



Public Data-based Report



# Validation Report Service

## Cryo-EM Map Validation Report

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

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

### Basic Entry Information:

**EMDB ID:** [EMD-9215](#)

**Title:** Cryo-EM structures and dynamics of substrate-engaged human 26S proteasome

**Authors:** [See EMDB entry link](#)

**Deposited on:** 2018-10-16T00:00:00

**Reported Resolution:** 2.8 Å

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Last update: **August 29, 2024, 7:24pm**

## 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).

This Validation Report Service is explained in more detail in this [paper](#). 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 seems to be a problem with the suggested threshold (see Sec. 2.2). There seems to be a problem with the map's background (see Sec. 2.3).

The average resolution of the map estimated by various methods goes from 3.5Å to 3.5Å with an average of 3.5Å. The resolution reported by the user was 2.8Å.

**The overall score (passing tests) of this report is 5 out of 8 evaluable items.**

|                         |                          |                            |
|-------------------------|--------------------------|----------------------------|
| 0.a Mass analysis       | Sec. <a href="#">2.1</a> | <a href="#">1 warnings</a> |
| 0.b Mask analysis       | Sec. <a href="#">2.2</a> | <a href="#">2 warnings</a> |
| 0.c Background analysis | Sec. <a href="#">2.3</a> | <a href="#">2 warnings</a> |
| 0.d B-factor analysis   | Sec. <a href="#">2.4</a> | <a href="#">OK</a>         |
| 0.e DeepRes             | Sec. <a href="#">2.5</a> | <a href="#">OK</a>         |
| 0.f LocBfactor          | Sec. <a href="#">2.6</a> | <a href="#">OK</a>         |
| 0.g LocOccupancy        | Sec. <a href="#">2.7</a> | <a href="#">OK</a>         |
| 0.h DeepHand            | Sec. <a href="#">2.8</a> | <a href="#">OK</a>         |

### Summary of the warnings across sections.

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

1. **The volume might be significantly decentered in X.**

#### Section 2.2 (0.b Mask analysis)

1. **There might be a problem of connectivity at this threshold because more than 5 connected components are needed to reach 95% of the total mask. Probably a smaller threshold will not cause this issue.**
2. **There might be a problem in the construction of the mask, because the overlap is smaller than 0.75. A common reason is that the suggested threshold causes too many disconnected components.**

#### 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=6796.05)**

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# 1 Input data

Input map: emd\_9215.map

SHA256 hash: 56ae27362820aad8d0f44ef7cbbe86464c135e0a0886296c198fd2b45e0ca562

Voxel size: 0.685000 (Å)

Visualization threshold: 0.012000

Resolution estimated by user: 2.8

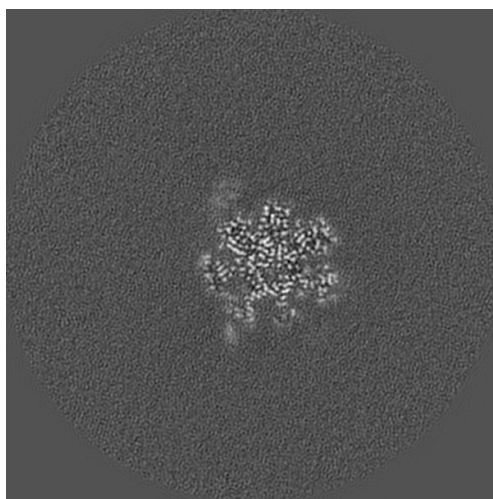
## Orthogonal slices of the input map

### **Explanation:**

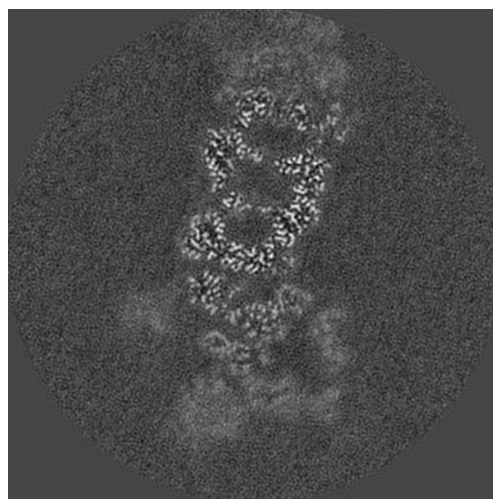
In the orthogonal slices of the map, the noise outside the protein should not have any structure (stripes going out, small blobs, particularly high or low densities, ...)

### **Results:**

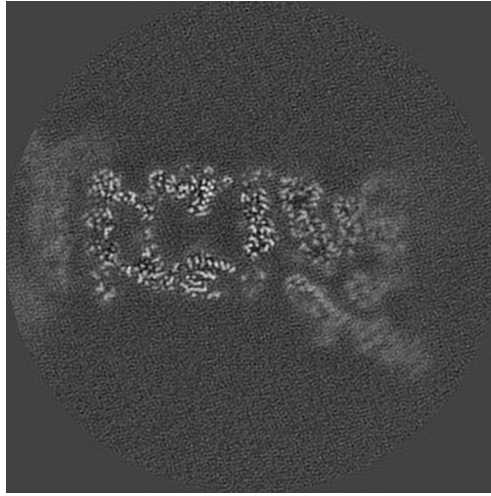
See Fig. 1.



