



Public Data-based Report



Validation Report Service

Cryo-EM Map Validation Report

Report to assess Cryo-EM Volume Map at Level(s) A, 0

This report has been generated based on data publicly available at [EMDB](#).

Basic Entry Information:

EMDB ID: [EMD-32284](#)

PDB ID: [7w3m](#)

Title: Structure of USP14-bound human 26S proteasome in substrate-inhibited state SD5_USP14

Authors: [See EMDB entry link](#)

Deposited on: 2021-11-25T00:00:00

Reported Resolution: 3.5 Å

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Context

Cryo-electron microscopy is currently one of the most active techniques in Structural Biology. The number of maps deposited at the [Electron Microscopy Data Bank](#) is rapidly growing every year and keeping the quality of the submitted maps is essential to maintain the scientific quality of the field. The ultimate quality measure is the consistency of the map and an atomic model. However, this is only possible for high resolution maps. Over the years there have been many suggestions about validation measures of 3DEM maps. Unfortunately, most of these measures are not currently in use for their spread in multiple software tools and the associated difficulty to access them. To alleviate this problem, we made available a validation grading system that evaluate the information provided to assess the map.

This system grades a map from 0 to 5 depending on the amount of information available. In this way, a map could be validated at Level 0 (the deposited map), 1 (two half maps), 2 (2D classes), 3 (particles), 4 (... + angular assignment), 5 (... + micrographs and coordinates). In addition, we can have three optional qualifiers: A (... + atomic model), W (... + image processing workflow), and O (... + other techniques). To know more about this service read this [paper](#)

This Validation Report Service uses Scipion (see this [link](#) for more detail) as workflow engine and ChimeraX (see this [link](#) for more detail) to generate the 3D views. For more information about the different methods and softwares used for this report, see the references [here](#).



Summarized overall quality

The map seems to have some problem in its centering or extra space (see Sec. [2.1](#)). There is no problem with the suggested threshold. There seems to be a problem with the map's background (see Sec. [2.3](#)).

The overall score (passing tests) of this report is 2 out of 4 evaluable items.

0.a Mass analysis	Sec. 2.1	1 warnings
0.b Mask analysis	Sec. 2.2	OK
0.c Background analysis	Sec. 2.3	2 warnings
0.d B-factor analysis	Sec. 2.4	OK

Summary of the warnings across sections.

Section [2.1](#) (0.a Mass analysis)

1. **There could be little space from X left to effectively correct for the CTF.**

Section [2.3](#) (0.c Background analysis)

1. **The null hypothesis that the background mean is 0 has been rejected because the p-value of the comparison is smaller than 0.001**
2. **There is a significant proportion of outlier values in the background (cdf5 ratio=6307.43)**

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1 Input data

Input map: emd_32284.map

SHA256 hash: ce092f633b876ed6d63a35c4959c97caad9b3e9280e0eec9bf0f9caef14b0adb

Voxel size: 0.685000 (Å)

Visualization threshold: 0.005000

Resolution estimated by user: 3.5

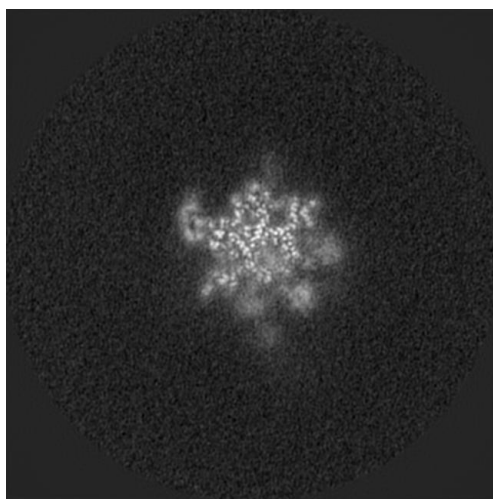
Orthogonal slices of the input map

Explanation:

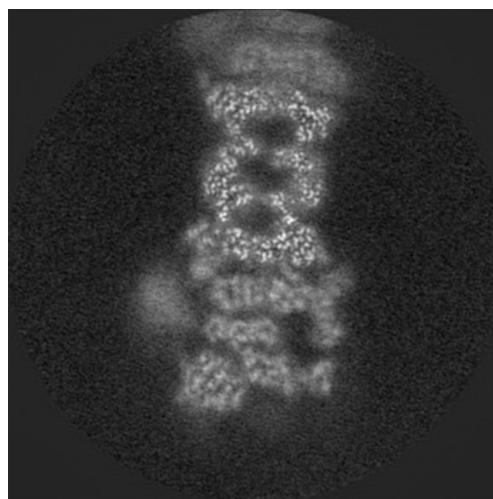
In the orthogonal slices of the map, the noise outside the protein should not have any structure (stripes going out, small blobs, particularly high or low densities, ...)

