artifact features in 3D volumes

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zachmaben

artifact features in 3D volumes

Hello,

I am a new user of cisTEM (and a novice to SPR cryoEM in general). I recently generated *ab initio* 3D volumes of my molecule of interest. These volumes have several features where a central spike is surrounded by a protruding ring, in a shape resembling a tulip. (see attached png file depicting two such features shown at center top)

Does anyone know what might cause this? I assume it is an artifact of the volume building process, possibly due to poor image or particle picking. And, more generally, is there a reference that I may use in the future to investigate unusual features that I might observe in the future?

Thank you for your help.

-Zach

File:
Maben_ab_initio_3D_volume.png

niko

It looks like this ab initio

It looks like this ab initio estimation did not work. The process sometimes fails, either when the signal is weak, for example when the particle is small, or when there is significant heterogeneity. If heterogeneity is the problem, maybe you could retry this with a subset of your data that you select based on 2D class averages that look consistent.

timgrant

Hi Zach,

Hi Zach,

To add to what Niko said, the artifacts you are seeing are severe overfitting artifacts (the ab-inito basically doesn't do anything to prevent overfitting). If the underlying structure looks right, you can try using this as an input for auto-refine starting from low resolution and see if this improves (the auto-refine should do a better job of limiting overfitting). If the structure is probably wrong, you will need to try and rub ab-inito with different options, e.g. doing more starts and perhaps not going to such high resolution.

Thanks,

Tim