



Figure 1. a) Expression test results. FSEC (fluorescence size exclusion chromatography) profile of rod CNG (eGFP tagged CNGA1/CNGB1 heterotetramer). Profiles of CNGA1 homotetramer and rod CNG heterotetramer are presented in blue and orange, respectively. b) Anti-FLAG tag western blot result. Anti-flag-HRP ab is used. c) Cryo-EM structures of CNGA1 (homotetramer) in lipid nanodiscs. We observed closed, intermediate, and open states. d–e) Western blot and purification results of Olfactory CNG heterotetramer (CNGA2/CNGA4/CNGB1). f) Cryo-EM structures of olfactory CNG channels in detergent. CNGA2, CNGA4, and CNGB1 subunits are colored in grey, blue, and red, respectively. g) SthK is cold-activated in POPE lipids but not in anionic lipids (POPA, CL, POPG). h) Cryo-EM structure of SthK in PE-containing nanodiscs reveals a PE lipid near the state-dependent intersubunit salt bridge. i) Previously reported cryo-EM structures of TRPM8, highlighting a state-dependent salt bridge at the water–lipid interface in the open (PDB: 8E4L) and closed (PDB: 8E4P) states. j) Size-exclusion chromatography and SDS-PAGE of purified mouse TRPM8. k) Single-channel recordings of TRPM8 showing activity in the presence of PIP₂ and menthol. l) Representative micrograph of BK proteoliposomes and 2D class averages obtained from initial data collection in Krios 300 kV microscope. m) Cryo-EM density maps of depolarized BK in proteoliposomes. Further dataset and processing are required for high resolution. n) SEC profile and SDS-PAGE results of purified BK-β2 complex. o) Representative micrograph of BK-β2 complex in detergent micelles under Ca²⁺ and EDTA conditions. Scale bar, 50 nm. And Ab initio reconstruction models of the BK-β2 complex in micelles with Ca²⁺ and EDTA, respectively.