Results:

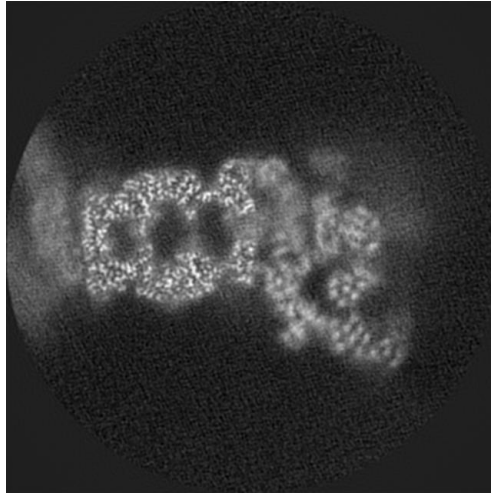
See Fig. 1.



(a) X Slice 320



(b) Y Slice 320



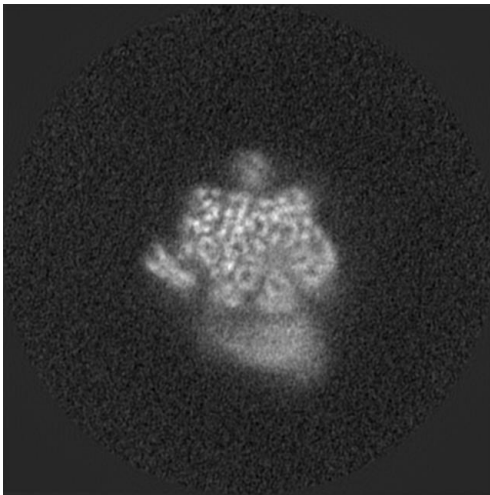
(c) Z Slice 320

Figure 1: Central slices of the input map in the three dimensions

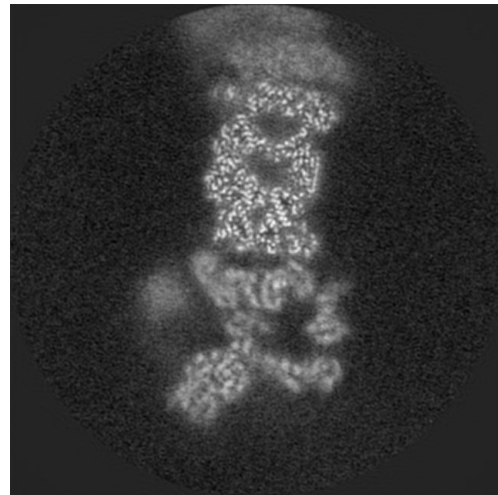
Orthogonal slices of maximum variance of the input map

Results:

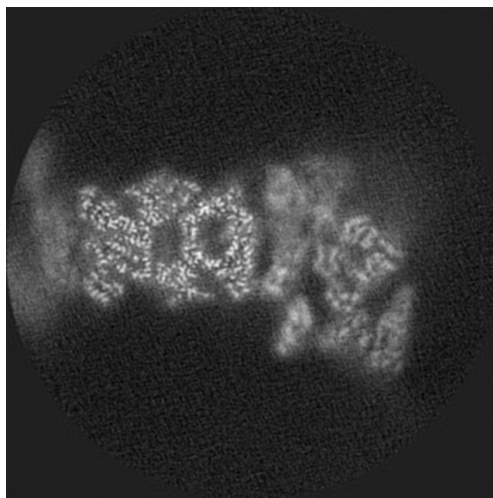
See Fig. 2.



