GUP1 proposal- LKR preliminary data

So far, we have been able to express and purify LKR constructs using Sf9 insect cells and baculovirus expression system. The figure below shows the large-scale purification of LKR-cb004. During the purification of LKR-cb004, after affinity chromatografy on Glutathione Sepharose 4B, 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 Quantifoil cu-carbon 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 same. Data processed with CryoSPARK already show promising results for the 2D classes and initial refinements, with resolutions up to 7.57 Å, even with a small dataset.

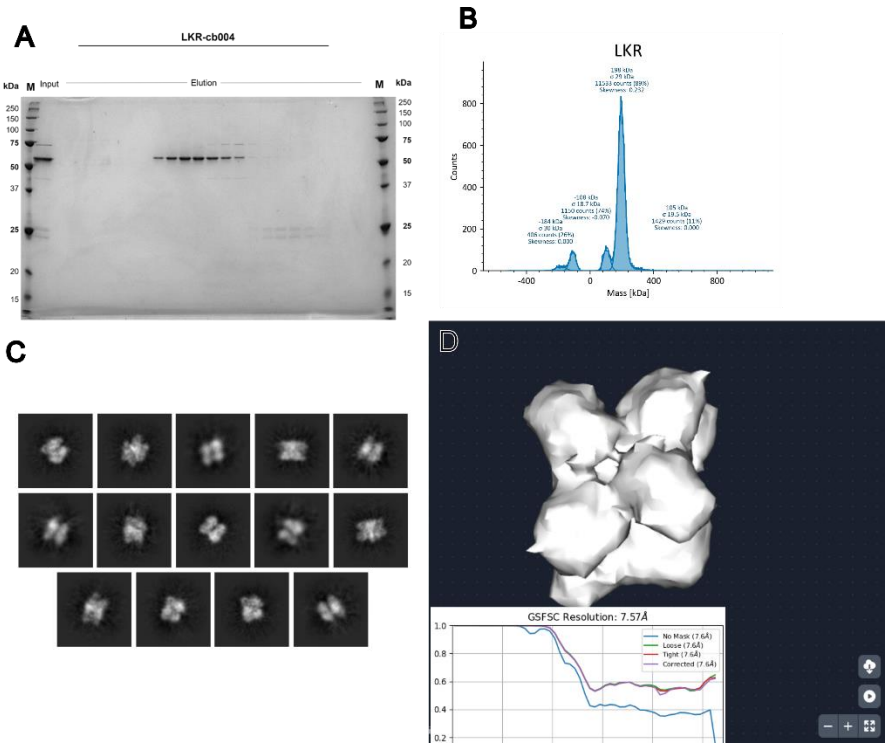

Figure 1: LKR-cb004 sample preparation and preliminary data analysis. High-resolution gel filtration (GF) of LKR fractions, the peak was analyzed by SDS-PAGE (A). ReFeyn Mass photometry homogeneity analysis, with an 89% of particles at the expected MW for the tetramer (approx. 200 kDa) (B). (C-D) 2D classification of data collected from at Glacios, (C) shows LKR homotetramer structure as predicted.