

Supplementary Information

Protein expression and purification of native CNGA3:CNGB3 and CNGA3:CNGB3-CaM complexes

Native CNGA3:CNGB3 and CNGA3:CNGB3-CaM complexes were heterologously expressed in HEK293F cells using the BacMaM expression system (Thermo Fisher Scientific). The

CNGA3:CNGB3 and CNGA3:CNGB3-CaM complexes were purified with good purity in a detergent condition (Fig. 1b and 2b). SDS-PAGE analysis shows the stoichiometry between CNGA3 and CNGB3 in both purified CNGA3:CNGB3 and CNGA3:CNGB3-CaM complexes was roughly 3:1 (Fig. 1b and 2b), indicating that these two complexes were properly assembled under our conditions.

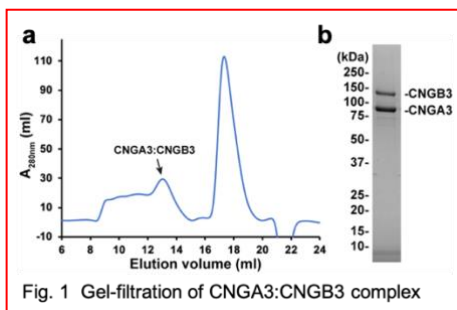


Fig. 1 Gel-filtration of CNGA3:CNGB3 complex

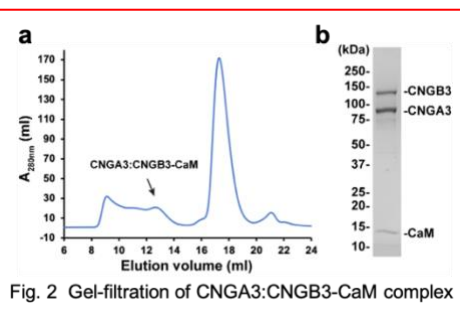


Fig. 2 Gel-filtration of CNGA3:CNGB3-CaM complex

Purified CNGA3:CNGB3 complexes are functional

To examine whether the purified CNGA3:CNGB3 complexes were functional, we reconstituted the purified protein into liposomes and performed single-channel recordings by patch-clamp. We observed robust cGMP-induced single-channel currents in many patches, as exemplified by the patch shown in Fig. 3. This result shows that the purified CNGA3:CNGB3 complex was functional. With this assurance, we prepared cryogenic grids with both purified CNGA3:CNGB3 and CNGA3:CNGB3-CaM samples.

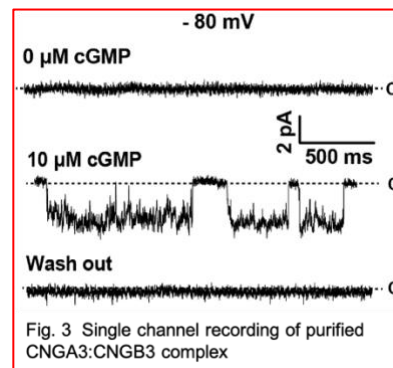


Fig. 3 Single channel recording of purified CNGA3:CNGB3 complex

Grid screening and data collection of CNGA3:CNGB3-CaM sample on a Glacios microscope

We made grids for the CNGA3:CNGB3-CaM sample first and screened them at NYSBC. A primary dataset of 1,326 micrographs were collected overnight on a Glacios microscope. A representative micrograph is shown in Fig. 4. After single particle analysis, we obtained a reasonable 2D classification with different orientations. The 3D map was reconstructed and refined to 7.5 angstrom (Fig. 4c). We expect a higher resolution map for the CNGA3:CNGB3-CaM sample, if enough micrographs can be collected.

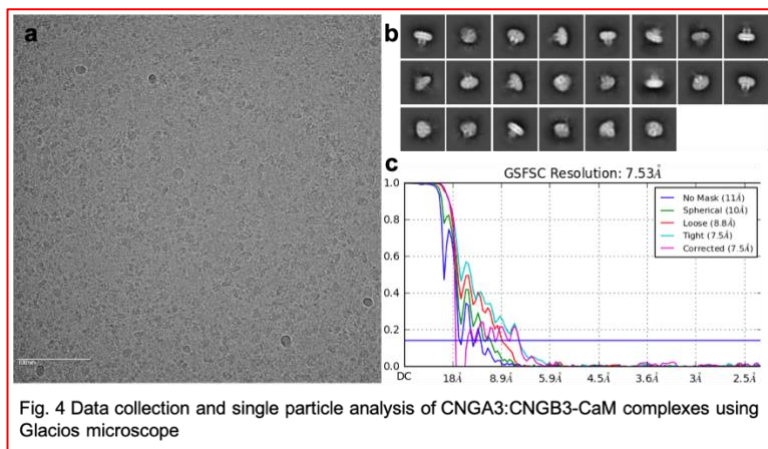


Fig. 4 Data collection and single particle analysis of CNGA3:CNGB3-CaM complexes using Glacios microscope