RESOLUTE

Research Empowerment on Solute Carriers

Use of biosensor as a tool for SLC26A9 and SLC9B2 assay development

Francesca Sassone[1], Giovanna Maresca[1], Loredana Redaelli[1], Lia Scarabottolo[1]

[1] Axxam S.p.A., OpenZone - via A. Meucci, 3 – 20091 Bresso (Milan, Italy)

Genetically Encoded Biosensors

Over the past decades, efforts were made for the development of Genetically Encoded (GE) fluorescent sensors derived from fluorescent proteins, which can undergo detectable changes in fluorescence in response to specific cell events. Among these, SuperClomeleon is a Cl^- sensitive ratiometric FRET (Fluorescence Resonance Energy Transfer) sensor obtained by fusing a YFP mutant (Yellow Fluorescent Protein, that is a yellow variant of the GFP with a high sensitivity towards halides) and the Cyan Fluorescent Protein (CFP, insensitive to halides). Intracellular increase of Cl^- finally leads to a decrease of the FRET ratio YFP/CFP. Another important GE fluorescent sensors are pHluorins, which are pH-sensitive variants of green fluorescent protein, that allow measurement of intracellular pH in living cells. At acidic pH, pHluorin is quenched, whereas at physiological pH, pHluorin fluorescence increases.

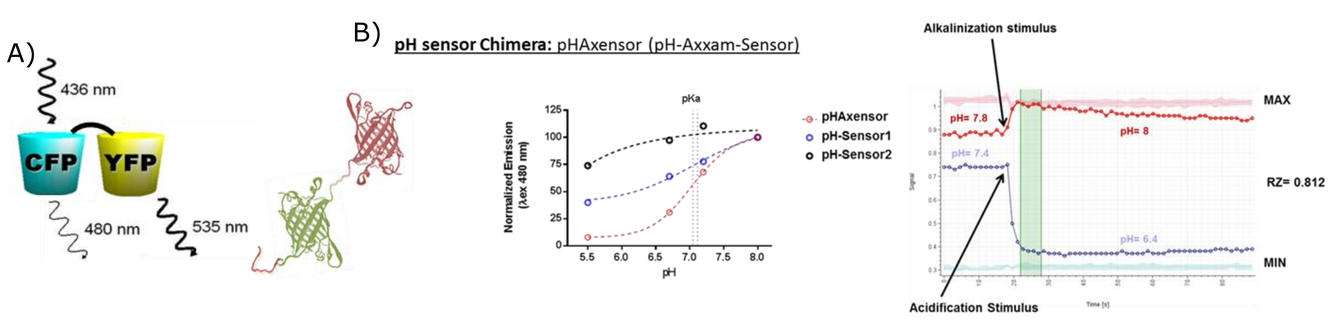


Figure 1 *– A) Schematic representation of FRET signal-based Superclomeleon biosensor: chloride fluxes detection depends on a FRET between CFP and chloride sensitive-YFP. B) The pH biosensor generated at Axxam (pHAxensor) is a chimera that stems form two fluorescent protein variants: a pH-insensitive Red Fluorescent Protein (RFP) and a pH-sensitive Green Fluorescent Protein (pHluorin) quenched at acidic pH. The pH sensitivity in a full linear range is between 6.4-7.7.*

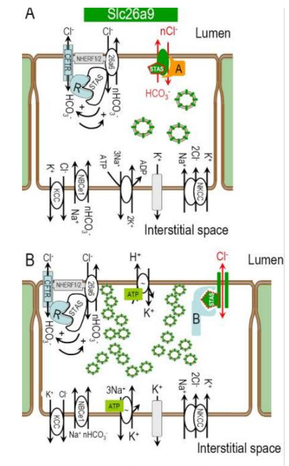
SLC26A9 assay development using SuperClomeleon biosensor

SLC26A9, localized in lung and upper gastrointestinal tract epithelia, is involved in volume control/apoptosis/migration/differentiation. It is not directly associated with any disease even though some SNPs have been reported to be related to the onset of disorders.

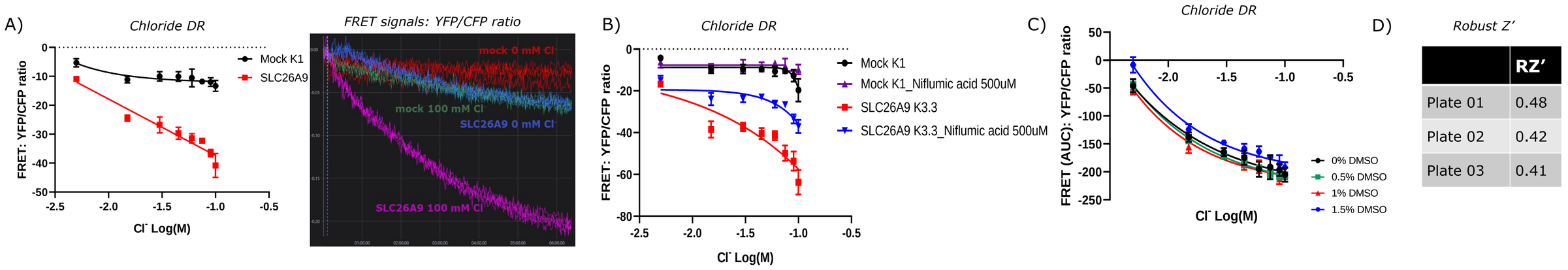
SLC26A9 acts using different transport modes in different systems: it is a highly selective Cl^- channel and $Cl^-/HCO3^-$ exchanger. For this reason, beside testing this transporter using the Membrane Potential sensitive dye for a preliminary proof of functionality, the assay development was performed using SuperClomeleon biosensor.

