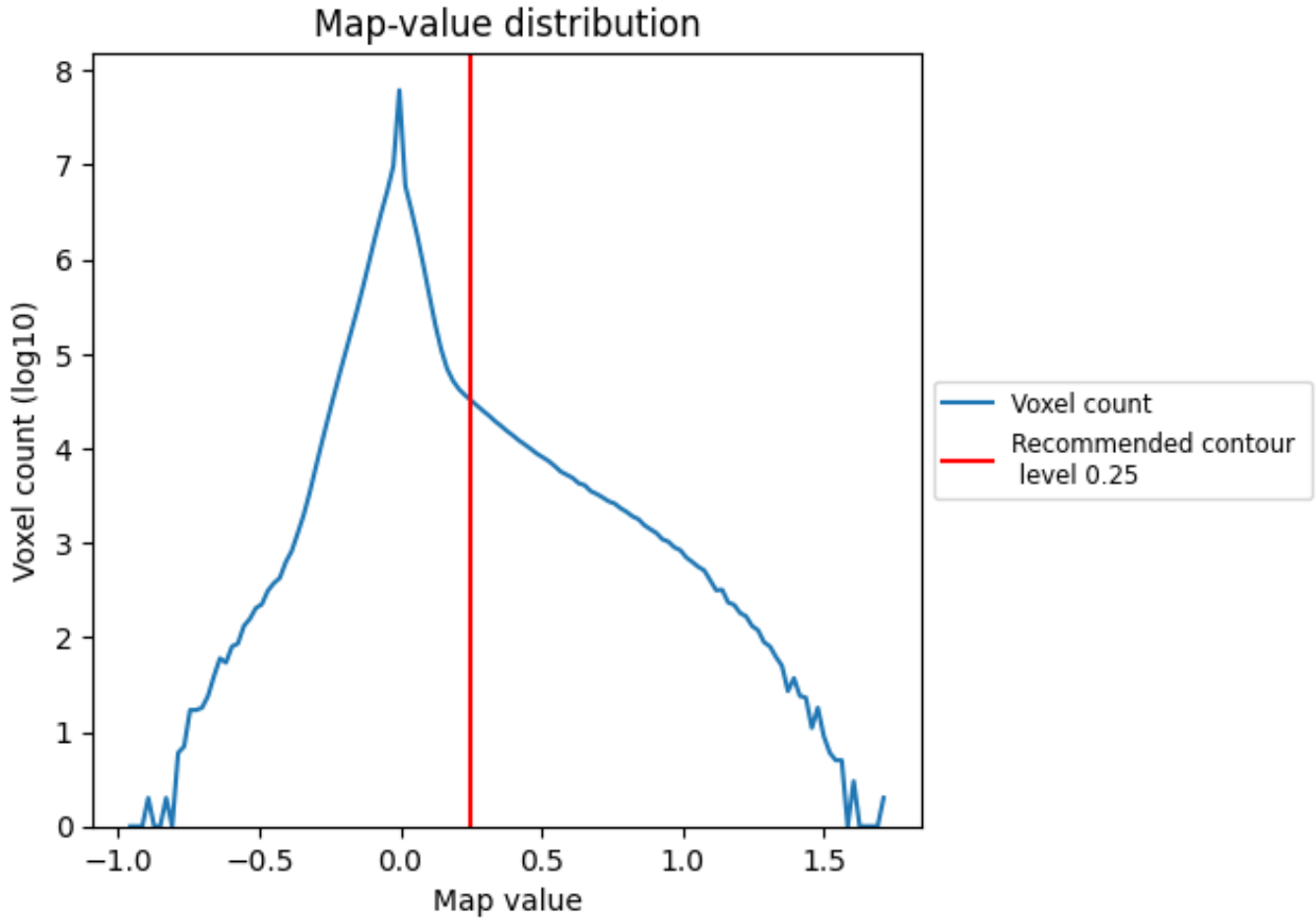


Z

7 Map analysis

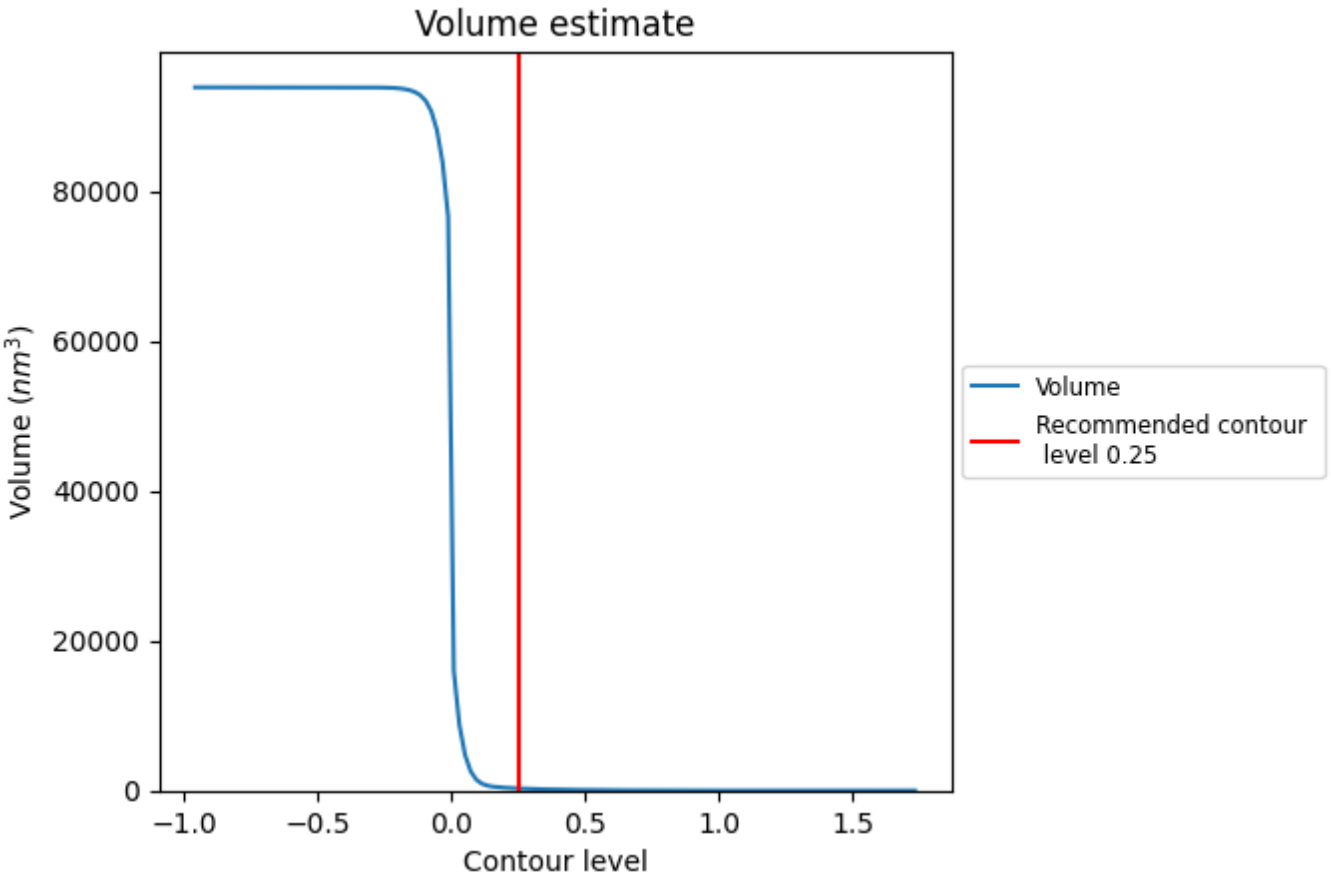
This section contains the results of statistical analysis of the map.

