

Supplementary Information

Protein purification and nanodisc reconstitution for FL-mTRPML2 apo and agonist-bound sample

FL-mTRPML2 protein was expressed in HEK293 cells. Protein was purified with detergent and reconstituted into nanodisc with MSP2N2 protein. Gel filtration and SDS-PAGE results indicate that apo TRPML2 sample is homogenous and good enough for cryo-EM study (Fig. 1). Agonist-bound samples were generated by incubating the protein with ML-SA1 and PIP2 after the protein is concentrated.

Image acquisition and processing for Trun-TRPML2 apo and agonist-bound states

We prepared cryogenic grids using vitrobot machine (FEI) for FL-mTRPML2 apo and agonist-bound samples. With apo mTRPML2, we were able to get 2.93 Å and 2.98 Å resolution structures on Krios microscope for each mML2 apo with pi-helix on S6 and alpha-helix on S6 (Fig. 2). Two different apo states of mTRPML2 show backbone structure difference on their S6 and different gate residue. With ML-SA1 added mTRPML2, we collected a 576

micrograph dataset on Glacios microscope. After image processing using cryoSPARC, we got very promising 2D classification result for ML-SA1 added sample. Representative views from 2D classification showed clear 4-fold symmetry feature and no preferred orientation. (Fig. 3b). Due to the small data set, we got an only one class of mTRPML2 map to 5.71 Å resolution (Fig. 3c). With a larger data set, it is expected to have several classes of map with pi and alpha-helix on S6 and even the open state.

Grid availability and session request

We have grids for both ML-SA1 and PIP2 added mTRPML2. They are available for data collection on Titan Krios. For session request, we would like to apply for 2 sessions with 24 hours for each session.

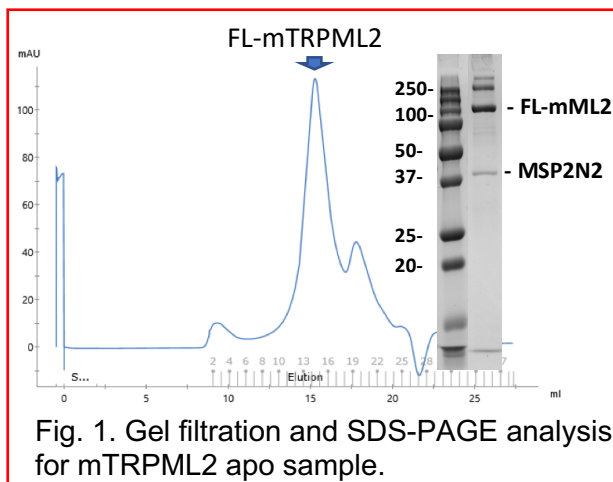


Fig. 1. Gel filtration and SDS-PAGE analysis for mTRPML2 apo sample.

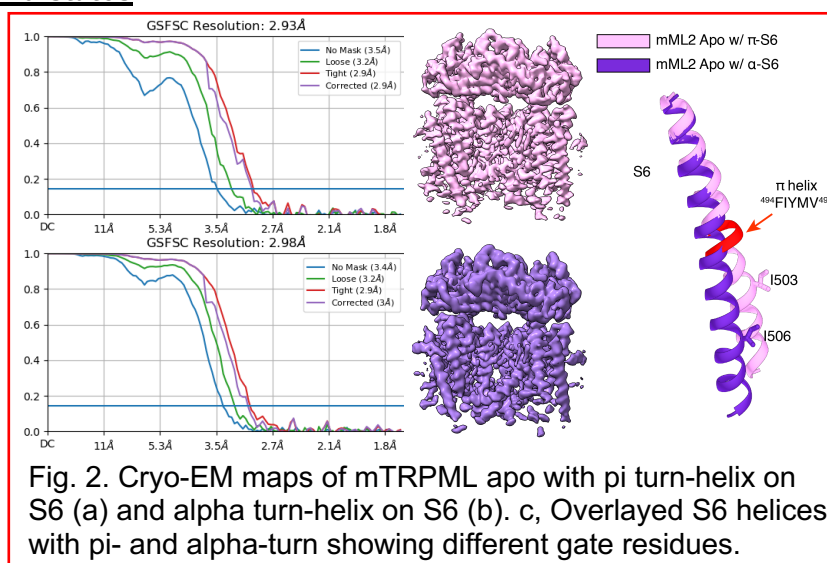


Fig. 2. Cryo-EM maps of mTRPML2 apo with pi turn-helix on S6 (a) and alpha turn-helix on S6 (b). c, Overlaid S6 helices with pi- and alpha-turn showing different gate residues.

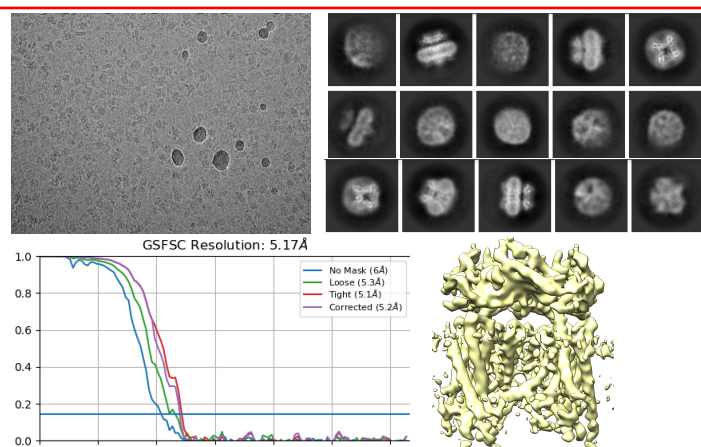


Fig. 3. Representative micrograph and data processing for ML-SA1 added mTRPML2