(a) X Slice 300



(b) Y Slice 300



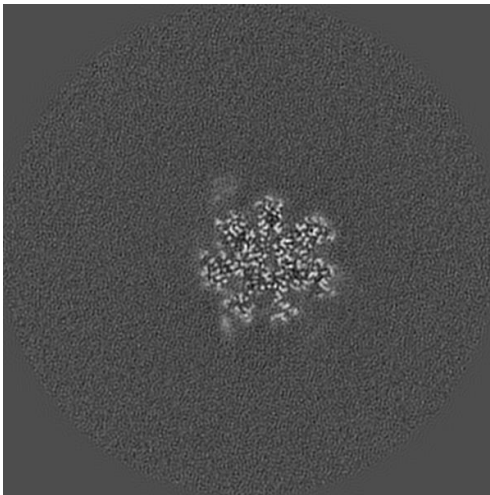
(c) Z Slice 300

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

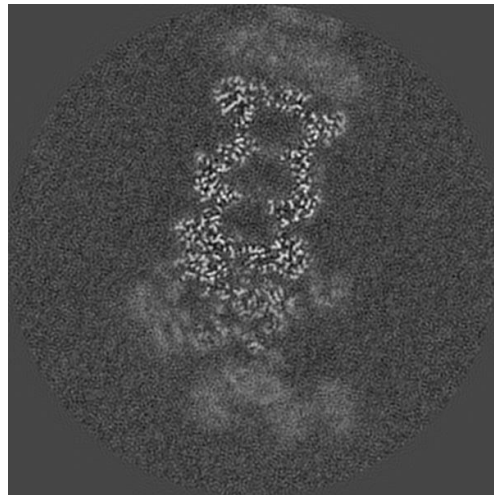
Orthogonal slices of maximum variance of the input map

**Results:**

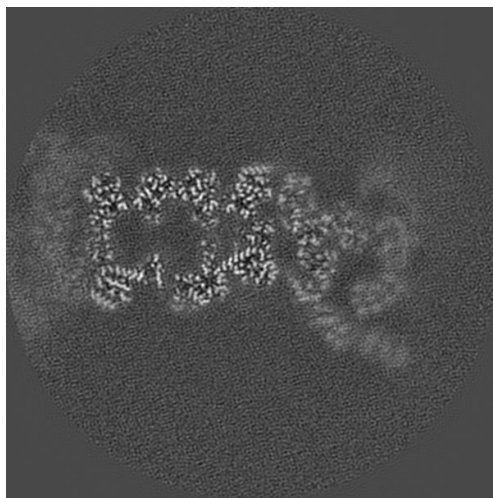
See Fig. 2.



(a) X Slice 296



(b) Y Slice 334



(c) Z Slice 318

Figure 2: Slices of maximum variation in the three dimensions

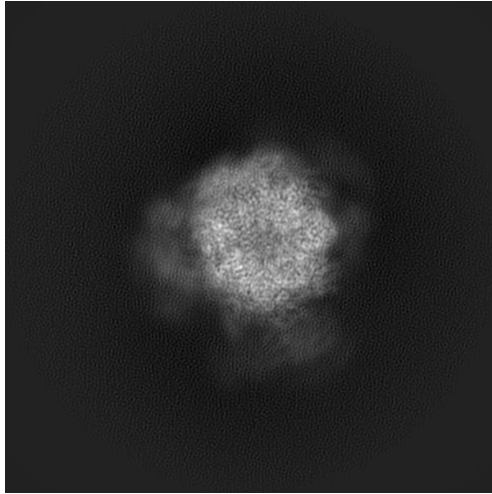
### Orthogonal projections of the input map

#### **Explanation:**

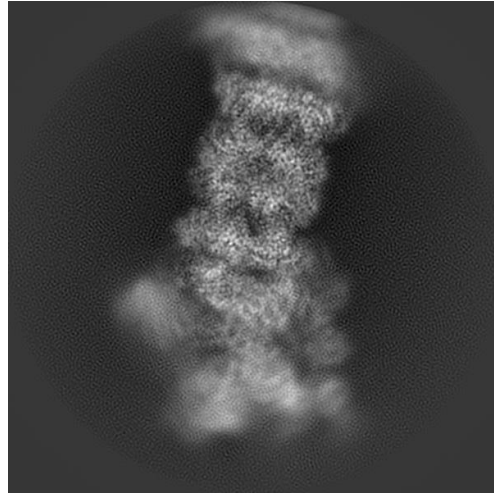
In the projections there should not be stripes (this is an indication of directional overweighting, or angular attraction), and there should not be a dark halo around or inside the structure (this is an indication of incorrect CTF correction or the reconstruction of a biased map).

#### **Results:**

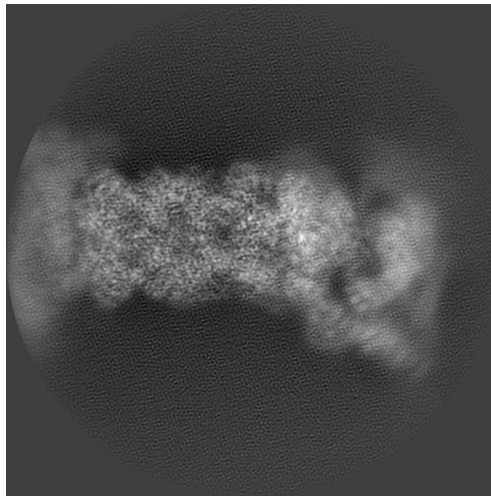
See Fig. [3](#).



(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.

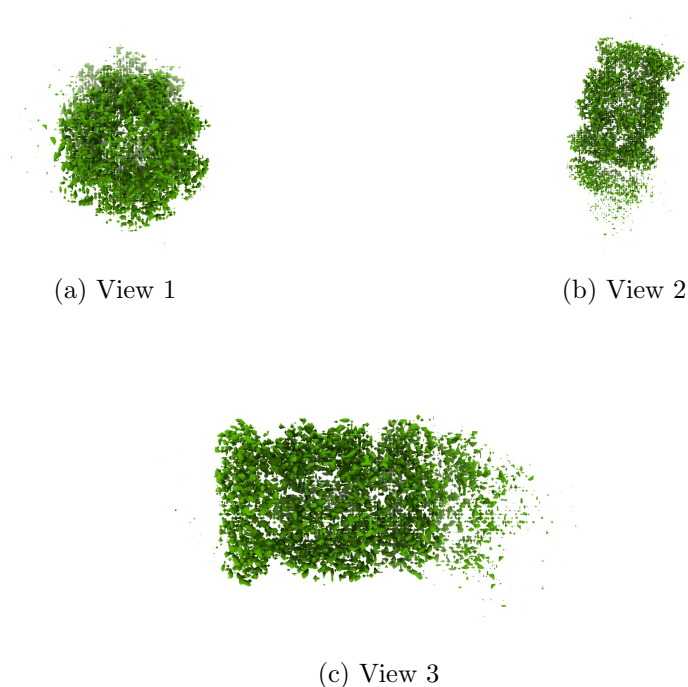


Figure 4: Isosurface at threshold=0.012000. Views generated by ChimeraX at a the following X, Y, Z angles: View 1 (0, -90, -90), View 2 (-90, 0, -90), View 3 (0, 0, 0).

