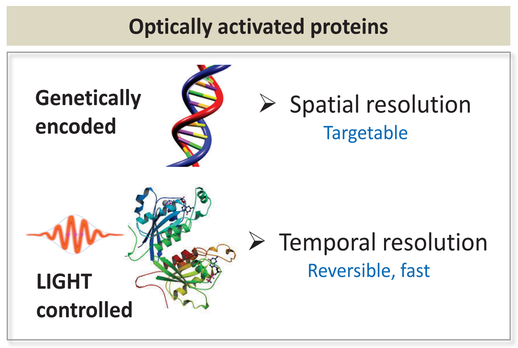
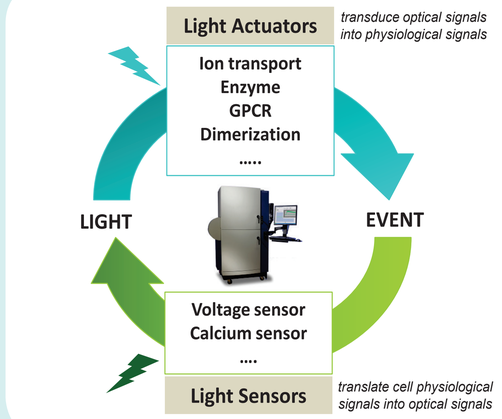
Optogenetics applied to the High Throughput Screening: novel cellular assays controlled by light

Viviana Agus, Alberto Di Silvio, Jean-Francois Rolland, Anna Mondini, Sara Tremolada, Katharina Montag, Lia Scarabottolo, Loredana Redaelli, Stefan Lohmer. AXXAM S.p.A, Bresso, Italy

Introduction

The recently developed Optogenetic technologies are based on genetically encoded elements which activity is controlled by light. By combining genetic targeting with optical stimulation, optogenetic tools can deliver a temporally precise stimulation or measurement of a biological process, in genetically defined group of cells or cell compartments.
Both "Light actuators" (proteins which transduce optical signals into physiological signals) and "Light sensors" (proteins which translate cell physiological signals into optical signals) have been expressed heterologously in different vertebrate systems, showing very good functionality even within intact tissues or behaving animals.
Due to the fact that they can be easily targetable to a specific cell type or cell compartment, and can be precisely and reversibly modulated by light pulses, they have been largely used to study complex cellular mechanisms, such as electrical networking of neuronal cells, or in general to use the light stimulation to induce or measure a biological event.
We investigated the possibility of using these optogenetic tools to create a new generation of cellular assays suitable for the High Throughput Screening, where the light is used to modulate and measure the functionality of a gene of interest in a more precise, reversible and physiological way compared to the use of chemicals. In particular we verified if these recombinant optogenetic assays can be adapted to a miniaturized 384-well plate format, and also if the optics of a standard HTS instrumentation like the FLIPR (FLuorescence Imaging Plate Reader) could efficiently induce or detect the light modulation of these optogenetic tools.

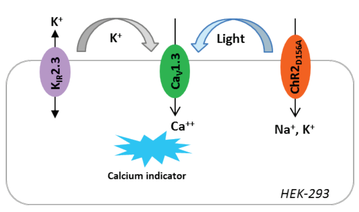




Case study 1
ChR2 ion channel to modulate $Ca_V1.3$

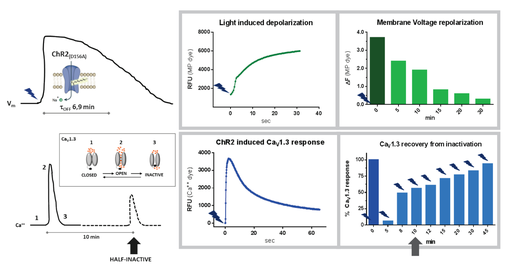
In the first case study, we could demonstrate that the light-activated cation channel ChR2 D156A (from *Chlamydomonas reinhardtii*) expressed in HEK-293 cells can be very efficiently stimulated by the blue LEDs of the FLIPR instrument; the membrane depolarization signal obtained upon blue light stimulation was very strong and sustained, and could be very well detected in the miniaturized 384-well plate format used. By the co-expression of the $Ca_V1.3$ voltage-gated calcium channel, we could also demonstrate that the membrane depolarization induced by the channelrhodopsin stimulation is sufficient to elicit a very strong calcium influx through the $Ca_V1.3$ channel; we could also setup a protocol where, upon repetitive blue light stimulations, we could study the $Ca_V1.3$ channel either in a "resting" or in an "inactivated" state. The optogenetic assay for $Ca_V1.3$ channel showed a very robust performance, and also pharmacological data in very good agreement with literature and with electrophysiology experiments.