(a) X Slice 360



(b) Y Slice 302



(c) Z Slice 299

Figure 2: Slices of maximum variation in the three dimensions

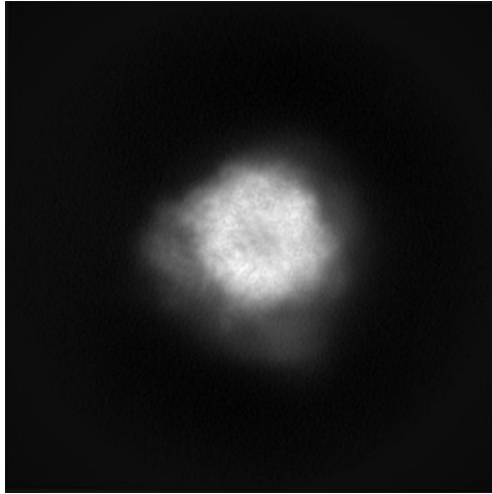
Orthogonal projections of the input map

Explanation:

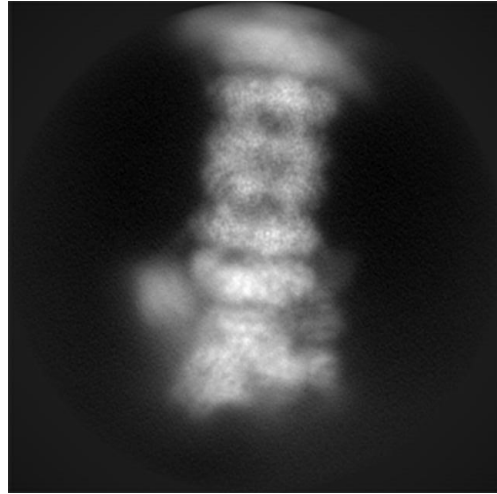
In the projections there should not be stripes (this is an indication of directional overweighting, or angular attraction), and there should not be a dark halo around or inside the structure (this is an indication of incorrect CTF correction or the reconstruction of a biased map).

Results:

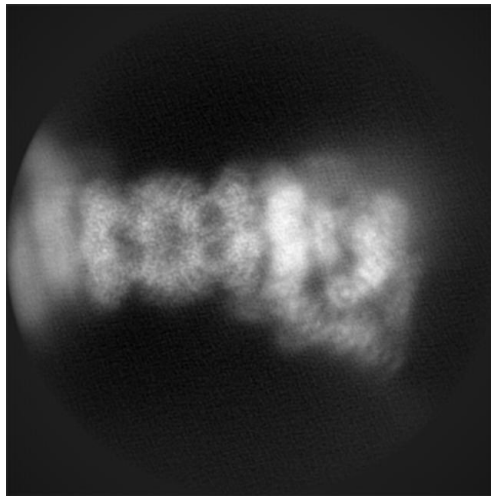
See Fig. [3](#).



(a) X Projection



(b) Y Projection



(c) Z Projection

Figure 3: Projections in the three dimensions

Isosurface views of the input map

Explanation:

An isosurface is the surface of all points that have the same gray value. In these views there should not be many artifacts or noise blobs around the map.

Results:

See Fig. 4.

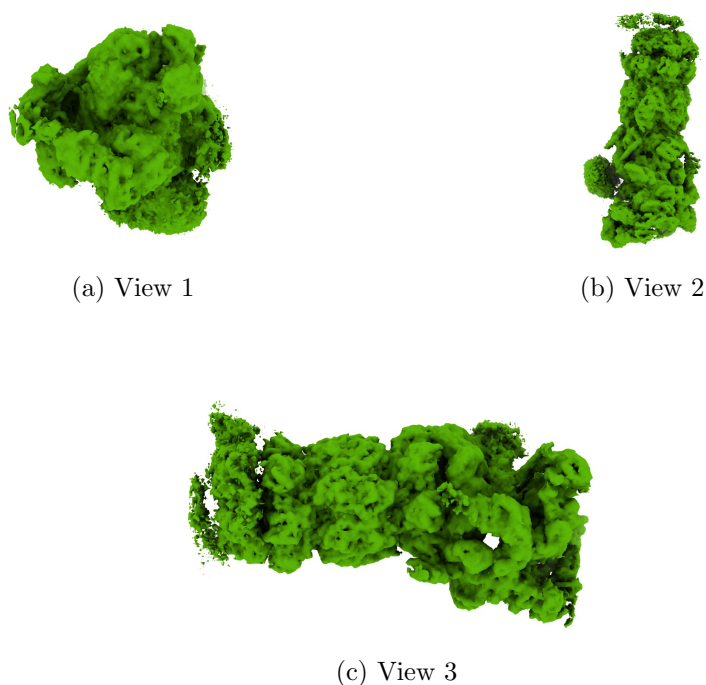


Figure 4: Isosurface at threshold=0.005000. Views generated by ChimeraX at a the following X, Y, Z angles: View 1 (0, -90, -90), View 2 (-90, 0, -90), View 3 (0, 0, 0).

