



Public Data-based Report



Validation Report Service

Cryo-EM Map Validation Report

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

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

Basic Entry Information:

EMDB ID: [EMD-1092](#)

Title: Cryo-EM visualization of a viral internal ribosome entry site bound to human ribosomes: the IRES functions as an RNA-based translation factor.

Authors: [See EMDB entry link](#)

Deposited on: 2004-08-10T00:00:00

Reported Resolution: 25.3 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 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). There seems to be a problem with its local B-factor (see Sec. 2.6).

The overall score (passing tests) of this report is 1 out of 5 evaluable items.

0.a Mass analysis	Sec. 2.1	1 warnings
0.b Mask analysis	Sec. 2.2	2 warnings
0.c Background analysis	Sec. 2.3	2 warnings
0.d B-factor analysis	Sec. 2.4	Does not apply
0.e DeepRes	Sec. 2.5	Does not apply
0.f LocBfactor	Sec. 2.6	1 warnings
0.g LocOccupancy	Sec. 2.7	OK
0.h Deep hand	Sec. 2.8	Does not apply

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 2.2 (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 with noise and artifacts, because the average noise blob has a volume of 49.027896 Å³.**

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=19988.51)**

Section 2.6 (0.f LocBfactor)

1. **The median B-factor is out of the interval [-300,0]**

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1 Input data

Input map: emd_1092.map

SHA256 hash: fc4c152f6cc0fa5d9268e1581f5b43132c11777f2f1e66b2bbbcdd49af7917a3

Voxel size: 3.660000 (Å)

Visualization threshold: 0.000084

Resolution estimated by user: 25.3

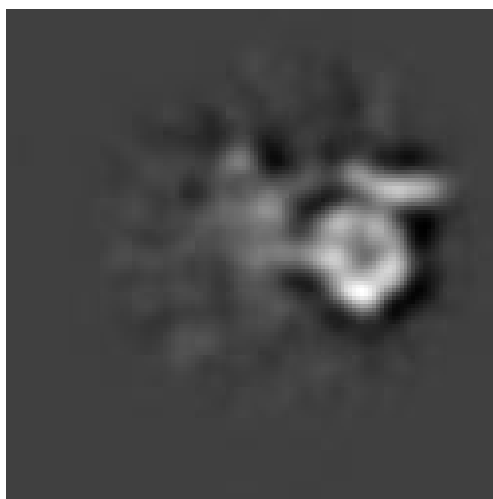
Orthogonal slices of the input map

Explanation:

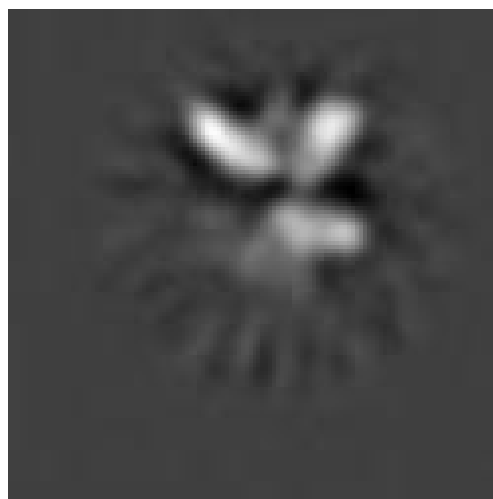
In the orthogonal slices of the map, the noise outside the protein should not have any structure (stripes going out, small blobs, particularly high or low densities, ...)

Results:

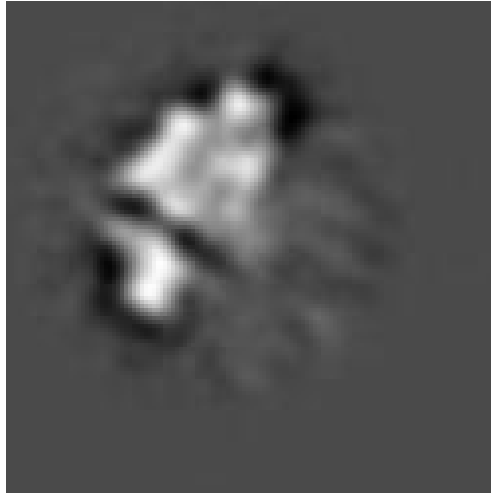
See Fig. 1.



