



Public Data-based Report



Validation Report Service

Cryo-EM Map Validation Report

Report to assess Cryo-EM Volume Map at Level(s) 1, 0

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

Basic Entry Information:

EMDB ID: [EMD-3769](#)

Title: Cryo-EM structure of a pre-catalytic human spliceosome primed for activation (B complex). Unmasked refinement, NON-sharpened map

Authors: [See EMDB entry link](#)

Deposited on: 2017-06-20T00:00:00

Reported Resolution: 9.9 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). There seems to be a problem with its local B-factor (see Sec. 2.6). The resolution does not seem to be uniform in all directions (see Sec. 4.6).

The average resolution of the map estimated by various methods goes from 5.6Å to 11.2Å with an average of 7.9Å. The resolution reported by the user was 9.9Å.

The overall score (passing tests) of this report is 5 out of 9 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	Does not apply
0.e DeepRes	Sec. 2.5	OK
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
1.a Global resolution	Sec. 4.1	OK
1.b FSC permutation	Sec. 4.2	OK
1.c Blocres	Sec. 4.3	Could not be measured
1.d Resmap	Sec. 4.4	Could not be measured
1.e MonoRes	Sec. 4.5	Could not be measured
1.f MonoDir	Sec. 4.6	1 warnings
1.g FSO	Sec. 4.7	Could not be measured
1.h FSC3D	Sec. 4.8	Could not be measured

Summary of the warnings across sections.

Section 2.1 (0.a Mass analysis)

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

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=18407.09)**

Section 2.6 (0.f LocBfactor)

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

Section 4.6 (1.f MonoDir)

1. **The distribution of best resolution is not uniform in all directions. The associated p-value is 0.000000.**

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1 Input data

Input map: emd_3769.map

SHA256 hash: 2a77a9f0df29b9f5c29775927f998e97725bcd641816727141b79262f1ac3ca1

Voxel size: 1.160000 (Å)

Visualization threshold: 0.040000

Resolution estimated by user: 9.9

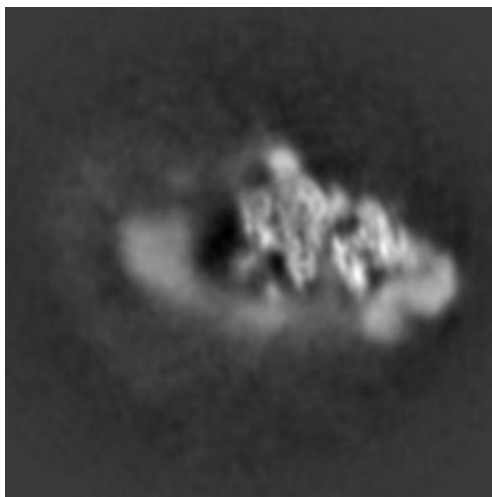
Orthogonal slices of the input map

Explanation:

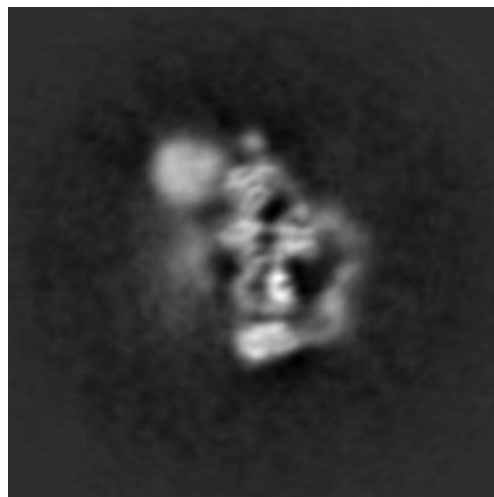
In the orthogonal slices of the map, the noise outside the protein should not have any structure (stripes going out, small blobs, particularly high or low densities, ...)

Results:

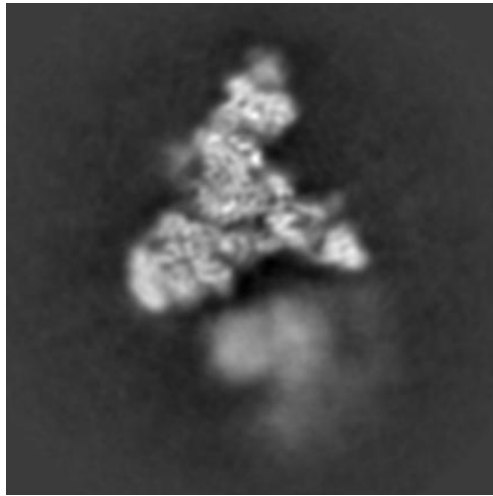
See Fig. 1.



(a) X Slice 216



(b) Y Slice 216



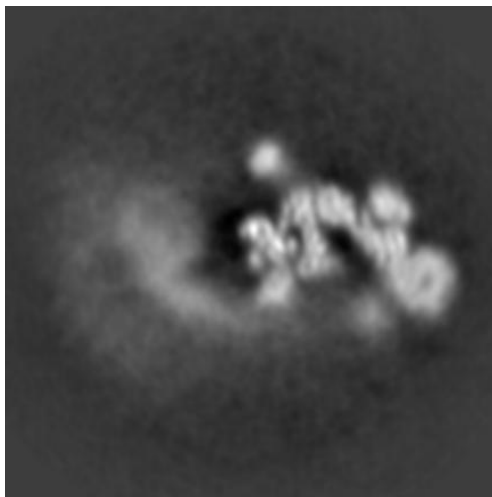
(c) Z Slice 216

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

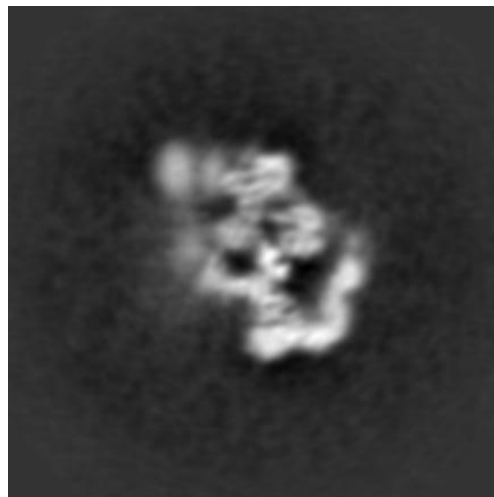
Orthogonal slices of maximum variance of the input map

Results:

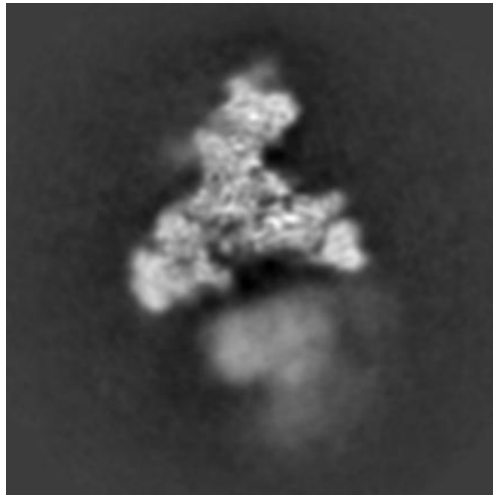
See Fig. 2.



