CFTR structure, stability, function and regulation

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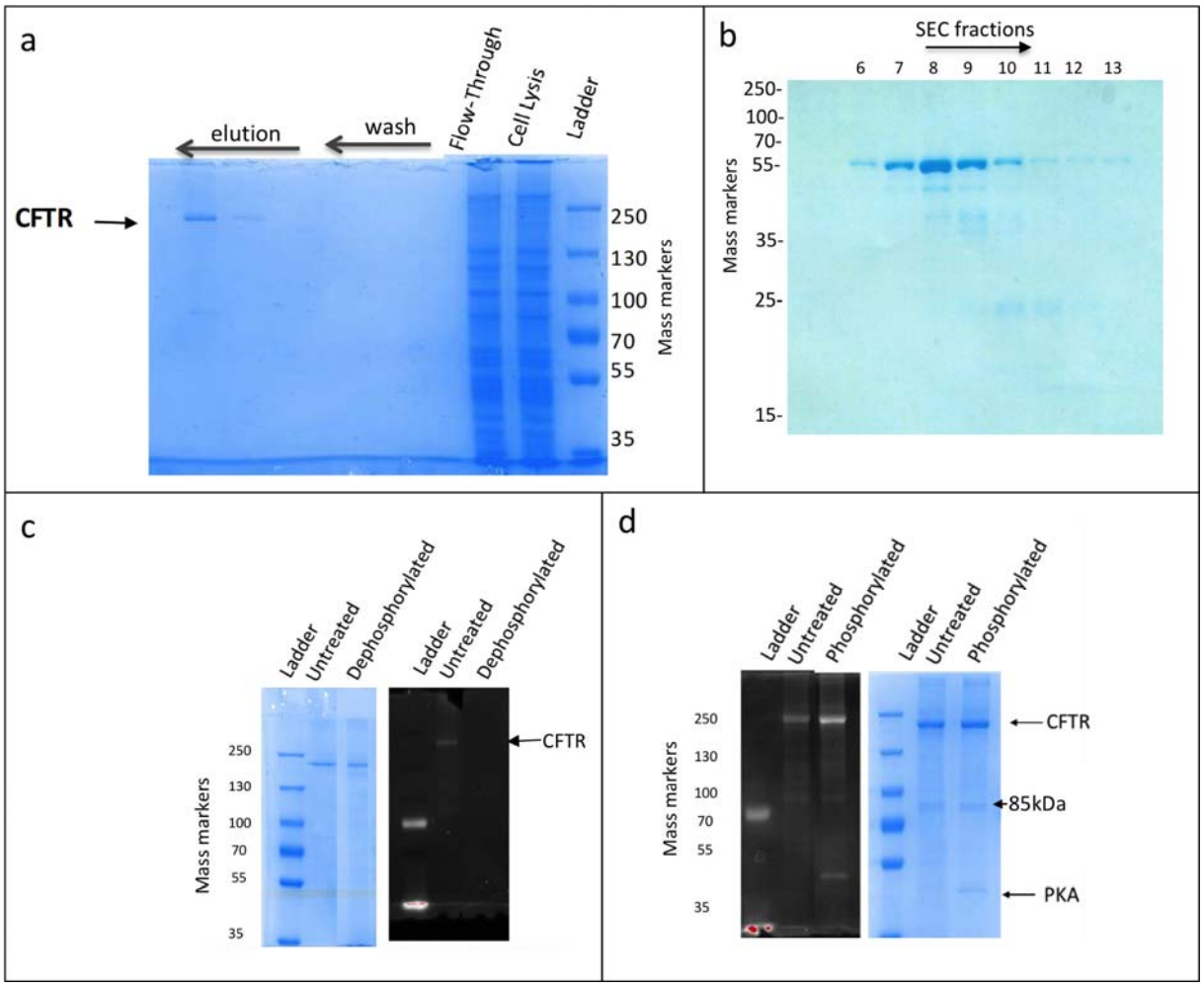
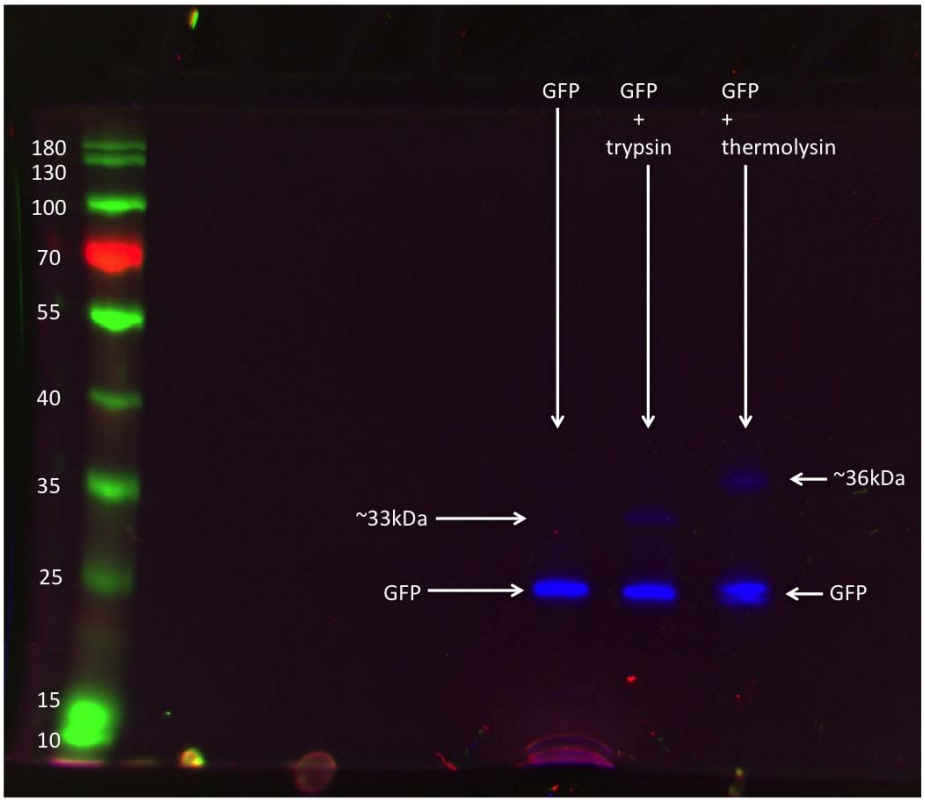
Supplementary material