(a) X Slice 62



(b) Y Slice 62



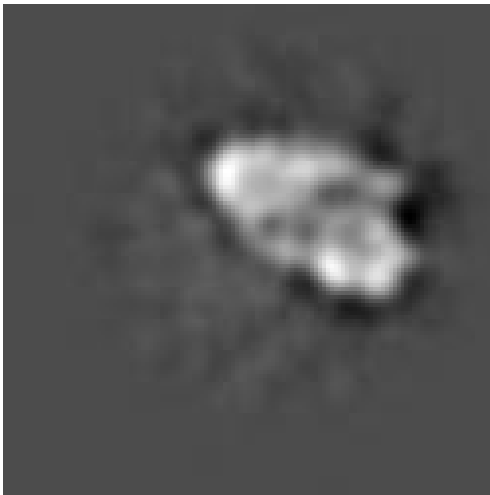
(c) Z Slice 62

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

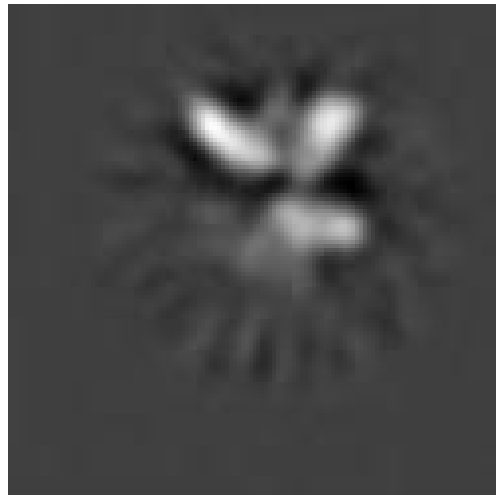
Orthogonal slices of maximum variance of the input map

Results:

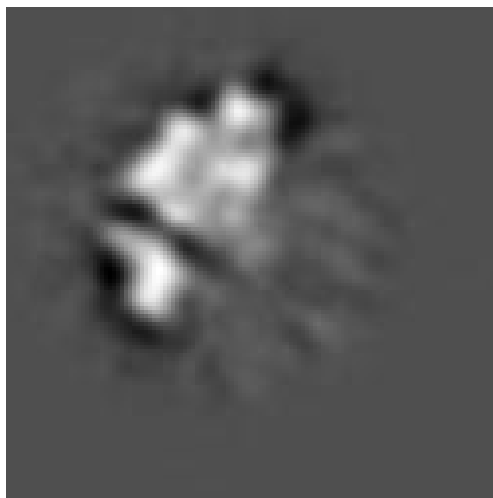
See Fig. 2.



(a) X Slice 56



(b) Y Slice 62



(c) Z Slice 61

Figure 2: Slices of maximum variation in the three dimensions

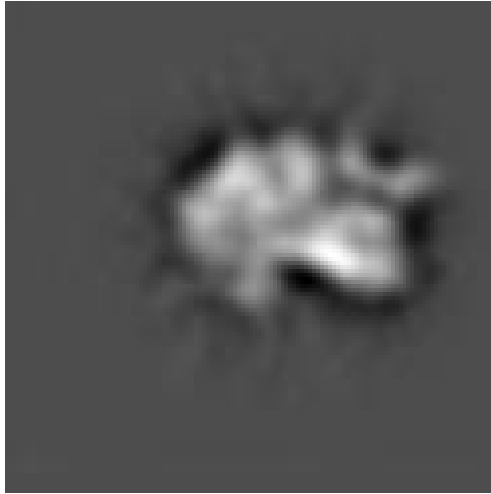
Orthogonal projections of the input map

Explanation:

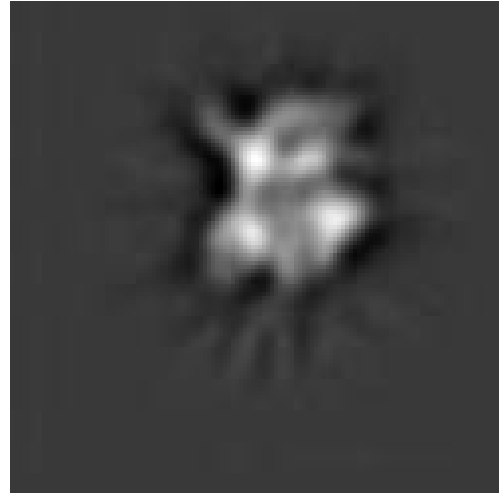
In the projections there should not be stripes (this is an indication of directional overweighting, or angular attraction), and there should not be a dark halo around or inside the structure (this is an indication of incorrect CTF correction or the reconstruction of a biased map).

