RESOLUTE

Research Empowerment on Solute Carriers

Fluorescent dyes and sensors for cell-based assays - WP3

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Introduction

Axxam's role in WP3 (**Task 3.1**) is performing proof of principle tests to assess the functionality of Solute Carrier Transporters (SLCs) by using **voltage/ion sensitive dyes** as well as **voltage sensors**. Axxam will participate within a consortium-wide effort combining the deorphanisation data/hypotheses generated in WP1 and WP2 and its know-how and technologies to investigate and provide further insights on electrogenic transporters.

Extrapolating from the percentage of known electrogenic transporters in the genome, we expect up to **65%** of SLCs to have electrogenic properties. We foresee therefore that this task will allow us to carry out functional screens for more than 100 SLCs over the course of the project.

Flowchart of activities

A collection of SLCs-overexpressing cell lines for >80% of all SLC family members will be generated in the **HEK-293 JumpIN T-REx** cell line at **CeMM** (transduction) and **Novartis** (clone isolation and characterization) (**WP1**)

A subset of these cell lines will be delivered to Axxam for testing. The choice of the transporters to be tested is going to take into account several factors:

1. Potential electrogenicity
2. Localization
3. Presence of a known substrate

The selected cell lines overexpressing SLCs will be delivered to Axxam as frozen cells

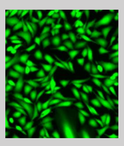
Cell handling at Axxam:

1. Cell thawing and expansion
2. Mycoplasma testing
3. Cell lines banking

SLCs cell lines will be tested using fluorescent dyes or sensors (see Figure 2) and using the substrates identified from the output of WP1 and WP2 or from previously available knowledge

Figure 1 *– Flowchart of activities highlighting the role of Axxam in WP3 (Task 3.1).*

Fluorescent dyes and sensors

Technology	SLCs to be targeted	Ions	Dyes	Screening technology
Fluorescent DYES  	Electrogenic/ cotransporter of substrate and ions	Any ion that determines changes in Membrane Potential (MP)	MP-sensitive dye	FLIPR^{TETRA} Hamamatsu FDSS
		Ca^{2+}	Ca^{2+}-sensitive dyes	FLIPR^{TETRA} Hamamatsu FDSS
		Na^{+}	Na^{+}-sensitive dye	FLIPR^{TETRA} Hamamatsu FDSS
		Organic cations	4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP^{+})	FLIPR^{TETRA} Hamamatsu FDSS
		Zn^{2+}	Zinquin / other dyes available on the market	FLIPR^{TETRA} Hamamatsu FDSS
		Mn^{2+}	Fura-2 AM	Hamamatsu FDSS

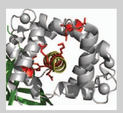
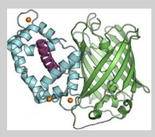
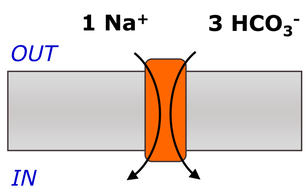
Technology	SLCs to be targeted	Ions	Dyes	Screening technology
Genetically encoded fluorescent SENSORS  	Electrogenic/ cotransporter of substrate and ions	I^{-}/Cl^{-}	Yellow fluorescent protein (EYFP) and SuperClomeleon	FLIPR^{TETRA} Hamamatsu FDSS
		Ca^{2+}	GCaMP2.1, GCaMP6, RCaMP	FLIPR^{TETRA} Hamamatsu FDSS
		Ca^{2+}/ Na^{+}/ H^{+}/ any ion that determines changes in MP	Arclight / pH sensor	FLIPR^{TETRA} Hamamatsu FDSS
	Transporter of ionic NAD^{+} substrate	NAD^{+}	SoNar, genetically encoded sensor for NAD^{+}/NADH	FLIPR^{TETRA} Hamamatsu FDSS

