RESOLUTE

Research Empowerment on Solute Carriers

Generation of robust cell-based assays for a set of SLCs – WP6

Salvatore Corallino, Francesca Sassone, Loredana Redaelli, Giovanna Maresca, Laura Montanini, Marcella De Silvestris and Lia Scarabottolo.

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

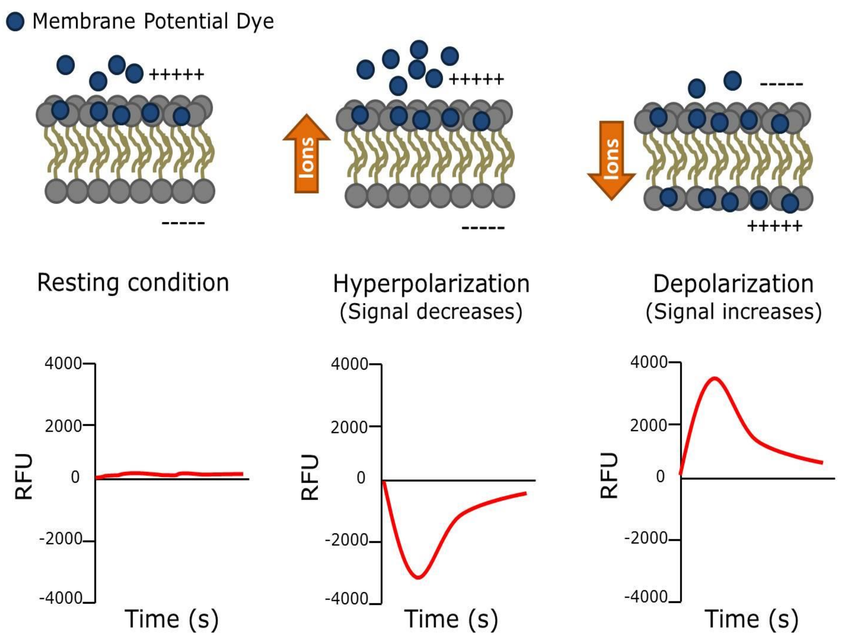
Introduction

Screening of large compound libraries represents a key strategy for developing pharmacotherapeutic interventions. To this aim, the goal of the WP6 is the development of reliable, reproducible and standardized SLCs assays suitable either for drug discovery campaigns on automated screening platforms or for in depth characterization of the selected targets. Axxam is generating cell-based assays targeting the transporters SLC2A9, SLC22A4, SLC6A8 and SLC29A1. The read-out systems adopted for developing this subset of functional assays rely on the use of Membrane Potential sensitive dye, in the case of electrogenic transporters, or on a fluorescent substrate when no variation in membrane potential can be detected. SLC2A9 and SLC22A4 have successfully concluded the assay development phase and are ready to enter the optimization phase. The functional results on second limiting dilution clones are reported. Assay development for the remaining targets is ongoing and different experimental strategies are under evaluation.