Results:

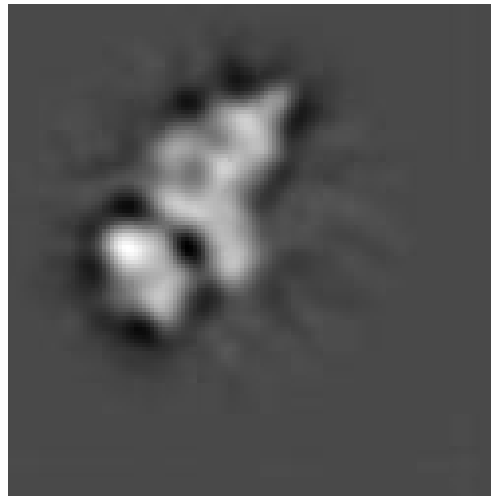
See Fig. [3](#).



(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.

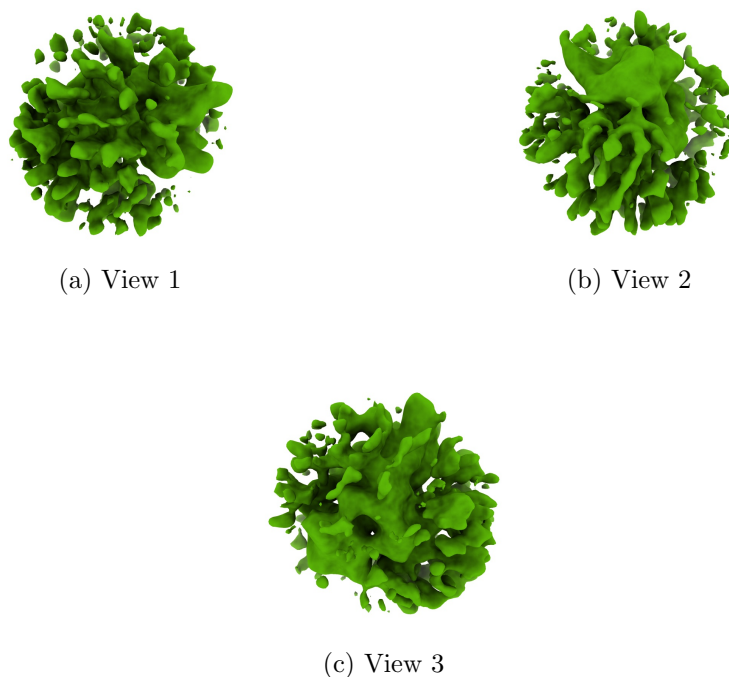


Figure 4: Isosurface at threshold=0.000084. Views generated by ChimeraX at a the following X, Y, Z angles: View 1 (0, -90, -90), View 2 (-90, 0, -90), View 3 (0, 0, 0).

Orthogonal slices of maximum variance of the mask with hard borders

Explanation:

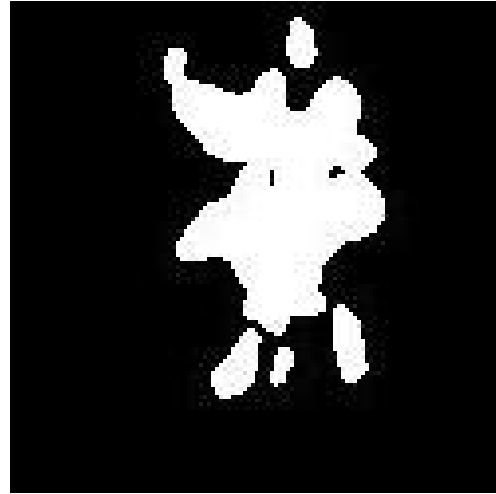
The mask with hard borders has been calculated at the suggested threshold 0.000084, the largest connected component was selected, and then dilated by 2Å.

Results:

See Fig. 5.



(a) X Slice 59



(b) Y Slice 67



(c) Z Slice 68

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

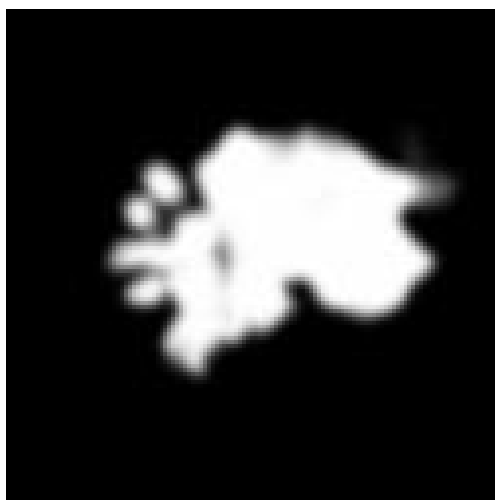
Orthogonal slices of maximum variance of the mask with soft borders