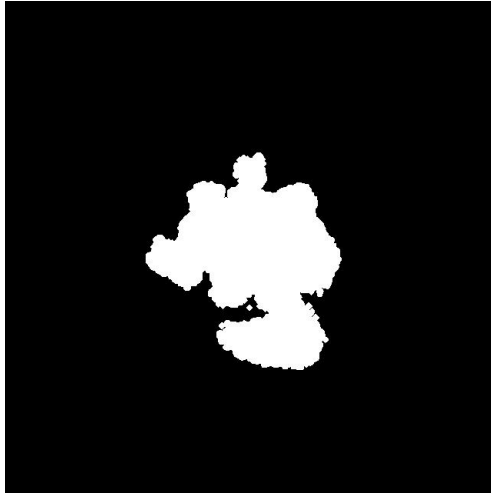
Orthogonal slices of maximum variance of the mask with hard borders

Explanation:

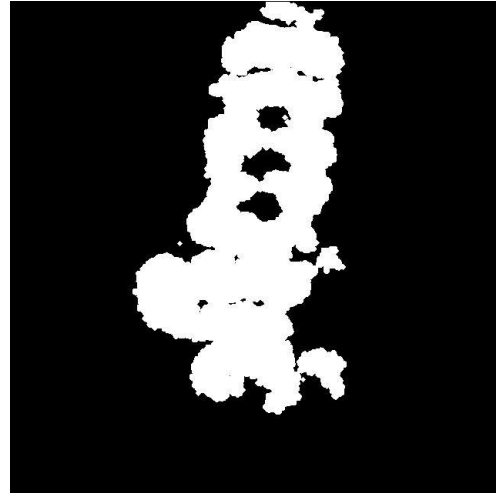
The mask with hard borders has been calculated at the suggested threshold 0.005000, the largest connected component was selected, and then dilated by 2Å.

Results:

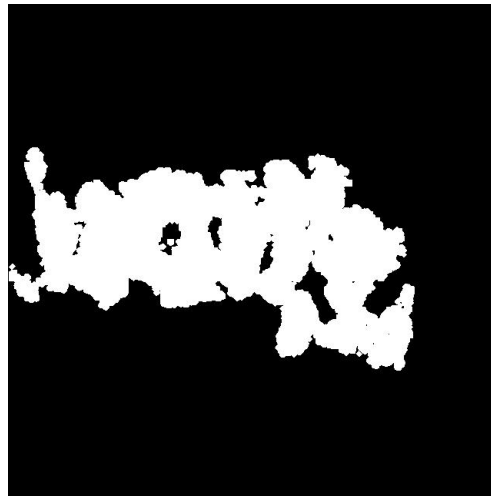
See Fig. 5.



(a) X Slice 364



(b) Y Slice 349



(c) Z Slice 304

Figure 5: Slices of maximum variation in the three dimensions of the mask with hard borders

Orthogonal slices of maximum variance of the mask with soft borders

Explanation:

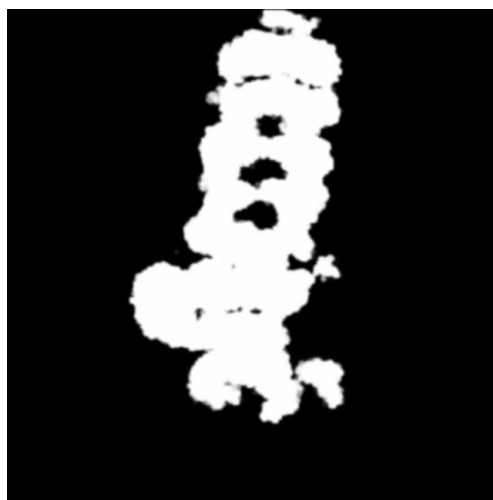
The mask with soft borders has been calculated at the suggested threshold 0.005000, the largest connected component was selected, and then dilated by

2Å.

Results:
See Fig. 6.