(a) X Slice 236



(b) Y Slice 229



(c) Z Slice 222

Figure 2: Slices of maximum variation in the three dimensions

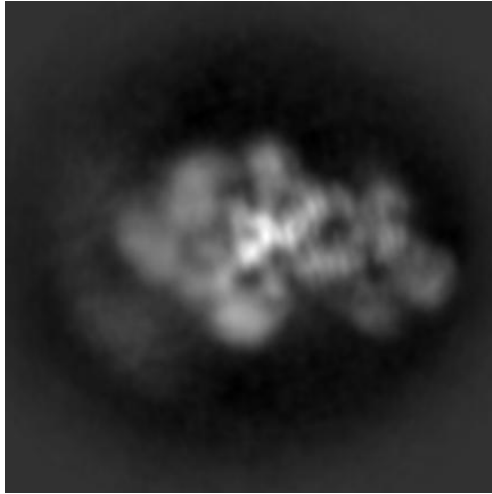
Orthogonal projections of the input map

Explanation:

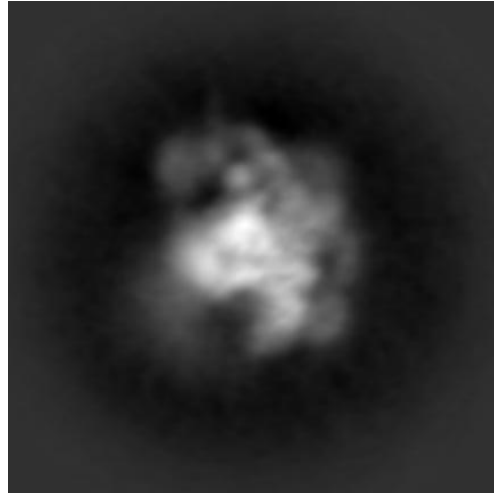
In the projections there should not be stripes (this is an indication of directional overweighting, or angular attraction), and there should not be a dark halo around or inside the structure (this is an indication of incorrect CTF correction or the reconstruction of a biased map).

Results:

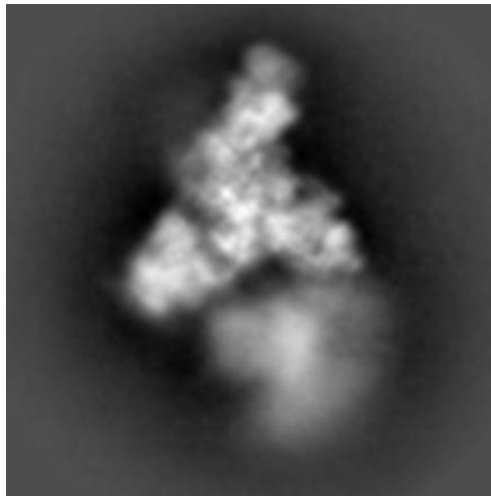
See Fig. 3.



(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.

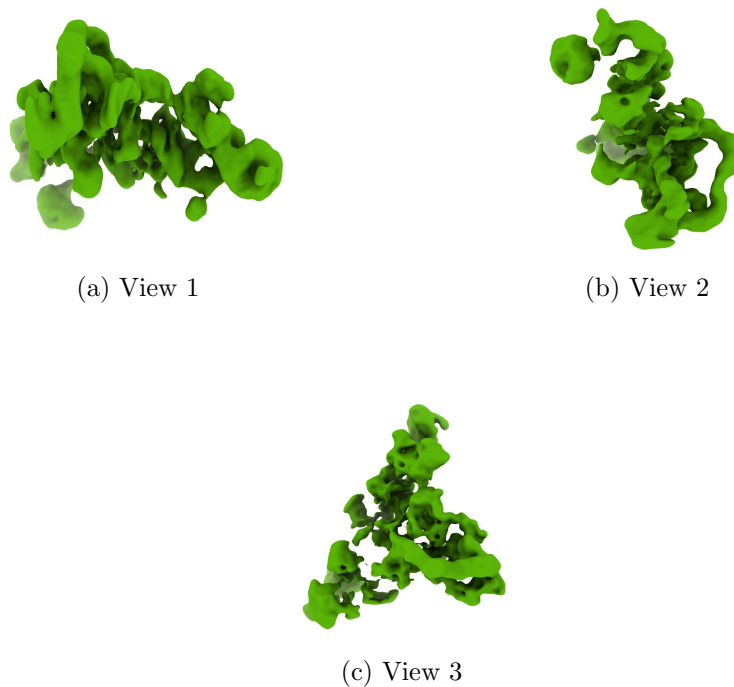


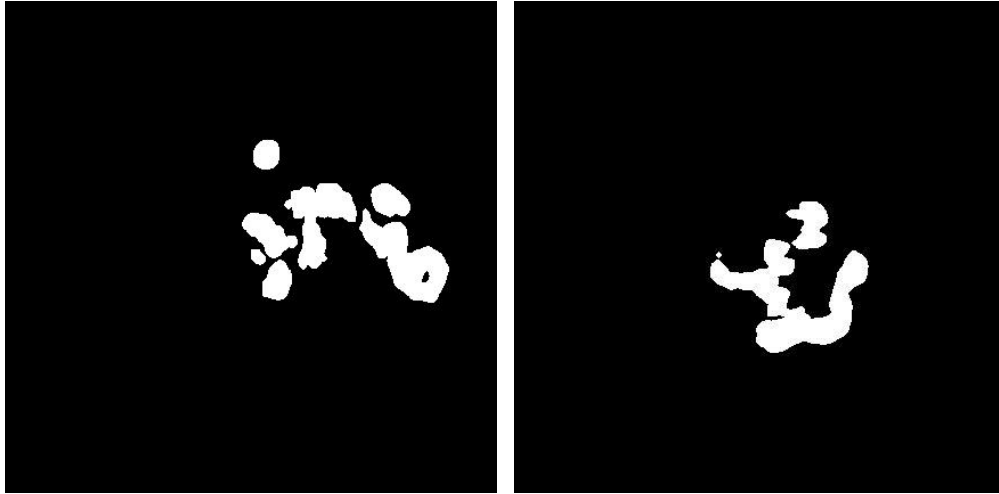
Figure 4: Isosurface at threshold=0.040000. Views generated by ChimeraX at a the following X, Y, Z angles: View 1 (0, -90, -90), View 2 (-90, 0, -90), View 3 (0, 0, 0).

Orthogonal slices of maximum variance of the mask with hard borders

Explanation:

The mask with hard borders has been calculated at the suggested threshold 0.040000, the largest connected component was selected, and then dilated by 2Å.

Results:
See Fig. 5.



(a) X Slice 237

(b) Y Slice 229



(c) Z Slice 225

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 0.040000, the largest connected component was selected, and then dilated by

2Å.

Results:
See Fig. 6.