Explanation:

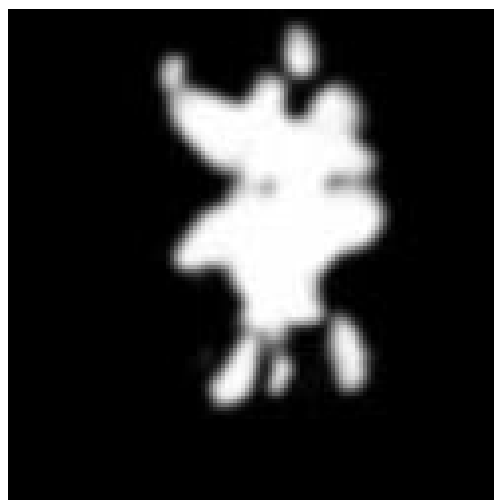
The mask with soft borders has been calculated at the suggested threshold 0.000084, the largest connected component was selected, and then dilated by

2Å.

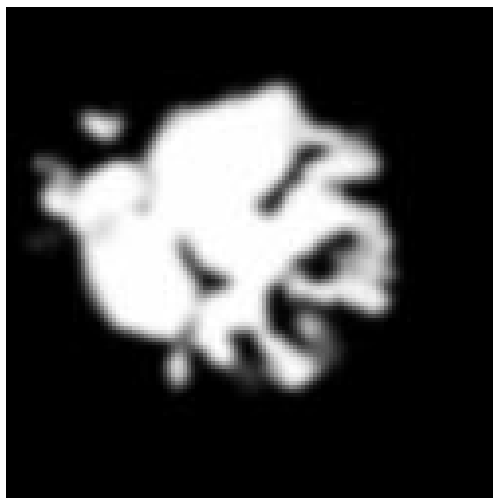
Results:
See Fig. 6.



(a) X Slice 58



(b) Y Slice 67



(c) Z Slice 68

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 14.64 and 91.50 $\text{\AA}$, respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 16.80%

The space from the left and right in Y are 91.50 and 29.28 $\text{\AA}$, respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 13.60%

The space from the left and right in Z are 95.16 and 58.56 $\text{\AA}$, respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 8.00%

The center of mass is at (x,y,z)=(50.29, 71.62, 69.63). The decentering of the center of mass (abs(Center)/Size)% is 9.77, 7.29, and 5.70, 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 0.000084, there are 92 connected components with a total number of voxels of 100275 and a volume of 4916272.27 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):

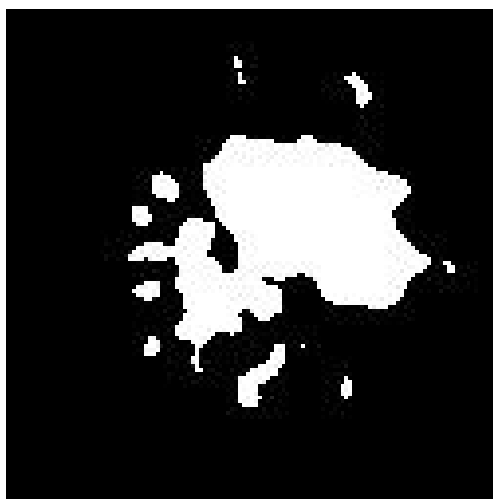
(90176 (4421139.55), 89.93, 89.93)(1980 (97075.23), 1.97, 91.90)(1094 (53636.52), 1.09, 92.99)(968 (47459.00), 0.97, 93.96)(672 (32946.75), 0.67, 94.63)(485

(23778.53), 0.48, 95.11)

Number of components to reach 95% of the mass: 6

The average size of the remaining 86 components is 56.98 voxels ($49.03 \text{ \AA}^3$).
Their size go from 485 voxels ($23778.53 \text{ \AA}^3$) to 1 voxels ($49.03 \text{ \AA}^3$).

