Every picture tells a story…

Quantifying the localization and gating function of CFTR

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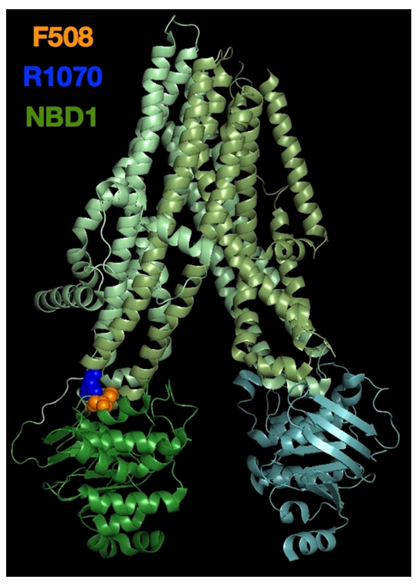
Introduction

Our group has developed an assay which allows for the study of both the localization and the gating function of CFTR. The assay involves collecting fluorescence images which are then processed with an image analysis tool, simply quantifying defects in CFTR function.

This assay can be used the study the effects of different Cystic Fibrosis-causing mutations, to study the effect of introducing second site mutations into CFTR-F508del and in the development of function-restoring compounds.

We here aim to shed light on the mechanism by which the introduction of mutation R1070W restores the function of CFTR-F508del.

CFTR



Cystic Fibrosis Transmembrane conductance Regulator

CFTR acts as an **ion channel** in the plasma membrane.

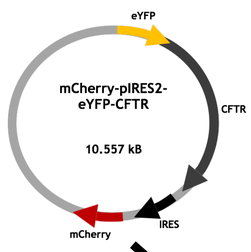
F508del is the most common mutation to cause cystic fibrosis.

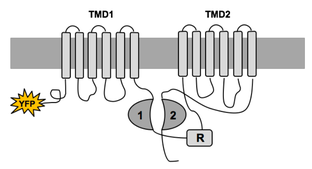
It has been shown that the introduction of a second mutation, **R1070W**, partially rescues the function of CFTR-F508del [1].

Methods

Three variants of CFTR are compared:
- CFTR-WT
- CFTR-F508del
- CFTR-F508del/R1070W

HEK293 cells are transfected with plasmids encoding for YFP-CFTR and mCherry.

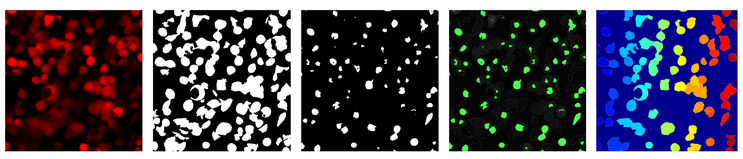




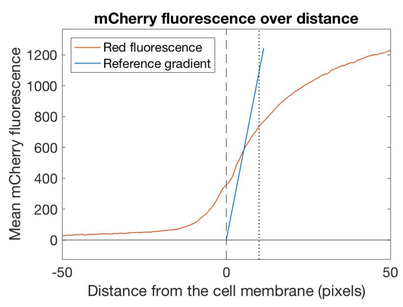
YFP tagged to the N-terminal of CFTR is sensitive to iodide ions

LOCALIZATION

Individual cells are located using marker-controlled watershed segmentation performed on the mCherry image.

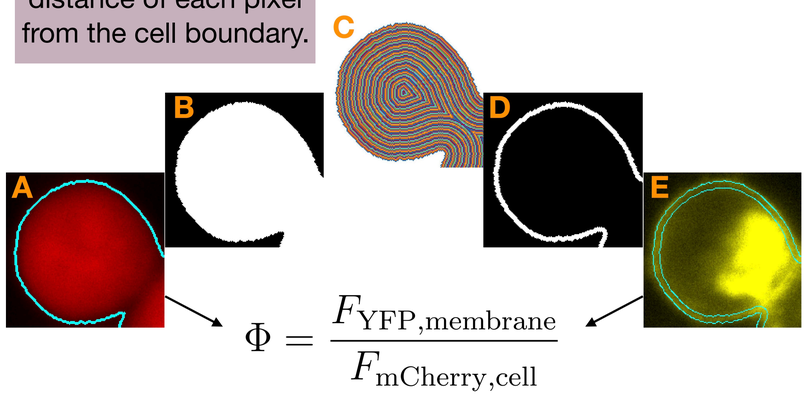


Cells unsuitable for analysis are discarded. Filtered cells include those:
- on the edge of a full image,
- too large or too small,
- with a large perimeter relative to their area.

