

Figure 1. Sample quality assessment of human CFTR proteins for cryo-EM study. (A) Size exclusion chromatography profile showing CFTR monomers purified in detergents. (B) SDS-PAGE gel indicating the deglycosylated CFTR so purified has the right molecular weight of ~150 kDa. (C) Negative staining image demonstrating well-separated CFTR protein molecules on a carbon film at room temperature. Note the correct size of the molecules indicated by the scale bar. (D) Cryo-EM image taken with the Krios microscope exhibiting notable mono-dispersity of our CFTR protein sample for single particle analysis.

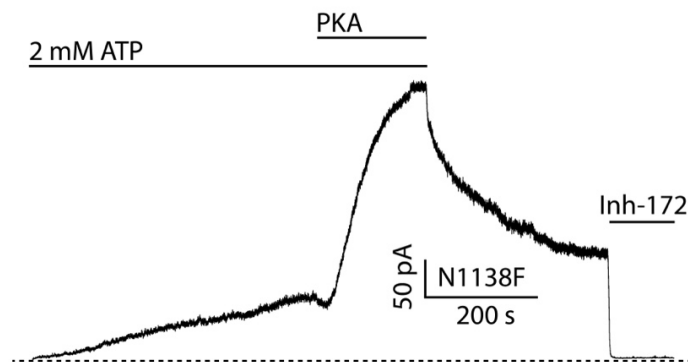


Figure 2. Spontaneous opening of N1138F-CFTR. In an inside-out patch, before phosphorylation, more and more N1138F-CFTR channels start to open simply under exposure to ATP, suggesting an opening mechanism different from normal CFTR channels which require phosphorylation. Subsequent PKA application further accelerates the openings. Strikingly, although the macroscopic current drops after removal of PKA and ATP, ~40% of current is still observed, suggesting significant number of channels can still open even without ATP and NBD dimerization. Application of CFTR inhibitor 172 (Inh-172) diminishes the current, confirming the current observed is from N1138F-CFTR channels.

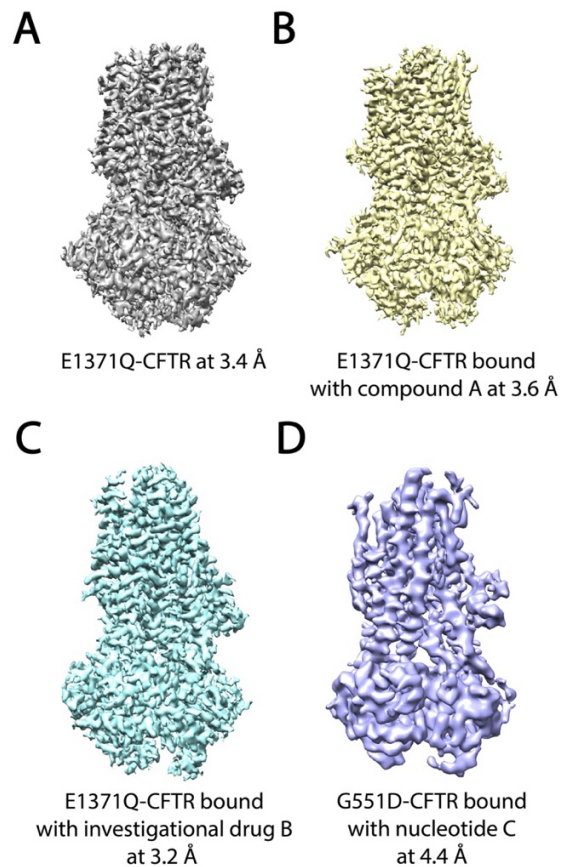


Figure 3. Examples of cryo-EM structures we have determined with the facilities at NCCAT. (A) – (D) A portion of data we have collected from NCCAT using microscope times allocated to us with our last BAG award. All the compounds' names are aliases due to either a manuscript being under review or the confidentiality agreement with our collaborators.

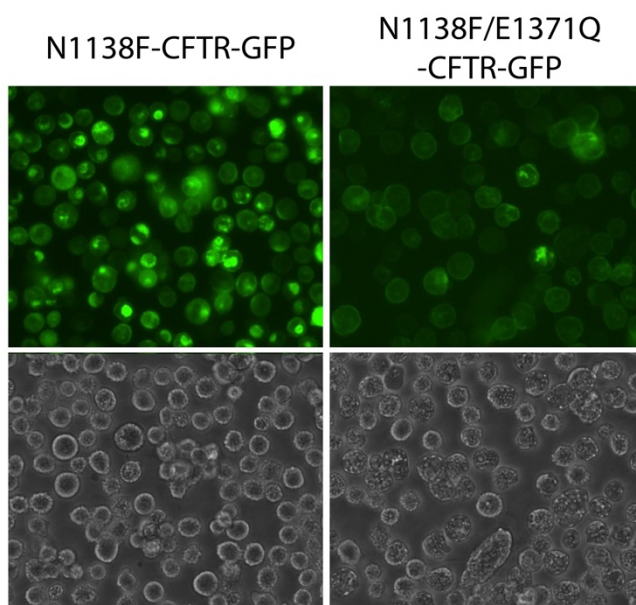


Figure 4. Fluorescence microscopy (top) and phase-contrast (bottom) images of GFP/CFTR expression for N1138F and N1138F/E1371Q cell lines. For N1138F cells (left, top and bottom), proteins are found both inside the cells, as indicated by the bright GFP spots, as well as on the cell membrane. For N1138F/E1371Q mutant (right, top and bottom), plasma-membrane compartmentalized CFTR is observed with GFP fluorescence on the circumference of the cells.