(a) X Slice 237



(b) Y Slice 230



(c) Z Slice 225

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\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 168.20 and 138.04 \AA , respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 6.02%

The space from the left and right in Y are 216.92 and 42.92 \AA , respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 34.72%

The space from the left and right in Z are 171.68 and 141.52 \AA , respectively. There is a decentering ratio $(\text{abs}(\text{Right-Left})/\text{Size})\%$ of 6.02%

The center of mass is at $(x,y,z)=(192.69,254.93,219.49)$. The decentering of the center of mass $(\text{abs}(\text{Center})/\text{Size})\%$ is 5.40, 9.01, and 0.81, 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 Y.**

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 $(\text{size}(\text{Raw AND Constructed})/\text{size}(\text{Raw}))$ that is a number between 0 and 1, the closer to 1, the more they agree.

Results:

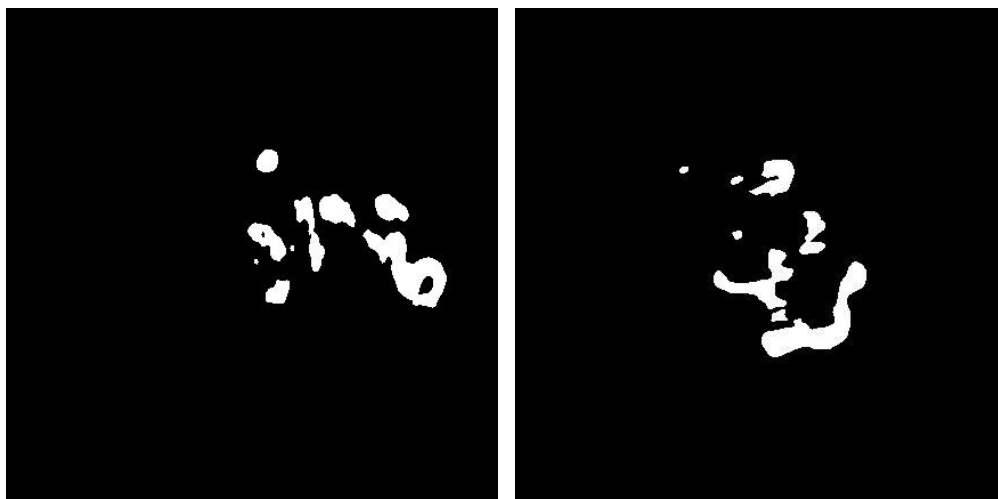
Raw mask: At threshold 0.040000, there are 10 connected components with a total number of voxels of 465828 and a volume of 727109.06 \AA^3 (see Fig. 7). The size and percentage of the total number of voxels for the raw mask are listed below (up to 95% of the mass or the first 100 clusters, whatever happens first), the list contains (No. voxels (volume in \AA^3), percentage, cumulated percentage):

(356420 (556334.55), 76.51, 76.51)(74943 (116978.23), 16.09, 92.60)(32218 (50288.95), 6.92, 99.52)

Number of components to reach 95% of the mass: 3

The average size of the remaining 7 components is 321.00 voxels (1.56 \AA^3).
Their size go from 32218 voxels (50288.95 \AA^3) to 1 voxels (1.56 \AA^3).

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



(a) X Slice 236

(b) Y Slice 230



(c) Z Slice 226

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
0.0032	5791552.00	7489.65	68087.75
0.0063	4486752.00	5802.28	52748.01
0.0095	3527205.00	4561.39	41467.20
0.0127	2832246.00	3662.67	33296.99
0.0159	2334642.00	3019.17	27446.96
0.0190	1988753.00	2571.86	23380.56
0.0222	1694193.00	2190.94	19917.59
0.0254	1415986.00	1831.16	16646.88
0.0286	1147302.00	1483.69	13488.13
0.0317	917885.00	1187.01	10791.01
0.0349	721998.00	933.69	8488.09
0.0381	558358.00	722.07	6564.27
0.0413	405716.00	524.67	4769.76
0.0444	265640.00	343.53	3122.97
0.0476	154228.00	199.45	1813.16
0.0508	81699.00	105.65	960.49
0.0540	39102.00	50.57	459.70
0.0571	16207.00	20.96	190.54
0.0603	5491.00	7.10	64.55
0.0635	2089.00	2.70	24.56
0.0667	917.00	1.19	10.78
0.0698	395.00	0.51	4.64
0.0730	178.00	0.23	2.09
0.0762	62.00	0.08	0.73

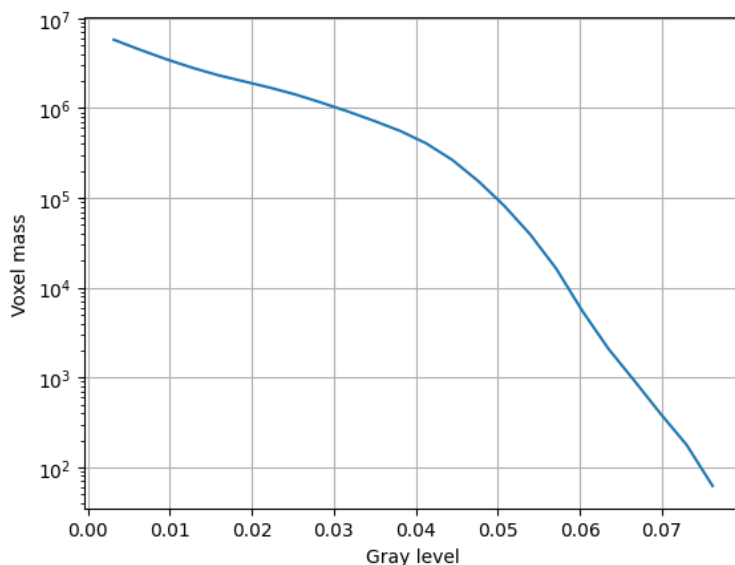


Figure 8: Voxel mass as a function of the gray level.

Constructed mask: After keeping the largest component of the previous mask and dilating it by 2\AA , there is a total number of voxels of 642474 and a volume of 1002835.10\AA^3 . The overlap between the raw and constructed mask is 0.77.

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\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

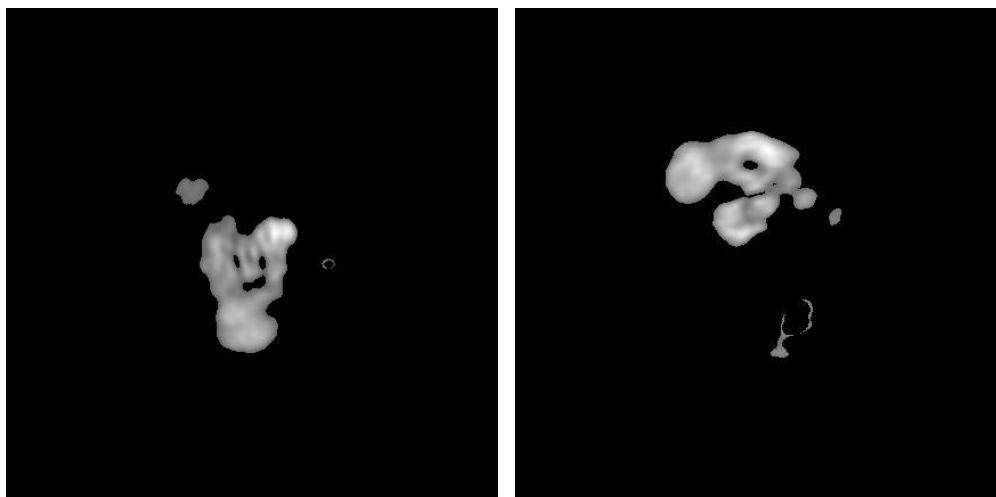
small and they should be randomly distributed without any specific structure. Sometimes, you can see some structure due to the symmetry of the structure.