(a) X Slice 365



(b) Y Slice 351



(c) Z Slice 305

Figure 6: Slices of maximum variation in the three dimensions of the mask with soft borders

2 Level 0 analysis

2.1 Level 0.a Mass analysis

Explanation:

The reconstructed map must be relatively well centered in the box, and there should be at least $30\text{\AA}$ (the exact size depends on the CTF) on each side to make sure that the CTF can be appropriately corrected.

Results:

The space from the left and right in X are 0.69 and $62.34\text{\AA}$, respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 14.06%

The space from the left and right in Y are 109.60 and $126.04\text{\AA}$, respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 3.75%

The space from the left and right in Z are 107.55 and $130.84\text{\AA}$, respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 5.31%

The center of mass is at (x,y,z)=(425.49,283.88,311.45). The decentering of the center of mass (abs(Center)/Size)% is 16.48, 5.64, and 1.34, respectively.

Automatic criteria: The validation is OK if 1) the decentering and center of mass less than 20% of the map dimensions in all directions, and 2) the extra space on each direction is more than 20% of the map dimensions. For local reconstruction, focused refinement, or similar, warnings are expected.

WARNINGS: 1 warnings

1. **There could be little space from X left to effectively correct for the CTF.**

2.2 Level 0.b Mask analysis

Explanation:

The map at the suggested threshold should have most of its mass concentrated in a single connected component. It is normal that after thresholding there are a few thousands of very small, disconnected noise blobs. However, their total mass should not exceed 10%. The raw mask (just thresholding) and the mask constructed for the analysis (thresholding + largest connected component + dilation) should significantly overlap. Overlap is defined by the overlapping coefficient (size(Raw AND Constructed)/size(Raw)) that is a number between 0 and 1, the closer to 1, the more they agree.

Results:

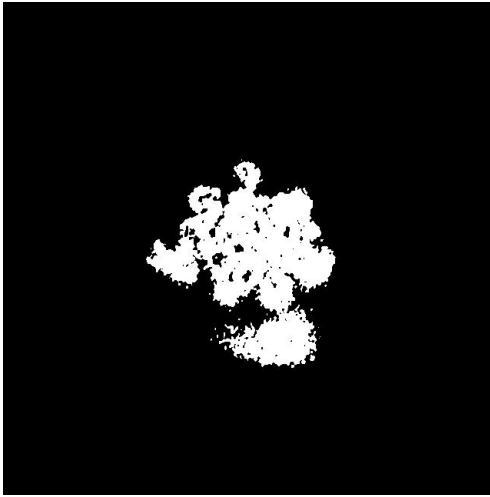
Raw mask: At threshold 0.005000, there are 2457 connected components with a total number of voxels of 7307498 and a volume of 2348769.61 Å³ (see Fig. 7). The size and percentage of the total number of voxels for the raw mask are listed below (up to 95% of the mass or the first 100 clusters, whatever happens first), the list contains (No. voxels (volume in Å³), percentage, cumulated percentage):

(7280251 (2340011.91), 99.63, 99.63)

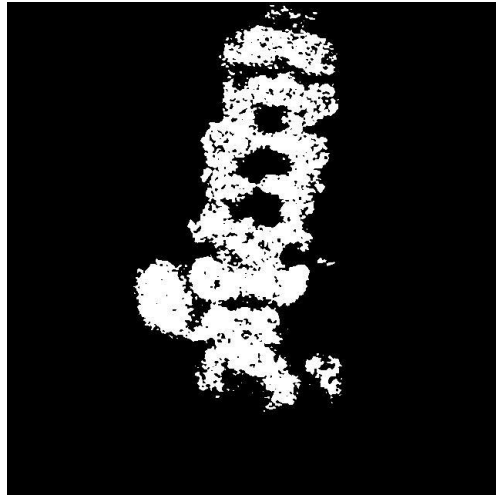
Number of components to reach 95% of the mass: 1

The average size of the remaining 2456 components is 11.09 voxels (0.32 $\text{\AA}^3$). Their size go from 7280251 voxels (2340011.91 $\text{\AA}^3$) to 1 voxels (0.32 $\text{\AA}^3$).

The slices of the raw mask can be seen in Fig. 7.