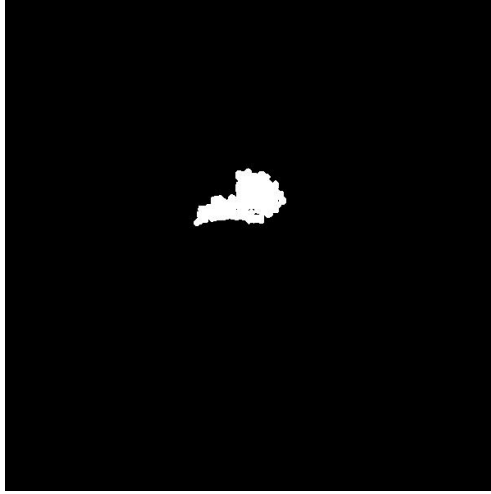
**Orthogonal slices of maximum variance of the mask with hard borders**

**Explanation:**

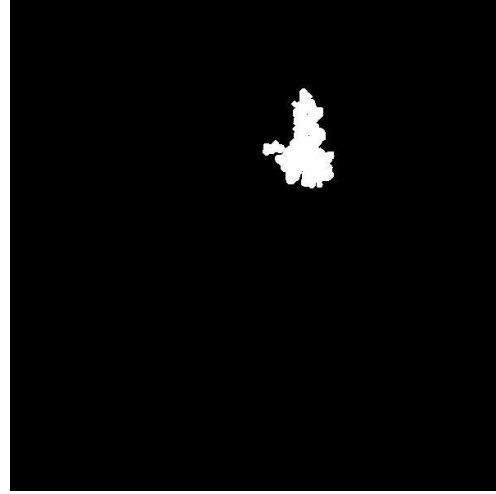
The mask with hard borders has been calculated at the suggested threshold 0.012000, the largest connected component was selected, and then dilated by 2Å.

**Results:**

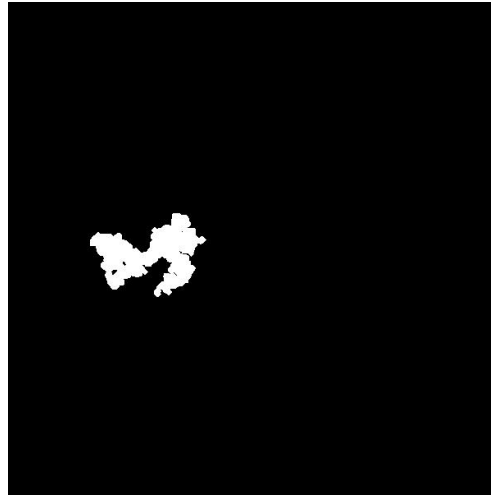
See Fig. 5.



(a) X Slice 202



(b) Y Slice 293



(c) Z Slice 355

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 0.012000, the largest connected component was selected, and then dilated by

2Å.

**Results:**  
See Fig. 6.



(a) X Slice 201



(b) Y Slice 293



(c) Z Slice 356

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 68.50 and 232.90  $\text{\AA}$ , respectively. There is a decentering ratio  $(\text{abs}(\text{Right-Left})/\text{Size})\%$  of 40.00%

The space from the left and right in Y are 157.55 and 176.73  $\text{\AA}$ , respectively. There is a decentering ratio  $(\text{abs}(\text{Right-Left})/\text{Size})\%$  of 4.67%

The space from the left and right in Z are 210.98 and 136.31  $\text{\AA}$ , respectively. There is a decentering ratio  $(\text{abs}(\text{Right-Left})/\text{Size})\%$  of 18.17%

The center of mass is at (x,y,z)=(311.55,298.93,305.39). The decentering of the center of mass (abs(Center)/Size)% is 1.93, 0.18, and 0.90, 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 and focused refinement, or similar, warnings are expected.

**WARNINGS:** 1 warnings

1. **The volume might be significantly decentered in X.**

## 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 0.012000, there are 1932 connected components with a total number of voxels of 615182 and a volume of 197731.26 Å<sup>3</sup> (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 Å<sup>3</sup>), percentage, cumulated percentage):

(50197 (16134.28), 8.16, 8.16)(37646 (12100.14), 6.12, 14.28)(31438 (10104.77), 5.11, 19.39)(20585 (6616.41), 3.35, 22.74)(19354 (6220.75), 3.15, 25.88)(18771 (6033.36), 3.05, 28.93)(18578 (5971.32), 3.02, 31.95)(18467 (5935.65), 3.00,