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 -882.67 and 0.000000, respectively.

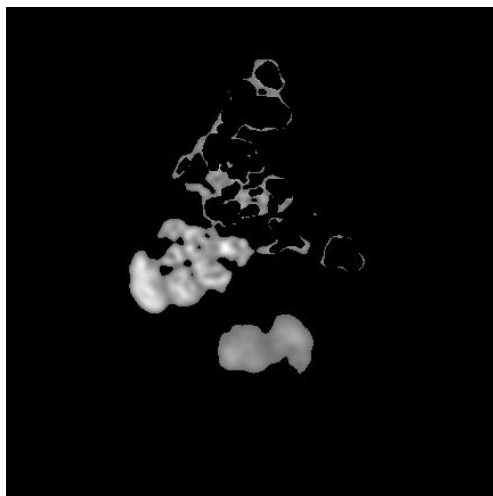
The mean and standard deviation (σ) of the background were -0.000487 and 0.004935. The percentage of background voxels whose absolute value is larger than 5 times the standard deviation is 1.06 % (see Fig. 9). The same percentage from a Gaussian would be 0.000057% (ratio between the two percentages: 18407.094636).

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



(a) X Slice 145

(b) Y Slice 196



(c) Z Slice 212

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=18407.09)**

2.4 Level 0.d B-factor analysis

Explanation:

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

Fig. 10 shows the histogram of the local resolution according to DeepRes. Some representative percentiles are:

Percentile	Resolution(Å)
2.5%	5.53
25%	7.74
50%	10.66
75%	12.90
97.5%	12.90

The reported resolution, 9.90 Å, is at the percentile 44.3. Fig. 11 shows some representative views of the local resolution.

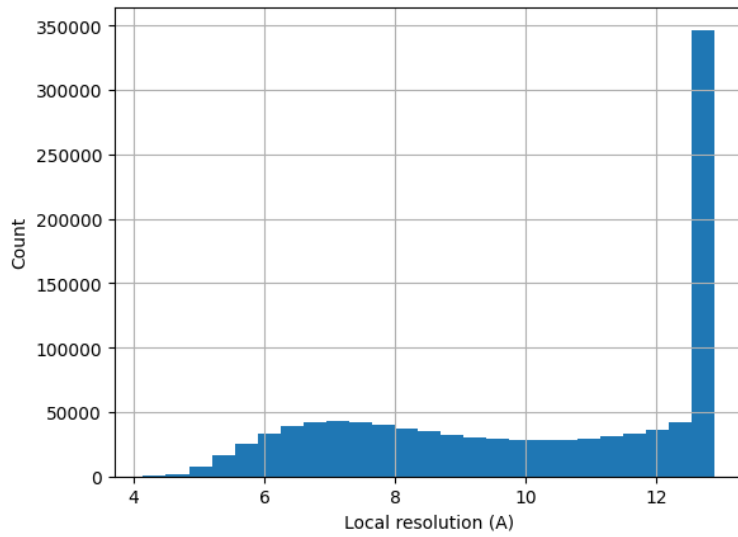
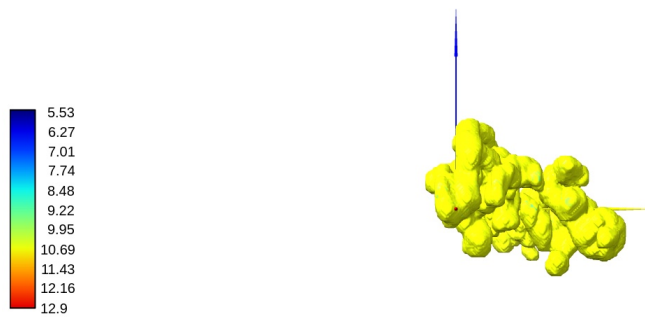
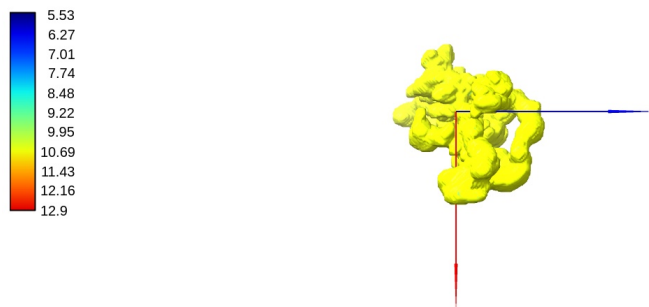


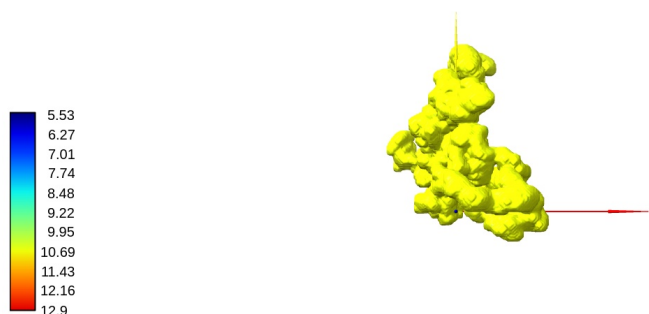
Figure 10: Histogram of the local resolution according to deepres.



(a) View 1



(b) View 2



(c) View 3

Figure 11: Local resolution according to DeepRes. 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 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. 12 shows the histogram of the local B-factor according to LocBfactor. Some representative percentiles are:

Percentile	Local B-factor (\AA^{-2})
2.5%	-2726.78
25%	-1711.37
50%	-1241.97
75%	-773.79
97.5%	153.37

Fig. 13 shows some representative views of the local B-factor.

