Development of high-content fluorescence assays to rapidly monitor CFTR-mediated bicarbonate flow - how do CFTR mutations and modulator drugs affect it?

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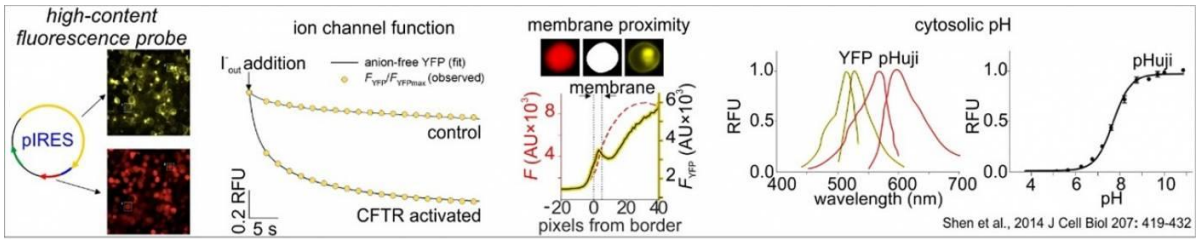
Funding: 1 Fully Funded PhD project.

As well as funding materials, consumables and use of imaging facilities, the award provides a generous tax-free stipend (£24,093, £26,057 and £26,839 per annum during the PhD years) and covers tuition fees (at home or overseas student rates). Funds are also available for attending specialised training workshops and relevant international scientific conferences to increase awareness of scientific advances, but also to provide opportunities for networking and team building.

Project details:



YFP-CFTR and cytosolic pHuji will be coexpressed from a bicistronic plasmid in HEK293 cells. Quenching of YFP fluorescence upon addition of extracellular I- informs on ion-channel function. Membrane proximity (ratio of YFP-CFTR fluorescence within 5 pixels from cell borders over final pHuji fluorescence throughout the cell) informs on the number of channels at the membrane. The pH sensitive pHuji fluorescence will inform on cytosolic pH.

1) Generation and optimization of a **fluorescence probe** to monitor CFTR-mediated anion flow.

The aim is to create a tool to rapidly quantify CFTR-dependent Cl^- and HCO_3^- flow, in the simplified HEK293 expression system. The new assay will be based on a recently developed high-content assay [1, 2]. Cells will be transformed with a bicistronic plasmid encoding a soluble red pH-sensitive fluorescent protein [3] and an N-terminal fusion of CFTR to the I^--sensitive yellow fluorescent protein, YFP [4]. Cells expressing these proteins will be grown in 96-well plates, and images will be acquired with a confocal screening microscope equipped with automated fluid-addition robotics. CFTR will be pre-activated in HCO_3^- -free vs. Cl^- -free solutions. Once steady-state activation is reached, a second addition will increase extracellular I^-. By monitoring quenching of YFP over time we will compare Cl^-/I^- vs. HCO_3^-/I^- exchange. Fluorescence timelines (on both red and yellow channels) following I^- addition will be fit using a mathematical model describing transmembrane ionic fluxes and intracellular and extracellular pH and buffers. Finally, the average red fluorescence throughout the cell (following cytosolic alkalinisation) will be used as a unit, to quantify how much CFTR is in proximity of the membrane. The assay will allow simultaneous monitoring of multiple readouts (CFTR Cl^- and HCO_3^- conductance, cytosolic pH, CFTR membrane proximity), at the level of individual cells, while automated image analysis will extract information from thousands of cells.

2) Generation and optimization of a cellular **anion flux biosensor system**

Results from expression and inhibitor studies (carried out in other SRC labs exploiting *ex-vivo* material), will help identify components likely to be necessary to recapitulate bile duct results: while ducts expressing WT-CFTR had a high HCO_3^- selectivity, CFTR modulator treatment of F508del-CFTR-expressing ducts increased Cl^- but not HCO_3^- secretion [5]. What protein(s) do we need to coexpress to recover this effect in HEK293 cells? By incorporating transport and regulatory proteins, we propose to build a relevant “minimal model” biosensor HEK293 cell-line. In support of the feasibility of this approach, coexpression of CFTR and the protein kinase WNK1, alone, was sufficient to reconstruct a system capable of modulating CFTR anion selectivity [6]. Mechanistic insight will emerge from building the system from its component parts.

3) Characterization of Cl^- vs. HCO_3^- fluxes in **mutant CFTR variants**, in the absence and presence of **CFTR modulator drugs**

The minimal model biosensor system will be used to profile how a panel of 62 CF-causing CFTR mutations [2] affect anion fluxes. Further, for each mutant, the effect of 20 hours’ treatment with

modulators will be quantified. How do modulators affect Cl^- and HCO_3^- transmembrane flow? Is there any mutant/modulator combination that differentially recovers one or the other? The empirical screens will provide material for generating hypotheses, and pinpoint compounds/residues/conditions requiring more detailed investigations in native systems (to be carried out in other SRC labs).

Intense cross-discipline communication and iterative interactions with other members of the SRC team will be essential, and enable a deeper understanding of how CFTR-mediated anion fluxes are modulated.

The PhD studentship is available to start in September 2023. Please contact Dr Paola Vergani (p.vergani@ucl.ac.uk) for informal enquiries.

References:

1. Prins, S., et al., *Can two wrongs make a right? F508del-CFTR ion channel rescue by second-site mutations in its transmembrane domains.* J Biol Chem, 2022. **298**(3): p. 101615.
2. Prins, S., et al., *Fluorescence assay for simultaneous quantification of CFTR ion-channel function and plasma membrane proximity.* J Biol Chem, 2020. **295**(49): p. 16529-16544.
3. Shen, Y., et al., *pHuji, a pH-sensitive red fluorescent protein for imaging of exo- and endocytosis.* J Cell Biol, 2014. **207**(3): p. 419-432.
4. Galietta, L., P. Haggie, and A. Verkman, *Green fluorescent protein-based halide indicators with improved chloride and iodide affinities.* FEBS Lett., 2001. **499**(3): p. 220-224.
5. Bijvelds, M.J.C., et al., *Rescue of chloride and bicarbonate transport by elexacaftor-ivacaftor-tezacaftor in organoid-derived CF intestinal and cholangiocyte monolayers.* J Cyst Fibros, 2022. **21**(3): p. 537-543.
6. Kim, Y., et al., *Regulation of CFTR Bicarbonate Channel Activity by WNK1: Implications for Pancreatitis and CFTR-Related Disorders.* Cell Mol Gastroenterol Hepatol. , 2020. **9**(1): p. 79-103.

Application deadline: 31/03/2023

Application details:
Please visit https://www.ucl.ac.uk/biosciences/cystic-fibrosis-bicarbonate-centre
The application will include:
- your latest CV with contact details for two referees
- a 2-3 paragraph statement explaining your interest in the project and what you feel you will bring to the role
- official University transcript(s).