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Every picture tells a story: a new assay for rapid assessment of function and localization of CFTR

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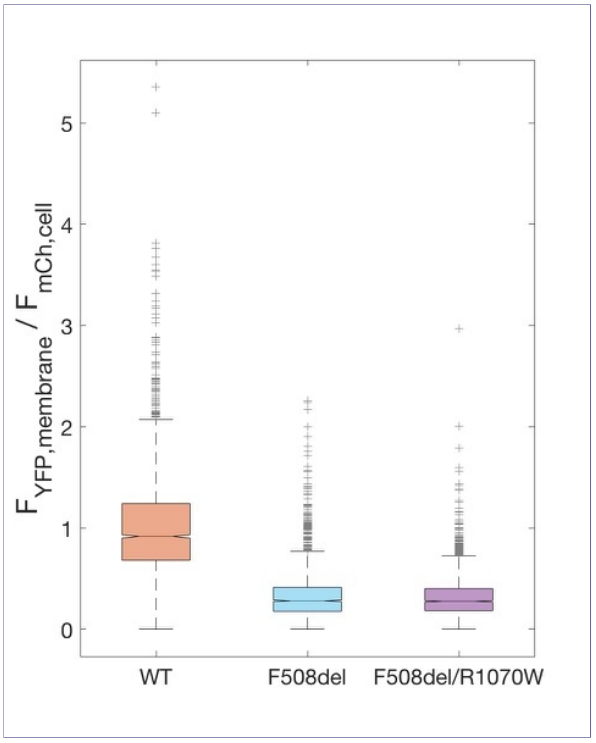
Abstract:
Introduction
People with cystic fibrosis (CF) have a mutation in the *CFTR* gene. Normally CFTR proteins regulate the movement of salt and water across epithelia. However, CFTR variants carrying CF-causing mutations have impaired gating, and/or they are mislocalized in the cell, with only a small proportion correctly reaching the plasma membrane. As a result, transepithelial fluid secretion is reduced, creating a range of problems, especially in the lungs, pancreas, and intestines.
There are many different mutations that cause CF: the most common, F508del, and >300 rarer ones. Unfortunately, the tests required to obtain genotype-specific information on CFTR are time consuming. Our aim is to speed up this process. Fluorescence assays to measure the ion-channel function of CFTR already exist. However, for many CFTR variants the main problem is their inability to reach the plasma membrane. Moreover, some drugs like Ivacaftor, whilst improving the gating of CFTR, can also increase mislocalization. Therefore, it is important to assess localization in addition to function, starting at early stages of the drug development process.
Methods
To enable rapid and simultaneous assessment of ion channel function and localization of CFTR, we modified the YFP-CFTR assay: a cell-based assay in which a halide-sensitive YFP (1), tagged to the N-terminal of CFTR (2) informs about ion channel function. A soluble, cytosolic mCherry is coexpressed with YFP-CFTR. The red mCherry fluorescence allows image segmentation, identification of cells suitable for analysis, and accurate positioning of the cell membrane. $\Phi = F_{YFP,membrane}/F_{mCherry,cell}$ (where $F_{protein,region}$ indicates mean fluorescence intensity per pixel for the stated protein, averaged over the membrane region or the entire cell) is used to quantify CFTR localized in the plasma membrane for each cell.
Results
We studied the effect of R1070W, a second-site mutation that, in cis, rescues defects caused by the F508del mutation. As expected, R1070W rescues gating of F508del-CFTR, measured as rate of YFP quenching upon iodide addition. However, per our measurements, R1070W does not significantly correct mislocalization (figure 1). This finding is inconsistent with published research.
Conclusions
Our readout might be more appropriate for quantifying CFTR membrane density than Western blots. If so, R1070W might not affect localization of F508del-CFTR as much as is currently believed.
This new assay can rapidly test many CFTR variants and their response to compounds and structural perturbations.
References
(1) Galietta *et al.* (2001). *Am.J.Physiol.Cell.Physiol.* **281**:C1734-C1742



(2) Langron *et al*. (2017). *Br.J.Pharmacol*. **174**:525:539.
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