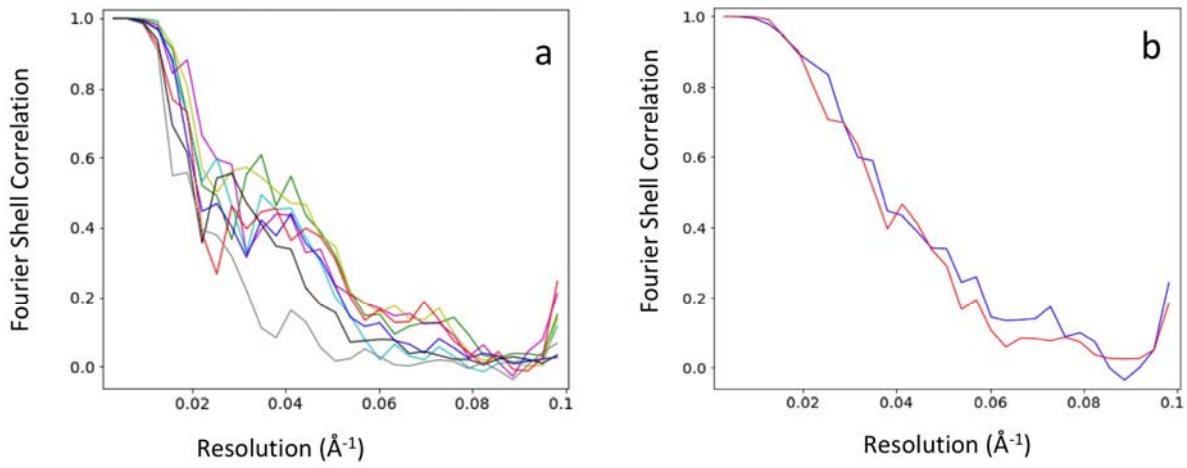


Supplementary Figure 1: Purification and characterisation of proteins: (a) Coomassie stained SDS-PAGE purification gel for CFTR, corresponding to cell lysis extract, flow-through, wash steps and the imidazole elution fractions from the Ni-NTA purification stage, with the major CFTR band eluting at 400mM imidazole. (b) Final purification gel for NHERF1 showing fractions from the 2nd step (size exclusion chromatography – SEC). (c,d) LPG- purified CFTR was treated with (c) lambda phosphatase twice for 1h at 22°C or (d) PKA and 0.2mM ATP for 1h at 30°C. The blue image in each panel represents the Coomassie -stained gel with before

and after treatment of CFTR samples. The greyscale image shows the Pro-Q Diamond stained SDS-PAGE counterpart, with phosphorylated protein bands showing up as white. The data shows that purified CFTR retains significant phosphorylation. A weak doublet band around 85kDa may be due to some proteolytic clipping at the R-region at 30°C which is also observed for the control untreated sample at this temperature.



Supplementary Figure 2: GFP-only controls: GFP was treated under limited proteolysis conditions in the same way as CFTR and then run on SDS-PAGE. GFP was resistant to degradation under the limited proteolysis conditions with both thermolysin and trypsin. Higher molecular mass fluorescent bands of ~36kDa and ~34kDa were formed in the presence of thermolysin and trypsin, respectively (indicated). A 36kDa fluorescent band was also detected after treatment of microsomes with high concentrations of thermolysin (Fig.3, main text). The formation of an SDS-stable complex between GFP and protease may be occurring.



Supplementary Figure 3: Fourier shell correlation plots for CFTR 3D refinements: Black – Dephosphorylated, inward facing state. Grey - Dephosphorylated, outward facing state. Dark blue – Phosphorylated, outward facing state. Red - Phosphorylated, inward facing state. Sky blue – Dehosphorylated + NHERF1, outward facing state. Magenta – Dephosphorylated + NHERF1, inward facing state. Yellow - Phosphorylated + NHERF1, outward facing state. Green - Phosphorylated + NHERF1, inward facing state. The Dephosphorylated, outward facing state (Grey) has the lowest resolution estimate and the fewest contributing particles (635). The highest resolution at FSC=0.5, the more conservative measure, is for the Dehosphorylated + NHERF1 inward-facing dataset (Magenta) with 2719 particles. (b) Fourier-shell correlation plots for global averages of the inward-facing (blue) and outward-facing (red) states.