(a) X Slice 365



(b) Y Slice 354



(c) Z Slice 303

Figure 7: Maximum variance slices in the three dimensions of the raw mask

The following table shows the variation of the mass enclosed at different thresholds (see Fig. 8):

Threshold	Voxel mass	Molecular mass(kDa)	# Aminoacids
0.0008	24832238.00	6612.72	60115.66
0.0015	18225210.00	4853.30	44120.89
0.0023	15016884.00	3998.93	36353.95
0.0031	12596395.00	3354.37	30494.26
0.0038	10402398.00	2770.12	25182.87
0.0046	8312568.00	2213.60	20123.66
0.0054	6378488.00	1698.57	15441.50
0.0061	4669251.00	1243.40	11303.66
0.0069	3281639.00	873.89	7944.43
0.0077	2266443.00	603.54	5486.77
0.0085	1559090.00	415.18	3774.36
0.0092	1066352.00	283.97	2581.50
0.0100	725095.00	193.09	1755.36
0.0108	489960.00	130.47	1186.13
0.0115	326452.00	86.93	790.30
0.0123	211182.00	56.24	511.24
0.0131	128730.00	34.28	311.64
0.0138	70579.00	18.79	170.86
0.0146	33589.00	8.94	81.31
0.0154	13217.00	3.52	32.00
0.0161	4077.00	1.09	9.87
0.0169	959.00	0.26	2.32
0.0177	159.00	0.04	0.38
0.0184	20.00	0.01	0.05
0.0192	0.00	0.00	0.00

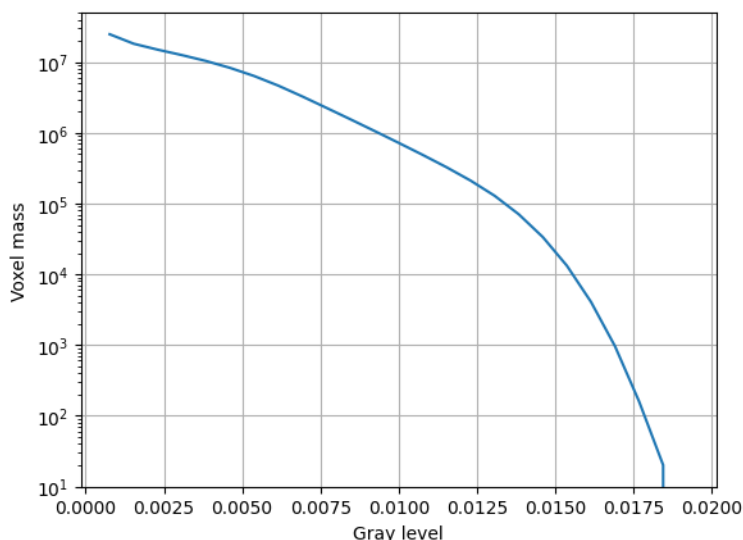


Figure 8: Voxel mass as a function of the gray level.

Constructed mask: After keeping the largest component of the previous mask and dilating it by $2\text{\AA}$, there is a total number of voxels of 11967842 and a volume of $3846693.30 \text{ \AA}^3$. The overlap between the raw and constructed mask is 1.00.

Automatic criteria: The validation is OK if 1) to keep 95% of the mass we need to keep at most 5 connected components; and 2) the average volume of the blobs outside the given threshold has a size smaller than $5\text{\AA}^3$; and 3) the overlap between the raw mask and the mask constructed for the analysis is larger than 75%.

STATUS: OK

2.3 Level 0.c Background analysis

Explanation:

Background is defined as the region outside the macromolecule mask. The background mean should be zero, and the number of voxels with a very low or very high value (below 5 standard deviations of the noise) should be very small and they should be randomly distributed without any specific structure.

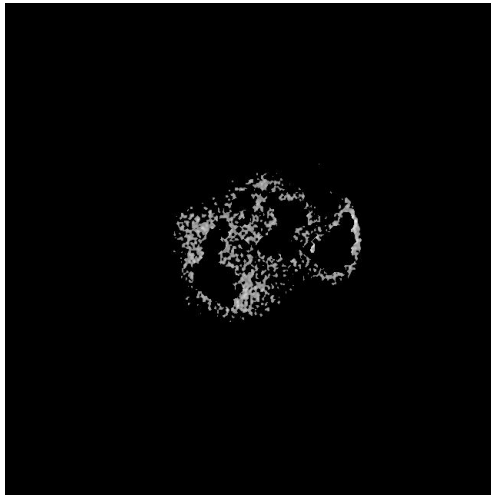
Sometimes, you can see some structure due to the symmetry of the structure.