34.95)(17926 (5761.76), 2.91, 37.87)(17790 (5718.05), 2.89, 40.76)(16429 (5280.59),  
 2.67, 43.43)(15964 (5131.13), 2.60, 46.03)(14447 (4643.54), 2.35, 48.37)(14219  
 (4570.26), 2.31, 50.69)(11554 (3713.68), 1.88, 52.56)(11517 (3701.78), 1.87,  
 54.44)(11358 (3650.68), 1.85, 56.28)(10496 (3373.62), 1.71, 57.99)(9924 (3189.76),  
 1.61, 59.60)(9739 (3130.30), 1.58, 61.18)(9626 (3093.98), 1.56, 62.75)(9527  
 (3062.16), 1.55, 64.30)(9401 (3021.66), 1.53, 65.83)(8840 (2841.35), 1.44,  
 67.26)(8564 (2752.63), 1.39, 68.66)(7388 (2374.64), 1.20, 69.86)(6491 (2086.33),  
 1.06, 70.91)(6207 (1995.05), 1.01, 71.92)(5917 (1901.84), 0.96, 72.88)(5758  
 (1850.73), 0.94, 73.82)(5455 (1753.34), 0.89, 74.71)(5029 (1616.42), 0.82,  
 75.52)(4976 (1599.38), 0.81, 76.33)(4215 (1354.78), 0.69, 77.02)(4176 (1342.25),  
 0.68, 77.70)(3900 (1253.53), 0.63, 78.33)(3814 (1225.89), 0.62, 78.95)(3663  
 (1177.36), 0.60, 79.54)(3569 (1147.14), 0.58, 80.13)(3409 (1095.72), 0.55,  
 80.68)(3230 (1038.18), 0.53, 81.20)(3092 (993.83), 0.50, 81.71)(3008 (966.83),  
 0.49, 82.20)(2937 (944.01), 0.48, 82.67)(2627 (844.37), 0.43, 83.10)(2611 (839.23),  
 0.42, 83.52)(2593 (833.44), 0.42, 83.95)(2529 (812.87), 0.41, 84.36)(2453 (788.44),  
 0.40, 84.76)(2446 (786.19), 0.40, 85.15)(2408 (773.98), 0.39, 85.55)(2257 (725.44),  
 0.37, 85.91)(2174 (698.77), 0.35, 86.27)(2018 (648.62), 0.33, 86.59)(1953 (627.73),  
 0.32, 86.91)(1919 (616.80), 0.31, 87.22)(1877 (603.30), 0.31, 87.53)(1804 (579.84),  
 0.29, 87.82)(1718 (552.20), 0.28, 88.10)(1686 (541.91), 0.27, 88.37)(1655 (531.95),  
 0.27, 88.64)(1653 (531.31), 0.27, 88.91)(1568 (503.99), 0.25, 89.17)(1546 (496.91),  
 0.25, 89.42)(1489 (478.59), 0.24, 89.66)(1418 (455.77), 0.23, 89.89)(1334 (428.77),  
 0.22, 90.11)(1308 (420.42), 0.21, 90.32)(1240 (398.56), 0.20, 90.52)(1209 (388.60),  
 0.20, 90.72)(1185 (380.88), 0.19, 90.91)(1128 (362.56), 0.18, 91.09)(1128 (362.56),  
 0.18, 91.28)(1088 (349.70), 0.18, 91.45)(1041 (334.60), 0.17, 91.62)(1039 (333.95),  
 0.17, 91.79)(1010 (324.63), 0.16, 91.96)(931 (299.24), 0.15, 92.11)(887 (285.10),  
 0.14, 92.25)(874 (280.92), 0.14, 92.39)(857 (275.46), 0.14, 92.53)(836 (268.71),  
 0.14, 92.67)(833 (267.74), 0.14, 92.81)(822 (264.21), 0.13, 92.94)(745 (239.46),  
 0.12, 93.06)(717 (230.46), 0.12, 93.18)(712 (228.85), 0.12, 93.29)(672 (215.99),  
 0.11, 93.40)(630 (202.49), 0.10, 93.50)(545 (175.17), 0.09, 93.59)(538 (172.92),  
 0.09, 93.68)(520 (167.14), 0.08, 93.76)(517 (166.17), 0.08, 93.85)(507 (162.96),  
 0.08, 93.93)(486 (156.21), 0.08, 94.01)(458 (147.21), 0.07, 94.08)(456 (146.57),  
 0.07, 94.16)(448 (144.00), 0.07, 94.23)(431 (138.53), 0.07, 94.30)

Number of components to reach 95% of the mass: 111

The average size of the remaining 1821 components is 16.76 voxels ( 0.32 Å<sup>3</sup>). Their size go from 326 voxels (104.78 Å<sup>3</sup>) to 1 voxels ( 0.32 Å<sup>3</sup>).