Cell-based assays under development

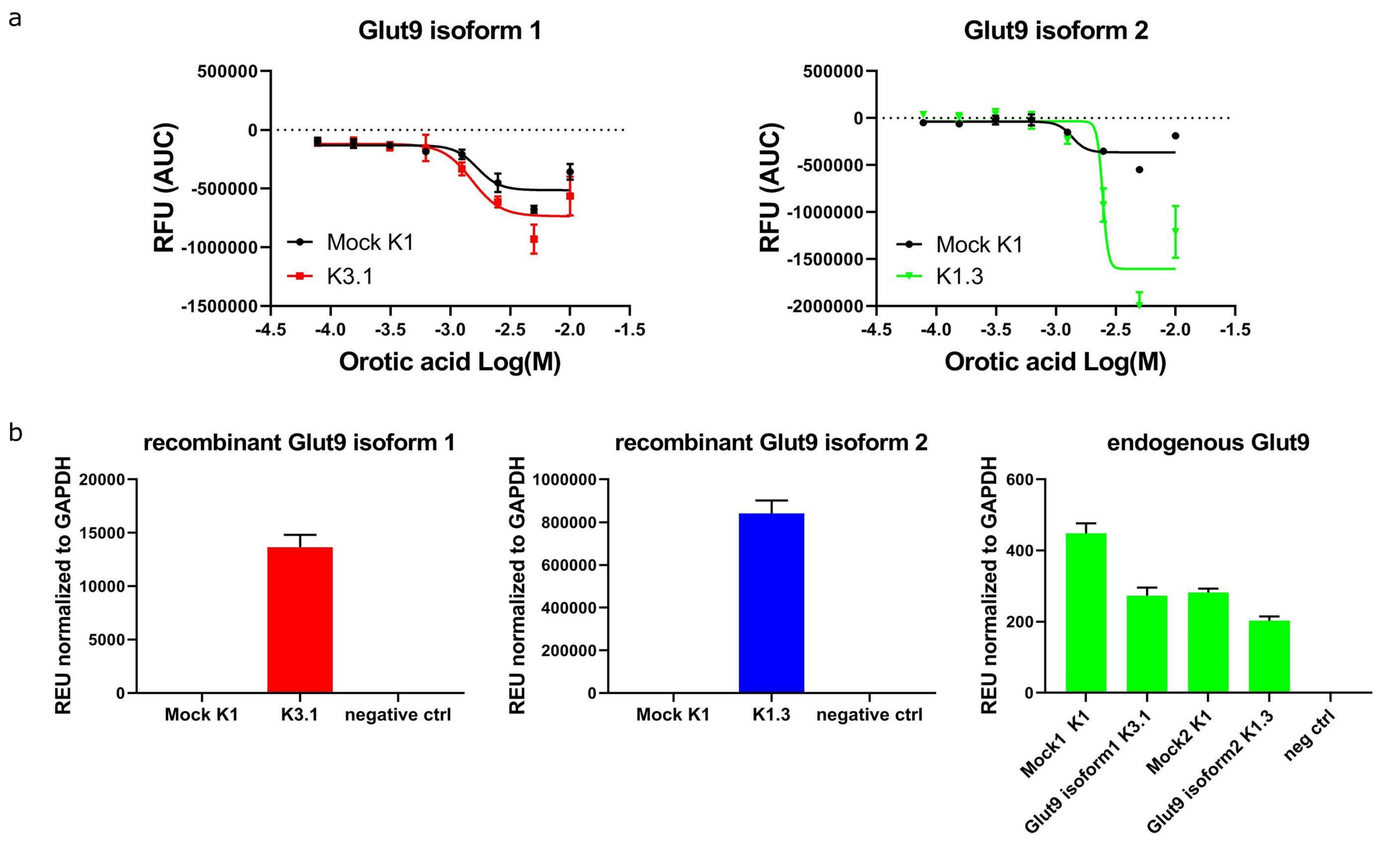
Target	Substrates	Reference inhibitors	Electrogenecity	Assay principle	Screening technology
SLC2A9 (GLUT9)	• Uric Acid • Orotic acid • Fructose	• Benzbromarone • Losartan	YES	Detection of membrane potential variation by Membrane Potential sensitive dye (Fluorescence)	• FLIPR^{TETRA} • Hamamatsu FDSS
SLC22A4 (OCTN1)	• Ergothioneine • cytarabine	• Verapamil • Disprocynium24	YES	Detection of membrane potential variation by Membrane Potential sensitive dye (Fluorescence)	• FLIPR^{TETRA} • Hamamatsu FDSS
SLC6A8 (CRTR)	• Creatine • Na+ • Cl-	• β guanidinopropionic acid • γ guanidinobutyrate • cyclocreatine	YES	Detection of membrane potential variation by Membrane Potential sensitive dye (Fluorescence)	• FLIPR^{TETRA} • Hamamatsu FDSS
SLC29A1 (ENT1)	• purine and pyrimidine nucleosides	• NBMPR (Nitrobenzylmercaptopurine ribonucleaside) • Dipyridamole • dilazep	NO	Detection of fluorescence signal variation upon fluorescent furopyrimidine (FuPmR) analogues transport	• Pherastar • Imaging

