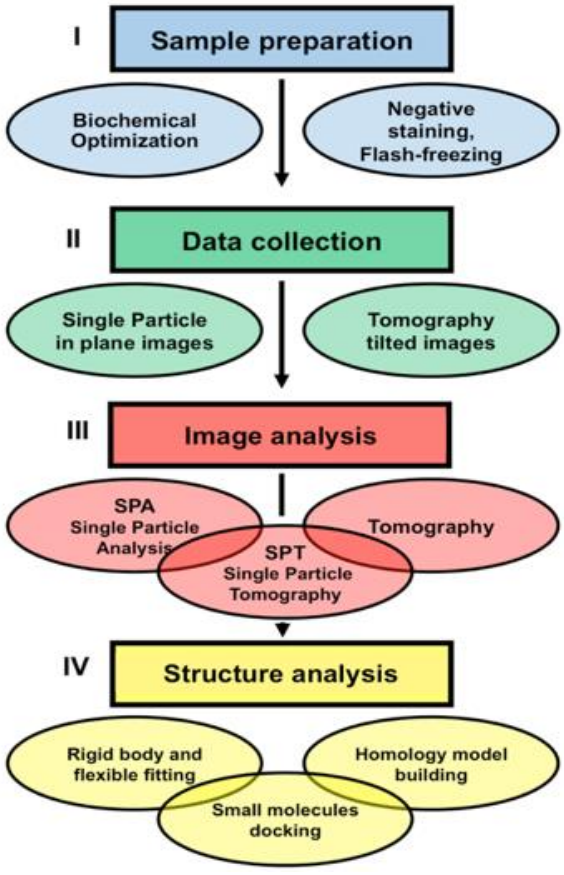
The Single Particle Cryo-EM workflow at the Center for Nanoscale Systems

Stoilova-McPhie, S.[1], Marks, C.[1], Graham, A.[2], Bell, D.[3,1] and Wilson, W.[1]

[1] Center for Nanoscale Systems, Harvard University, United States, [2] Center for Nanoscale Systems, Harvard University, United States, [3] Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, United States

Biological Imaging by Cryogenic Transmission Electron Microscopy (cryoEM) is currently undergoing a technological transformation, revolutionizing structural biology. CryoEM combined with single particle analysis; aided by advances in computational techniques, have in many ways eclipsed x-ray crystallography as the biological structure determination tool of choice. Near atomic resolution structures have been revealed for a number of challenging protein complexes. Nearly 20% of the EM maps deposited in the Electron Microscopy Data Bank (EMDB) over the last few years have sub 5 Å resolution. Driving this revolution are advancements in imaging technologies, as TEM design (Cs correctors, phase plates, automatic loading), advances in high performance direct electron detectors, and most importantly, the development of techniques to prepare and stabilize the vitreous sample of interest captured in their native state. Finally, the exploitation of complex single particle image analysis paradigms with advanced image artifact correction strategies, such as motion correction and dose weighting, dramatically improve image contrast and resolution.



Resolving the structures of macromolecular assemblies to gain fundamental insights into biological mechanisms and function remains a major challenge in structural biology. The methods are complex and under constant development, so in depth, advanced training of new users is essential to ensure future users can adapt new technologies to expand into new areas and solve new biological problems. The cryoEM core at CNS has been effective at assisting new users to develop cryoEM expertise, enabling them to transition to independent work. CNS has two field emission gun (FEG) equipped 200 kV TEM systems fully devoted to cryoEM: a FEI Tecnai F20 which enables HRTEM imaging at cryogenic temperatures using custom holders and a fully automated FEI Arctica cryo-TEM, with high tilt capability (+/- 70 degrees), a 12-sample auto-loader and equipped with a K2 summit direct electron detector capable of near atomic (2.5 Å) resolution. These instruments have been made available to the entire CNS user base (internal and external) and are housed in state-of-the-art imaging suites; spaces optimized low stray fields and low mechanical interference, enabling high resolution, high performance cryoEM imaging. The goal of the single particle cryoEM workflow is to enable an expanding fraction of the scientific community to perform high-

resolution structure determination, leading to transformational impact on a wide diversity of biological, biomedical, and soft materials research grand challenges. The cryoEM core provides theoretical and practical training encompassing the full cryoEM workflow from sample preparation to data collection and image processing. Effective training of users involves several levels. The initial level is an overview of the techniques and practical considerations. Hands on training follows in a small group environment by experienced staff. Users are allowed then to operate the microscope after passing a basic certification. Experienced users are given 24hr access to complete their projects. We plan to expand our cryoEM abilities with a 300kV fully automated cryo-TEM instruments to enable state-of-the-art single paticle cryoEM structure determination work at atomic resolution and more usage bandwidth for the userbase.

This work was performed at the Center for Nanoscale Systems (CNS), a member of the National Nanotechnology Coordinated Infrastructure Network (NNCI), which is supported by the National Science Foundation under NSF award no. 1541959.