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

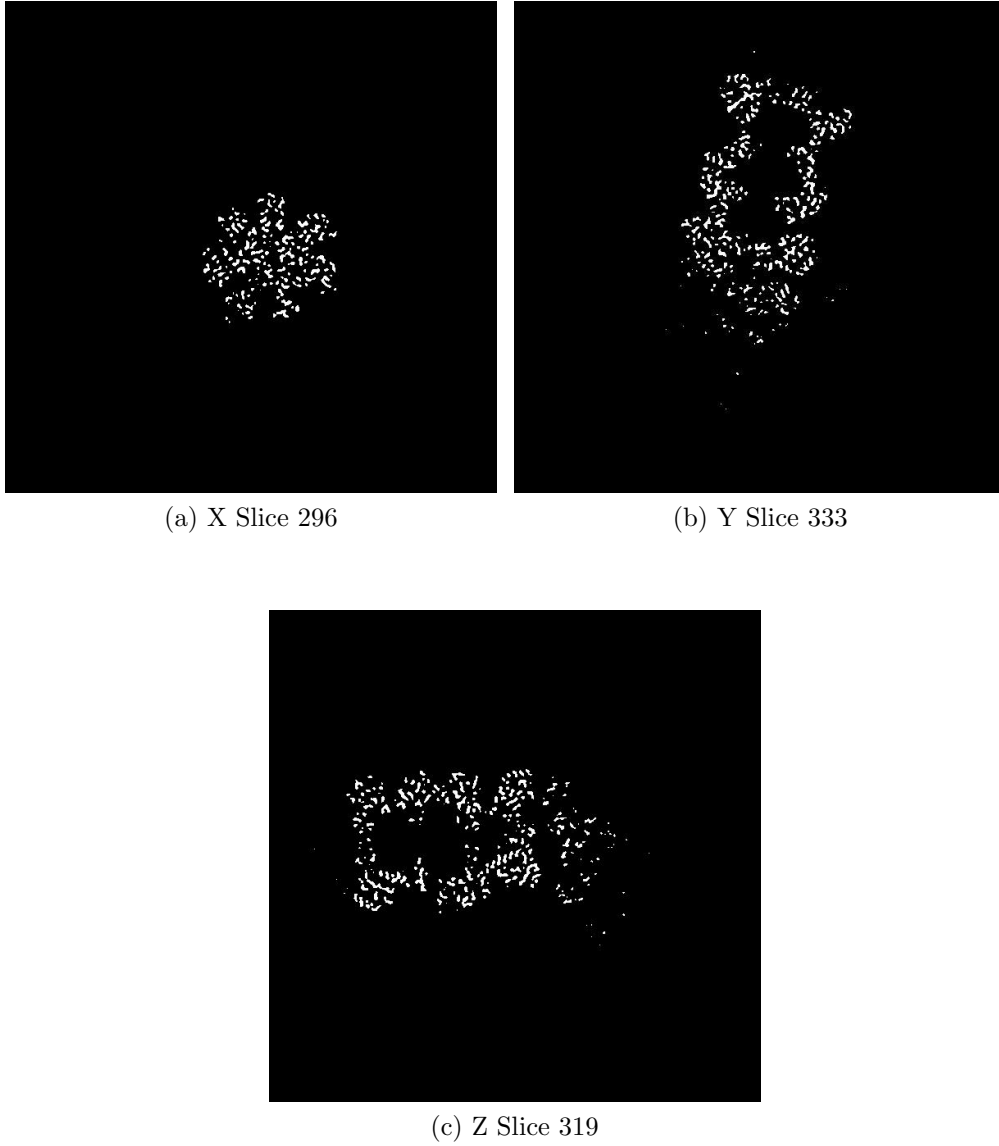


Figure 7: Maximum variance slices in the three dimensions of the raw mask

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

| Threshold | Voxel mass  | Molecular mass(kDa) | # Aminoacids |
|-----------|-------------|---------------------|--------------|
| 0.0016    | 27732024.00 | 7384.92             | 67135.67     |
| 0.0032    | 10495934.00 | 2795.02             | 25409.31     |
| 0.0048    | 4124604.00  | 1098.37             | 9985.14      |
| 0.0065    | 2070012.00  | 551.24              | 5011.23      |
| 0.0081    | 1284579.00  | 342.08              | 3109.80      |
| 0.0097    | 908005.00   | 241.80              | 2198.16      |
| 0.0113    | 688206.00   | 183.27              | 1666.06      |
| 0.0129    | 531705.00   | 141.59              | 1287.19      |
| 0.0145    | 408863.00   | 108.88              | 989.80       |
| 0.0161    | 309329.00   | 82.37               | 748.85       |
| 0.0178    | 227622.00   | 60.61               | 551.04       |
| 0.0194    | 162199.00   | 43.19               | 392.66       |
| 0.0210    | 110183.00   | 29.34               | 266.74       |
| 0.0226    | 70637.00    | 18.81               | 171.00       |
| 0.0242    | 41808.00    | 11.13               | 101.21       |
| 0.0258    | 22664.00    | 6.04                | 54.87        |
| 0.0274    | 10844.00    | 2.89                | 26.25        |
| 0.0291    | 4469.00     | 1.19                | 10.82        |
| 0.0307    | 1527.00     | 0.41                | 3.70         |
| 0.0323    | 449.00      | 0.12                | 1.09         |
| 0.0339    | 100.00      | 0.03                | 0.24         |
| 0.0355    | 21.00       | 0.01                | 0.05         |
| 0.0371    | 8.00        | 0.00                | 0.02         |
| 0.0387    | 3.00        | 0.00                | 0.01         |

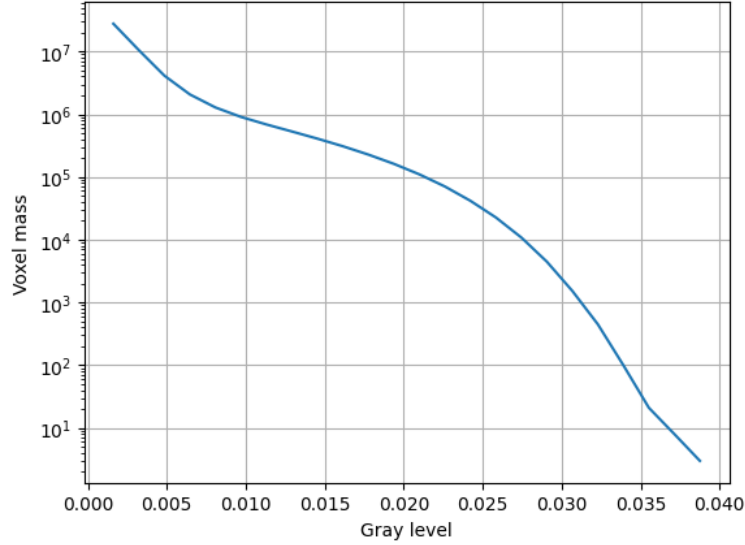


Figure 8: Voxel mass as a function of the gray level.

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 316675 and a volume of  $101785.40 \text{ \AA}^3$ . The overlap between the raw and constructed mask is 0.08.

**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%.

**WARNINGS:** 2 warnings

1. **There might be a problem of connectivity at this threshold because more than 5 connected components are needed to reach 95% of the total mask. Probably a smaller threshold will not cause this issue.**
2. **There might be a problem in the construction of the mask, because the overlap is smaller than 0.75. A common reason is that the suggested threshold causes too many disconnected**

**components.**

## 2.3 Level 0.c Background analysis

### Explanation:

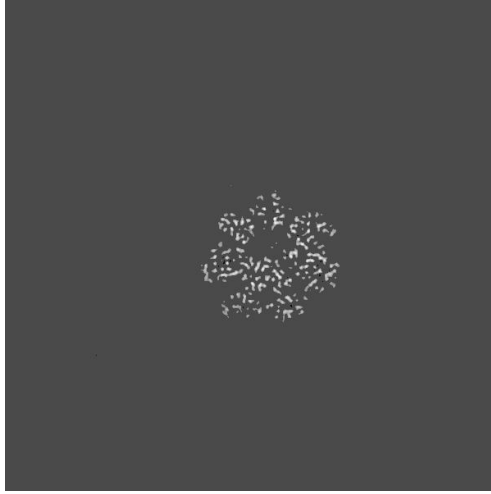
Background is defined as the region outside the macromolecule mask. The background mean should be zero, and the number of voxels with a very low or very high value (below 5 standard deviations of the noise) should be very small and they should be randomly distributed without any specific structure. Sometimes,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

### Results:

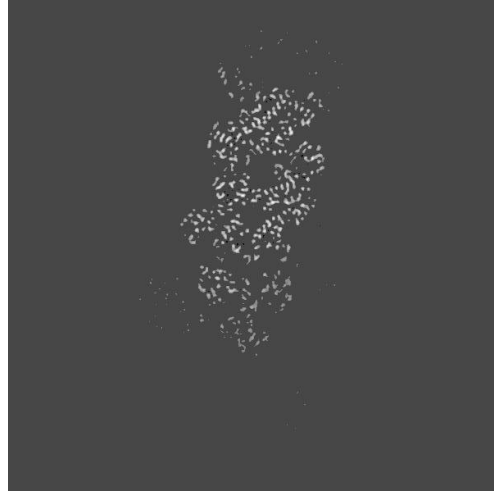
The null hypothesis that the background mean is 0 was tested with a one-sample Student's t-test. The resulting t-statistic and p-value were 626.85 and 0.000000, respectively.