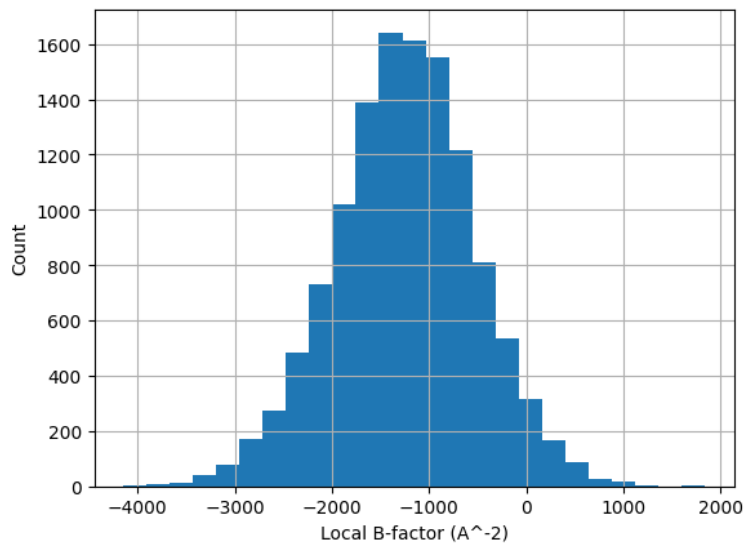
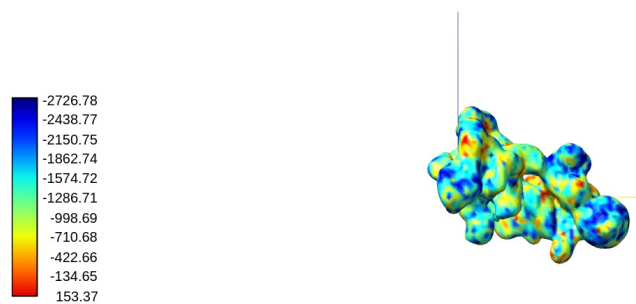
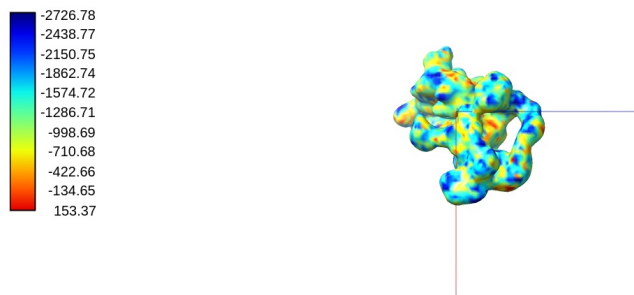


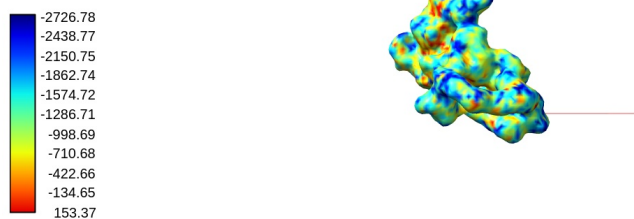
Figure 12: Histogram of the local B-factor according to LocBfactor.



(a) View 1



(b) View 2



(c) View 3

Figure 13: 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. 14 shows the histogram of the local occupancy according to LocOccupancy. Some representative percentiles are:

Percentile	Local Occupancy [0-1]
2.5%	0.00
25%	0.67
50%	1.00
75%	1.00
97.5%	1.00

Fig. 15 shows some representative views of the local occupancy.

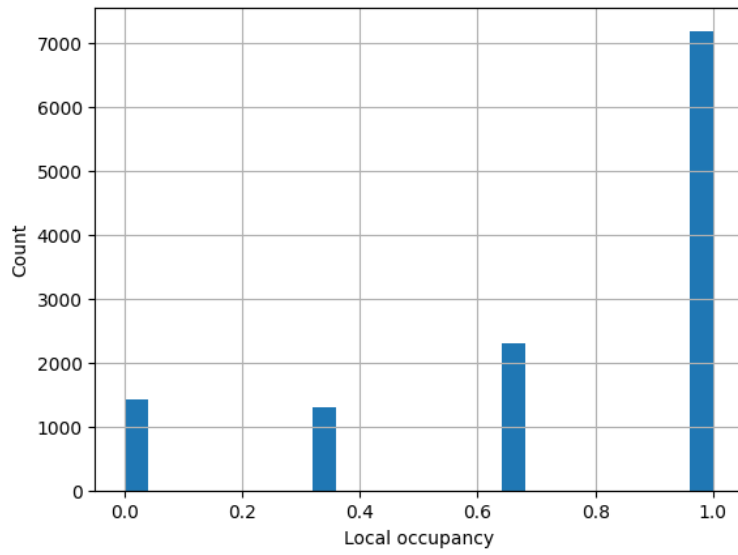
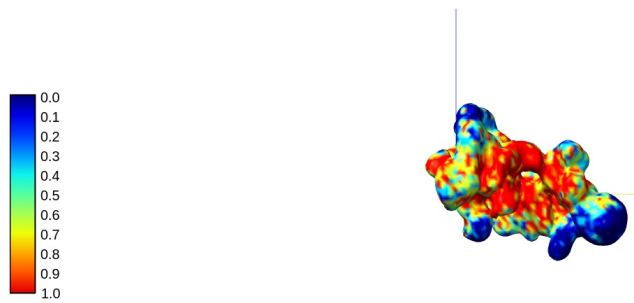
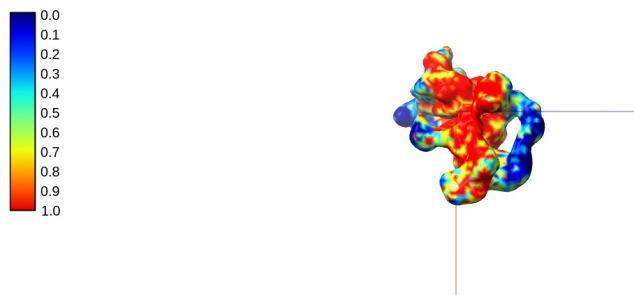


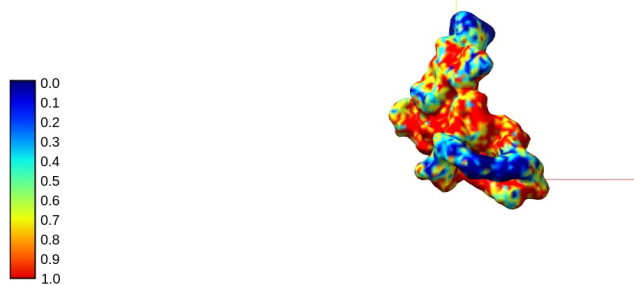
Figure 14: Histogram of the local occupancy according to LocOccupancy.



(a) View 1



(b) View 2



(c) View 3

Figure 15: 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](#)

3 Half maps

Half map 1: emd_3769_half_map_1.map

SHA256 hash: c63ca2a8e05e73733207144bbae436efa500be472fb8f356ec47b33cd6262175

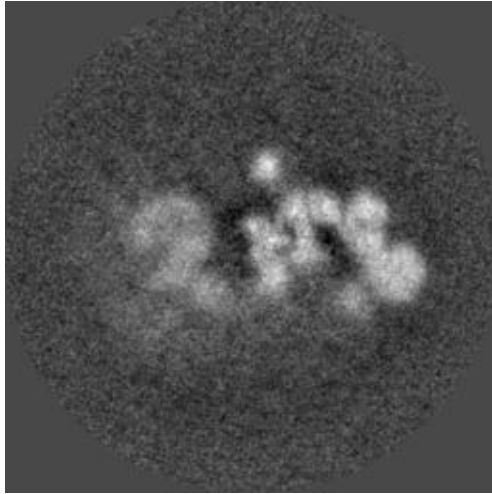
Half map 2: emd_3769_half_map_2.map

SHA256 hash: be8aa0f848a8aa489304807539aaa67b4813b7917f1c1cb7e4866d4ebeaf6c03

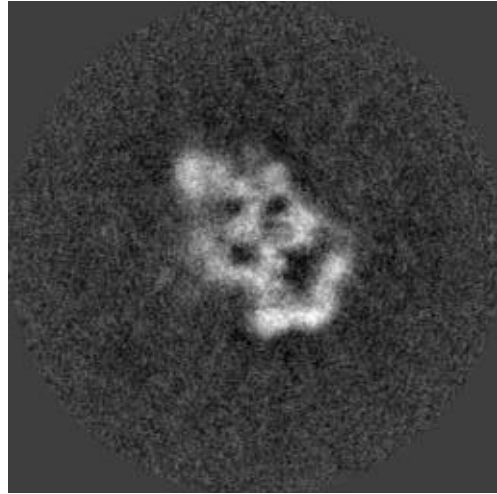
Slices of the first half map can be seen in Fig. [16](#).

