
Supplementary File III: Generation of initial model by cryoSPARC

Generate the initial model using CryoSPARC by following these steps: Click on the **Builder** button located at the top right of the panel, then find and select the **Ab-Initio reconstruction** option within the panel. Open the job for importing the particle stack from the fifth iteration (or any desired iteration) in the main panel on the left. Drag the **Imported particles** module from the right side of the main panel and drop it into the **Particle stacks** section of the **Builder** on the right. Initiate the ab-initio reconstruction by clicking the **Queue Job** button, followed by clicking **Queue** button. Repeat for any additional iterations needing re-estimation. It should be noted that applying symmetry higher than C1 for ab-initio reconstruction is generally discouraged.