7.1 Map-value distribution



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

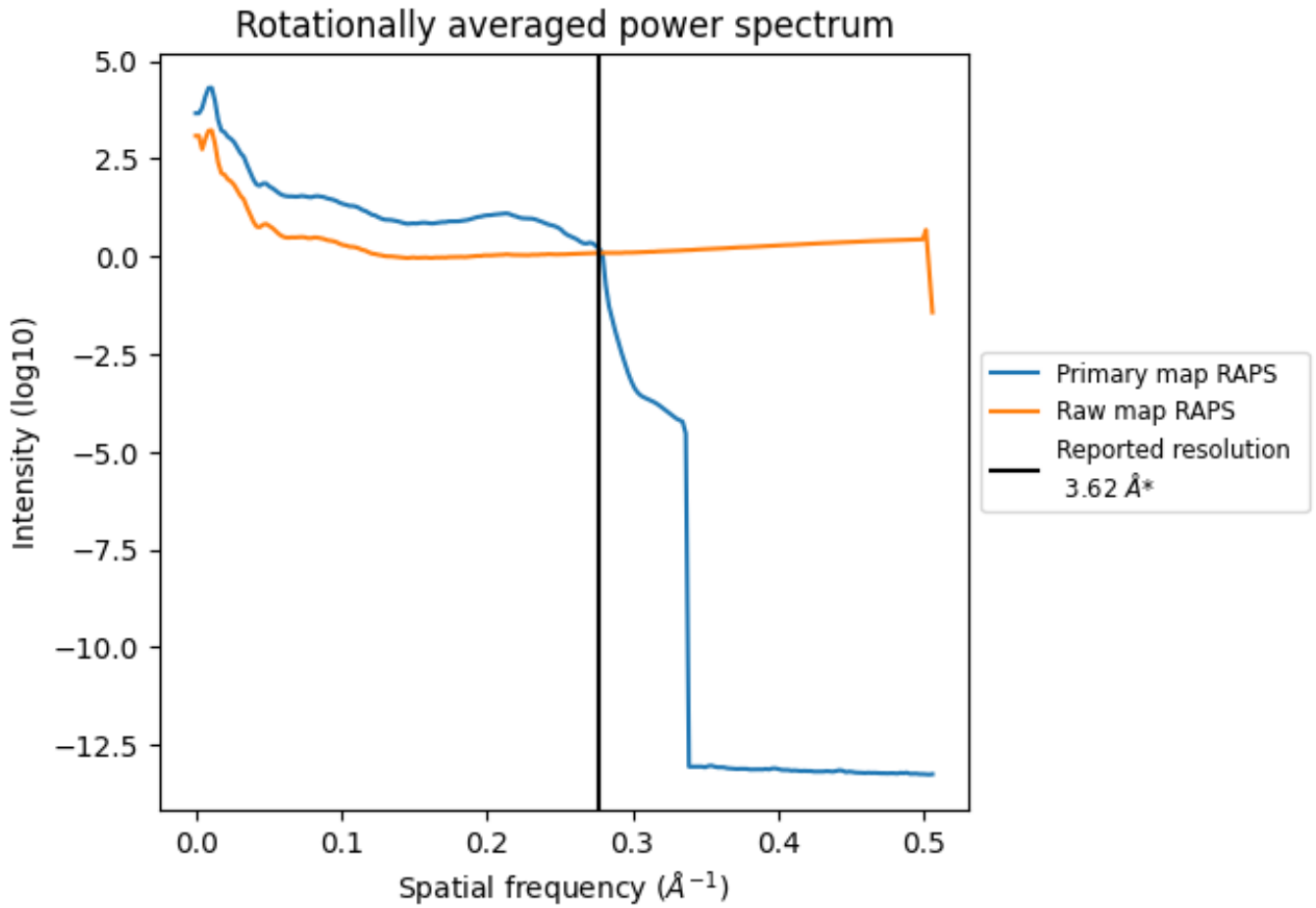
7.2 Volume estimate (i)



The volume at the recommended contour level is 288 nm^3; this corresponds to an approximate mass of 260 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)