FLIPR Membrane Potential dye Assay Principle



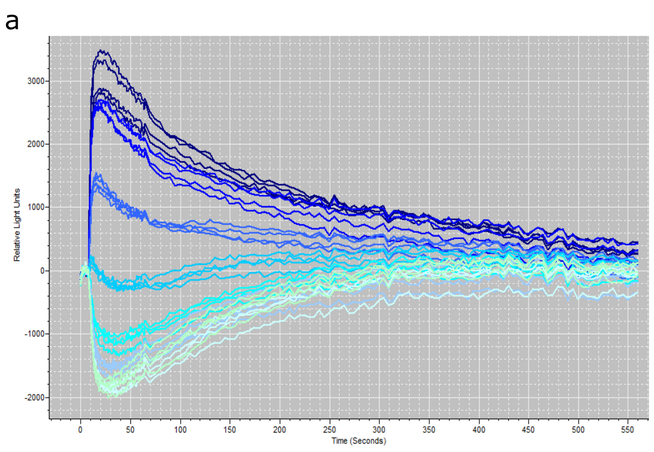
FLIPR Membrane Potential dye detects variations in membrane potential by following the movement of positively charged ions inside and outside the cell. A decrease in fluorescence intensity is observed upon membrane hyperpolarization whereas a depolarization event is characterized by an increase in fluorescence signal.

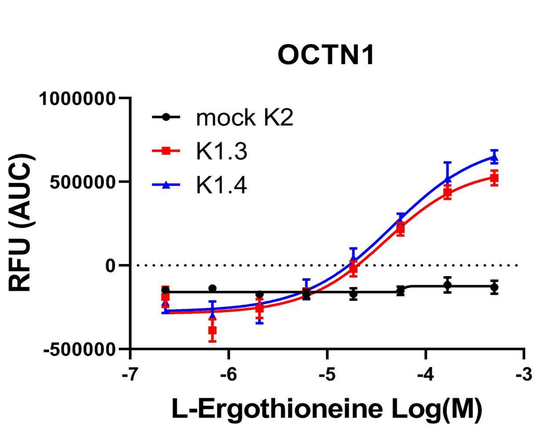
GLUT9: pharmacological characterization and expression analysis



(a) The indicated 2nd LD clones were functionally tested at FLIPR^{TETRA} using orotic acid as substrate and Membrane Potential dye as readout. The resulting D/R curves are reported. (b) The expression of endogenous and recombinant GLUT9 genes (isoform 1 and 2) was evaluated by qPCR analysis.

OCTN1: pharmacological characterization





	EC50
K1.3	3.916e-005
K1.4	4.575e-005

(a) Example of Kinetic traces obtained at FLIPR^{TETRA} after the injection of a D/R of ergothioneine (clone K1.4). (b) The indicated 2nd LD clones were functionally tested at FLIPR^{TETRA} using ergothioneine as substrate and Membrane Potential dye as readout. The resulting D/R curves and EC50 values are reported.

Concluding remarks

- AXXAM is carrying out the assay development for the following solute carriers: SLC2A9 (isoform 1 and 2), SLC22A4, SLC6A8 and SLC29A1
- The functional cell-based assays for SLC2A9 and SLC22A4 are going to be optimized in order to prove their suitability for HTS
- Preliminary results have been obtained for the transporters SLC6A8 and SLC29A1. These functional assays need to be finely tuned and alternative experimental approaches will also be considered

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www.re-solute.eu
Loredana.Redaelli.LR@axxam.com
Salvatore.Corallino.SC@axxam.com