The mean and standard deviation (sigma) of the background were 0.000083 and 0.001946. The percentage of background voxels whose absolute value is larger than 5 times the standard deviation is 0.39 % (see Fig. 9). The same percentage from a Gaussian would be 0.000057% (ratio between the two percentages: 6796.052530).

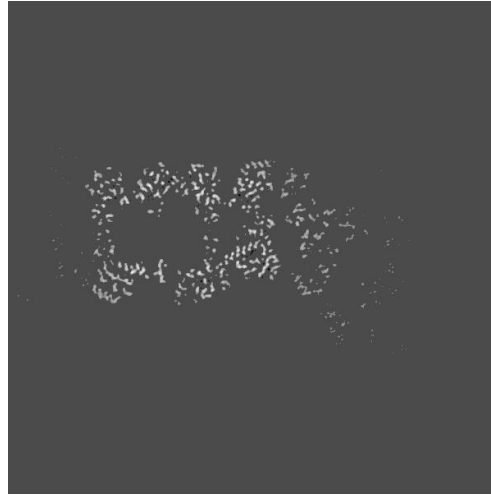
Slices of the background beyond 5\*sigma can be seen in Fig. 9.



(a) X Slice 289



(b) Y Slice 357



(c) Z Slice 319

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

**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=6796.05)**

## 2.4 Level 0.d B-factor analysis

### **Explanation:**

The B-factor line (see this [link](#) for more details) fitted between 15Å and the resolution reported should have a slope that is between 0 and 300 Å<sup>2</sup>.

### **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 -3.6. The fitted line was  $\log(|F|^2) = -0.9/R^2 + (-14.6)$ .

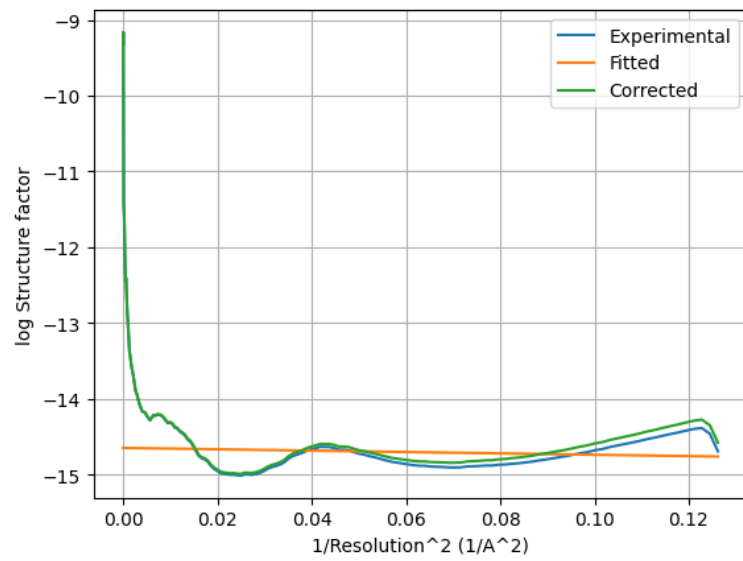
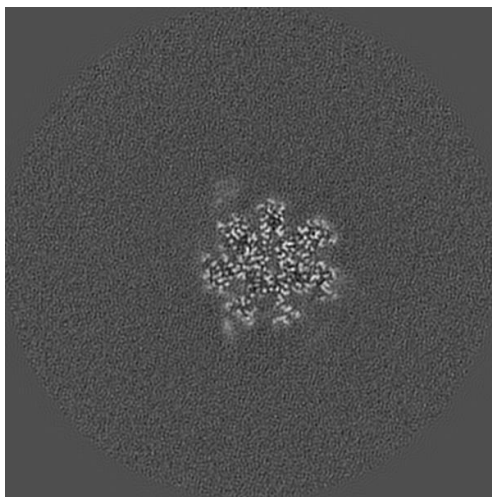
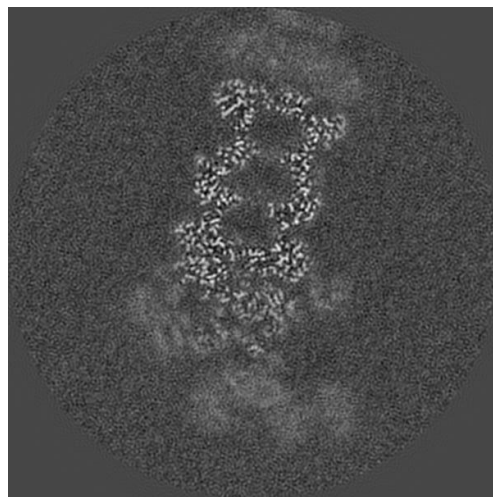


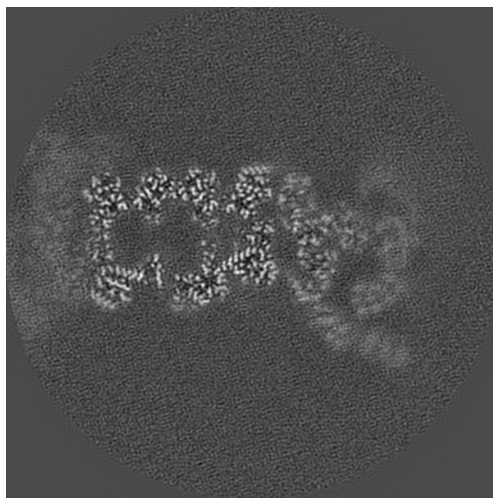
Figure 10: Guinier plot. The X-axis is the square of the inverse of the resolution in Å.



