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Benchmarking Cryo-EM Single Particle Analysis Workflows at CEMRC

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Recent technological and methodological advances in single-particle cryo-electron microscopy (cryo-EM) have paved the way for the determination of high-resolution structures of biological macromolecules at unprecedented resolutions. Cryo-EM is becoming increasingly popular as a research tool for structural biology studies. Despite the significant developments in cryo-EM in recent years, opportunities for improvement remain in various aspects of the cryo-EM single-particle analysis workflow (e.g., sample preparation, image acquisition and processing, and structure validation).

The CEMRC is home to three transmission electron microscopes (EMs), each of which is routinely benchmarked for performance. These instruments include a 300 keV Titan Krios G3i and a 200 keV Talos Arctica, each equipped with a Gatan K3 detector with Bio Quantum energy filter, and a 120 keV Talos L120C. Our workflow was optimized using several biological samples, and includes the full process from specimen preparation through imaging and data processing. Using this benchmarking process, we may discover possible difficulties and bottlenecks that could arise for users learning biological EM techniques, and with our instrumentation and data collection strategies. These experiments also provide us with the opportunity to optimize single particle analysis (SPA) workflows.

In this study, we demonstrate that high-resolution cryo-EM data can be acquired using minimally processed, commercially available biological standards. We used rabbit muscle aldolase, a homotetrameric enzyme of 160kDA; bovine liver catalase, a tetramer composed of four equal subunits, each with a molecular weight of 60 kDa; and mouse apoferritin, 487 kDa. Our benchmarking technique is an easy to follow, step-by-step workflow that can be replicated with other standardized or experimental samples and conditions. Our protocol was successfully tested on a Titan Krios, Talos Arctica, and Talos L120C microscopes. Following our workflow, we generated a 2.6 Å reconstruction of rabbit muscle aldolase, 3.3 Å reconstruction of bovine liver catalase from the data sets collected from Talos Arctica 200keV, and 1.57 Å reconstruction of mouse apoferritin from the data set collected from Titan Krios G3i. This workflow, as demonstrated with the CEMRC instruments and three unique biological specimens, produced structural benchmarks of sufficiently high-resolution to validate instrument performance, data collection techniques, and data processing pipelines [5].