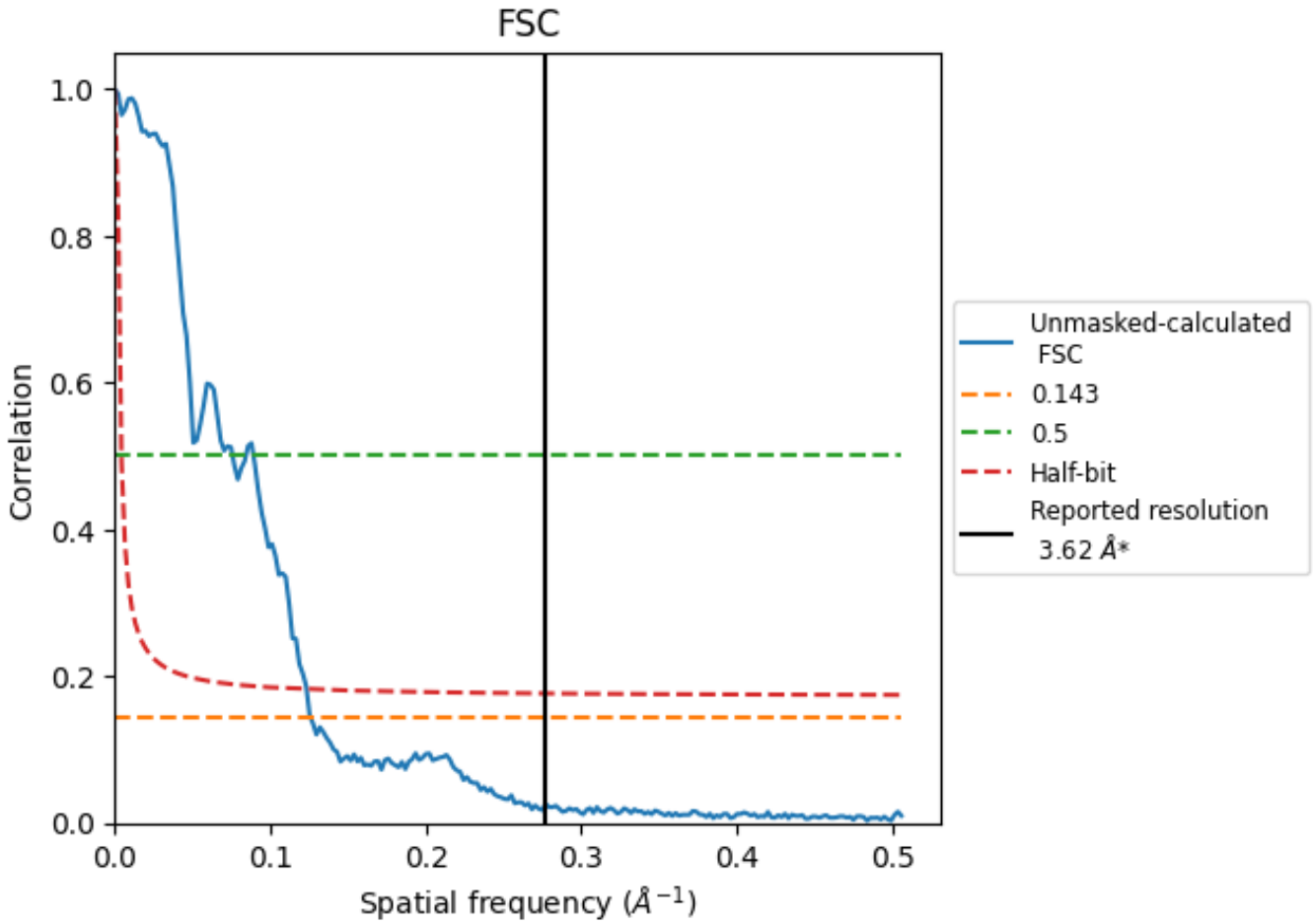


*Reported resolution corresponds to spatial frequency of 0.276 $Å^{-1}$

8 Fourier-Shell correlation

Fourier-Shell Correlation (FSC) is the most commonly used method to estimate the resolution of single-particle and subtomogram-averaged maps. The shape of the curve depends on the imposed symmetry, mask and whether or not the two 3D reconstructions used were processed from a common reference. The reported resolution is shown as a black line. A curve is displayed for the half-bit criterion in addition to lines showing the 0.143 gold standard cut-off and 0.5 cut-off.

8.1 FSC



*Reported resolution corresponds to spatial frequency of 0.276 Å^{-1}

8.2 Resolution estimates (i)

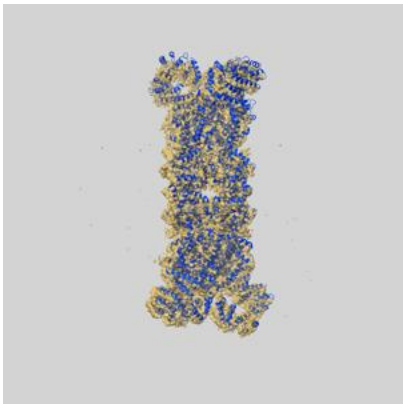
Resolution estimate (Å)	Estimation criterion (FSC cut-off)		
	0.143	0.5	Half-bit
Reported by author	3.62	-	-
Author-provided FSC curve	-	-	-
Unmasked-calculated*	7.93	13.18	8.10

*Resolution estimate based on FSC curve calculated by comparison of deposited half-maps. The value from deposited half-maps intersecting FSC 0.143 CUT-OFF 7.93 differs from the reported value 3.62 by more than 10 %

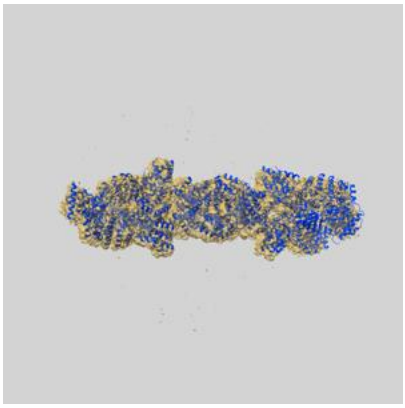
9 Map-model fit

This section contains information regarding the fit between EMDB map EMD-37921 and PDB model 8WYA. Per-residue inclusion information can be found in section 3 on page 4.