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



(a) X Slice 57



(b) Y Slice 64



(c) Z Slice 68

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
0.0001	84755.00	3442.72	31297.43
0.0002	58499.00	2376.21	21601.89
0.0003	49577.00	2013.80	18307.27
0.0004	43976.00	1786.29	16238.99
0.0006	39289.00	1595.90	14508.22
0.0007	34953.00	1419.78	12907.07
0.0008	30911.00	1255.59	11414.49
0.0009	27015.00	1097.34	9975.81
0.0010	23301.00	946.48	8604.35
0.0011	19647.00	798.05	7255.04
0.0012	16221.00	658.89	5989.92
0.0013	13128.00	533.25	4847.77
0.0015	10388.00	421.96	3835.97
0.0016	7986.00	324.39	2948.99
0.0017	6086.00	247.21	2247.37
0.0018	4624.00	187.83	1707.50
0.0019	3340.00	135.67	1233.36
0.0020	2302.00	93.51	850.06
0.0021	1493.00	60.65	551.32
0.0022	870.00	35.34	321.26
0.0023	411.00	16.69	151.77
0.0025	163.00	6.62	60.19
0.0026	51.00	2.07	18.83
0.0027	16.00	0.65	5.91

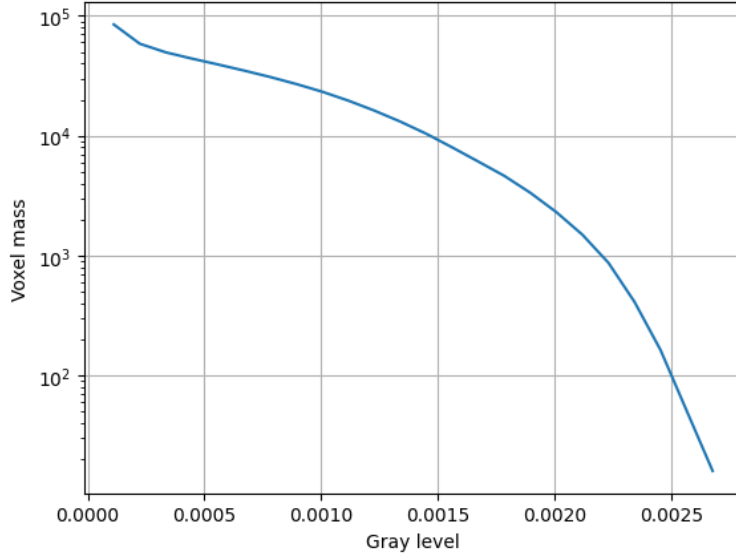


Figure 8: Voxel mass as a function of the gray level.

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 134368 and a volume of $6587780.33 \text{ \AA}^3$. The overlap between the raw and constructed mask is 0.90.

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 with noise and artifacts, because the average noise blob has a volume of $49.027896 \text{ \AA}^3$.**

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 -288.89 and 0.000000, respectively.

The mean and standard deviation (sigma) of the background were -0.000016 and 0.000074. The percentage of background voxels whose absolute value is larger than 5 times the standard deviation is 1.15 % (see Fig. 9). The same percentage from a Gaussian would be 0.000057% (ratio between the two percentages: 19988.508558).

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



(a) X Slice 46



(b) Y Slice 104



(c) Z Slice 58

Figure 9: Maximum variance slices in the three dimensions of the parts of the background beyond 5σ

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=19988.51)**

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:

This method cannot be applied to maps with a resolution worse than 8Å.

STATUS: Does not apply

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:

This method cannot be applied to maps with a resolution worse than 13Å.

STATUS: Does not apply

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. 10 shows the histogram of the local B-factor according to LocBfactor. Some representative percentiles are:

Percentile	Local B-factor ($\text{\AA}^{-2}$)
2.5%	-6253.77
25%	-4591.21
50%	-3809.07
75%	-2955.92
97.5%	-1261.56

Fig. 11 shows some representative views of the local B-factor.

