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Mon, 02/10/2020 - 03:02

#1

abhisek

remove micelle/lipid belt

Hi,

I'm refining a structure at 3.4Å with a huge belt of micelle wrapped around the transmembrane part.

Is there a way to subtract the micelle density from the transmembrane part and re-align all particles to improve map features in cisTEM ?

Please advise.

Thank you

Abhisek

timgrant

Hi Abhisek,

Hi Abhisek,

In the released version of cisTEM you are unable to subtract micelles. However, we have had very good results low pass filtering them. I gave a brief description previously here :-

<https://cistem.org/get-rid-micelle>

I am happy to answer any questions.

Tim

Tue, 02/11/2020 - 22:44 (Reply to #2)

abhisek

Hi,

Hi,

Thank you for the suggestion. However, I could not be able to binarise using eman2's e2proc3d program.

The program I'm trying to use is "e2proc3d.py input.mrc output.mrc bin=(threshold)".

The program gives no output. Also could not found -bin flag in program manual.

Is it the right way to go?

Abhisek

timgrant

Hi Abhisek,

Hi Abhisek,

For binarizing, you should use :-

```
e2proc3d.py input.mrc output.mrc --  
process=threshold.binary:value=(threshold)
```

Thanks!

Tim

Wed, 02/12/2020 - 18:24 (Reply to #4)

abhisek

Hi Tim,

Hi Tim,

Thank you for the response. I have two major concern about this way of filtering:

1. Despite increaring the voxel threshold (in chimera) it is still almost impossible to remove ceritan part of micelle belt, as they are quite sticky to few transmembrane helices (and have higher threshold than even certain transmembrane helices). Is there any other way to remove unwanted micells in specific regions ? I was thinking of volume eraser but have enough doubt regarding whether it should be used in this porpose.

2. While binarising the mask should we be expanding it by 3-5 voxels and follwed by adding a soft mask about 3-5 voxels ? I found a cisTEM presentation in c-cina website that recommends this way, however, I have no idea how to achieve this in cisTEM.

Would you please care to comment.

Thanks again

Abhisek

timgrant

Hi Abhisek,

Hi Abhisek,

1. You can use the volume eraser to remove harder to reach parts. Remember, you are not masking out areas outside of the mask, you are just low-pass filtering, so it's not quite as dangerous as pure masking (although you should still be a bit cautious).

2. cisTEM will add a mask edge that you specify, but that edge will be half in the volume and half out. So if your mask is very very close, you should definitely expand it a bit before applying it. E.g. if you had a 10 pixel fall-off, and a very close mask, you would want to expand it by 5 pixels or so. One way to expand the mask is to low-pass filter it and then re-binarise it.

Thanks,

Tim

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