Slices of the second half map can be seen in Fig. [17](#).

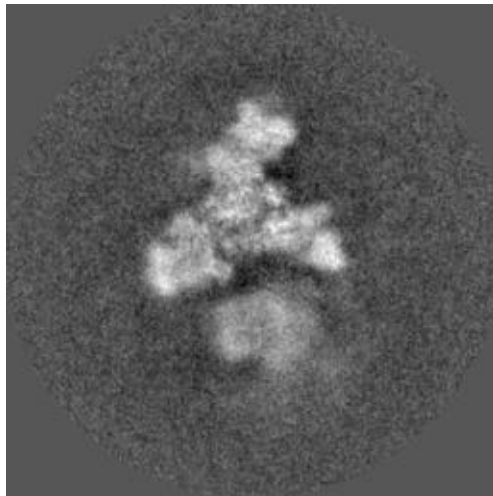
Slices of the difference between both maps can be seen in Fig. [18](#). There should not be any structure in this difference. Sometimes some patterns are seen if the map is symmetric.



(a) X Slice 139

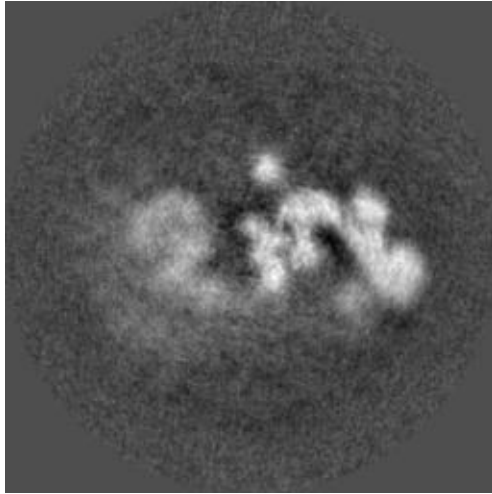


(b) Y Slice 134

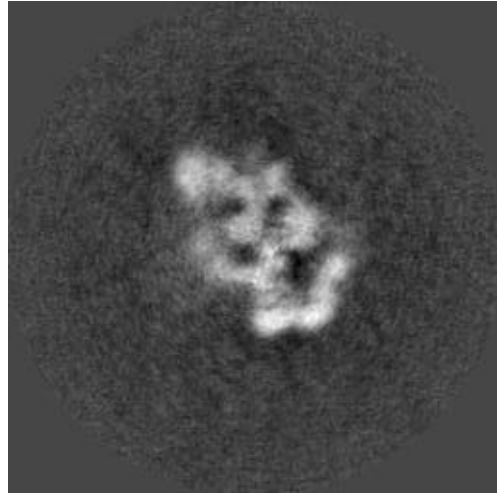


(c) Z Slice 131

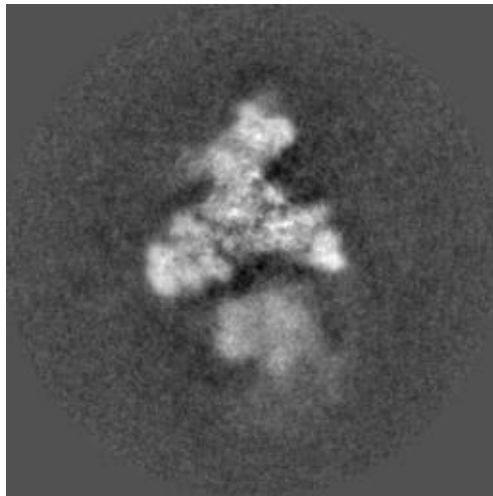
Figure 16: Slices of maximum variation in the three dimensions of Half 1



(a) X Slice 140

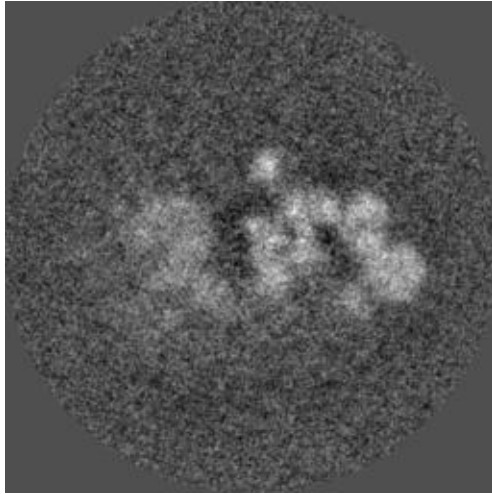


(b) Y Slice 134

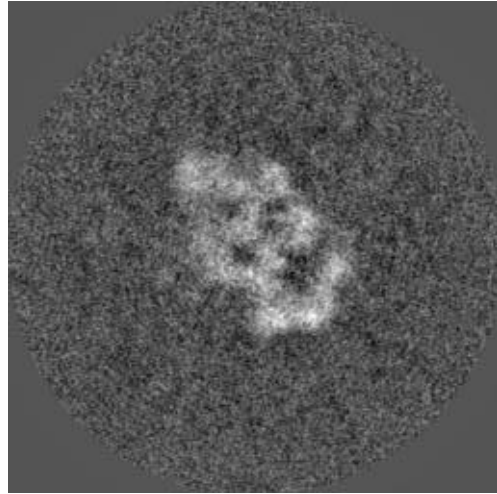


(c) Z Slice 131

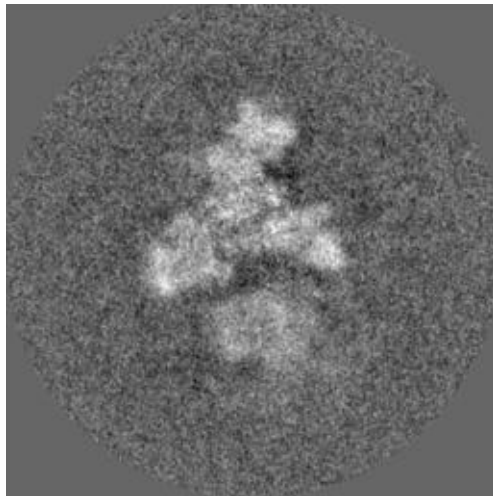
Figure 17: Slices of maximum variation in the three dimensions of Half 2



(a) X Slice 139



(b) Y Slice 135



(c) Z Slice 131

Figure 18: Slices of maximum variation in the three dimensions of the difference Half1-Half2.

