METHOD DEVELOPMENT OF COMBINATION OF ATOMIC FORCE AND ELECTRON MICROSCOPY DATA TO OBTAIN BETTER 3D RECONSTRUCTIONS.

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3D electron microscopy (3D−EM) allows to obtain a 3D reconstruction of biological specimens from a series of micrographs taken with the electron microscope. The different projections from the particle under study are properly combined by a 3D reconstruction algorithm such as ART, SIRT or Weighted Backprojection producing a density volume. In this work, an extension of the ART algorithm[1] intended to improve the quality of reconstructions by means of merging protein surface data with the EM projections is presented. The surface data may be obtained by an atomic force microscope (AFM)[2], using the metal shadowing technique[3] or even by simulation. Briefly, the method imposes extra conditions to the reconstructed volume using the knowledge steaming from the surface data. These new constraints are translated into new equations forcing voxels outside the known surface to have no electronic density. The surface restriction can be specially helpful in the case of knowing the surface of the protein in volumetric regions which have not been adequately captured by the EM projections due to a missing region in the projection space.

A study of the method capabilities has been carried out following the objective comparison methodology proposed by Marabini et al.[4] based on phantoms and Figures of Merit (FOMs). Phantoms have been chosen from the Protein Data Bank (PDB) modified entries stored in PQS for the bacteriorhodopsin (1BRD), human aquaporin (1HWO), tubulin (1TUB) and DNA polymerase sliding clamp (1B8H) to density volumes at their highest resolution. The corresponding surfaces have been theoretically computed by thresholding the phantoms, these surfaces are referred to as "perfect" surfaces. Eliminating the central slices to simulate the real AFM process, AFM-like surfaces are obtained (see fig. 1). 1000 noiseless projections of the phantom volumes with randomly distributed directions have been simulated leaving a missing cone of 35º around the vertical axis. The evaluation FOMs are the correlation index and the mean squared error between the voxels in the phantom and the reconstruction measured inside the perfect restriction surface. Five realizations of each experiment have been done to assess statiscal significance to the results.

Three different cases have been compared: reconstruction without surface, with AFM-like surface and with perfect surface. Figure 2 shows the differences between the reconstruction without surface knowledge and with AFM-like surface for the bacteriorhodopsin, improvements mainly concentrate near the given surface. Evaluation results for the four proteins and three reconstruction cases are shown in table 1. It can be seen that reconstructions using surface restrictions are better than reconstructions without them: they offer an improvement between 0.5-2% in the correlation index and between 5-12 % in the mean squared error FOM. FOM differences among different reconstruction cases of the same protein are significant (at a 95% confidence level) as applying a Student t−test for mean differences. Furthermore, it must be noticed the reproducibility of the experiments indicated by the low standard deviations at the FOM measures.

A more detailed analysis of improvements reveals that these tend to be more often in those regions associated to protein side chains. This effect is stronger as the surface topology becomes more complex (deep inlets and high protuberantions) as it is providing more information.

It should be pointed out that for some specimens the perfect surface is not improving significantly the reconstruction results. This can be explained by the fact that the main gain is done on the region where projection data is missing in Fourier space (the missing cone). This information is mainly provided by the top and bottom surface of the protein, thus, supplying the whole surface is not giving more information as most of it is also captured by the EM projections.

Concluding, we have shown that *a priori* surface information can be used to improve the quality of 3D reconstructions in electron microscopy, specially in those cases where there exists a missing cone in the projection space. The amount of information contained in the protein surface depends on the specific specimen under study but significant improvements should be expected. Further investigations must be done in order to quantify the effect of the surface accuracy on the reconstruction process and its application to real experimental data.

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PERFECT SURFACE RESTRICTION

	Correlation index		Squared errors mean	
PDB code	**mean.**	**confidence interval of 95%**	**mean.**	**confidence interval of 95%**
1BRD	**1,06%**	**±0,01%**	**10,79%**	**±0,25%**
1HWO	**0,71%**	**±0,01%**	**6,85%**	**±0,13%**
1TUB	**0,61%**	**±0,06%**	**4,46%**	**±0,26%**
1B8H	**2,43%**	**±0,07%**	**8,73%**	**±0,27%**

AFM SURFACE RESTRICTION

	Correlation index		Squared errors mean	
PDB code	**mean.**	**confidence interval of 95%**	**mean.**	**confidence interval of 95%**
1BRD	**1,22%**	**±0,02%**	**12,11%**	**±0,36%**
1HWO	**0,69%**	**±0,02%**	**7,13%**	**±0,17%**
1TUB	**−0,05%**	**±0,08%**	**−2,58%**	**±0,39%**
1B8H	**1,57%**	**±0,06%**	**5,11%**	**±0,15%**

Table 1. Percentaje of improvement in figures respect to a reconstruction without surface restriction

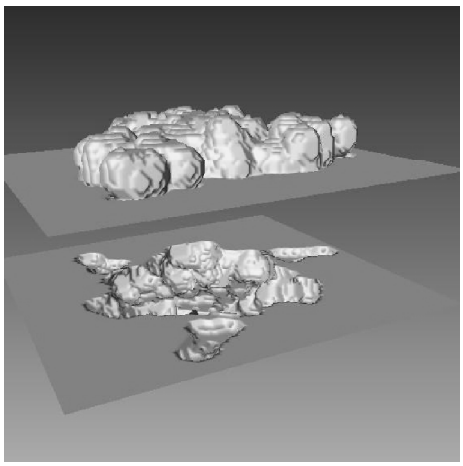

Fig. 1. Simulated AFM-like surface for the bacteriorhodopsin

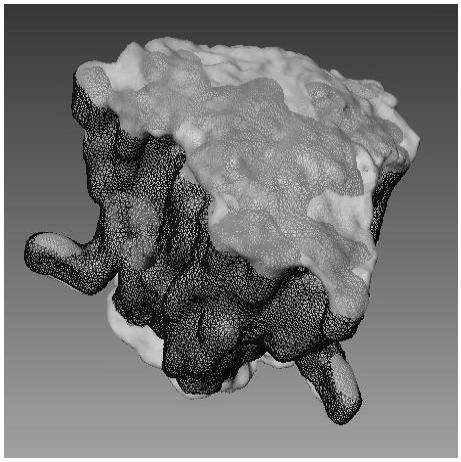

Fig. 2. Comparison of bacteriorhodopsin volumes reconstructed with (dark) and without (clear) restriction surface.