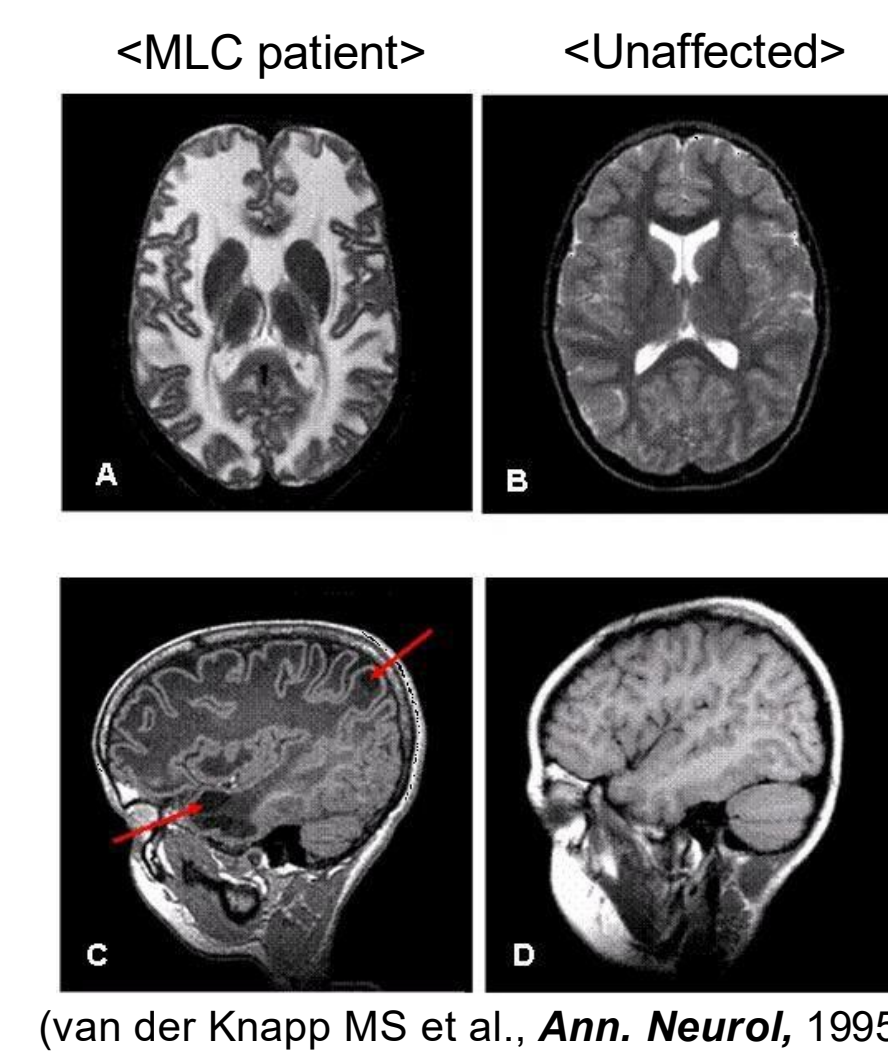


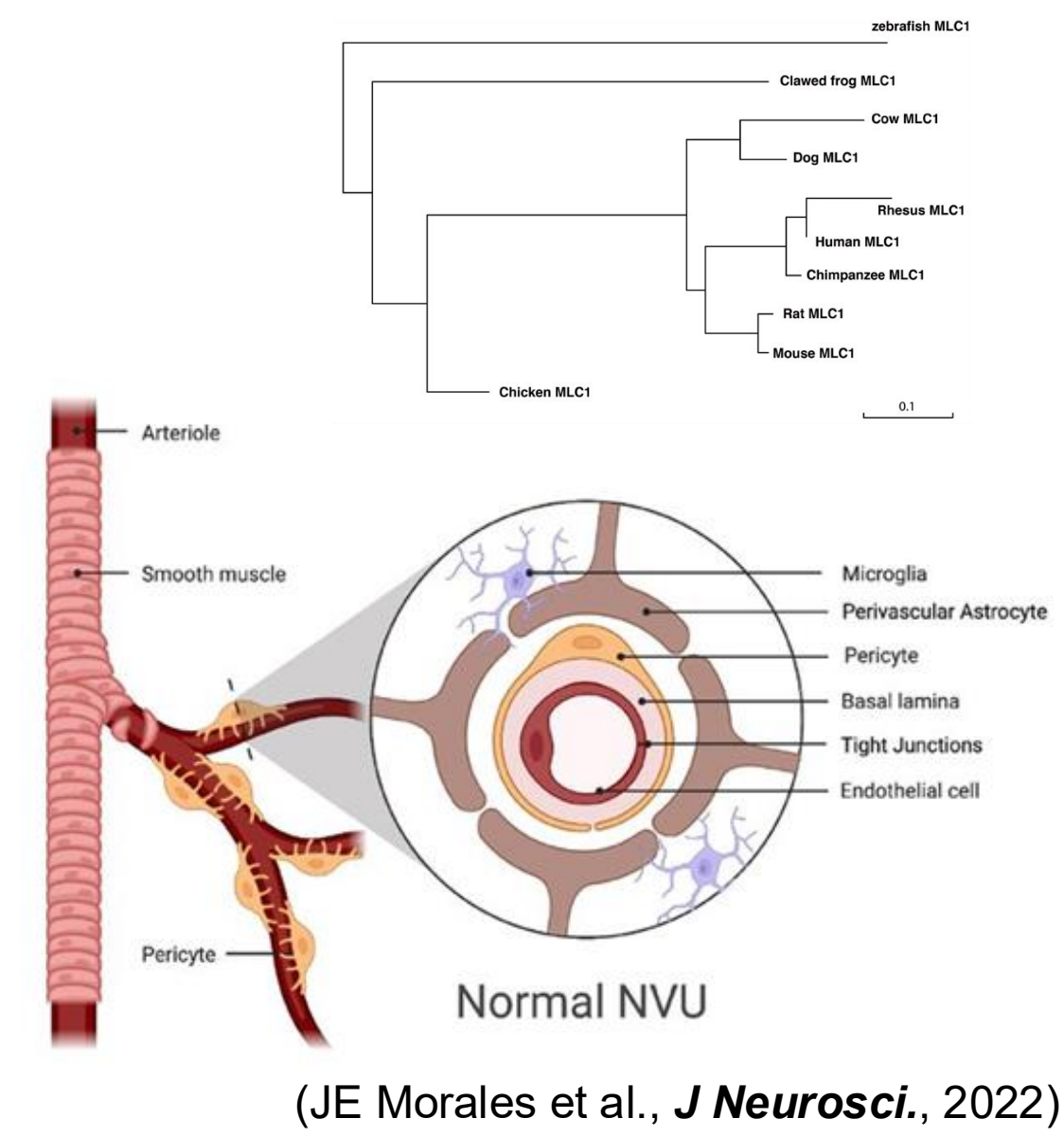
MLC1 is a membrane protein with multi-transmembrane domains and is mainly expressed in astrocytes. Genetic mutants on Mlc1 are associated with an early-onset rare genetic neurological disease called Megalencephalic Leukoencephalopathy with Subcortical Cysts (MLC), which leads to progressive neurodegeneration. MLC typically presents with infantile macrocephaly, followed by progressive gait disturbances and cognitive impairment. However, despite its clinical importance, the structure and function of MLC1 remain poorly understood. In our previous study, Wild-type MLC1 localized to the plasma membrane, altered cell morphology and motility, and increased filopodia about threefold compared to GFP controls. In contrast, pathogenic mutants such as S280L and P92S failed to induce these effects. And we showed that wild-type MLC1 efficiently localizes to the plasma membrane, where it induces marked changes in cell morphology and motility, in contrast, pathogenic mutants remain trapped within the cell and are functionally inactive. Structural and biochemical analyses, including epitope mapping, cross-linking, electron microscopy, and single-molecule photobleaching, further revealed that MLC1 adopts an eight-transmembrane topology and assembles into oligomers.

