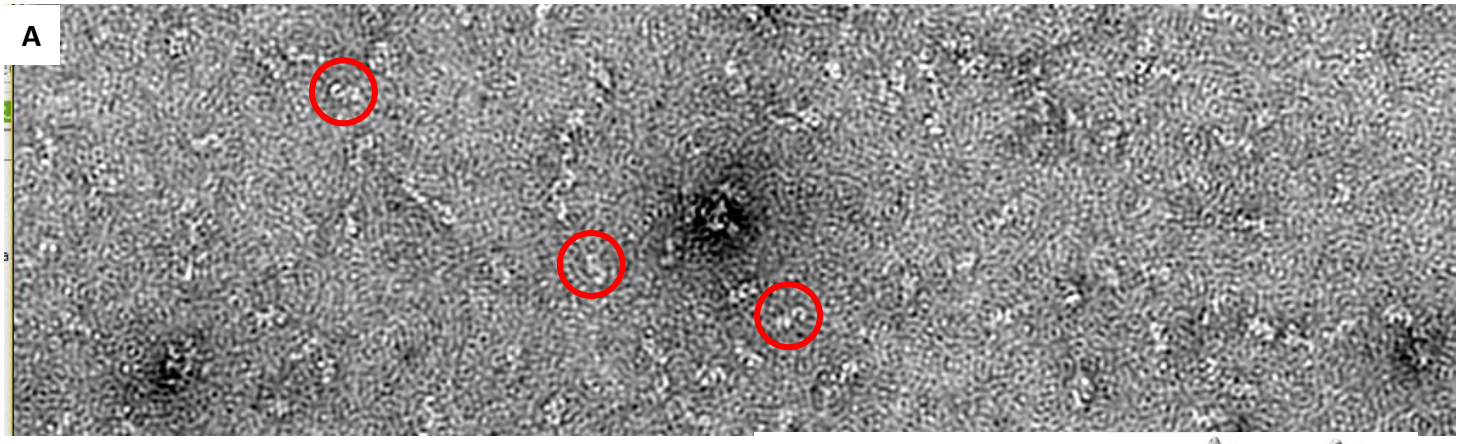
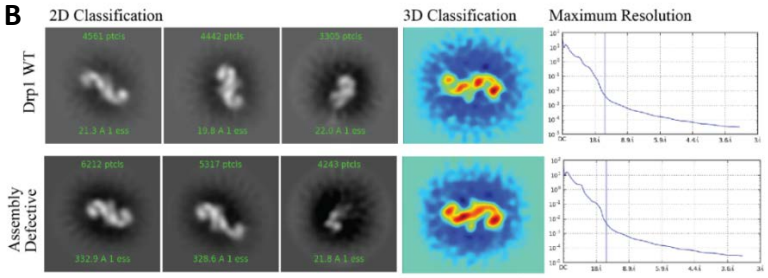
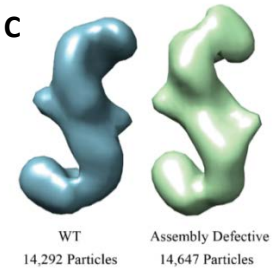
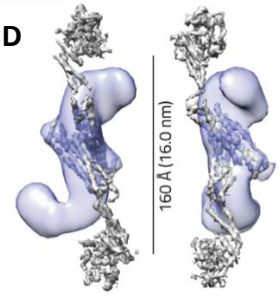
Using negative stain microscopy and a gradient fixation (GraFix) process, we resolved a sub-20 Å structure of both wild type Drp1 (WT) and a Drp1 mutant which yields an assembly defective dimer. When compared to the crystal structure, our EM structures demonstrate a shift in conformation of the GTPase domains. These results suggest that the GTPase domain may be in an inhibited state that shields the stalk from forming intermolecular contact. Below is a sample image from the micrographs (A), examples of the 2D class averages (B), the ab initio structures (C), and the solved crystal structure docked in the WT density (D).

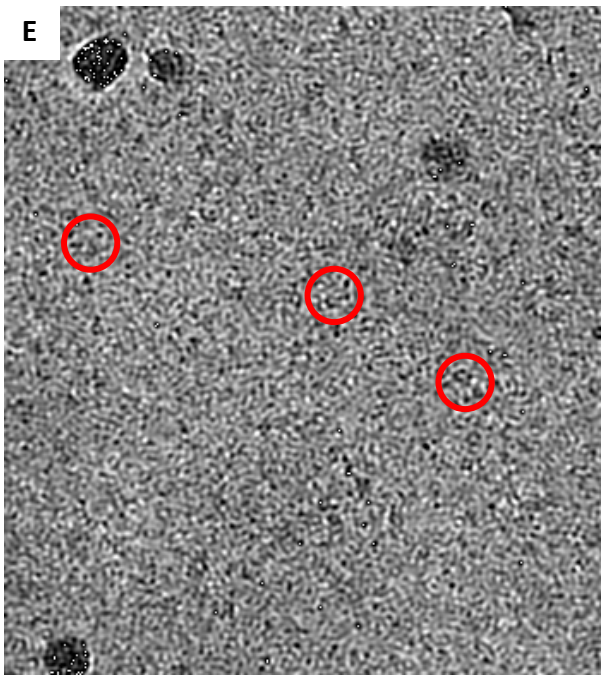


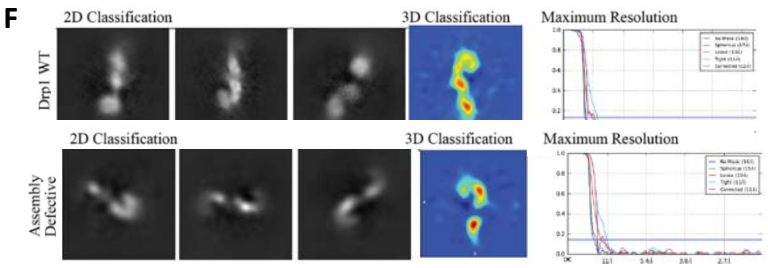


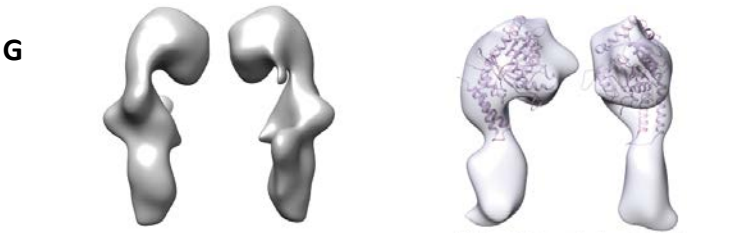




Using cryo-EM, the WT and assembly defective mutant was imaged using a 200 kV Talos Arctica with a K2 detector. The resolution has not improved compared to the negative due to several suspected issues: 1) air-water interface challenges, 2) preferred orientation, and 3) heterogeneity within the dimer structure. For each sample, one million particles have been selected; however, through sorting and refinement, the heterogeneity results in only tens of thousands of particles contributing to the density. We suspect the only way to overcome this challenge is by collected thousands of micrographs. Below is a sample image from the micrographs (E), examples of the 2D class averages (F), and the ab initio structures (G).







WT: 31,478 particles
1.6M Particles Picked Excluded
3,613 micrographs

Assembly Defective: 58,433 particles
944,374 Particles Picked Excluded
1,657 micrographs