Plasma membrane-specific interactome analysis reveals calpain 1 as a druggable modulator of rescued Phe508del-CFTR cell surface stability

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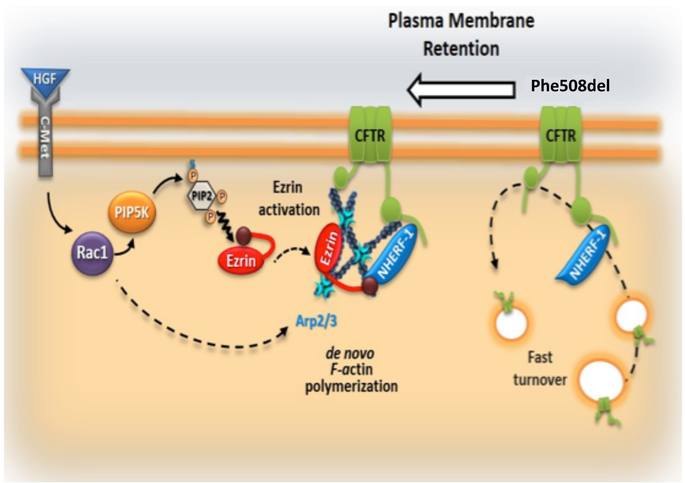
Background

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene encoding CF transmembrane conductance regulator (CFTR), a chloride channel normally expressed at the surface of epithelial cells. The most frequent mutation, resulting in Phe-508 deletion, causes CFTR misfolding and its premature degradation. Low temperature or pharmacological correctors (e.g., VX-809) can partly rescue the Phe508del-CFTR processing defect and enhance trafficking of this channel variant to the plasma membrane (PM). Nevertheless, the rescued channels have an increased endocytosis rate, being quickly removed from the PM by the peripheral protein quality-control pathway. We previously reported that rescued Phe508del-CFTR (rPhe508del) can be retained at the cell surface by stimulating signaling pathways that coax the adaptor molecule ezrin (EZR) to tether rPhe508del–Na+/H+-exchange regulatory factor-1 (NHERF1) complexes to the actin cytoskeleton, thereby averting the rapid internalization of this channel variant. But why…?

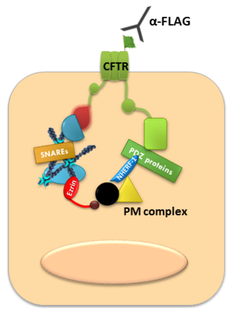


Main Questions

- ✓ What prevents active EZR from associating with rPhe508del-CFTR anchoring complexes at the PM?
- ✓ Are there differences between wt-CFTR and rPhe508del-CFTR interactors at the PM?

Proteomics approach to identify the components of CFTR PM molecular complexes

Selective IP of CFTR membrane association complexes

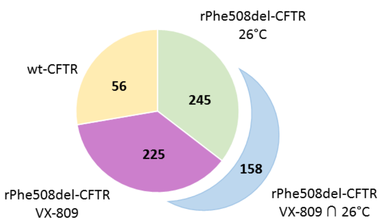


- wt-CFTR — **w/o Ab** 37˚C · DOX · DMSO
- wt-CFTR — **w/ Ab** 37˚C · DOX · DMSO
- Phe508del-CFTR — **w/ Ab** 37˚C · DOX · DMSO
- Phe508del-CFTR — **w/ Ab** (Pharmacological Correction) 37˚C · VX809 · DOX
- Phe508del-CFTR — **w/ Ab** (Low Temperature Correction) 26˚C · DOX · DMSO

Experimental Protocol

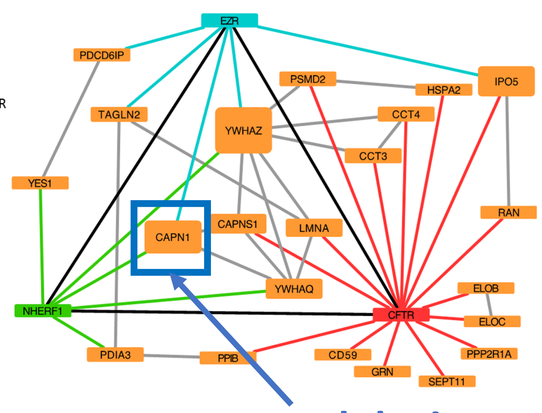
1. Incubate live cells with α-Flag to selectively label CFTR at the PM
2. Incubate cells with cross-linkers (DSP + SPDP)
3. Isolate α-Flag-labelled CFTR with G-protein beads
4. Identify co-precipitated proteins by MS

Bioinformatic analysis of MS data highlights CAPN1 promising hit



Number of high confidence proteins co-detected with wt-CFTR or rPhe508del-CFTR at the PM

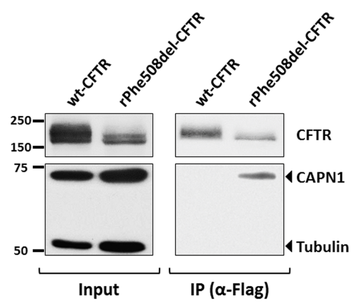
Subnetwork of 22 proteins with direct interactions to CFTR, EZR or NHERF1



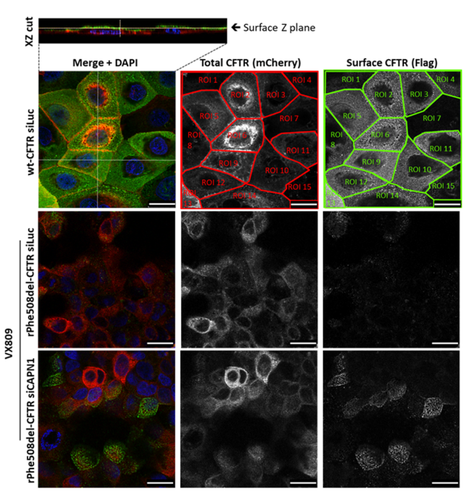
Druggable!