(a) X Slice 296



(b) Y Slice 334



(c) Z Slice 318

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

## 2.5 Level 0.e Local resolution with DeepRes

### Explanation:

DeepRes (see this [link](#) for more details) measures the local resolution using a neural network that has been trained on the appearance of atomic structures at different resolutions. Then, by comparing the local appearance of the input map to the appearance of the atomic structures a local resolution label can be assigned.

### Results:

Fig. [12](#) shows the histogram of the local resolution according to DeepRes. Some representative percentiles are:

| Percentile | Resolution( $\text{\AA}$ ) |
|------------|----------------------------|
| 2.5%       | 2.50                       |
| 25%        | 3.16                       |
| 50%        | 3.47                       |
| 75%        | 3.76                       |
| 97.5%      | 4.38                       |

The reported resolution, 2.80  $\text{\AA}$ , is at the percentile 8.3. Fig. 13 shows some representative views of the local resolution.

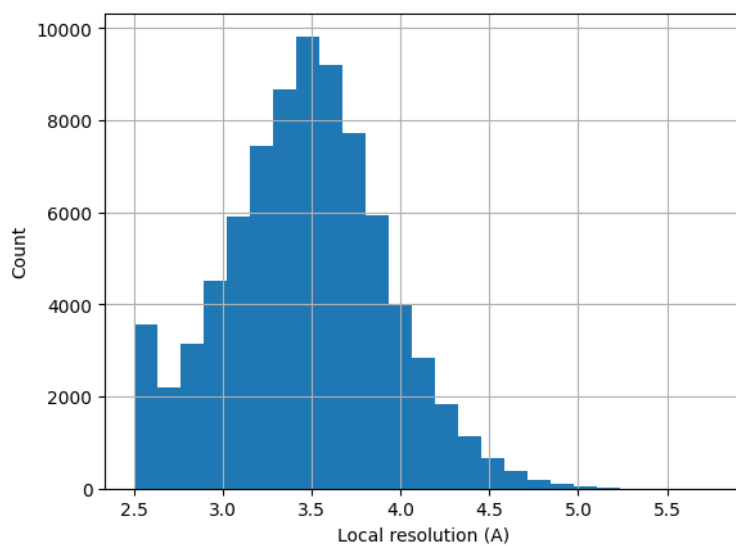
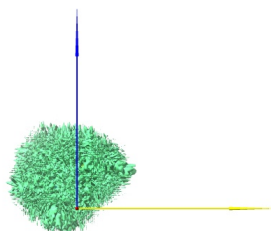
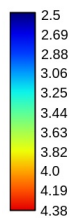
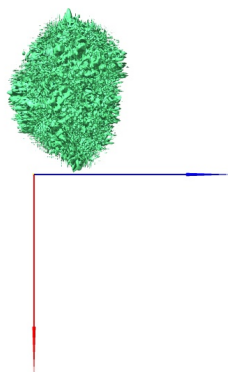
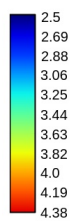


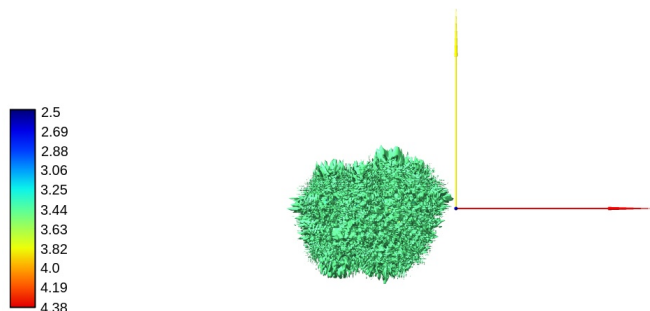
Figure 12: Histogram of the local resolution according to deepres.



(a) View 1



(b) View 2



(c) View 3

Figure 13: Local resolution according to DeepRes. 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).

**Automatic criteria:** The validation is OK if the percentile of the user provided resolution is larger than 0.1% of the percentile of the local resolution as estimated by DeepRes.

STATUS: **OK**

## 2.6 Level 0.f Local B-factor

### Explanation:

LocBfactor (see this [link](#) for more details) estimates a local resolution B-factor by decomposing the input map into a local magnitude and phase term using the spiral transform.

### Results:

Fig. [14](#) shows the histogram of the local B-factor according to LocBfactor. Some representative percentiles are:

| Percentile | Local B-factor ( $\text{\AA}^{-2}$ ) |
|------------|--------------------------------------|
| 2.5%       | -179.14                              |
| 25%        | -149.29                              |
| 50%        | -135.28                              |
| 75%        | -121.72                              |
| 97.5%      | -96.88                               |

Fig. 15 shows some representative views of the local B-factor.

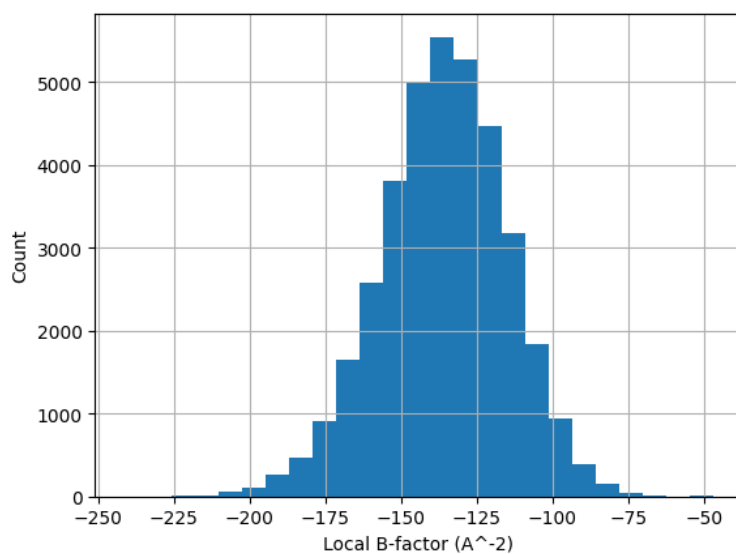
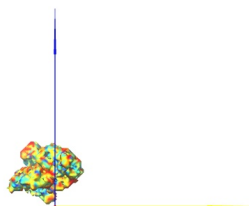
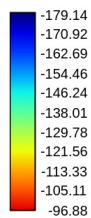
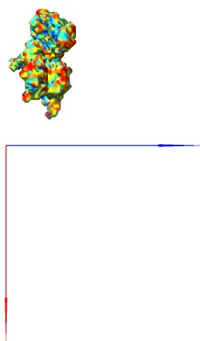
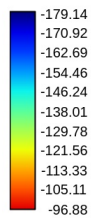