Setup of an optogenetic assay for $Ca_V1.3$

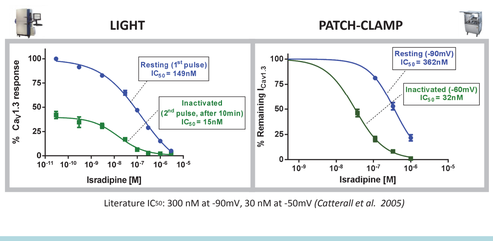


HEK-293 cells expressing human $K_{IR}2.3$ inward rectifying potassium channel were stably transfected with human $Ca_V1.3$ ($\alpha1 + \alpha2\delta1 + \beta3$ subunits) and *Chlamydomonas reinhardtii* Channelrhodopsin-2 (ChR-2) D156A mutant.
ChR2 was stimulated by FLIPR-TETRA LEDs (Exc. 470-495nM) then membrane depolarization or calcium influx were measured using fluorescent organic dyes (Molecular Devices).

Light can control membrane voltage and $Ca_V1.3$ channel states



State-dependent blockers pharmacology

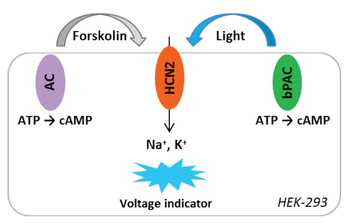


Literature IC_{50}: 300 nM at -90mV, 30 nM at -50mV *(Catterall et al. 2005)*

Case study 2
bPAC enzyme to modulate HCN2

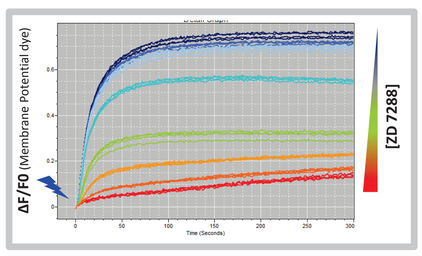
In the second case study, we stably expressed a light-activated enzyme, the Photoactivated Adenylyl Cyclase from *Beggiatoa* bacterium (bPAC) in HEK-293 cells.
By the co-expression with the HCN2 cyclic nucleotide gated channel, we could demonstrate that the blue LEDs of the FLIPR instrument can activate in a very fast and robust way the cAMP production through bPAC enzyme, resulting in a strong and sustained cation influx through the HCN2 channel.
The optogenetic assay for HCN2 channel showed an improved response window compared to the classical Forskolin stimulation of endogenous adenylyl cyclases, and pharmacological data in good agreement with literature.

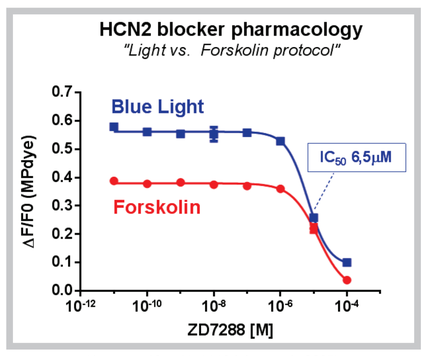
Setup of an optogenetic assay for HCN2



HEK-293 cells were stably transfected with human Hyperpolarization-activated Cyclic Nucleotide-gated cation channel (HCN2) and *Beggiatoa* Photo-activated Adenylyl cyclase (bPAC). Blue light stimulation of bPAC was obtained by FLIPR-TETRA LEDs (Exc. 470-495nM) then membrane depolarization induced by HCN2 was measured using fluorescent organic dyes (Molecular Devices).

Light-induced cAMP production can activate HCN2 channel



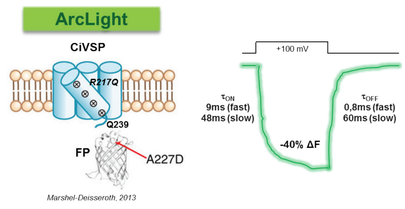


Literature IC_{50}: 41μM (*Stieber et al., 2005*)

Case study 3
ArcLight and RCaMP for simultaneous voltage and calcium imaging

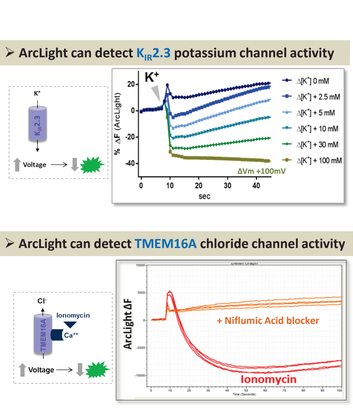
In the third case study we co-expressed the Genetically Encoded Voltage Indicator ArcLight (from *Ciona intestinalis*) with the Genetically Encoded Calcium Indicator RCaMP in HEK-293 cells, and we demonstrated that this cell line can efficiently detect membrane voltage and intracellular calcium changes induced by different ion channel targets. Both the green fluorescent ArcLight and red fluorescent RCaMP sensors showed a robust and homogeneous fluorescent signal, which can be very well detected by the FLIPR instrument LEDs, in a miniaturized 384-well plate assay format. Thanks to the nice spectral separation of these two sensors, we could develop a protocol for the simultaneous detection of both membrane voltage and intracellular calcium changes in the same well, generating a multi-color, multi-parametric HTS-compatible cellular assay with improved content and reduced costs compared to the use of organic dyes.

