

Fig. 1. CRAC Channel activation and structural information of Orai and STIM. X-ray and cryo-EM structures of Orai from our previous studies as well as the minimal CRAC channel activation domain (CAD) from human STIM1 are shown as cartoons. Biophysical and cellular studies suggest that CAD folds back with CC1s at the resting state and exposes to the cytosol upon store depletion. Question marks and dotted arrows highlight the unaddressed questions regarding channel activation.

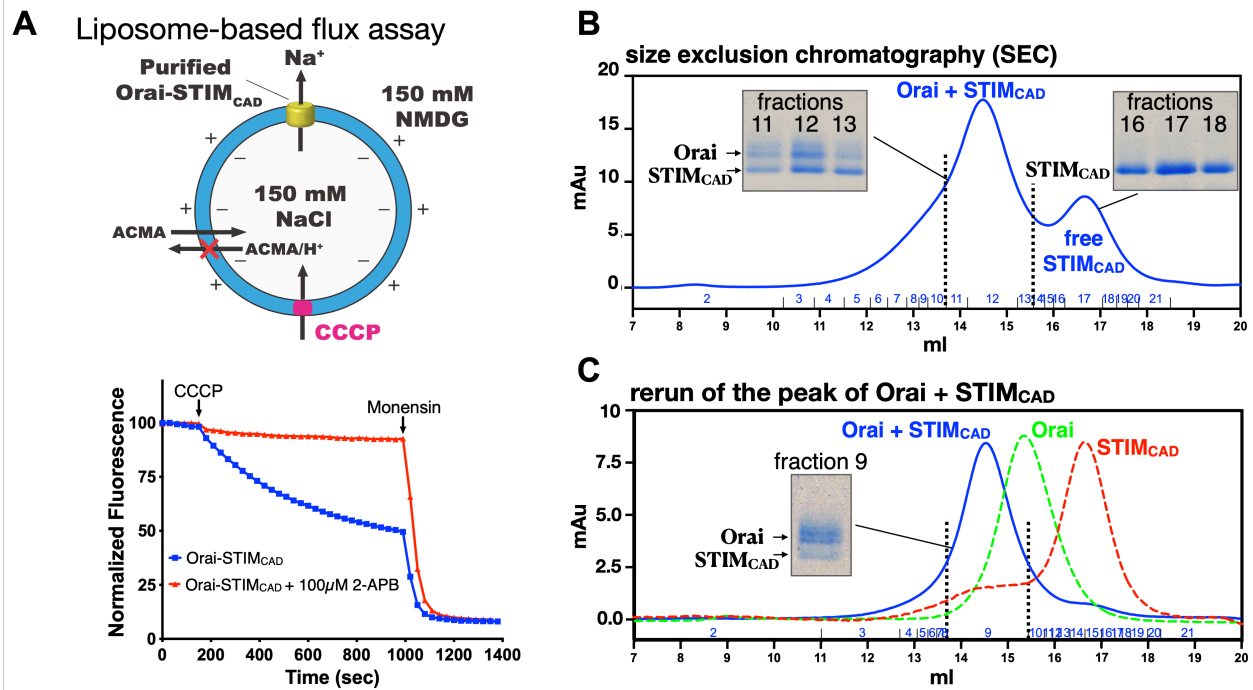


Fig. 2. Functional and biochemical assessment of Orai1-STIM1_{CAD} complexes. **A.** The complex was active in liposomes and was inhibited by the CRAC channel inhibitor 2-APB. **B.** After removing the linker, a portion of STIM_{CAD} stayed with Orai during purification. **C.** The two proteins remained together in a complex, confirmed by the fact that they eluted earlier on the SEC column than Orai or STIM_{CAD} alone.