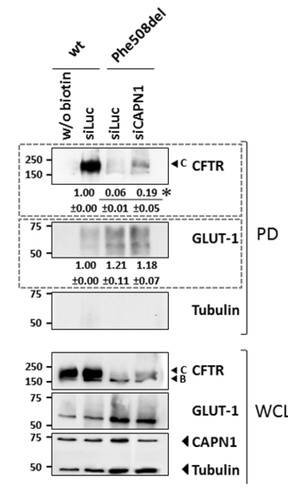
Effect of CAPN1 on the PM stability of rPhe508del-CFTR

Validation of calpain 1 (CAPN1) as an exclusive PM-rPhe508del interactor

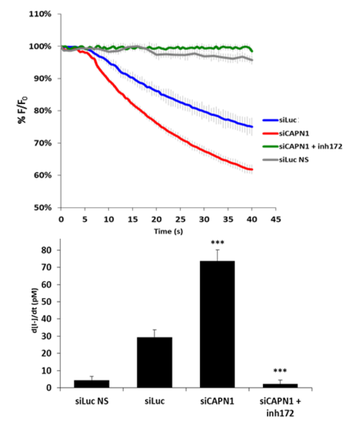


CAPN1 downregulation increases the PM abundance and function of VX-809-rPhe508del-CFTR



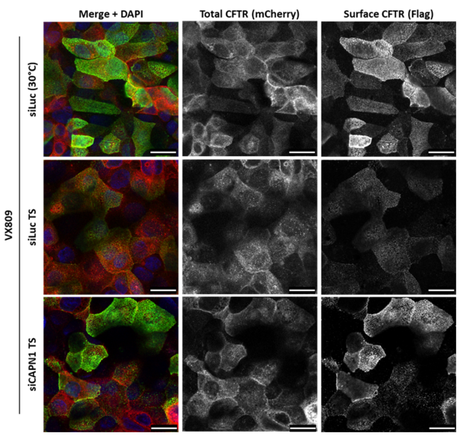


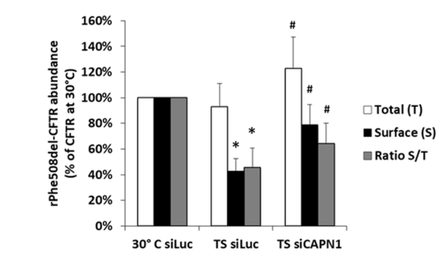
WB analysis of biotinylated cell surface proteins (biotin pull-down – PD) and whole-cell lysates (WCL)



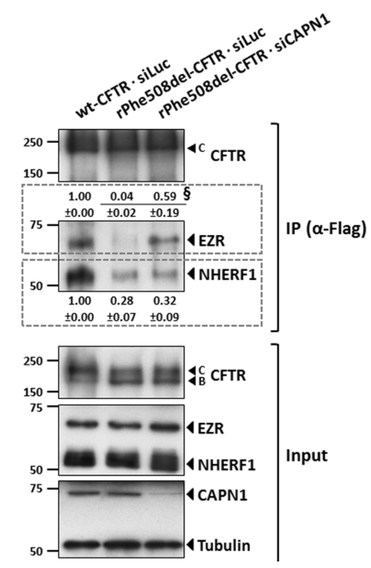
Iodide-induced HS-YFP fluorescence decay assay of Phe508del-CFTR CFBE cells treated with VX-809

Knockdown of CAPN1 improves the PM stability of rPhe508del-CFTR through enhanced EZR binding



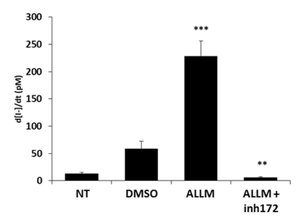


Thermal-shift assay of mCherry-Flag-Phe508del-CFTR CFBE cells by confocal microscopy (Graph – quantification of fluorescence signals)

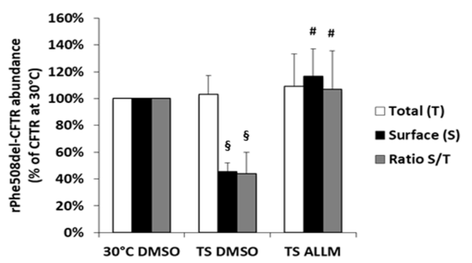


WB analysis of EZR and NHERF1 levels co-precipitating with wt- or Phe508del-CFTR at the PM

Acute chemical inhibition of CAPN1 increases VX-809-mediated Phe508del-CFTR functional rescue



Iodide-induced HS-YFP fluorescence decay assay of Phe508del-CFTR CFBE cells treated with VX-809 (influx rates calculated by fitting the iodide assay results to exponential decay curves; ALLM – CAPN1 inhibitor)



Thermal-shift assay of single-cell fluorescence signals of mCherry-Flag-Phe508del-CFTR CFBE cells

Conclusions

- ✓ **rPhe508del-CFTR has a much more complex network of PM interactors than wt-CFTR.**
- ✓ **The innovative PM-CFTR IP approach allowed the identification of calpain 1 (CAPN1) protease as a key player in destabilizing rPhe508del-CFTR anchoring at the PM by interfering with EZR binding.**
- ✓ **We showed that the chemical inhibition of Calpain1 has potential in the pharmacological context, namely to enhance the efficacy of current drugs as part of CF combination therapies.**

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