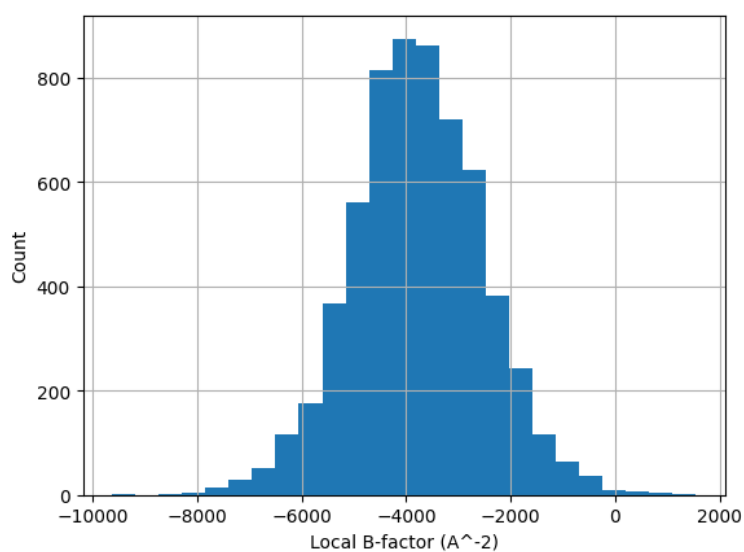
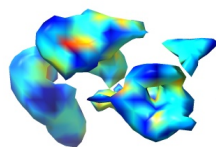
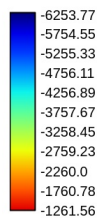
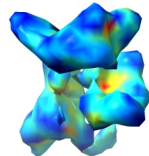
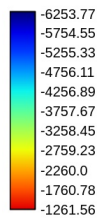


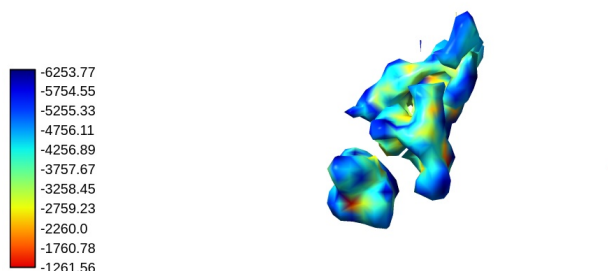
Figure 10: Histogram of the local B-factor according to LocBfactor.



(a) View 1



(b) View 2



(c) View 3

Figure 11: Local B-factor according to LocBfactor. 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 median B-factor is in the range $[-300,0]$.

WARNINGS: 1 warnings

1. **The median B-factor is out of the interval $[-300,0]$**

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

Percentile	Local Occupancy [0-1]
2.5%	0.00
25%	0.50
50%	1.00
75%	1.00
97.5%	1.00

Fig. 13 shows some representative views of the local occupancy.

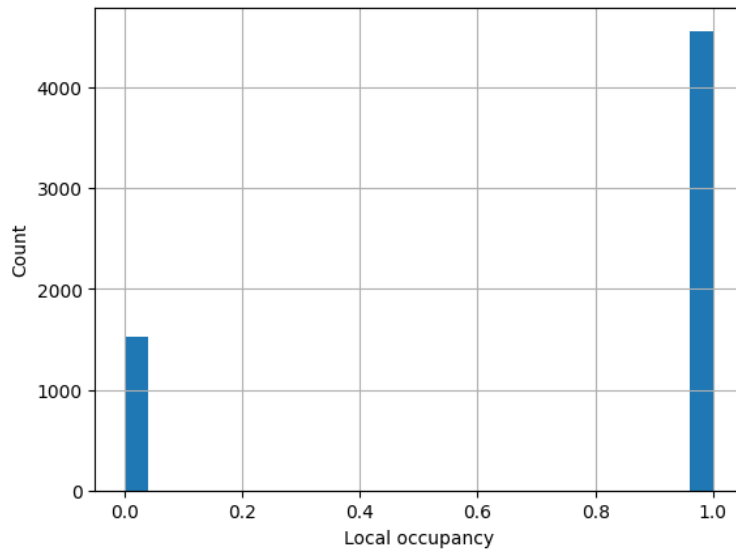
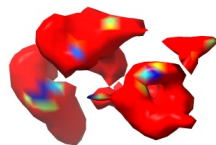
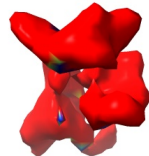


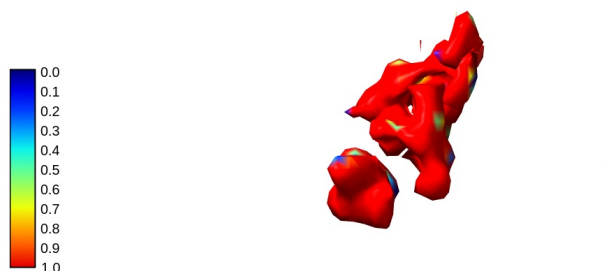
Figure 12: Histogram of the local occupancy according to LocOccupancy.



(a) View 1



(b) View 2



(c) View 3

Figure 13: 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 5Å. The method calculates a value between 0 (correct hand) and 1 (incorrect hand) using a neural network to assign its hand.

Results:

This method cannot be applied to maps with a resolution worse than 5Å.

STATUS: Does not apply