4 Level 1 analysis

4.1 Level 1.a Global resolution

Explanation: The Fourier Shell Correlation (FSC) between the two half maps is the most standard method to determine the global resolution of a map. However, other measures exist such as the Spectral Signal-to-Noise Ratio and the Differential Phase Residual. There is a long debate about the right thresholds for these measures. Probably, the most clear threshold is the one of the SSNR (SSNR=1). For the DPR we have chosen 103.9° and for the FSC, the standard 0.143. For a deep discussion of all these thresholds, see this [link](#). Note that these thresholds typically result in resolution values that are at the lower extreme of the local resolution range, meaning that this resolution is normally in the first quarter. It should not be understood as the average resolution of the map.

Except for the noise, the FSC and DPR should be approximately monotonic. They should not have any “coming back” behavior. If they have, this is typically due to the presence of a mask in real space or non-linear processing.

Results:

Fig. 19 shows the FSC and the 0.143 threshold. The resolution according to the FSC is 11.16Å. The map information is well preserved (FSC>0.9) up to 20.41Å.

Fig. 20 shows the DPR and the 103.9° threshold. The resolution according to the DPR is 5.87Å.

Fig. 21 shows the SSNR and the SSNR=1 threshold. The SSNR does not cross the 1 threshold.

The mean resolution between the three methods is 8.52Å and its range is within the interval [5.87,11.16]Å.

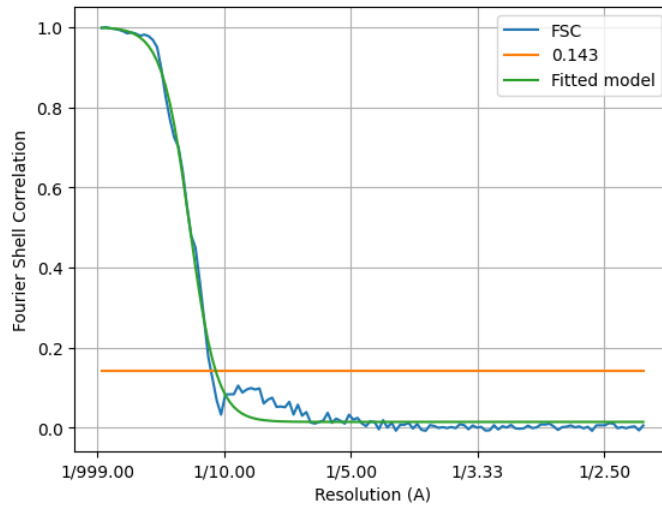


Figure 19: Fourier Shell correlation between the two halves.

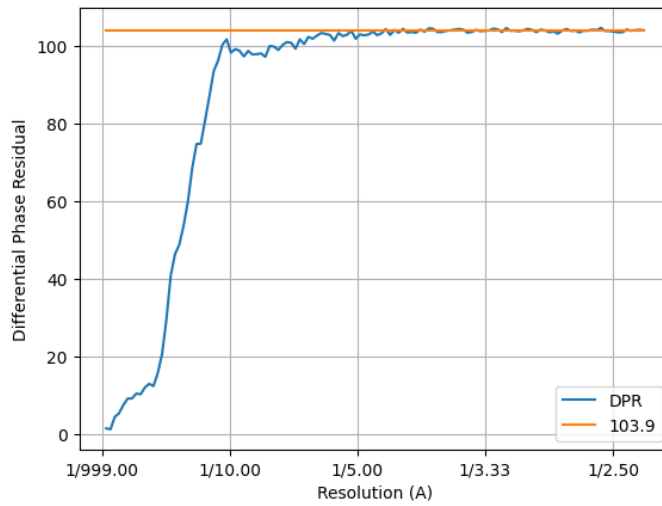


Figure 20: Differential Phase Residual between the two halves.

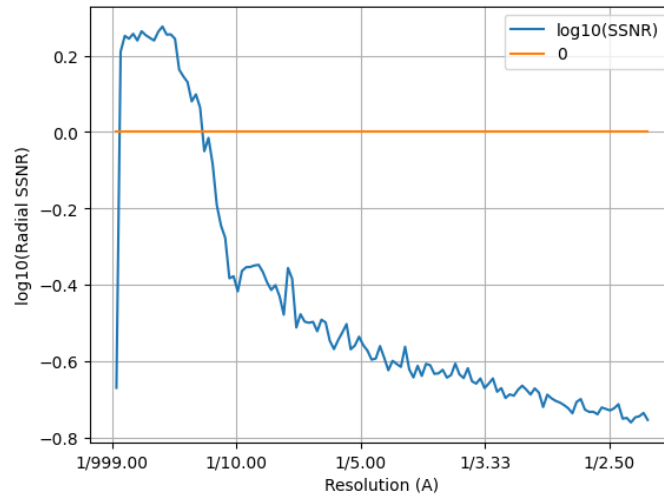


Figure 21: Spectral Signal-to-Noise Ratio estimated from the two halves.

Automatic criteria: The validation is OK if the user provided resolution is larger than 0.8 times the resolution estimated by 1) FSC, 2) DPR, and 3) SSNR.

STATUS: OK

4.2 Level 1.b FSC permutation

Explanation:

This method (see this [link](#) for more details) calculates a global resolution by formulating a hypothesis test in which the distribution of the FSC of noise is calculated from the two maps.

Results:

The resolution at 1% of FDR was 6.1. The estimated B-factor was -174.15. Fig. 22 shows the estimated FSC and resolution.

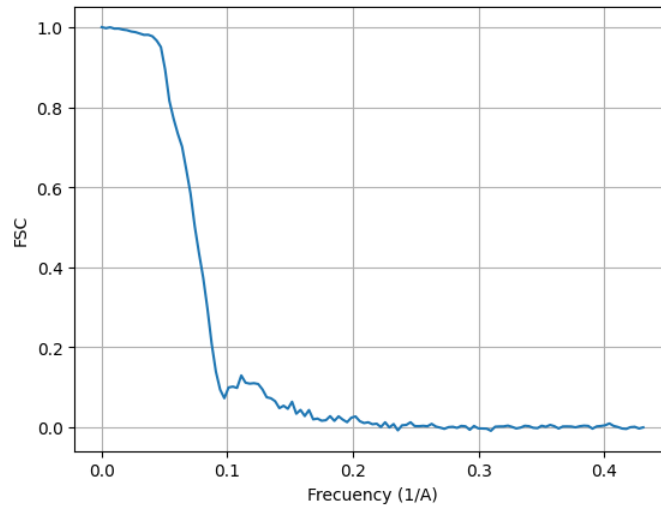


Figure 22: FSC and resolution estimated by a permutation test.

Automatic criteria: The validation is OK if the user provided resolution is larger than 0.8 times the resolution estimated by FSC permutation.

STATUS: OK

4.3 Level 1.c Local resolution with Blocres

Explanation:

This method (see this [link](#) for more details) computes a local Fourier Shell Correlation (FSC) between the two half maps.

Results:

ERROR: The protocol failed.

STATUS: Could not be measured

4.4 Level 1.d Local resolution with Resmap

Explanation:

This method (see this [link](#) for more details) is based on a test hypothesis testing of the superiority of signal over noise at different frequencies.

Results:

ERROR: The protocol failed.