To gain structural insights, we performed cryo-EM analysis of human MLC1. The protein was expressed in HEK293S GnT1⁻ cells, purified under optimized conditions, and imaged by cryo-EM. Although a ~3.3 Å dataset was obtained, preferred particle orientations limited high-resolution reconstruction. To address this issue, we tested strategies to improve orientation distribution, including the addition of trace CTAB to LMNG micelles and reconstitution into lipid nanodiscs, both of which enhanced particle orientation diversity and image quality.

MLC, Megalencephalic Leukoencephalopathy with subcortical Cysts



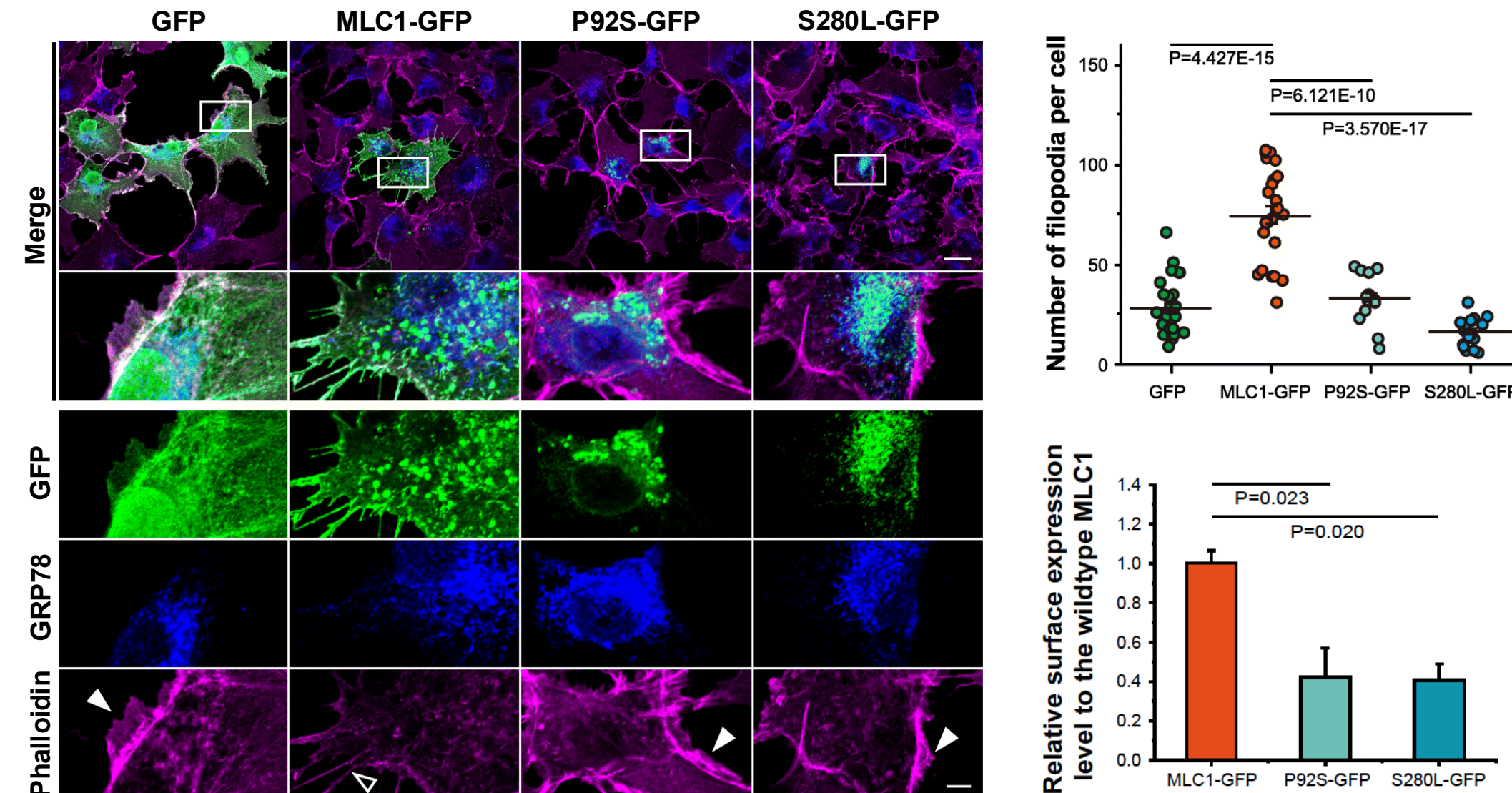
- Early childhood** onset with progressive neurological decline, prevalence: ~1/500,000^{UK}, ~1/1,000,000^{Japan}.
- Macrocephaly (enlarged brain), diffuse white matter swelling, subcortical cysts, myelin vacuolation and atrophy.
- Slow deterioration of motor functions, with **ataxia** (difficulty coordinating movements) and **spasticity** (stiffness of muscles and muscle spasms), **seizures** (well-controlled with medication).



