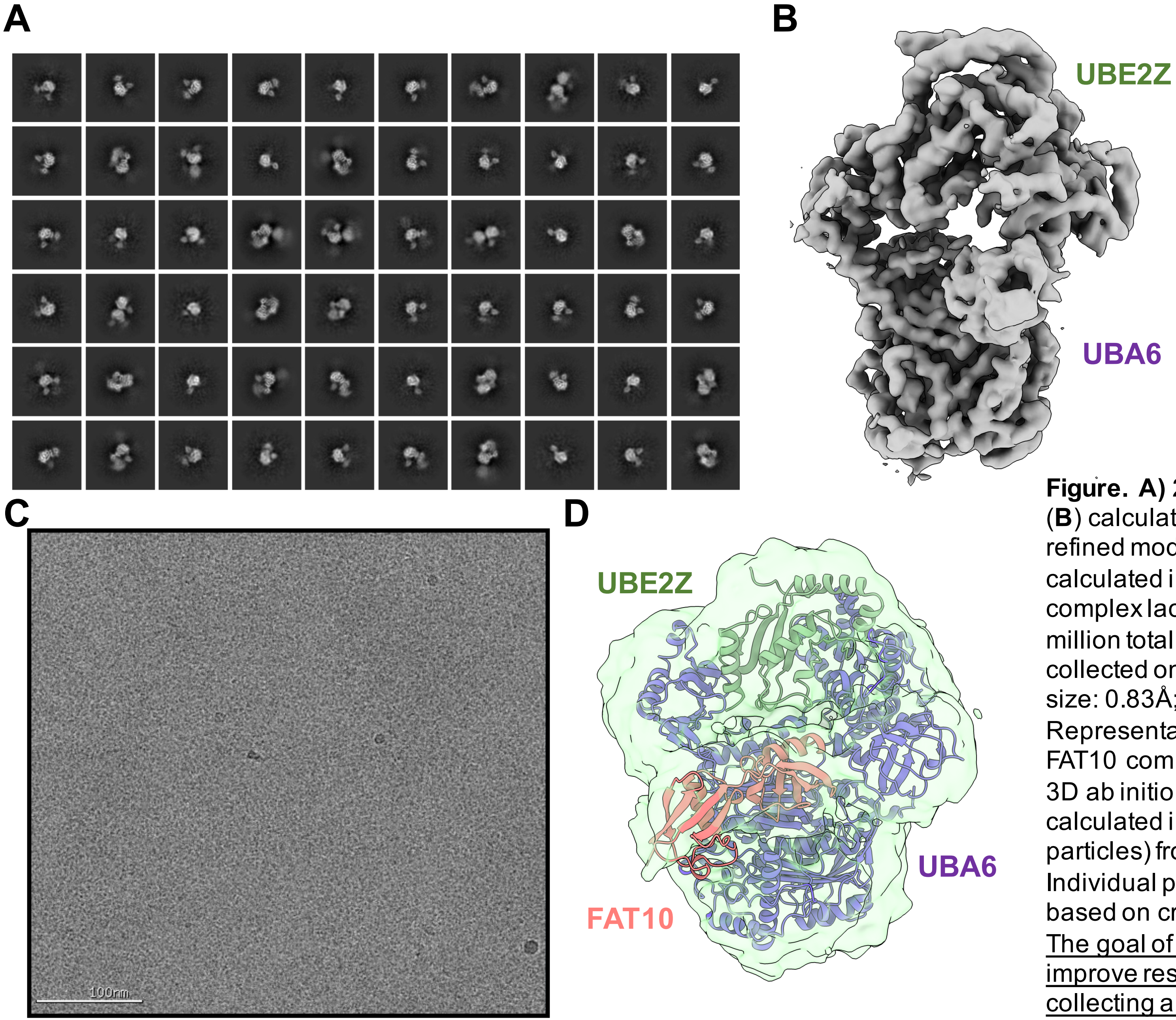

Figure. A) 2D classification from dataset in (**B**) calculated in Cryosparc. **B)** 3D ab initio / refined model (3.7Å, 77,973 particles) calculated in Cryosparc of UBA6-UBE2Z complex lacking FAT10, derived from 1.6 million total particles from 7,651 micrographs collected on a Titan Krios/Falcon 4 (Pixel size: 0.83Å; total dose: 56.14e^-/Å^2. **C)** Representative micrograph of UBA6-UBE2Z-FAT10 complex acquired on Glacios/K2. **D)** 3D ab initio / refined model of complex in (**C**) calculated in Cryosparc to 6.5Å (34,767 particles) from 110,030 total particles. Individual proteins are docked into density based on crystal structures of homologues. The goal of this GUP1 application is to improve resolution of this complex by collecting a 4-5 day dataset at 300 kV.