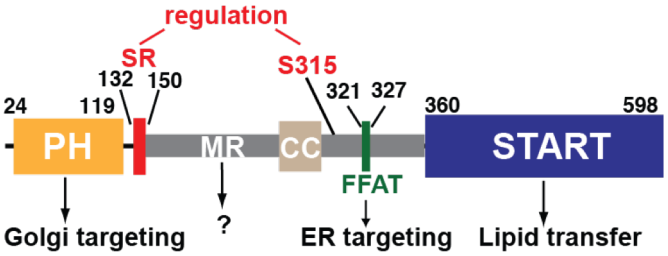

Figure 1. CERT domains and regions. The N-terminal pleckstrin homology (PH) domain is responsible for binding to phosphatidylinositol 4-phosphate (PI4P) that is enriched in the *Trans*-Golgi. Hyperphosphorylated of the serine repeat (SR) leads to inhibition of CERT. The C-terminal steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain carries our ceramide transfer. Between the SR and the START domain is a ~ 200-residue region named the middle region (MR), which is predicted to contain a 35-residue coiled-coil (CC). The MR also harbors a short FFAT (two phenylalanine in an acidic tract) motif that associates with an ER-resident type II membrane protein, vesicle-associated membrane protein (VAMP)-associated protein A (VAP-A)

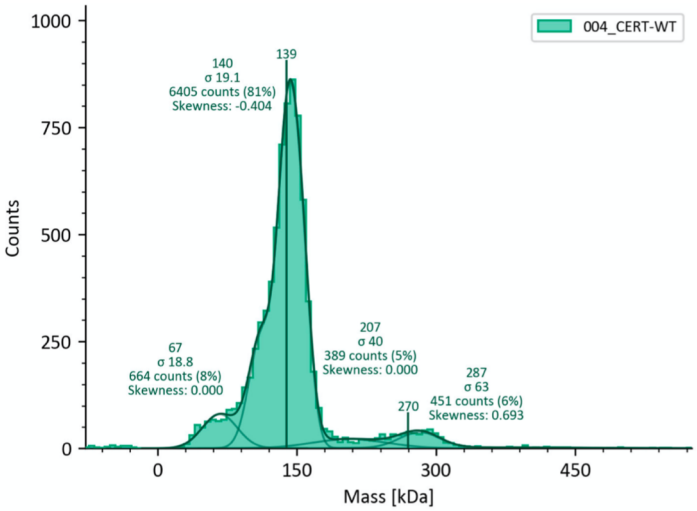

Figure 3. Mass photometry analysis of CERT protein. Mass photometry measures single particle size and distribution in solution. This result indicates 80% of the species has a molecular weight of 140 kDa, which is consistent with a dimer of the 68 kDa CERT protein.

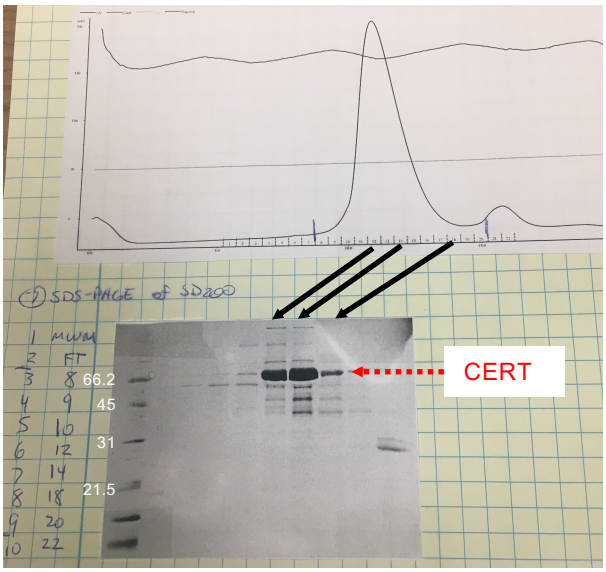

Figure 2. A representative trace of size exclusion chromatography (24 ml SD200) of CERT protein with the SDS-PAGE gel of some fractions. Only fractions in the center of the peak are collected. CERT protein is 68 kDa. Mass spectrometry analysis indicate the lower bands underneath the main peak are degradations.

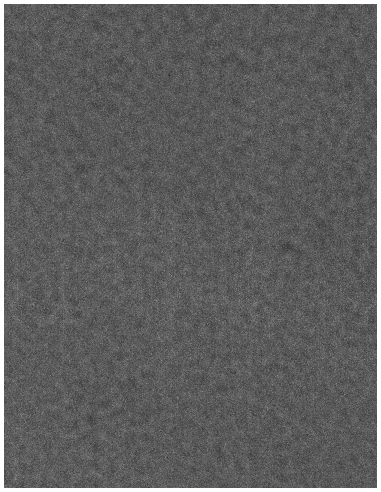

Figure 4. Preliminary cryo-EM image of CERT.