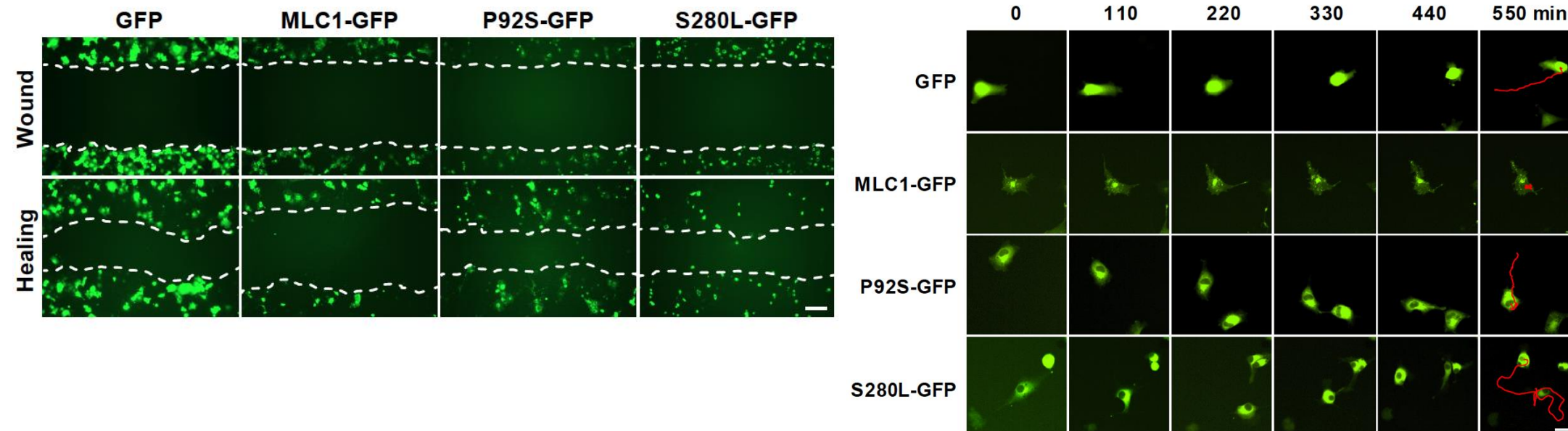
Cellular Function and Architecture of MLC1

I. MLC1 regulates cellular morphology and motility

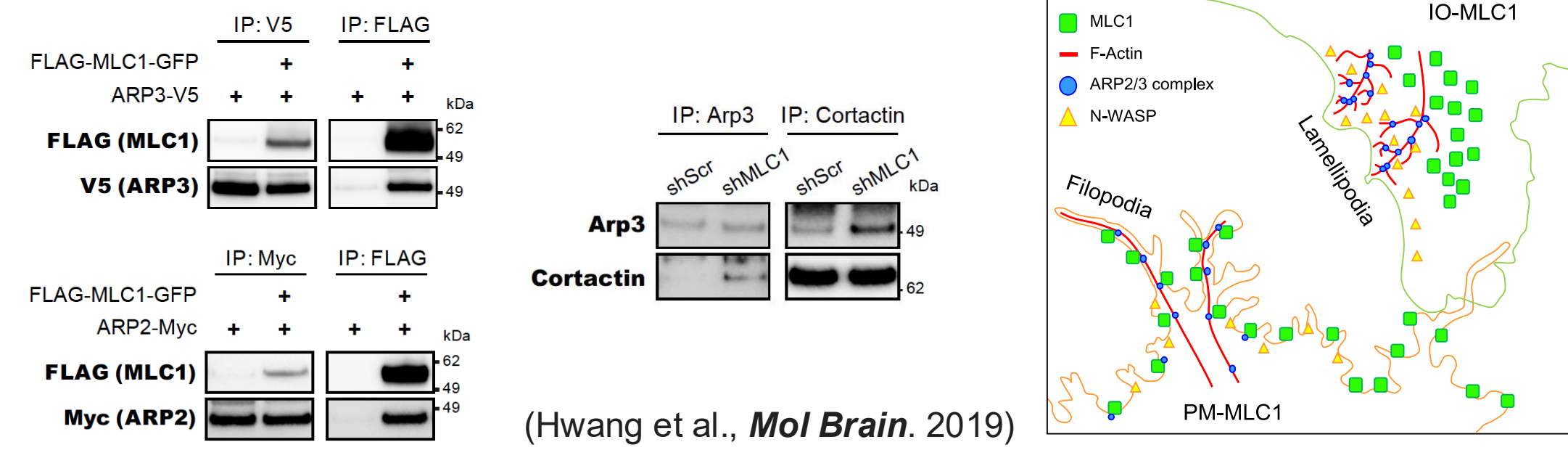
I-1. PM expression of MLC1 induces filopodia protrusion



