Directional Resolution of Cryo-EM Maps – Using the 3DFSC Server

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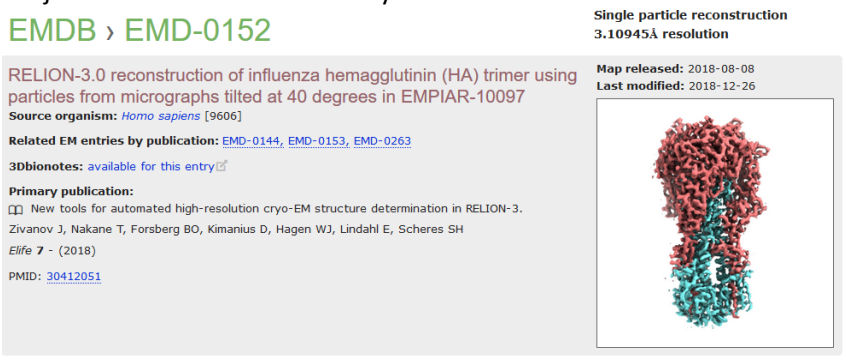
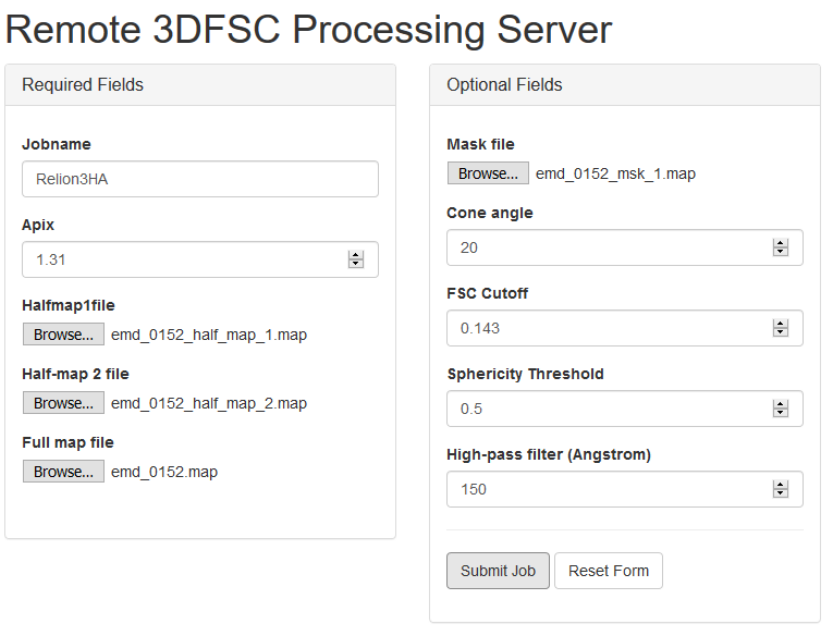
Requirements: Web browser (To download datasets)
UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) for visualization

Instructions for Practical

Map Validation – Local Resolution (Directional FSC)

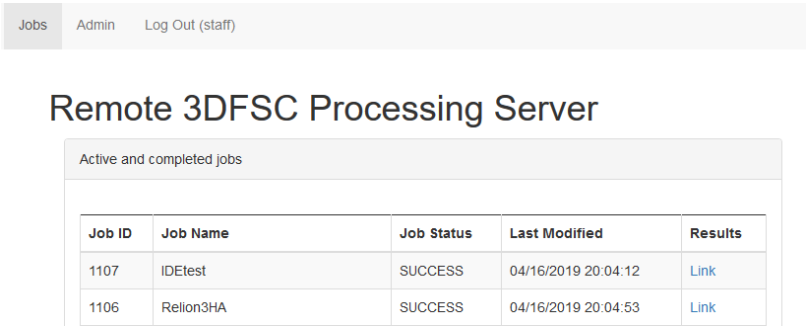
1. Maps usually have variations in resolution, both in real space and in Fourier space. Variation of resolution in Fourier Space can be due to preferred orientation issues
2. To explore directional anisotropy in resolution, go to this online server: https://3dfsc.salk.edu
    a. Register for an account and login
3. Start a new job with another EMDB entry
    b.





    c.
4. Submit the job. It will take about 1 minute to run, but if there is a queue you might have to wait a bit longer
5. Once it is done, navigate to Jobs > Link for the results

d.

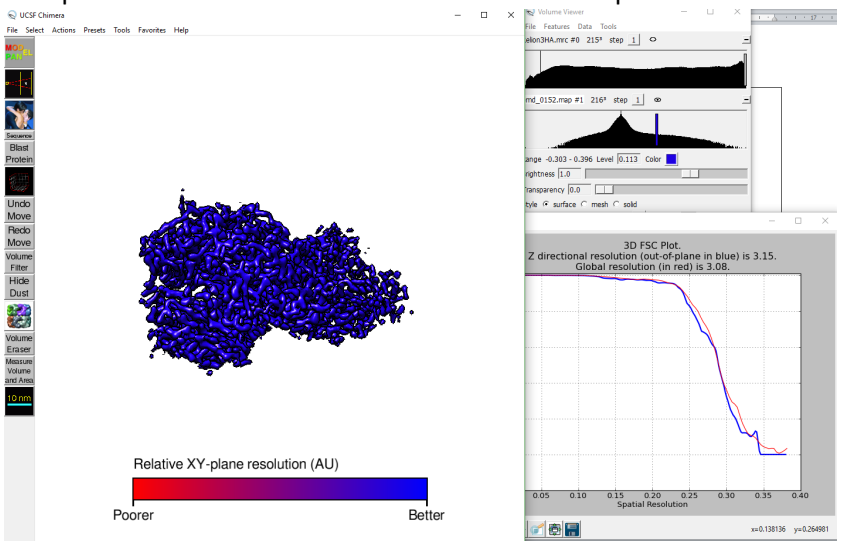


6. Answer the following questions based on your results

What is the sphericity value? Sphericity indicates how isotropic the 3DFSC is. If you do not have preferred orientation, your 3DFSC is a perfect sphere and the sphericity is 1. Values from 0.9 to 1.0 indicate good sphericity, while values from 0.8 to 0.9 are acceptable. Values below that are usually map plagued by severe preferred orientation.	

7. Now download the (compressed) results onto your computer. When unzipping the files, make sure you have the password copied.
8. Once you have download the results, open another Chimera session. Now go to File > Open and open the file called "3DFSCPlot_Chimera". This will automatically open up the relevant maps in Chimera for you to view the directional resolution of the map
9. Move your map around and notice how the color changes. The more blue the map is, the better the XY-plane resolution the view has. This also corresponds (roughly) to having more views in that particular direction – i.e. this is the location of preferred orientation.

e.



10. Answer the following questions.

Which is the preferred view of the protein?	
What is the Z directional resolution of the best view?	

What is the Z directional resolution of the worst view?	