Results:

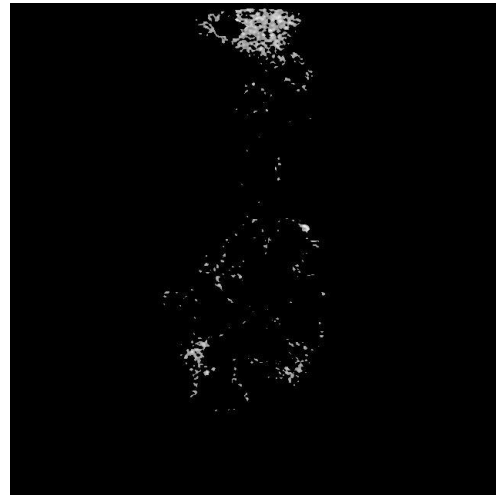
The null hypothesis that the background mean is 0 was tested with a one-sample Student's t-test. The resulting t-statistic and p-value were -7792.01 and 0.000000, respectively.

The mean and standard deviation (σ) of the background were -0.000334 and 0.000678. The percentage of background voxels whose absolute value is larger than 5 times the standard deviation is 0.36 % (see Fig. 9). The same percentage from a Gaussian would be 0.000057% (ratio between the two percentages: 6307.427479).

Slices of the background beyond 5σ can be seen in Fig. 9.



(a) X Slice 33



(b) Y Slice 252



(c) Z Slice 317

Figure 9: Maximum variance slices in the three dimensions of the parts of the background beyond $5 \times \sigma$

Automatic criteria: The validation is OK if 1) the p-value of the null hypothesis that the background has 0 mean is larger than 0.001; and 2) the number of voxels above or below 5 sigma is smaller than 20 times the amount expected for a Gaussian with the same standard deviation whose mean is 0.

WARNINGS: 2 warnings

1. **The null hypothesis that the background mean is 0 has been rejected because the p-value of the comparison is smaller than 0.001**
2. **There is a significant proportion of outlier values in the background (cdf5 ratio=6307.43)**

2.4 Level 0.d B-factor analysis

Explanation:

The B-factor line (see this [link](#) for more details) fitted between $15\text{\AA}$ and the resolution reported should have a slope that is between 0 and $300\text{ \AA}^2$.

Results:

Fig. 10 shows the logarithm (in natural units) of the structure factor (the module squared of the Fourier transform) of the experimental map, its fitted line, and the corrected map. The estimated B-factor was -65.7. The fitted line was $\log(|F|^2) = -16.4/R^2 + (-15.0)$.

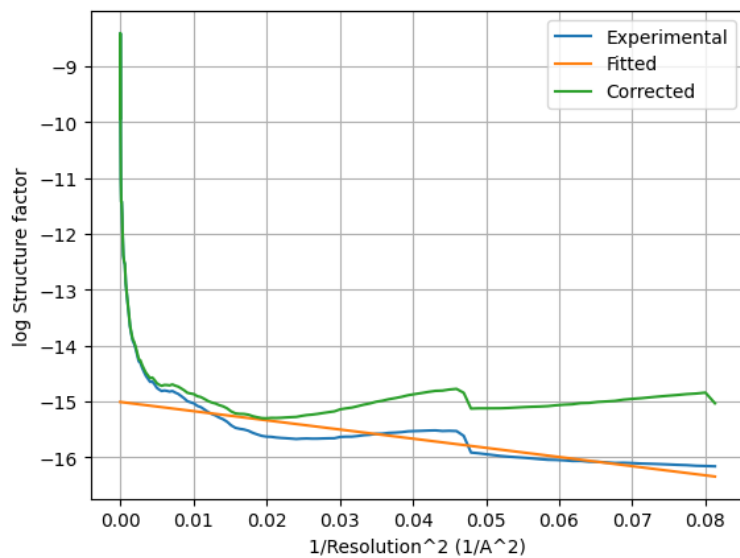
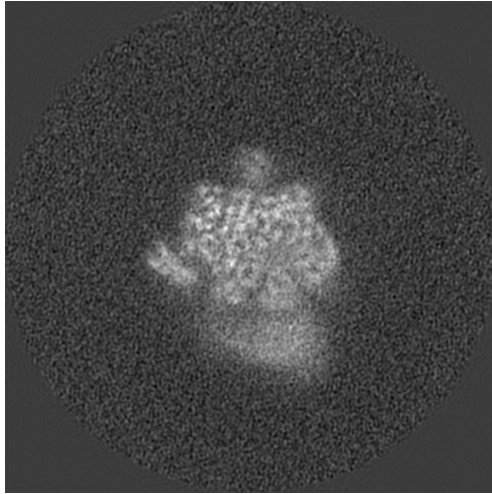
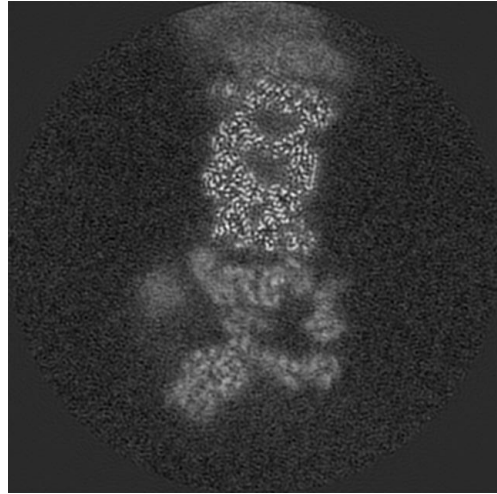


