GUP3 proposal- ASCC3 preliminary data

So far, we have been able to express and purify two ASCC3 constructs using Sf9 insect cells and baculovirus expression system. The figure below shows the large-scale purification of ASCC3_cb014 (aa 401-2022). During the purification of ASCC3_cb014, after IMAC (Immobilized Metal Affinity Chromatography) on Ni2+ Resin, the elution fraction was concentrated and applied to high-resolution gel filtration using a Superdex 200 Increase 10/300 column, which has improved protein homogeneity and reduced protein aggregates.

Samples were plunge-frozen in a VitroBot onto UltrAUfoil R1.2/1.3, 300 mesh grids, and screened with a 200 kV Glacios microscope equipped with a Falcon 3 camera. Initial data was collected on a Titan Krios microscope equipped with a Gatan K3 direct detector. Data processed with CryoSPARK already show promising results for the 2D classes and initial refinements.

Construct informations

ASSC3A_cb014 (His401-Lys2202), with His-Flag C-terminal tag. Expected Mass with Tag: 209 KDa

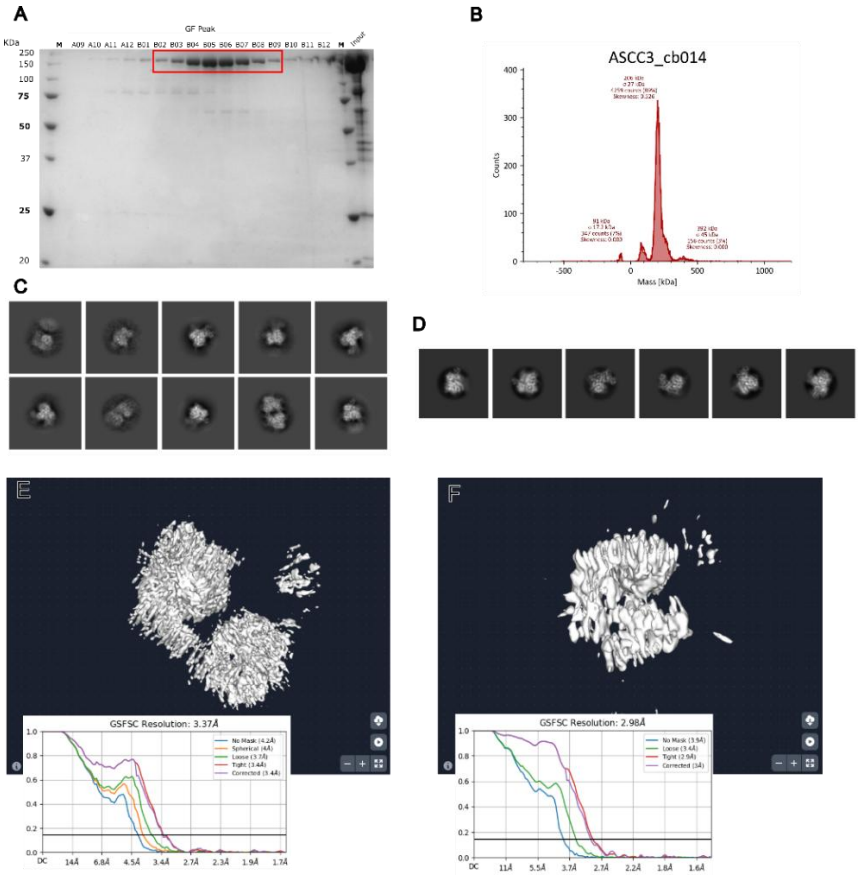

Figure 1: ASCC3 sample preparation and preliminary data analysis. High-resolution gel filtration (GF) of ASCC3 fractions, the peak was analyzed by SDS-PAGE (A). ReFeyn Mass photometry homogeneity analysis, with an 89% of particles at the expected MW (206 kDa) (B). (C-D) 2D classification of data collected from 2 different grids at Titan Krios, (C) show ASCC3 bounded to one of the inhibitors (a fragment), and (D) ASCC3 without inhibitor.