Get rid of micelle

Tue, 01/02/2018 - 14:38

#1

zhho

Get rid of micelle

Hi,

After sharpeninging the 3D volume, I find that the micelle looks really bad. Do cistem have any method to get rid of the micelle? Thanks.

Hongtao

timgrant

Hi Hongtao,

If you are worried about the micelle affecting your refinement you can provide a mask in the manual refinement panel and do some refinement with that. The way we typically deal with micelles is to use a mask, but with filtering instead of true masking. If you start with a mask that is quite tight to the protein, and does not contain the micelle, import this and select it as the active mask in the manual refinement panel. Then in expert options under masking - set the "outside weight" to 1.0. Change "Low-pass filter outside of mask" to YES, and then set the resolution to something like 20 or 30A. This leaves low resolution signal in the reference from the micelle which can actually be useful for alignment, but removes high resolution signal and should therefore prevent overfitting within the micelle.

If you simply want to apply a mask to remove the micelle for visualisation you can use the apply_mask command line program that comes with cisTEM. cisTEM doesn't yet have a "mask creator", and so you will have to generate the mask yourself. I typically use the following procedure, but you can use any you want, in cisTEM anything that is 0 and under will be considered outside the mask, anything that is above zero will be considered inside the mask :-

1. Take your reconstruction and open in chimera - in chimera turn on "hide dust" and adjust the threshold until your structure remains, but the noise and micelle are gone (hopefully this is possible, you may need to play with the sharpening) - you want the lowest value where this is true, record this number.

2. Run make_size_map, it is a command line program included with cisTEM. when it asks for a binarisation threshold enter the value you figured out above. This creates a new map where the values for each voxel are replaced with the number of connected voxels. You should now be able to binarise this program such that it keeps on the density for your protein. You cannot do this in cisTEM currently, you will need to use another program such as EMAN, IMAGIC, TIGRIS etc.. You could use this directly as a very tight mask, but i prefer to dilate it a little bit (maybe a couple of voxels), you can do this by filtering it and rebinarising it for example.

Cheers,

Tim

Tue, 01/02/2018 - 18:24 <u>(Reply to #2)</u>

zhho

Hi Tim,

Thanks a lot for your suggestions. I will give a try.

Source URL: https://cistem.org/get-rid-micelle