refinement always goes to nyquist (over-refinement)

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#1

mrhansenable

refinement always goes to nyquist (over-refinement)

hiya

Have seen this a few times in the past. My data when refining will go to 2.1A very easily (pixel size 1.05). Dataset is small (10k particles), somewhat noisy, and definitely not 2.1A resolution limit. The data itself is fine I think, since I got to 8A ab initio which looks nice. The problem is when I then try to refine the data against the ab initio model.

See attached screenshot, things seem to refine alright up to a certain point then the next round it just maxes out to nyquist. I don't think this is a small # of particles problem because I have seen it with larger datasets. The output map for the penultimate step looks reasonable, then the final map is way jacked up with noise and clearly over-refined/sharpened.

Have tried doing a global manual refine and limiting resolution but it takes ages to make any progress, which makes no sense since it shoul drun quicker. I've tried with just doing local refine and the nyquist problem occurs again. Haven't yet ruled out that manual refining is the solution and I am just setting it up incorrectly.

thanks!

File:
🖼 refinement.jpg

timgrant

Hi,

This does look weird! Can you answer the following so we can try and get to the bottom of it?

1. What molecular weight and particle size did you enter for your refinement package?

2. How did you pick the particles? Are they very densely packed?

3. Is the particle very elongated?

4. Is the circular mask cutting into density? i.e. in the orth views that are shown in the refinement panel, where does the circular mask cut?

Can you send a screenshot of the orth views?

Thanks!

Tim

mrhansenable

hi Tim,

This is with a helical protein. There are 5 protomers in the helix and so it is quite long.

1) 1250kda for molecular weight and 500A largest dimension.

2) the particles were auto-picked in cisTEM. They are pretty dense yes, since the helices tend to bundle.

3/4) yes, because it is a helical protein it extends to the edge of the box and the mask cuts it off on either side.

I'm running it again with five times as many particles and will report back if it works. The orth views go from being reasonably dark background to very fuzzy and noisy the next round.

timgrant

Hi,

So although some people are using cisTEM for helical reconstructions, right now it is not something that is "officially supported". One thing that you have to be careful about that can lead to inflated FSCs is putting the same segment of helix in both of the half maps, this will lead to very inflated FSCs. Right now, as helical reconstructions are not well supported, the only way you can limit this is by setting the "Search Range X/Y" to something that is quite a bit smaller than your helical repeat.   This is what I would try first to see if it helps with your weird FSCs.

Cutting into the density can also cause problems with the FSC measurement, you might want to try writing out the half maps (select yes in the Generate3D panel), and calculating the FSC yourself with a soft mask.

Please let us know if either of these things solves the problem.

Thanks!

Tim

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hi Tim, it actually DID help

hi Tim, it actually DID help to do the very X/Y range for refinement.
Thank you! Things look normal now.

Much appreciated.

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by the way, is it possible to

by the way, is it possible to retrieve data about from which micrographs the particles originated? Exporting stacks gives "unknown.mrc"; it might be a good addition in future versions to have this include the micrograph name.

Thanks again for your help, that reduced X/Y really did the trick!

timgrant

Hi,

I thought this was included in the exported star file? If not, you can get this information from the database. An example is given on the following page :-

https://cistem.org/documentation#tab-1-16

Cheers,

Tim

Source URL:https://cistem.org/refinement-always-goes-nyquist-over-refinement?page=0