I-2. MLC1 expression greatly reduces cellular motility

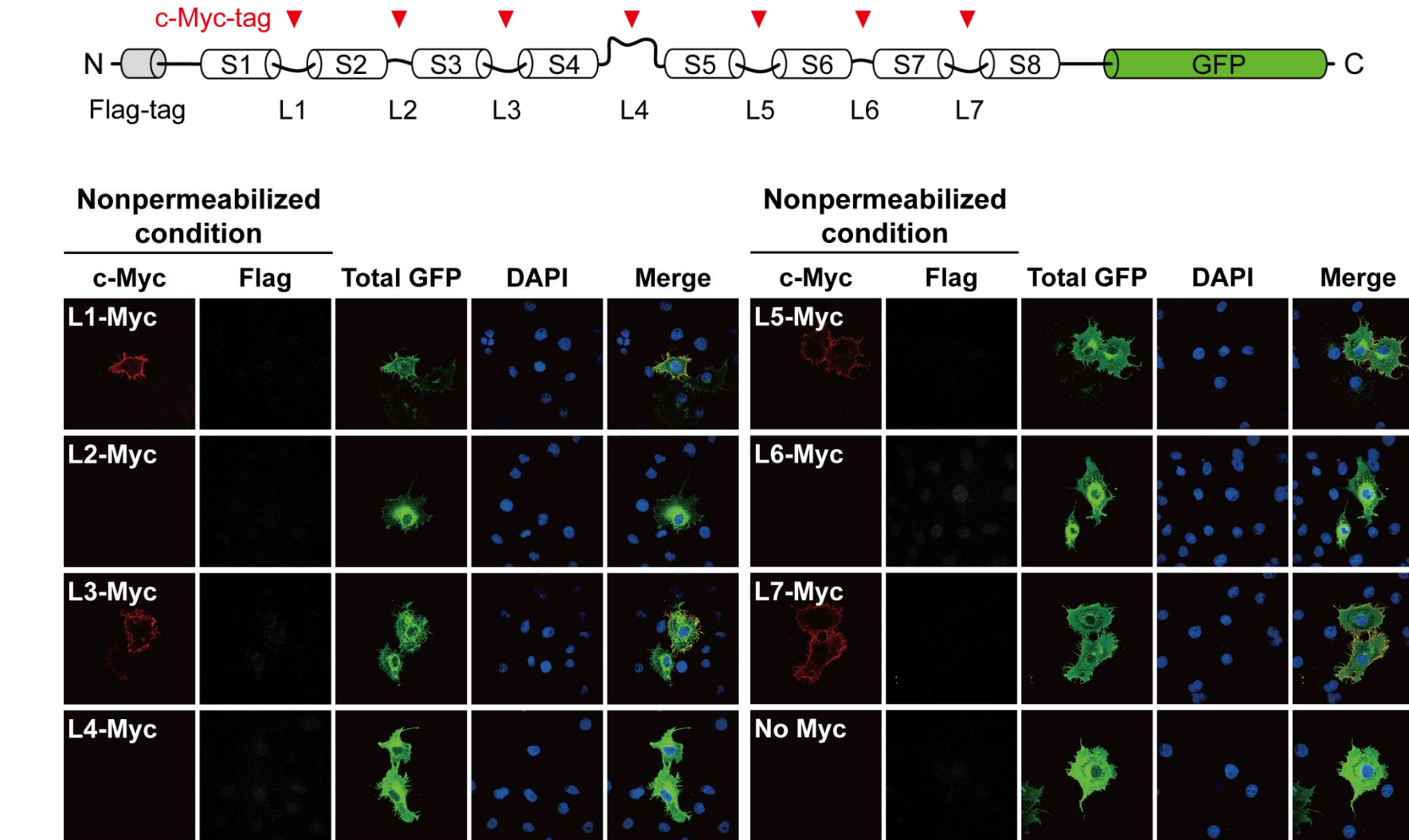


I-3. MLC1 may regulate actin dynamics via ARP2/3