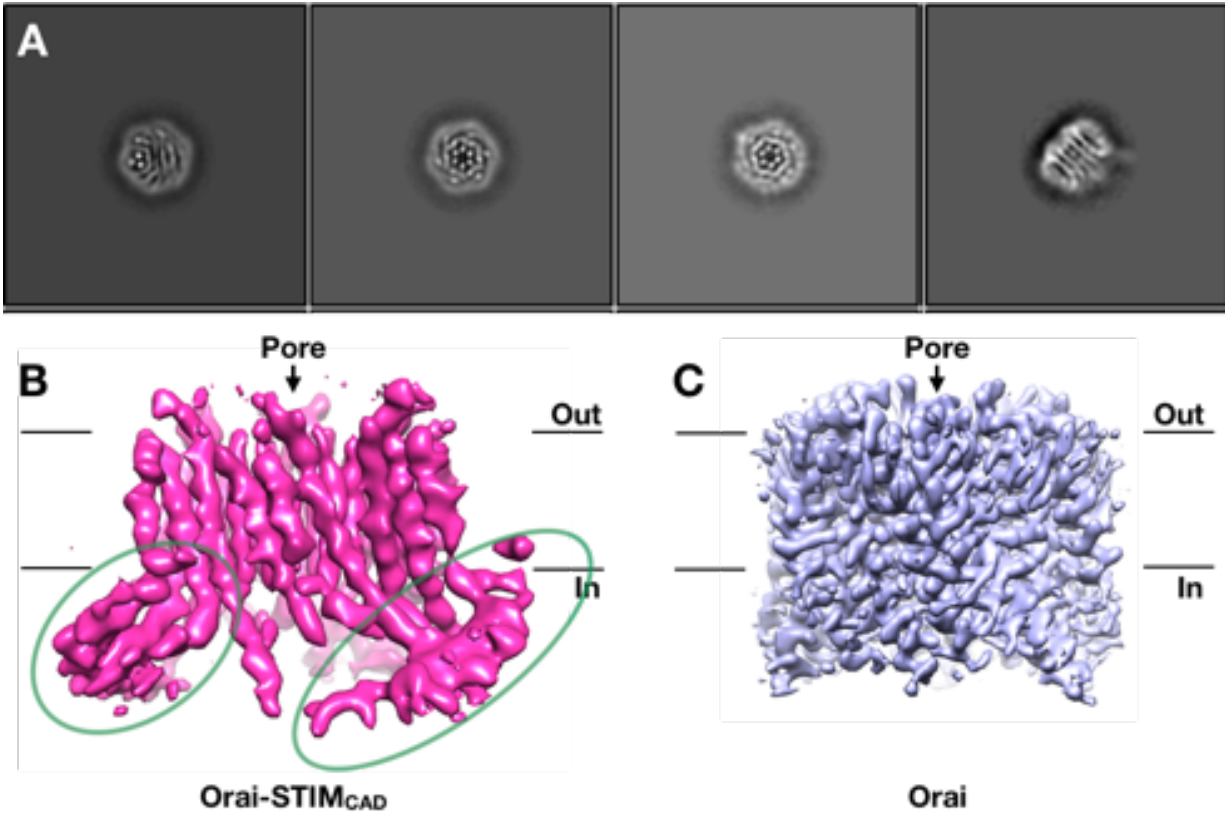


Fig. 3. Preliminary cryo-EM studies. **A.** Representative 2D classes of the Orai-STIM_{CAD} fusion. Two major structures emerge from the data, including **B**, ~4 Å 3D reconstruction of Orai-STIM_{CAD} (green ovals highlight the densities ascribed to STIM) and **C**, 2.8 Å reconstruction with only Orai resolved.

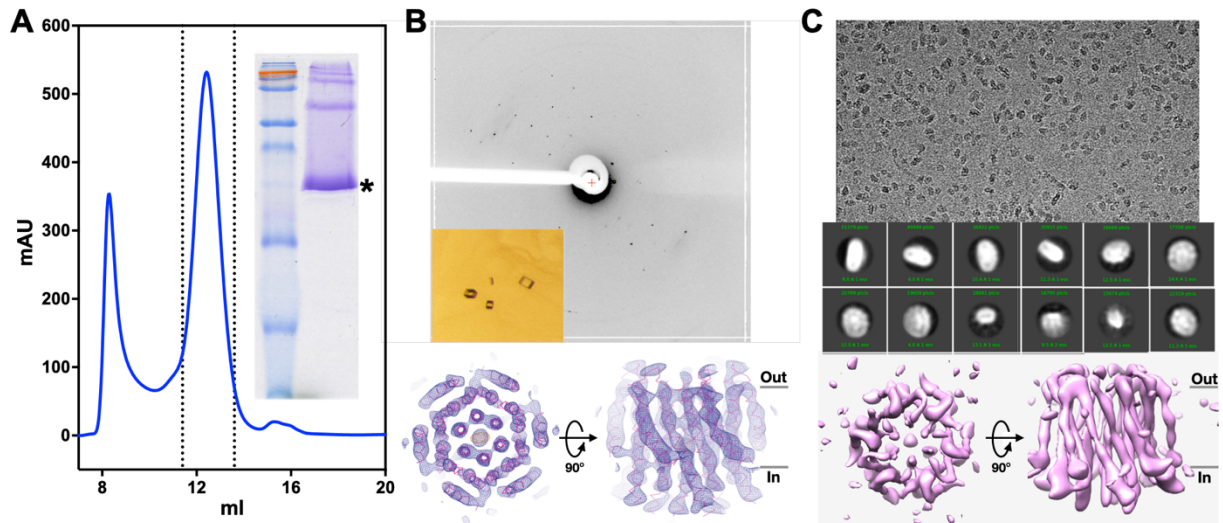


Fig. 4. Purification and structures of human Orai1. **A.** Current protein construct gives a sharp and monodisperse peak (within the vertical dashed lines) on SEC column. Coomassie stain of the purified protein is indicated with asterisk. **B.** Crystal diffraction and $\sim 7\text{\AA}$ electron density map of human Orai1. **C.** Representative cryo-EM micrograph and 2D classes, and $\sim 7\text{\AA}$ 3D reconstruction of human Orai1.