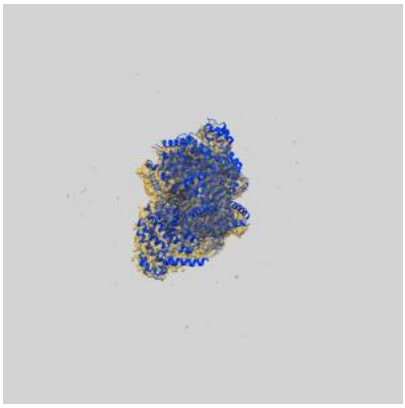
9.1 Map-model overlay



X



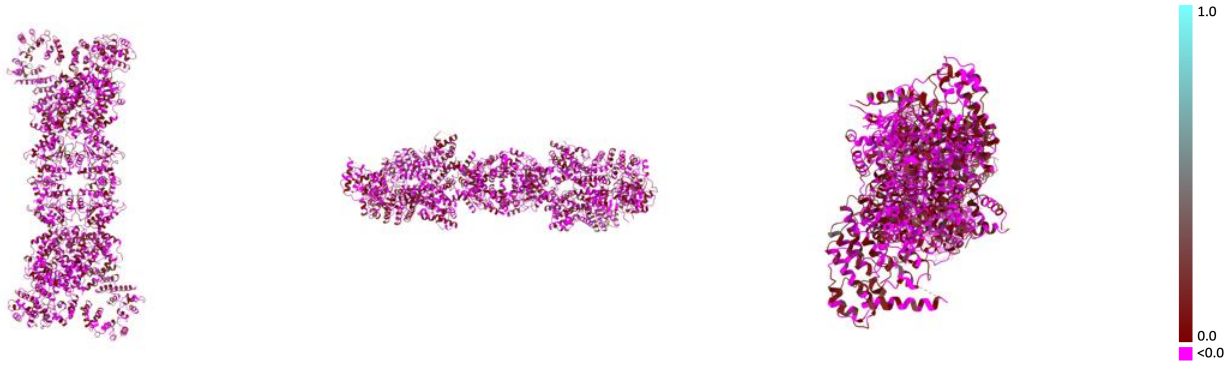
Y



Z

The images above show the 3D surface view of the map at the recommended contour level 0.25 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.

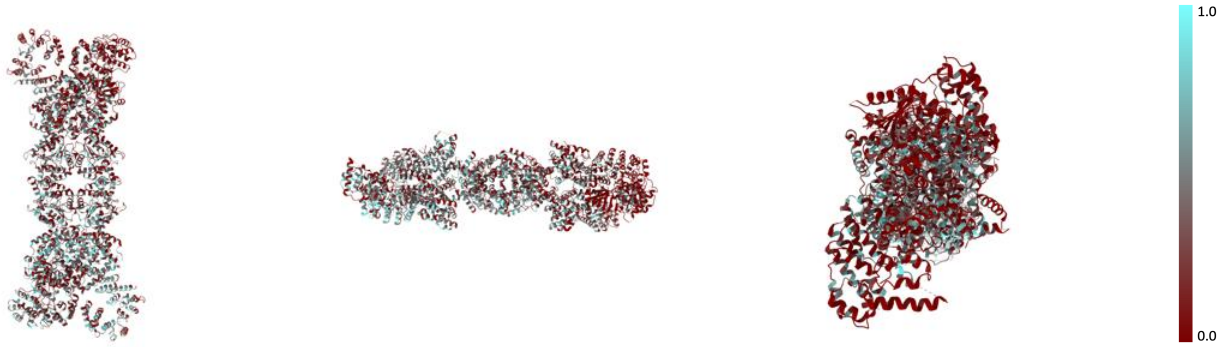
9.2 Q-score mapped to coordinate model (i)



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.