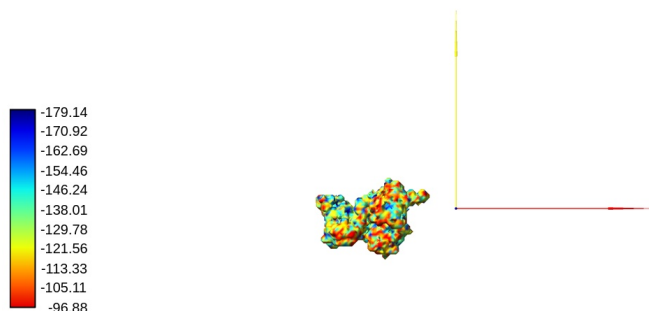
Figure 14: Histogram of the local B-factor according to LocBfactor.



(a) View 1



(b) View 2



(c) View 3

Figure 15: Local B-factor according to LocBfactor. Views generated by ChimeraX at the following X, Y, Z angles: View 1 (0, -90, -90), View 2 (-90, 0, -90), View 3 (0, 0, 0).

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

**STATUS:** OK

## 2.7 Level 0.g Local Occupancy

### Explanation:

LocOccupancy (see this [link](#) for more details) estimates the occupancy of a voxel by the macromolecule.

### Results:

Fig. 16 shows the histogram of the local occupancy according to LocOccupancy. Some representative percentiles are:

| Percentile | Local Occupancy [0-1] |
|------------|-----------------------|
| 2.5%       | 0.38                  |
| 25%        | 0.67                  |
| 50%        | 0.79                  |
| 75%        | 0.88                  |
| 97.5%      | 0.96                  |

Fig. 17 shows some representative views of the local occupancy.

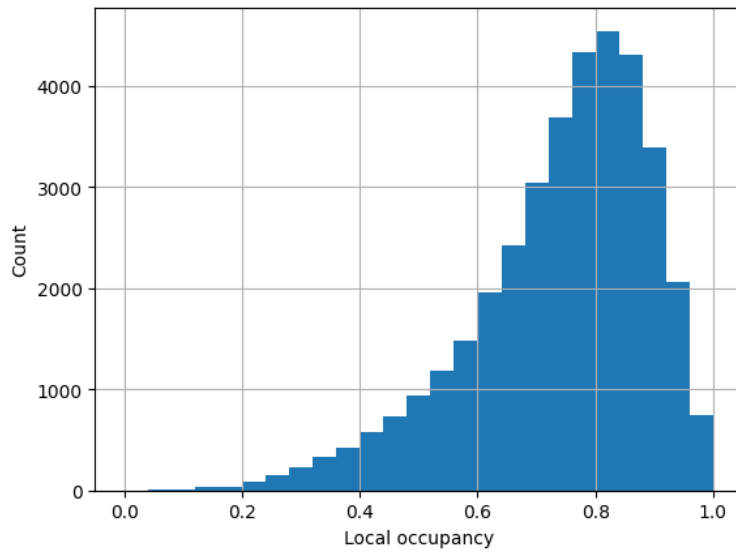
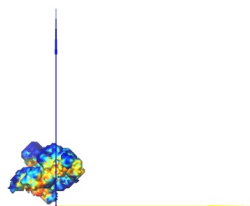
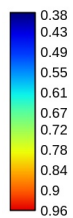
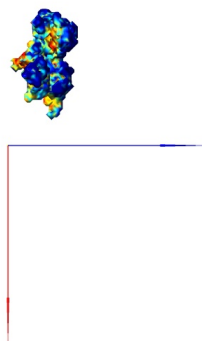
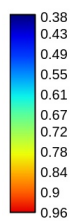


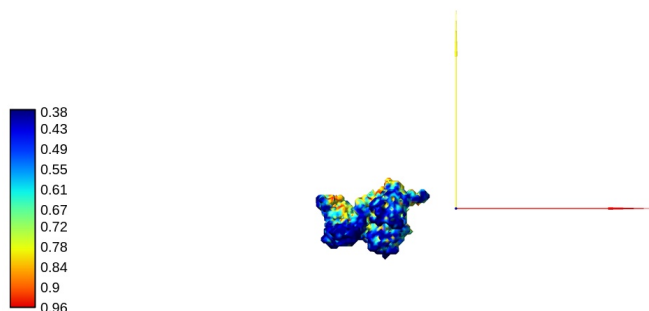
Figure 16: Histogram of the local occupancy according to LocOccupancy.



(a) View 1



(b) View 2



(c) View 3

Figure 17: Local occupancy according to LocOccupancy. Views generated by ChimeraX at the following X, Y, Z angles: View 1 (0, -90, -90), View 2 (-90, 0, -90), View 3 (0, 0, 0).

**Automatic criteria:** The validation is OK if the median occupancy is larger than 50%.

**STATUS:** OK

## 2.8 Level 0.h Hand correction

### Explanation:

Deep Hand (see this [link](#) for more details) determines the correction of the hand for those maps with a resolution smaller than  $5\text{\AA}$ . The method calculates a value between 0 (correct hand) and 1 (incorrect hand) using a neural network to assign its hand.

### Results:

Deep hand assigns a score of 0.215 to the input volume.

**Automatic criteria:** The validation is OK if the deep hand score is smaller than 0.5.

STATUS: OK