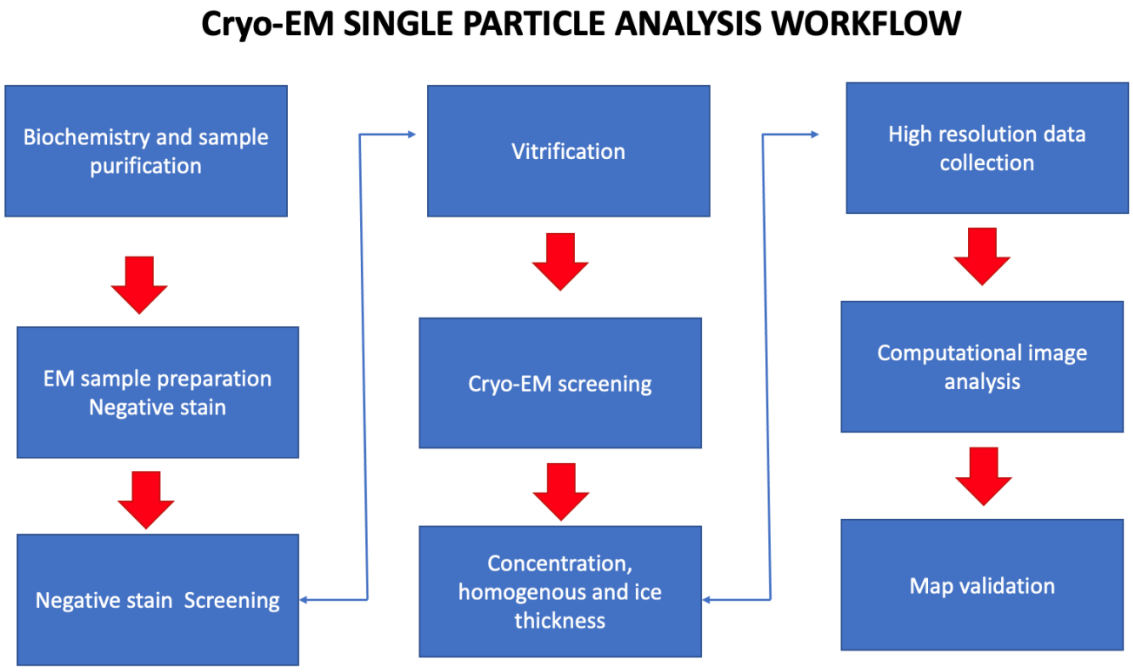

Figure 1. Single Particle Cryo-Electron Microscopy workflow

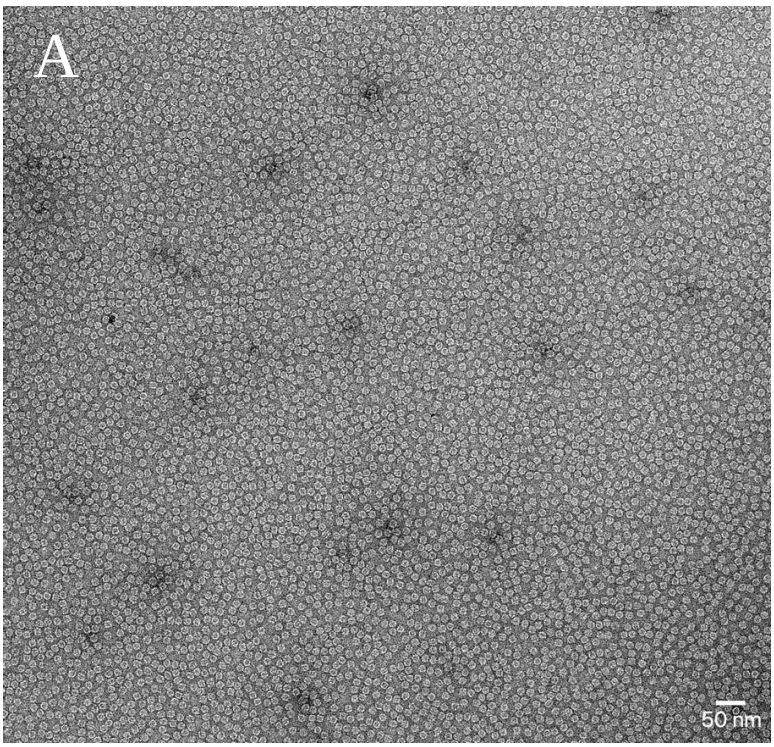

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Figure 2. Negative Stain TEM Analysis: (A) Micrograph of mouse apoferritin that was stained with 2% uranyl acetate, 50 nm scale bar.

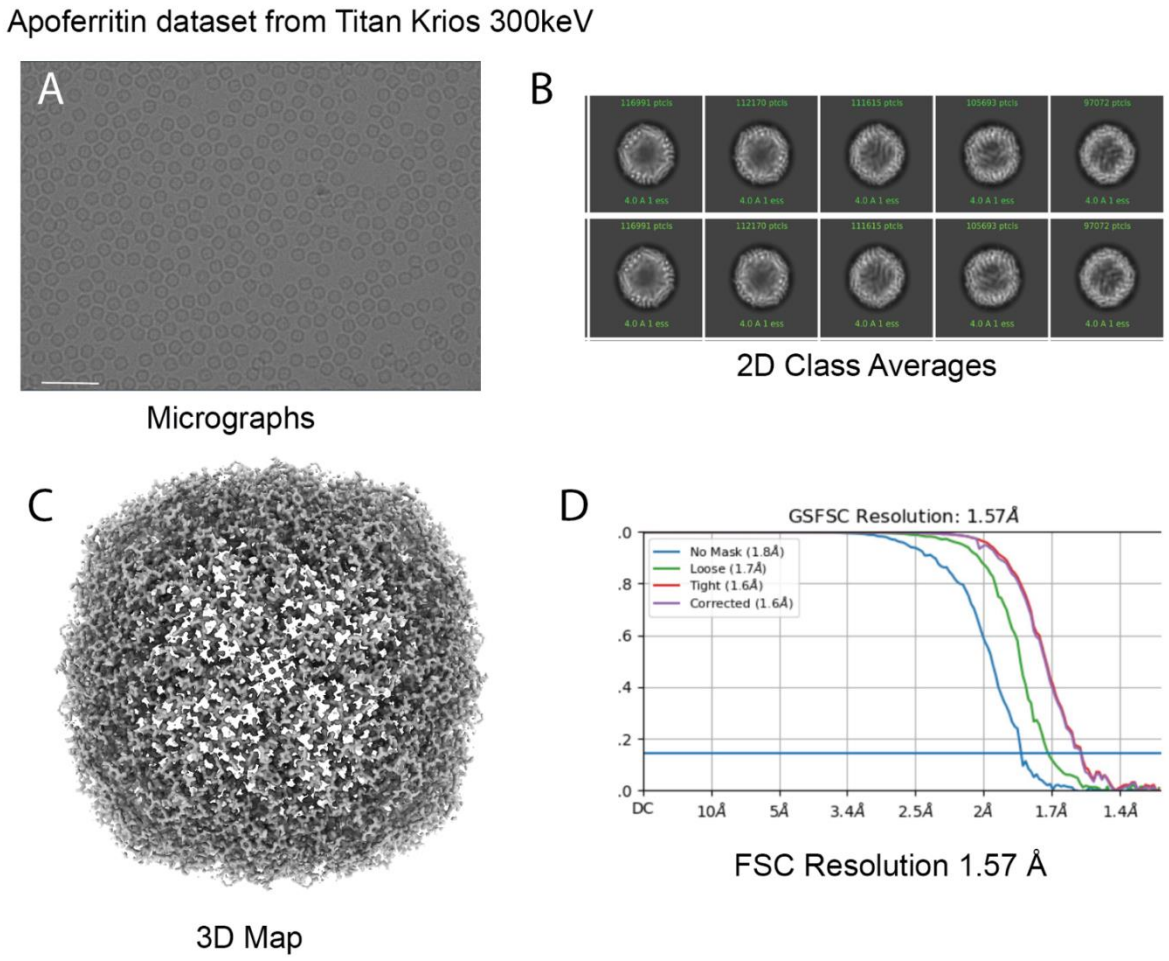

Figure 3. Cryo-Electron Microscopy Single Particle Analysis workflow: (A) Representative micrograph and (B) 2D class averages of apoferritin. (C) 3D Map of apoferritin. (D) Fourier shell correlation (FSC) at FSC = 0.143. Scale bar 50 nm.

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