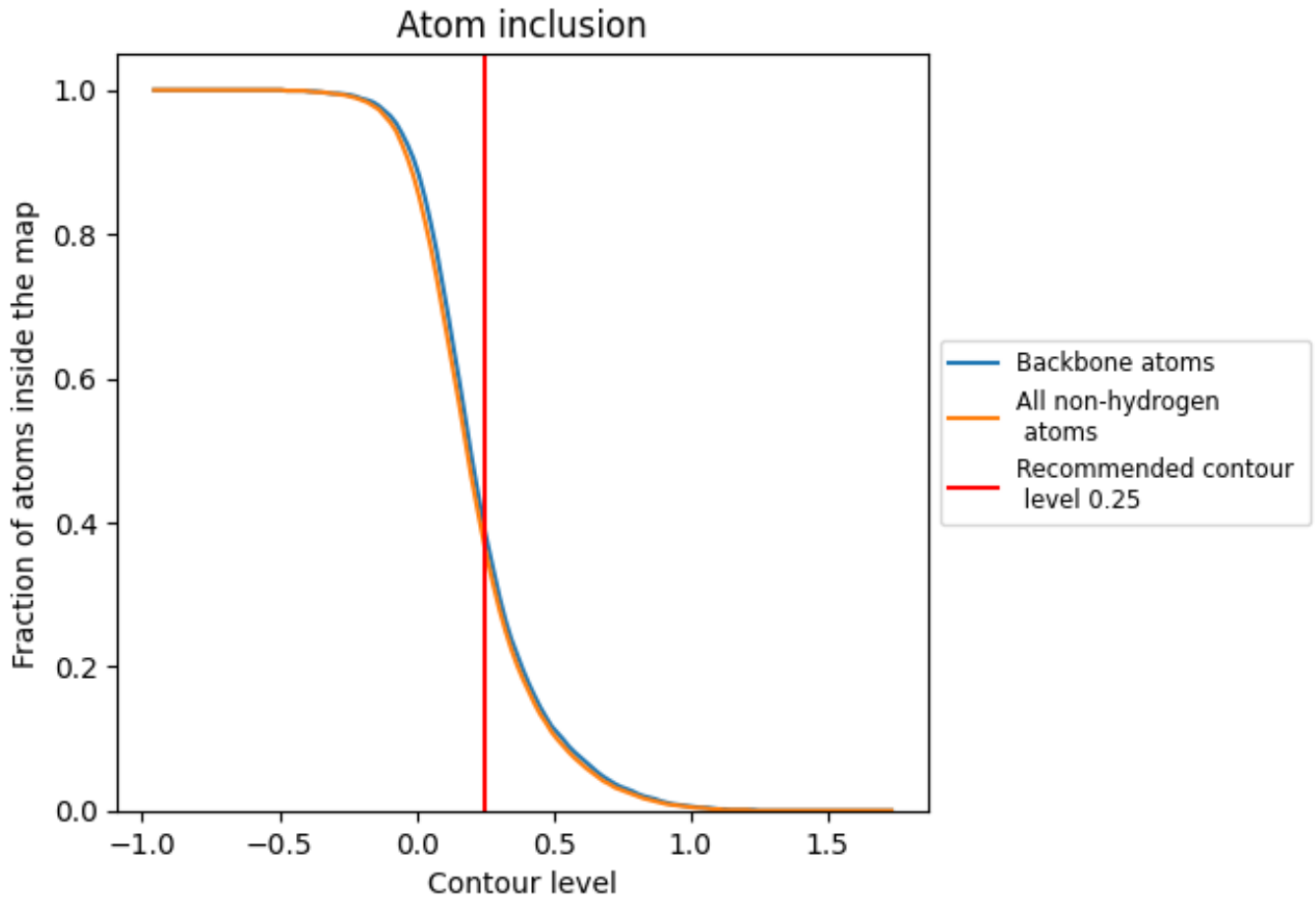
9.3 Atom inclusion mapped to coordinate model (i)



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 (0.25).

9.4 Atom inclusion (i)



At the recommended contour level, 39% of all backbone atoms, 36% 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 (0.25) and Q-score for the entire model and for each chain.

Chain	Atom inclusion	Q-score
All	0.3640	0.0110
A	0.4410	0.0090
B	0.4560	0.0130
C	0.2300	-0.0050
D	0.3100	0.0190
E	0.3070	0.0110
F	0.0240	-0.0250