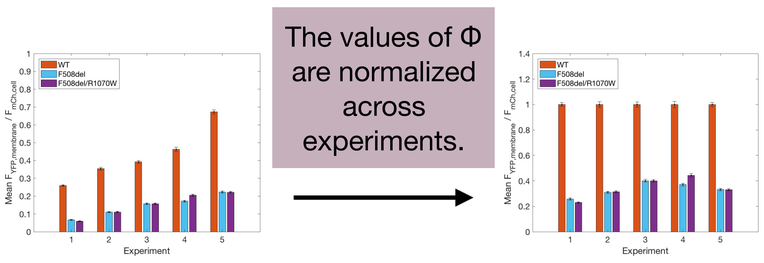


A quality control is imposed on the segmentation. The rate of increase in mCherry fluorescence must be above a threshold within the cell membrane.

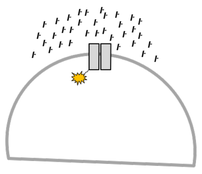
A distance map is created, giving the distance of each pixel from the cell boundary.



The values of Φ are normalized across experiments.



GATING FUNCTION



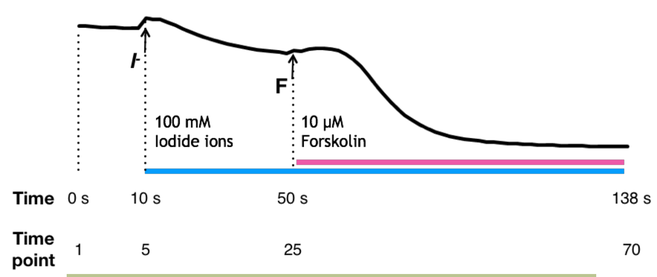
Cells are flushed with 100 mM iodide.

10 µM Forskolin in DMSO

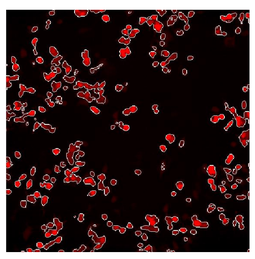
DMSO only as control

CFTR is activated, if functional.

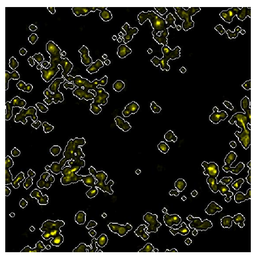
CFTR is not activated.



Images are collected every 2 seconds.



The cells are selected by applying a threshold to the mCherry image.



The selection is morphologically dilated and the YFP signal is defined to be the mean fluorescence inside minus outside.

The YFP signal is normalized against the fluorescence just before iodide is added.

Rearranging a Hill—Langmuir-type equation, allows the calculation of the **maximum change in iodide concentration**.

$$F/F_{\max} = 1 - \frac{[\mathrm{I}^-]_{\mathrm{in}}}{K_1 + [\mathrm{I}^-]_{\mathrm{in}}}$$

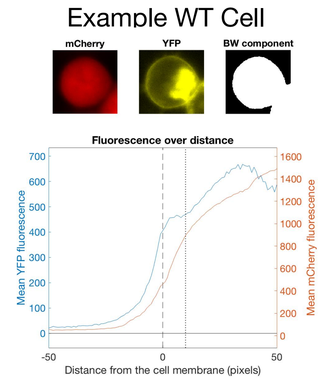
K_1 is the binding affinity of CFTR for iodide ions. We use K_1 = 1.9. [2]

Results

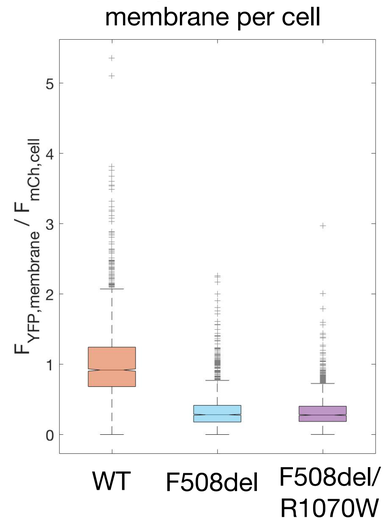
LOCALIZATION

It can be seen that, for some individual cells, there is an observable ring of increased YFP fluorescence around the border of the cell.

