

### **Protein Purification of TAX-4 in 5mM EGTA**

TAX-4 WT proteins are expressed in Sf9 cells and extracted using detergent and packed into nanodisc. Gel filtration and SDS-PAGE analysis indicate that the protein is good enough for cryo-EM study.

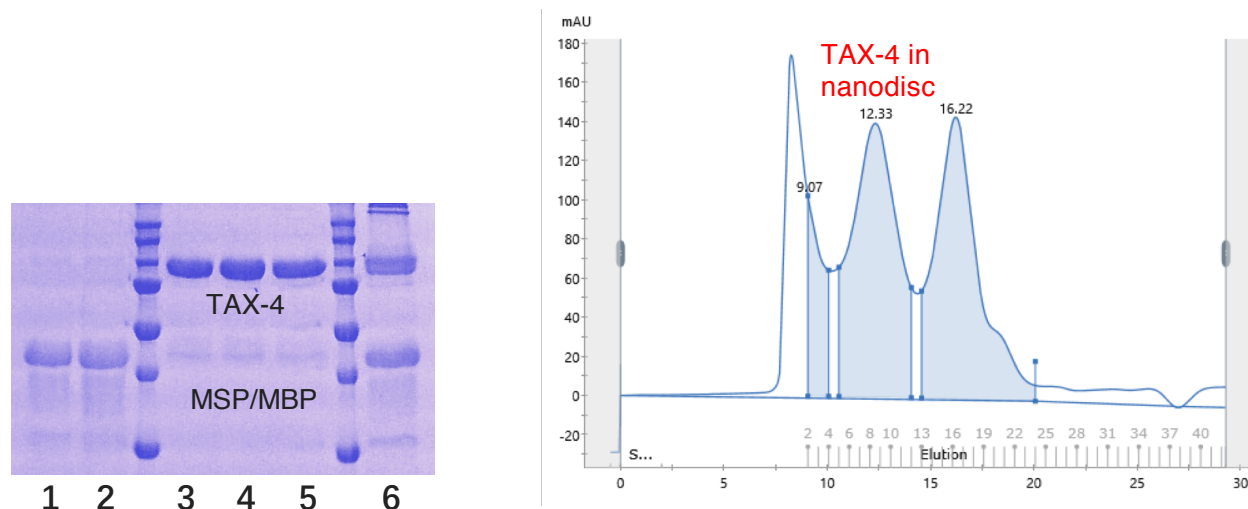


Fig. 1. Gel filtration and SDS-PAGE analysis of TAX-4 WT with EGTA.

### **Image acquisition on F20 and processing for TAX-4 WT in 5mM EGTA with cGMP**

I prepared cryo-grids using vitrobot for TAX-4 WT with added cGMP to achieve open state. A 460-micrograph dataset was collected using F20 microscope with a 3Å pixel size. After image processing using Relion and cryoSPARC, I got very promising 2D classification result. Representative views showed clear C4 symmetry. After 3D refinement, I get a 6.14Å structure due to the pixel size of the microscope and limited number of micrographs.

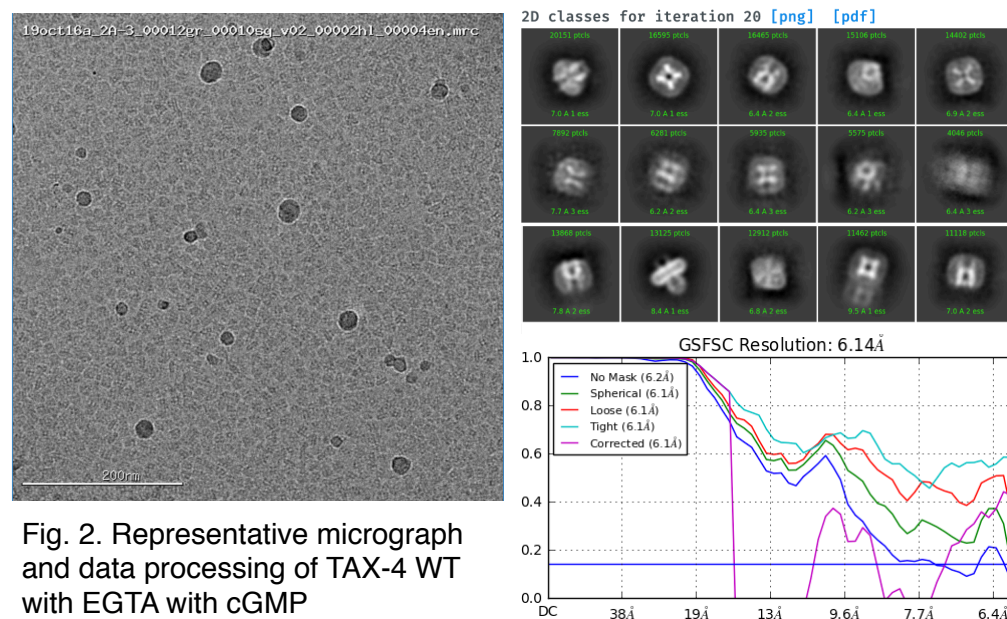


Fig. 2. Representative micrograph and data processing of TAX-4 WT with EGTA with cGMP