3D reconstruction of disk-shaped particle... or lack thereof

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cg

3D reconstruction of disk-shaped particle... or lack thereof

I have collected relatively good data on my disk-shaped, trimeric the protein, but I cannot get good 3D reconstruction and I cannot figure out why. My classes look good (see attached), I think, and there are over 30,000 particles chosen. I have run many 3D classifications to clean out any bad particles and I think it is a relatively clean picking.

Ab initio 3D gives me very poor results (see attached), appearing like it simply cannot properly overlay the particles in a reasonable way. Also, when I try to refine against a 20-angstrom map of the known structure of a nearly identical protein, the resolution never goes beyond 14 angstroms, and the slices only get worse from the start.

I have tried making the refinement package in C1 and C3. I have tried different size/location parameters in the Expert Settings in both Ab initio and AutoRefinement. I have tried to add a class in the refinement package and it gives me ~1% for the second particle, so I assume that means there is really only 1.

I am really out of ideas and I cannot seem to make progress. If anyone has any input, I would appreciate it.

Here is some extra information on the shape and size of the protein: The protein has C3 symmetry so that each wedge (monomer) of the disk are equivalent. On one side of the disk, there is a bump on each of the three wedges. The other side is pretty flat If considered a disk, the protein is about 300 angstroms in diameter and the thickness is ~40 angstroms, not including the bump region on the one side which would add another ~15-20 angstroms.

File:
Abinitio.png
2Dclasses.jpg

timgrant

Hi,

Hi,

Is this a protein in a micelle? It looks to me like there is a lot of "disordered" density around as the classums lack high resolution features. If it is a smallish protein in a large micelle it makes it much harder to solve. In general, I tell people that if they see secondary structure in their classums, the ab-inito should work - and I think you don't see that level of detail.

The only tips I can give is to try multiple times with different parameters. Try increasing the number of starts, you could also try not go all the way to 8A, stop at 15 or 12 or something. If you are sure that you will have C3 (or very close) to it symmetry, I would use a refinement package with C3. You could also try clicking the "always use symmetry" option in the expert options to see if it helps.

Thanks!

Tim

cg

Hi Tim,

Hi Tim,

It's a large protein (~100 kDa) with a micelle, yes. Okay, i'm going to keep trying various parameters. Hopefully I will get back with a success story.

-Chris

timgrant

Hi Chris

Hi Chris

Definitely try it a few times with always apply symmetry set to yes.

Tim