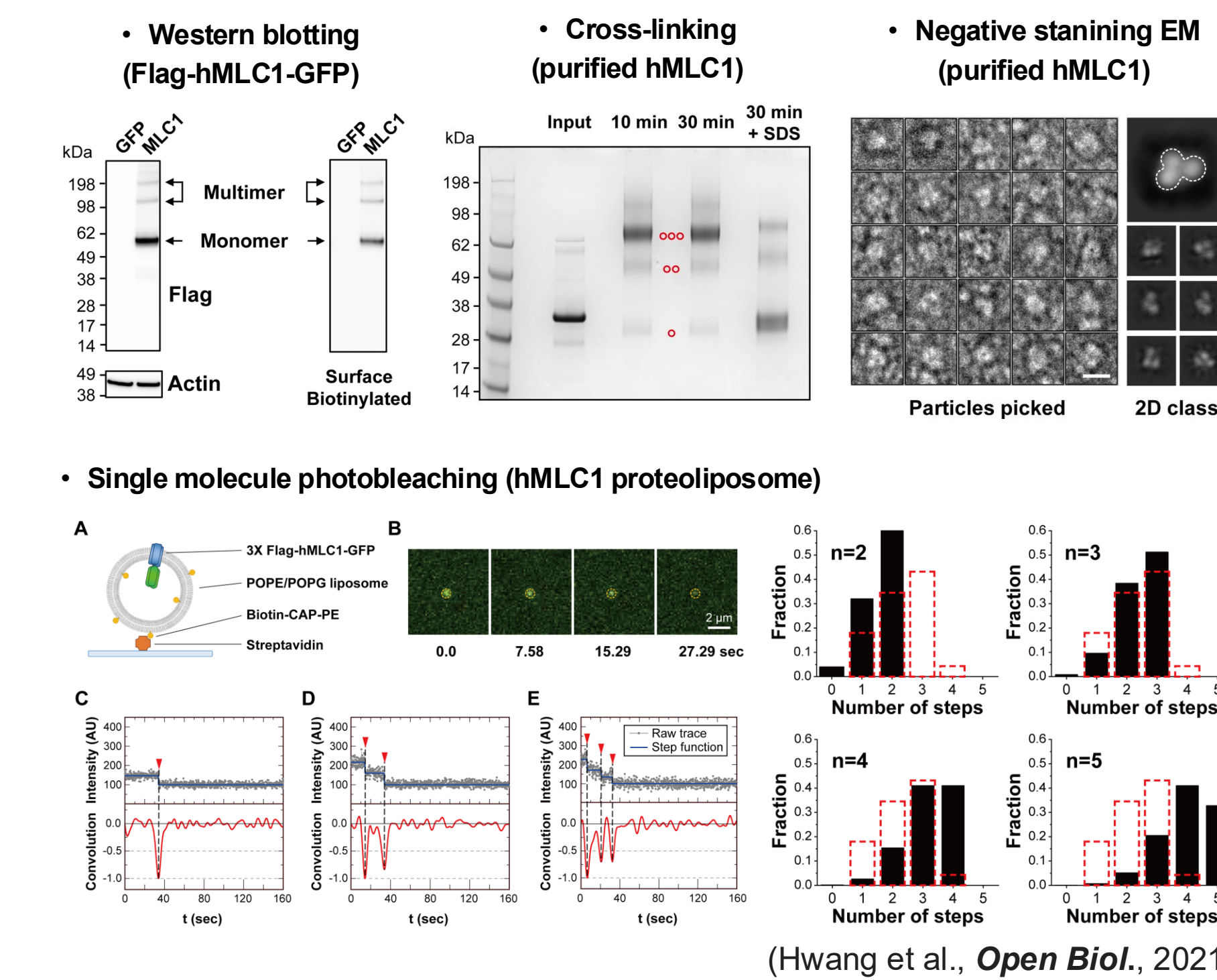


II. TM topology and oligomeric state

II-1. MLC1 is an eight-TM membrane protein



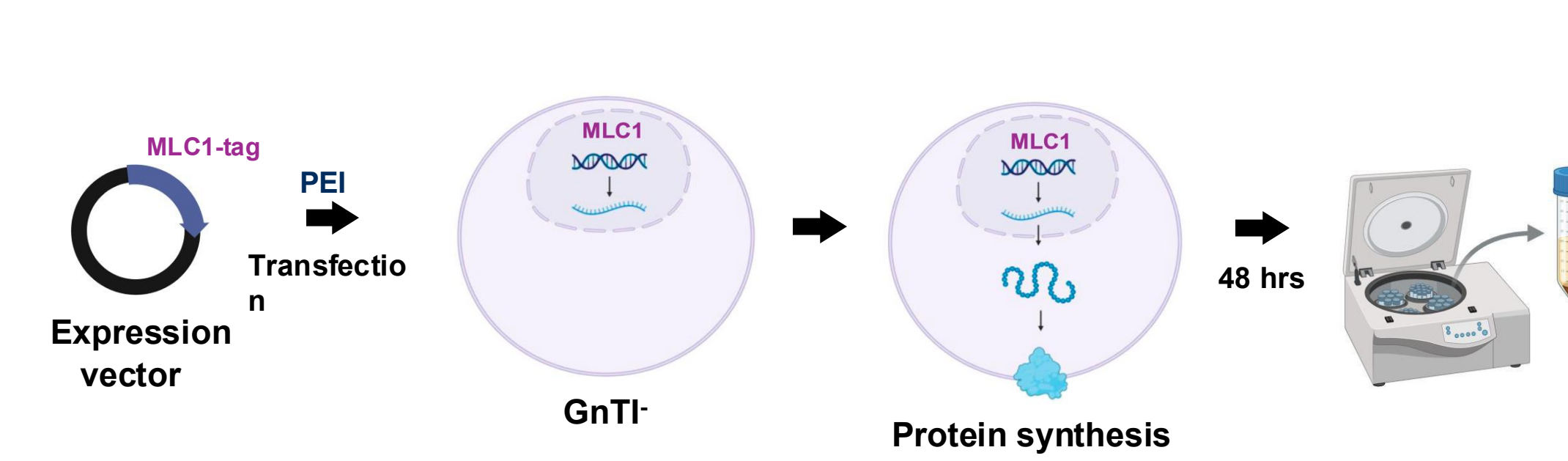