The biosensor was transiently transfected in HEK-293 stably expressing SLC26A9 or in the mock clone.

HEK-293/SLC26A9 showed good FRET signal decrease upon stimulation with Cl^- (with very low effect detected in mock cells), suggesting an **SLC26A9-mediated chloride influx** (Figure 2A). In the presence of Niflumic acid (described as unspecific and partial SLC26A9 inhibitor) the FRET signal decrease is weaker, suggesting that **SLC26A9-mediated chloride influx is partially inhibited by Niflumic acid** (Figure 2B). The presence of **DMSO** up to 1.5% **is not affecting assay pharmacology** (Figure 2C). A multiplate test was carried out on 3 plates to assess the robustness of the assay and its eligibility for HTS purposes. **Assay quality criteria are fulfilled** (Figure 2D).



Chang et al., 2009



	RZ'
Plate 01	0.48
Plate 02	0.42
Plate 03	0.41

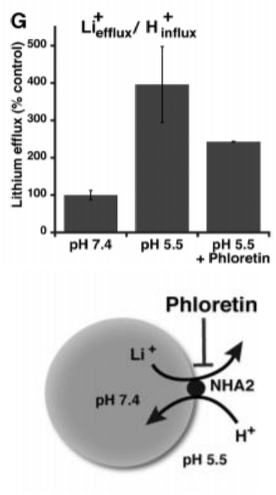
Figure 2 *– A) To test the effect of chloride on FRET signal, cell were incubated in Cl^- free buffer and then Cl^- dose-response was injected at Hamamatsu FDSS plate reader and both CFP and YFP fluorescent signals recorded. FRET signal was calculated as ratio between YFP and CFP recordings. B) Effect of Niflumic acid inhibitor was tested pre-incubating the cells in the presence of 500 µM Niflumic acid. C) Effect of DMSO presence was tested. D) Multiplate test was performed in single injection protocol, using as stimulus Chloride EC100 (100 mM) in HEK-293/SLC26A9 K3.3.*

SLC9B2 assay development using pH biosensor pHAxensor

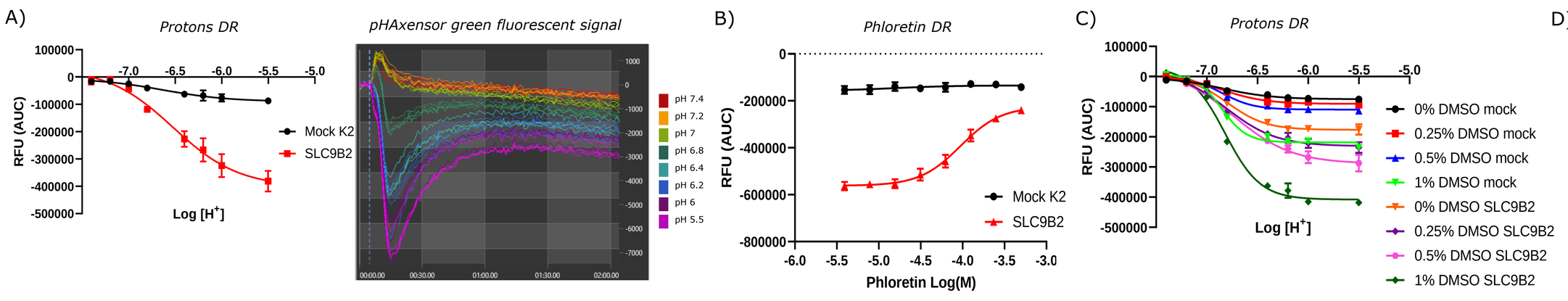
SLC9B2, expressed in a wide range of tissues with high levels in nephron distal tubules and in the renal collecting duct, belongs to the Cation/Proton Antiporter (CPA) superfamily. It couples inward transport of protons to mediate sodium (or lithium) efflux from the cells. The transporter is sensitive to Phloretin. Human SLC9B2 has been implicated as a marker of essential hypertension.

To detect pH changes upon SLC9B2 activation, a pH biosensor developed at Axxam (pHAxensor) was used. This biosensor was expressed in HEK-293 cells, where SLC9B2 was stably transfected, mock were generated in parallel.

HEK-293/pHAxensor/SLC9B2 showed sensor quenching in response to cell acidification (with very slight effect in mock cells) suggesting the **presence of SLC9B2-mediated protons influx** (Figure 3A). In the presence of Phloretin, biosensor fluorescence quenching inhibition was observed suggesting that **SLC9B2 is partially inhibited by Phloretin** (Figure 3B). The presence of **DMSO** affected the assay in both SLC9B2 expressing clone and mock, suggesting a DMSO sensitivity of the pH sensor itself. **DMSO is tolerated up to 0.5%** (Figure 3C). A multiplate test was carried out on 3 plates to assess the robustness of the assay and its eligibility for HTS purposes. **Assay quality criteria are fulfilled** (Figure 3D).



Kondapalli et al., 2012



	RZ'
Plate 01	0.48
Plate 02	0.55
Plate 03	0.48

Figure 3 *– A) To test SLC9B2-mediated protons influx, pH D/R in sodium free buffer was injected at Hamamatsu FDSS plate reader and fluorescent signal recorded (green fluorescence, pH sensitive part of the biosensor). B) Effect of Phloretin inhibitor was tested with a double injection protocol: cells were first injected with Phloretin D/R and then with protons to reach final pH of 6.2 (absence of sodium) and fluorescence recorded. C) Effect of DMSO presence was tested. D) Multiplate test was performed in single injection protocol, using as stimulus pH 6 in HEK-293/SLC9B2.*

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www.re-solute.eu
francesca.sassone.fs@axxam.com