Figure 2 *– Fluorescent dyes and sensors that can be used by Axxam to assess the functionality of electrogenic transporters.*

Pilot study: HEK-293 FlipIn T-REx/NBCe1 and NBCE

Two HEK-293 FlipIn T-REx cell lines overexpressing the sodium-coupled bicarbonate transporters encoded by the genes ***SLC4A4*** and ***SLC4A10*** were delivered to Axxam from CeMM and included in a preliminary pilot study, in which the **Membrane Potential dye** was used as read-out to test their functionality:

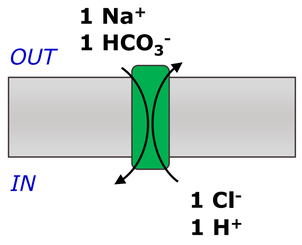
NBCe1 (*SLC4A4*)



NBCe1 moves a net negative charge in the direction of the transport (**electrogenic**)

Figure 3 *– Stoichiometry of the NBCe1 transporter.*

NBCE (*SLC4A10*)



NBCE transports extracellular Na^{+} and HCO_3^{-} into cells in exchange for intracellular Cl^{-} and H^{+} (**electroneutral**)

Figure 4 *– Stoichiometry of the NBCE transporter.*

PROTOCOL: Cells were seeded in a 384 multiwell format and induced (Doxycycline, 1 µg/mL). 24h after seeding, cells were loaded with MP dye in a buffer Na^{+}/HCO_3^{-} free for 30 min at room temperature. MP dye can detect changes in membrane potential by increasing (depolarization) or decreasing (hyperpolarization) the fluorescent signal. Stimulus ($NaHCO_3$) was then injected (10 mM; 1:2 dilution steps) and fluorescence was recorded for 5 min at FLIPR^{TETRA}

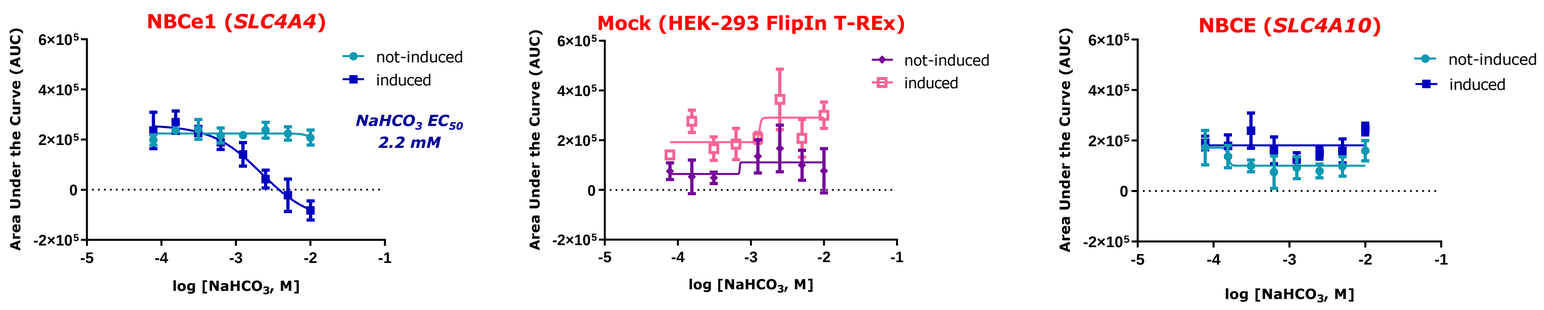


Figure 5 *– Results of the cell lines testing with MP dye.*

- **A specific response** of **NBCe1** was detected upon stimulation with the substrates (detected as a reduction of the fluorescent signal, due to hyperpolarization of the cell membrane)
- **No specific response** of **NBCE** was detected upon stimulation with the substrates
- **NBCe1** was confirmed to be **electrogenic**; **NBCE** was confirmed to be **electroneutral**
- **MP dye was proven to be a useful tool to characterize electrogenic transporters.**

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