II-2. Homotrimeric assembly



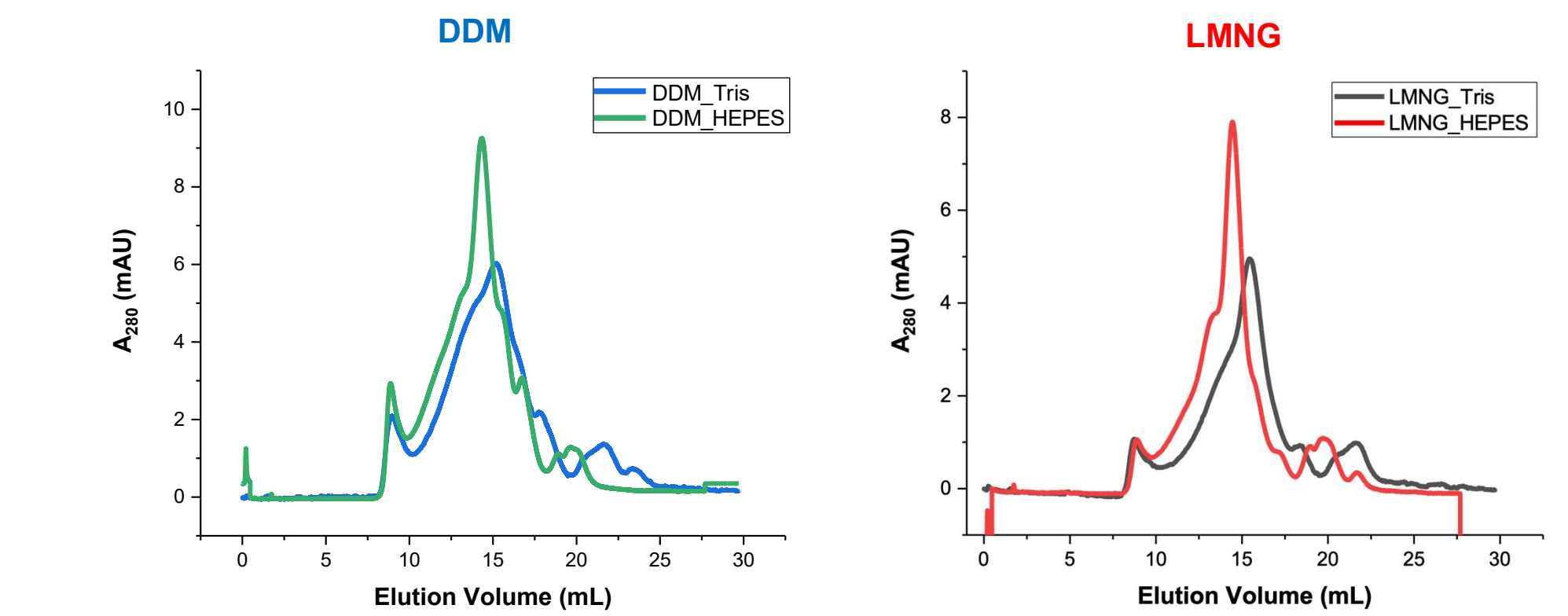
Cryo-EM Trials for Determination of MLC1 Structure

I. Expression and purification