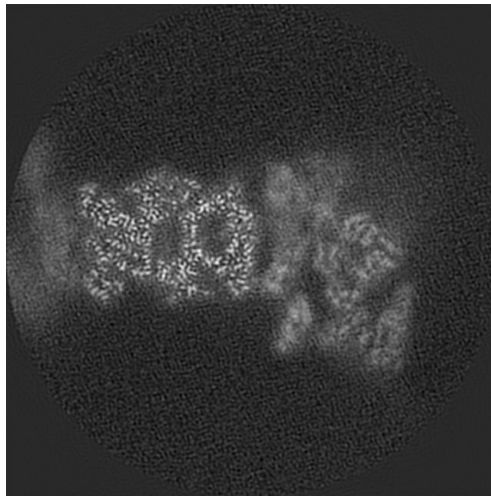
Figure 10: Guinier plot. The X-axis is the square of the inverse of the resolution in Å.



(a) X Slice 360



(b) Y Slice 302



(c) Z Slice 299

Figure 11: Slices of maximum variation in the three dimensions of the B-factor corrected map

Automatic criteria: The validation is OK if the B-factor is in the range $[-300,0]$.

STATUS: OK

3 Atomic model

Atomic model: 7w3m.cif

See Fig. 12.

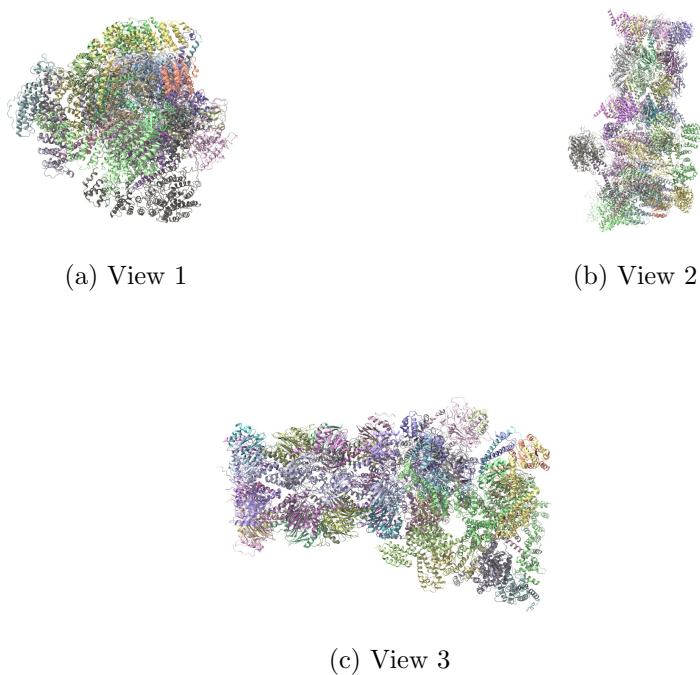


Figure 12: Input atomic model Views generated by ChimeraX at a the following X, Y, Z angles: View 1 (0, -90, -90), View 2 (-90, 0, -90), View 3 (0, 0, 0).

4 Level A Analysis