Example WT Cell



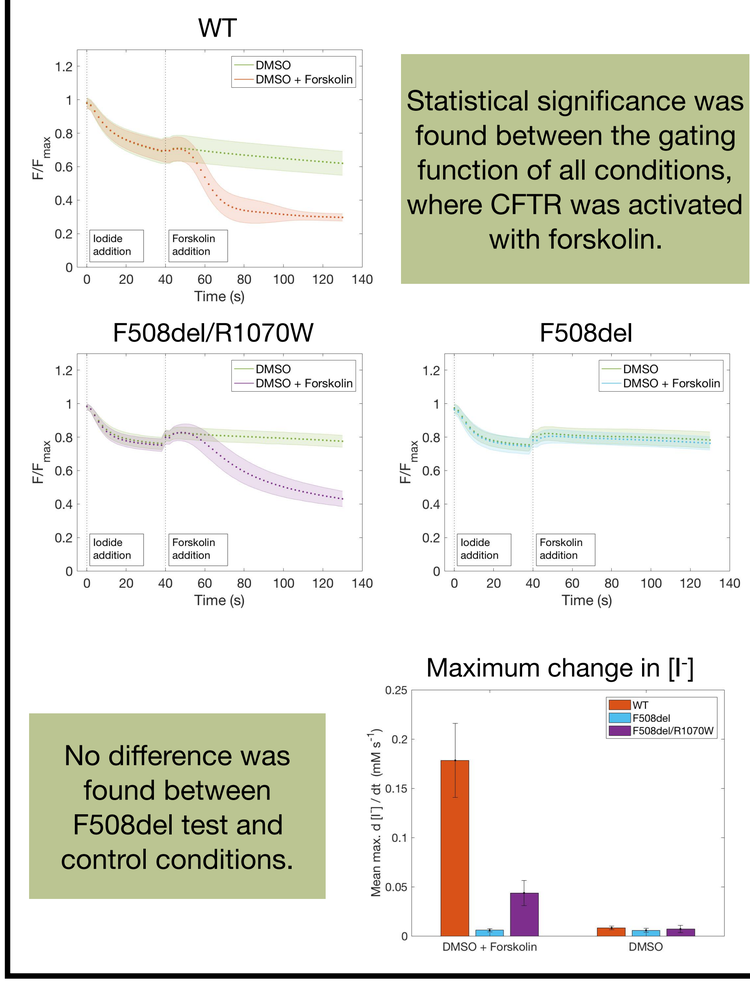
Density of CFTR at the membrane per cell



Statistical significance was found between the CFTR density at the membrane for WT and both F508del and F508del/R1070W.

However, there was no difference between F508del and F508del/ R1070W.

GATING FUNCTION



Statistical significance was found between the gating function of all conditions, where CFTR was activated with forskolin.

No difference was found between F508del test and control conditions.

Maximum change in $[I^-]$

Summary

We conclude that our assay can successfully quantify the localisation and gating function of CFTR.

It can be seen that there is significantly less CFTR-F508del at the membrane than wild-type CFTR, and that its gating function is significantly decreased.

We show that the introduction of R1070W into CFTR-F508del does not increase the density of CFTR at the membrane, but that it does significantly rescue the gating function. However, gating function is not restored to that of CFTR-WT.

References

[1] P H Thibodeau, J M Richardson, W Wang, L Millen, J Watson, J L Mendoza, K Du, S Fischman, H Senderowitz, G L Lukacs, K Kirk, and P J Thomas. The cystic fibrosis-causing mutation ΔF508 affects multiple steps in cystic fibrosis transmembrane conductance regulator biogenesis. *J. Biol. Chem.*, 285(**46**): 35825–35835, 2010.

[2] L J V Galietta, P M Haggie, and A S Verkman. Green fluorescent protein-based halide indicators with improved chloride and iodide affinities. *FEBS Letters*, 499(**3**): 220–224, 2001.