of MLC1 in HEK293 GnT1⁻ cells

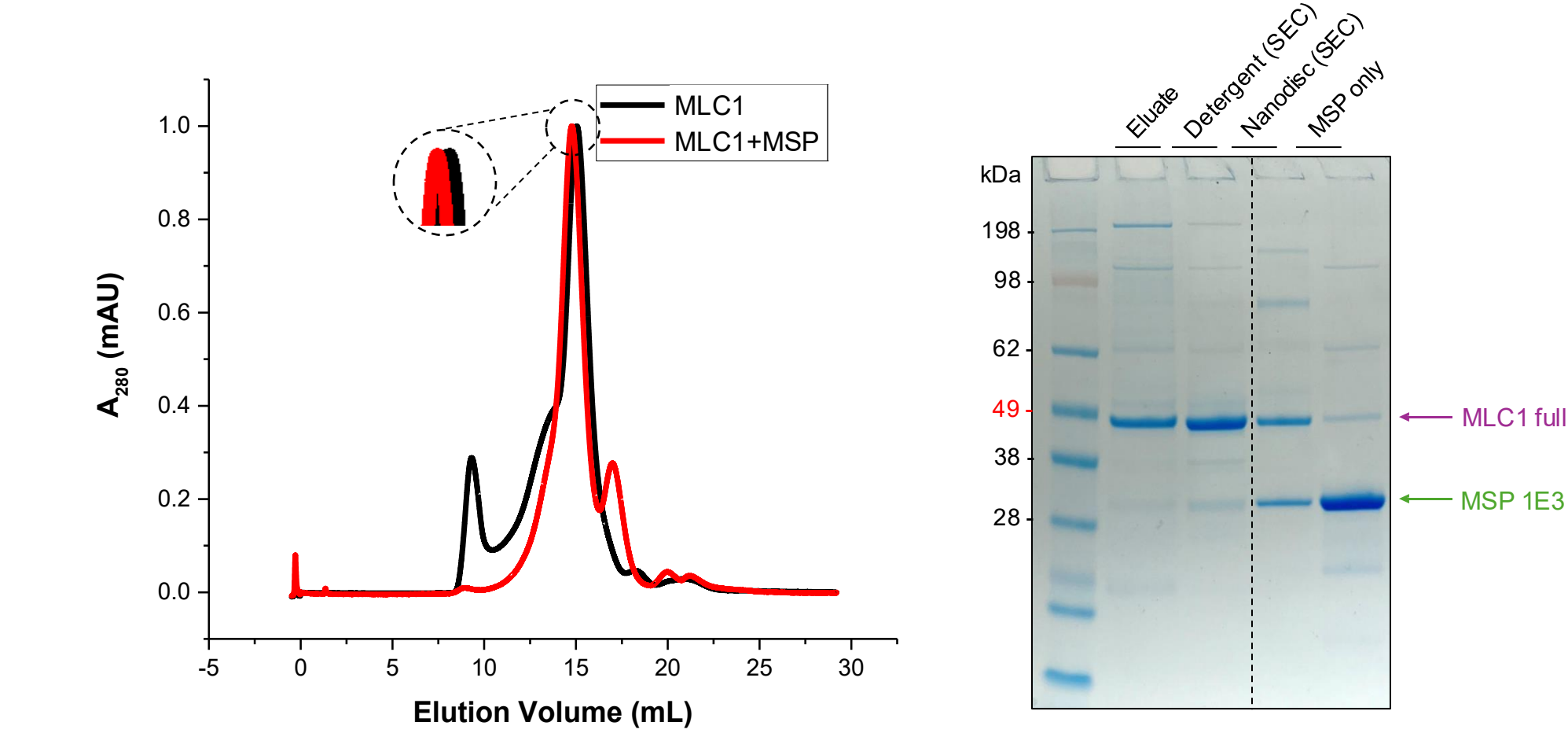
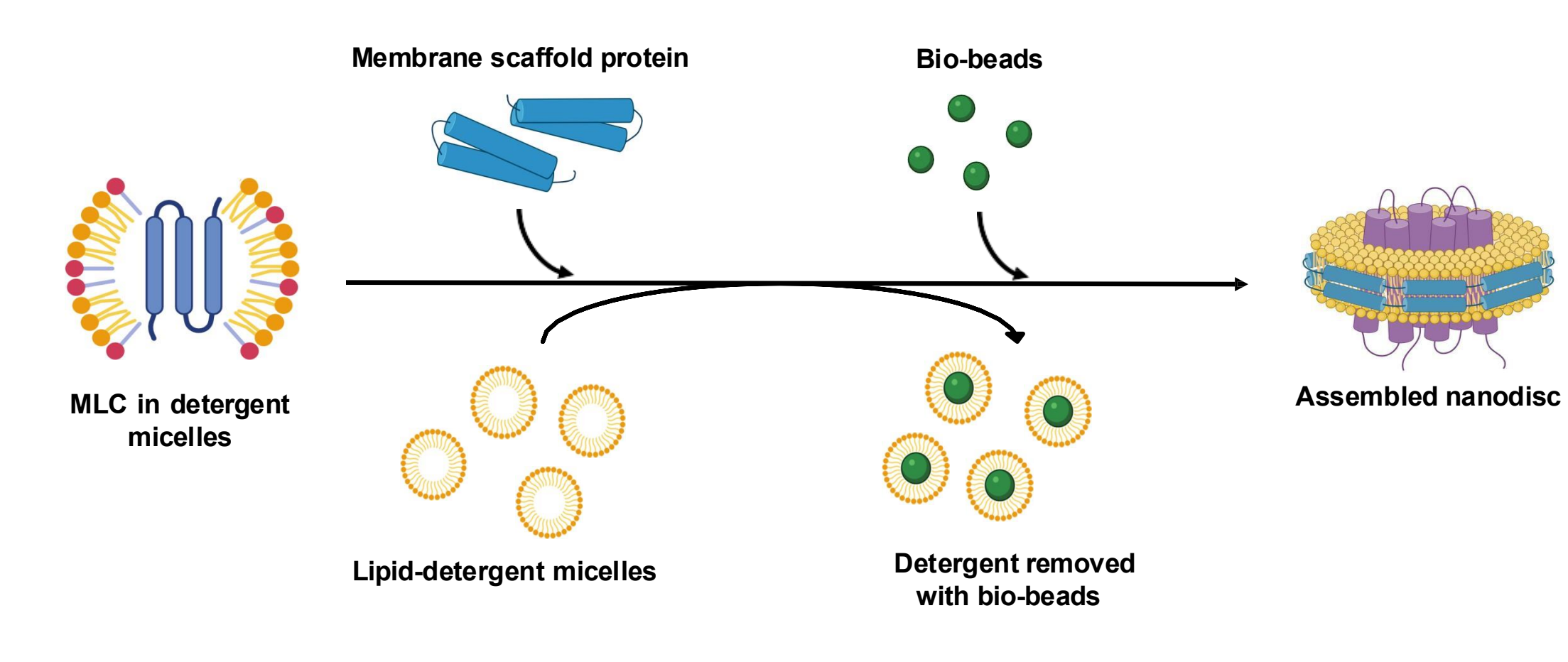
* Expression in mammalian cell line