Genetically Encoded Voltage Indicator

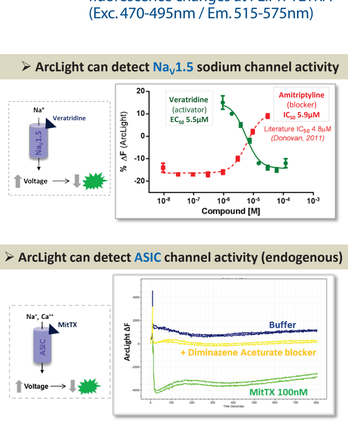


The *Ciona intestinalis* "ArcLight" fluorescent voltage probe was stably transfected in HEK-293 cells expressing different ion channel targets: the human $K_{IR}2.3$ inward rectifying potassium channel, the human $Na_V1.5$ voltage gated sodium channel, the human TMEM16A calcium activated chloride channel or the ASIC acid sensing (proton gated) ion channel (endogenouse). Membrane voltage changes were measured as ArcLight fluorescence changes at FLIPR-TETRA (Exc. 470-495nm / Em. 515-575nm)

➢ ArcLight can detect $K_{IR}2.3$ potassium channel activity



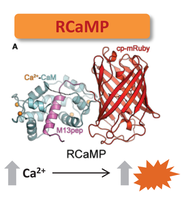
➢ ArcLight can detect $Na_V1.5$ sodium channel activity



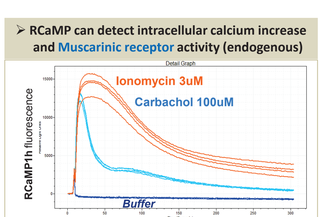
➢ ArcLight can detect TMEM16A chloride channel activity

➢ ArcLight can detect ASIC channel activity (endogenous)

Genetically Encoded Calcium Indicator

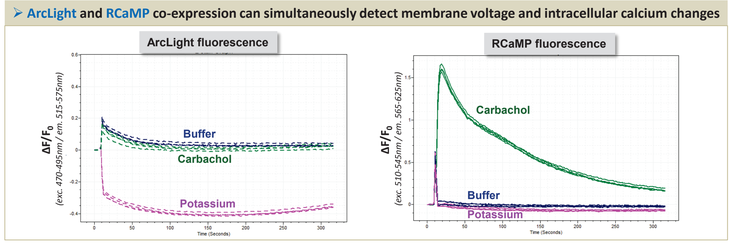


➢ RCaMP can detect intracellular calcium increase and Muscarinic receptor activity (endogenous)



The "RCaMP" fluorescent calcium probe was stably transfected in HEK-293 cells. Intracellular calcium changes were measured as RCaMP fluorescence changes at FLIPR-TETRA (Exc. 510-545nm / Em. 565-625nm)

➢ ArcLight and RCaMP co-expression can simultaneously detect membrane voltage and intracellular calcium changes



Multi-color multi-parametric measurements were performed on HEK-293_$K_{IR}2.3$ cells stably co-expressing «ArcLight» green fluorescent voltage indicator and «RCaMP» red fluorescent calcium indicator; membrane voltage changes and intracellular calcium changes were measured **simultaneously in the same well**, running a «Dual Read Mode» protocol at FLIPR-TETRA (ReadMode1 (ArcLight) = Exc. 470-495nm / Em. 515-575nm; ReadMode2 (RCaMP) = Exc. 510-545nm / Em. 565-625nm)

Concluding remarks

- A new generation of recombinant cellular assays have been developed, by the use of light activated proteins and genetically encoded calcium or voltage indicators.
- The light-activated cation channel Channelrhodopsin 2 was proven to efficiently modulate the membrane voltage and the $Ca_V1.3$ calcium channel activity, allowing a robust and reliable detection of $Ca_V1.3$ state-dependent blockers.
- The light-activated adenylyl cyclase bPAC was proven to efficiently activate the HCN2 cyclic nucleotide gated cation channel, through the production of cAMP second messenger. The induced membrane depolarization was very fast and robust, showing higher performance compared to the classical Forskolin stimulation and good pharmacological behaviour.
- The Genetically Encoded Voltage Indicator ArcLight was proven to be able to detect the membrane voltage changes induced by a potassium, a sodium or a chloride ion channel target.
- The Genetically Encoded Calcium Indicator RCaMP was proven to be able to detect the intracellular calcium changes induced by a muscarinic receptor.
- The co-expression of ArcLight and RCaMP indicators can be used to generate a multi-color, multi-parametric reporter cell line suitable for the simultaneous detection of membrane voltage and intracellular calcium changes in the same well.
- Our results demonstrated that different optogenetic tools can be efficiently modulated by the FLIPR instrument optics, in a miniaturized 384-well plate format.
- The possibility to use optogenetic tools in an High Throughput Screening-compatible instrumentation, might deliver significant improvements in the development of future functional assays, allowing a temporal and spatial precision of stimulation and detection that would be superior to any other chemical or electrical methodology.

Axxam SpA • OpenZone
via Meucci 3 • 20091 Bresso, Milan (Italy) • phone +39 02 210561 • fax +39 02 2105602 • **www.axxam.com**