STATUS: Could not be measured

4.5 Level 1.e Local resolution with MonoRes

Explanation:

MonoRes (see this [link](#) for more details) evaluates the local energy of a point with respect to the distribution of energy in the noise. This comparison is performed at multiple frequencies and for each one, the monogenic transformation separates the amplitude and phase of the input map. Then the energy of the amplitude within the map is compared to the amplitude distribution observed in the noise, and a hypothesis test is run for every voxel to check if its energy is significantly above the level of noise.

Results:

ERROR: The protocol failed.

STATUS: Could not be measured

4.6 Level 1.f Local and directional resolution with MonoDir

Explanation:

MonoDir (see this [link](#) for more details) extends the concept of local resolution to local and directional resolution by changing the shape of the filter applied to the input map. The directional analysis can reveal image alignment problems.

The histogram of best resolution voxels per direction (Directional Histogram 1D) shows how many voxels in the volume have their maximum resolution in that direction. Directions are arbitrarily numbered from 1 to N. This histogram should be relatively flat. We perform a Kolmogorov-Smirnov test to check its uniformity. If the null hypothesis is rejected, then the directional resolution is not uniform. It does not mean that it is wrong, and it could be caused by several reasons: 1) the angular distribution is not uniform, 2) there are missing directions, 3) there is some anisotropy in the data (including some preferential directional movement).

Ideally, the radial average of the minimum, maximum, and average resolution at each voxel (note that these are spatial radial averages) should be flat and as low as possible. If they show some slope, this is associated with inaccuracies in the angular assignment. These averages make sense when the shells are fully contained within the protein. As the shells approach the outside of the protein, these radial averages make less sense.

Results:

Fig. 23 shows the 1D directional histogram and Fig. 24 the 2D directional histogram. We compared the 1D directional histogram to a uniform distribution using a Kolmogorov-Smirnov test. The D statistic was 0.185526, and the p-value of the null hypothesis 0.000000.

The radial average of the minimum, maximum and average resolution at each voxel is shown in Fig. 25. The overall mean of the directional resolution is 5.57

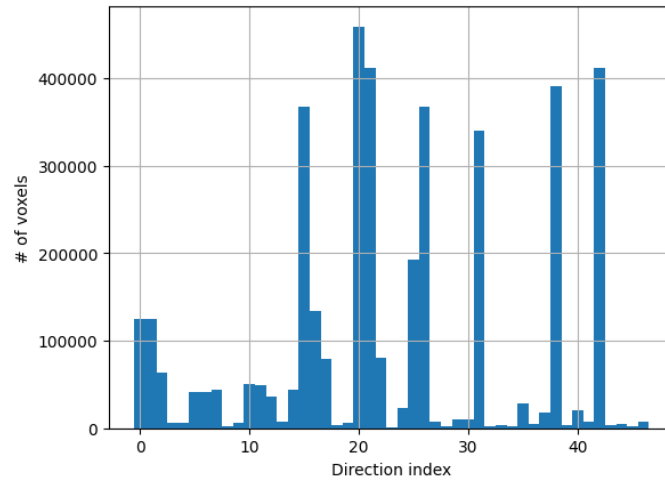


Figure 23: Histogram 1D of the best direction at each voxel.

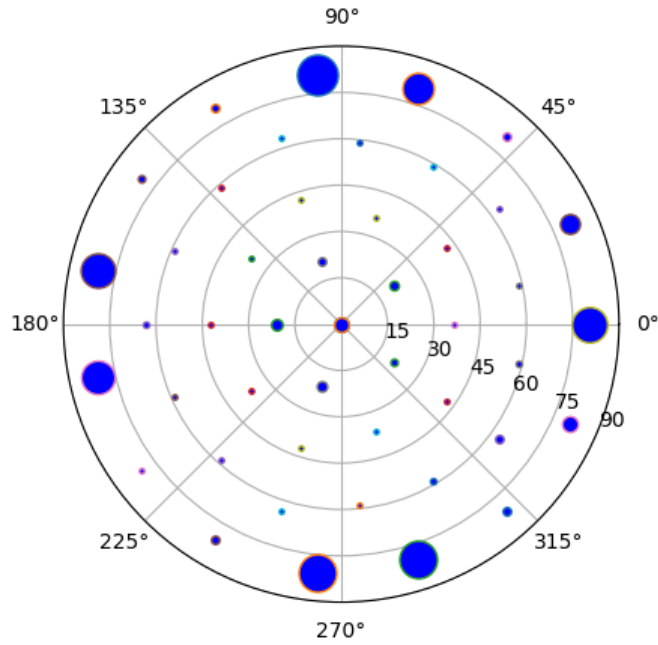


Figure 24: Histogram 2D of the best direction at each voxel. The azimuthal rotation is circular, while the tilt angle is the radius. The size of the point is proportional to the number of voxels whose maximum resolution is in that direction (this count can be seen in Fig. 23).

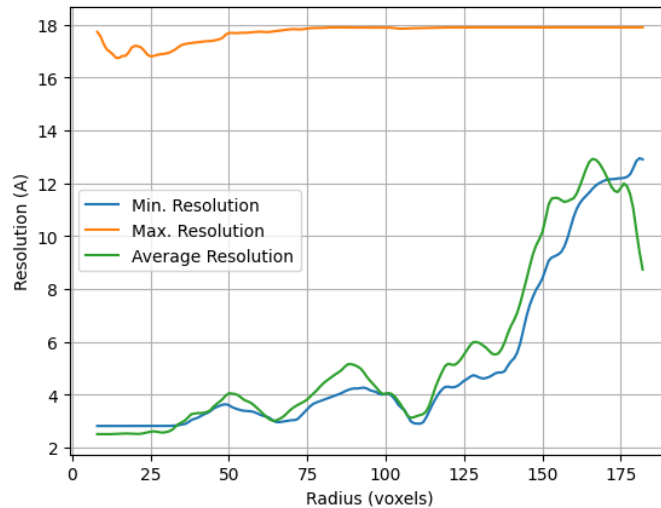


Figure 25: Radial averages (in space) of the minimum, maximum and average resolution at each voxel.

Automatic criteria: The validation is OK if 1) the null hypothesis that the directional resolution is not uniform is not rejected with a threshold of 0.001 for the p-value, and 2) the resolution provided by the user is not smaller than 0.8 times the average directional resolution.

WARNINGS: 1 warnings

1. **The distribution of best resolution is not uniform in all directions. The associated p-value is 0.000000.**

4.7 Level 1.g Fourier Shell Occupancy

Explanation:

This method (see this [link](#) for more details) calculates the anisotropy of the energy distribution in Fourier shells. This is an indirect measure of anisotropy of the angular distribution or the presence of heterogeneity. A natural threshold for this measure is 0.5. However, 0.9 and 0.1 are also interesting values that define the frequency at which the occupancy is 90% and 10%, respectively. This region is shaded in the plot.

Results:

ERROR: The protocol failed.

STATUS: Could not be measured

4.8 Level 1.h Fourier Shell Correlation 3D

Explanation:

This method (see this [link](#) for more details) analyzes the FSC in different directions and evaluates its homogeneity.

Results:

ERROR: The protocol failed.

STATUS: Could not be measured