* Buffer and detergent screening

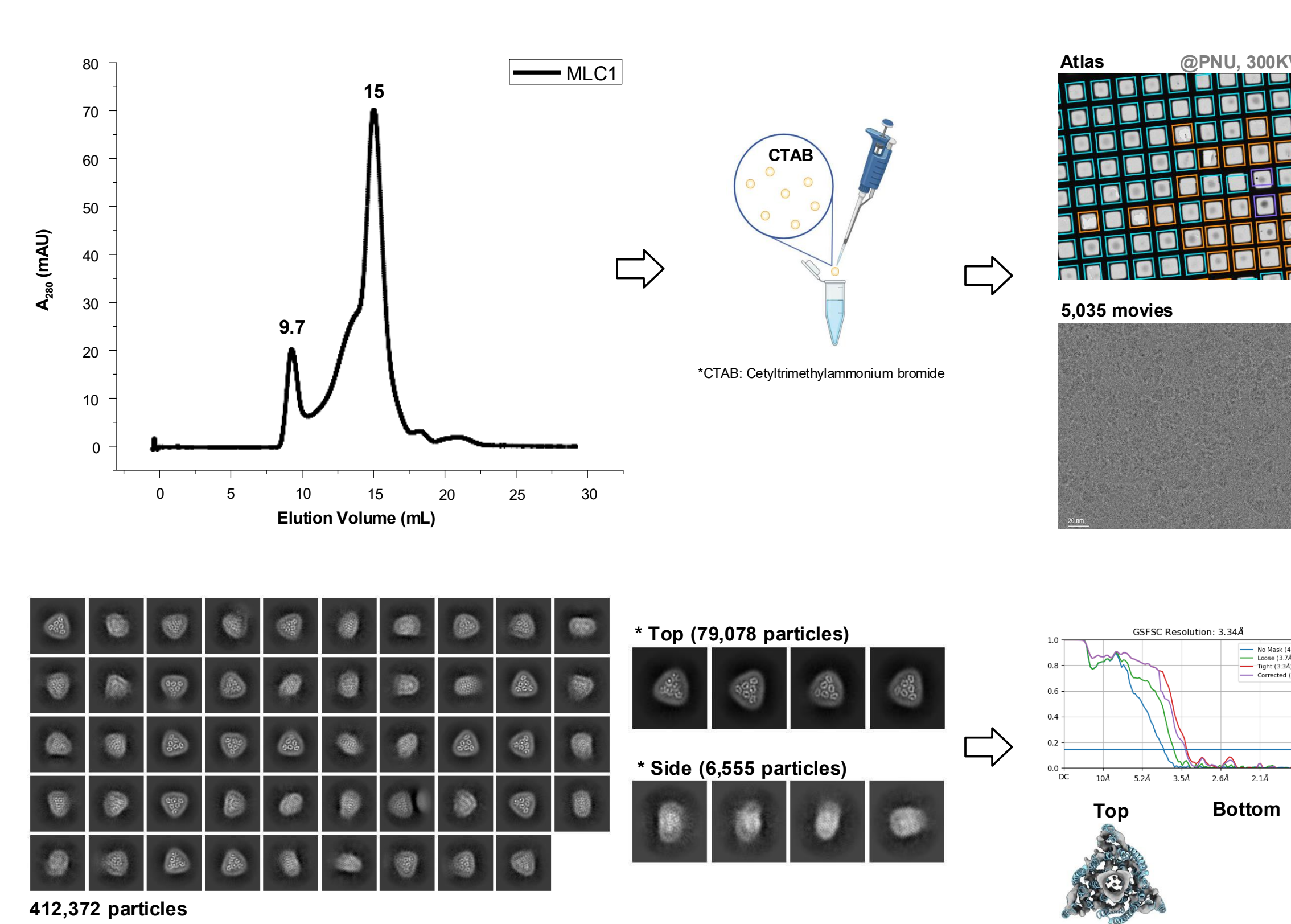


II. Nanodisc reconstitution for mimicking membrane environment

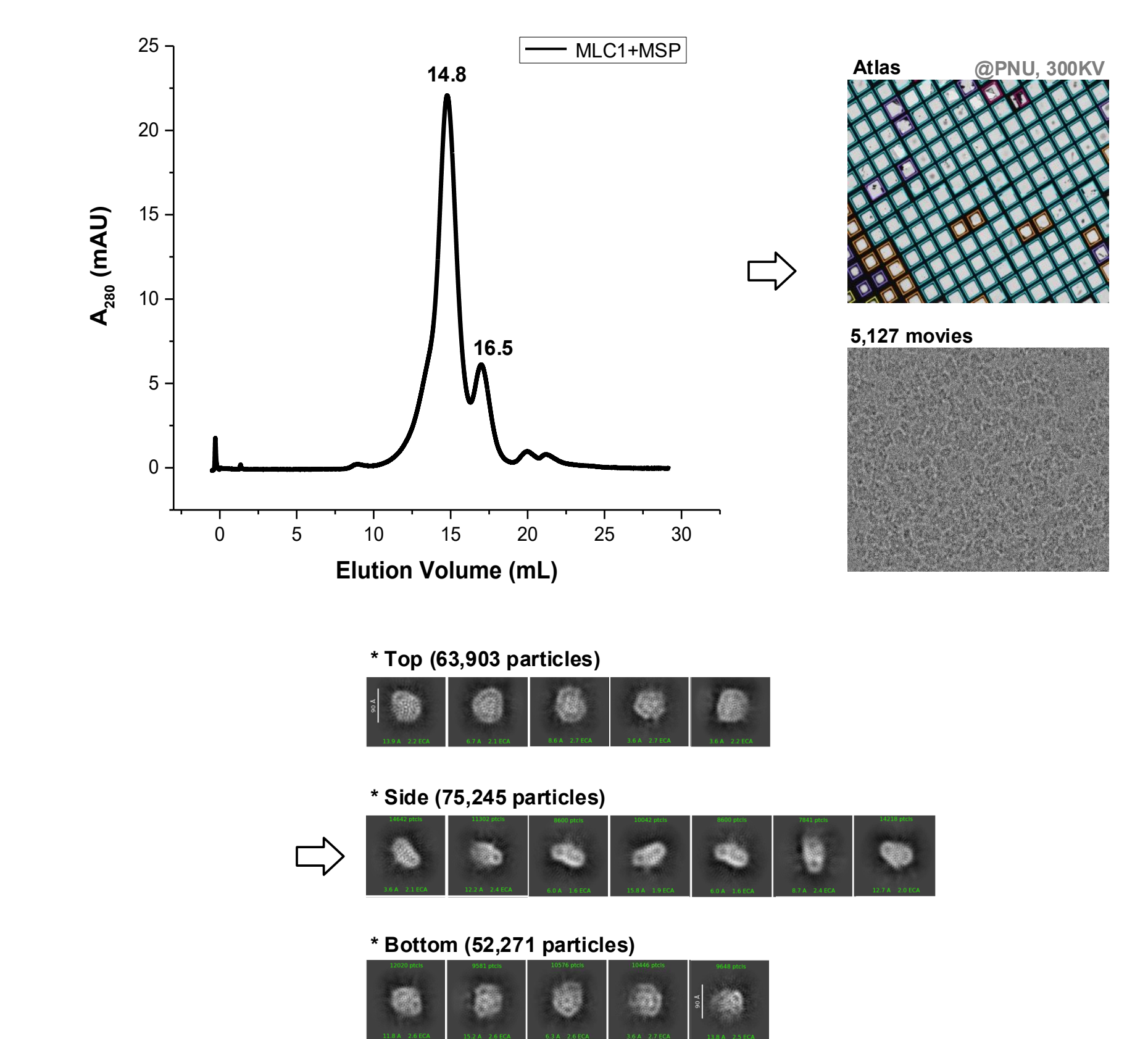


III. Cryo-EM screening and preliminary data analysis

* Detergent-solubilized MLC1



* Nanodisc-reconstituted MLC1



Summary and on-going research

To investigate the structural characteristics of the MLC1 protein, we expressed the protein in a mammalian cell line and systematically optimized the purification conditions. However, cryo-EM imaging of purified MLC1 showed preferred orientation, which limited high resolution structural determination. To overcome preferred orientation issues, we have tested additive treatment (varying concentration of CTAB) and nanodisc reconstitution. These approaches showed a possibility of sample a wider variety of particle orientations, but it was not enough to determine the atomic resolution structure of the MLC1 protein. We are currently optimizing conditions for obtaining additional views, particularly the side view of the protein, to achieve a more complete and detailed understanding of its structural architecture.