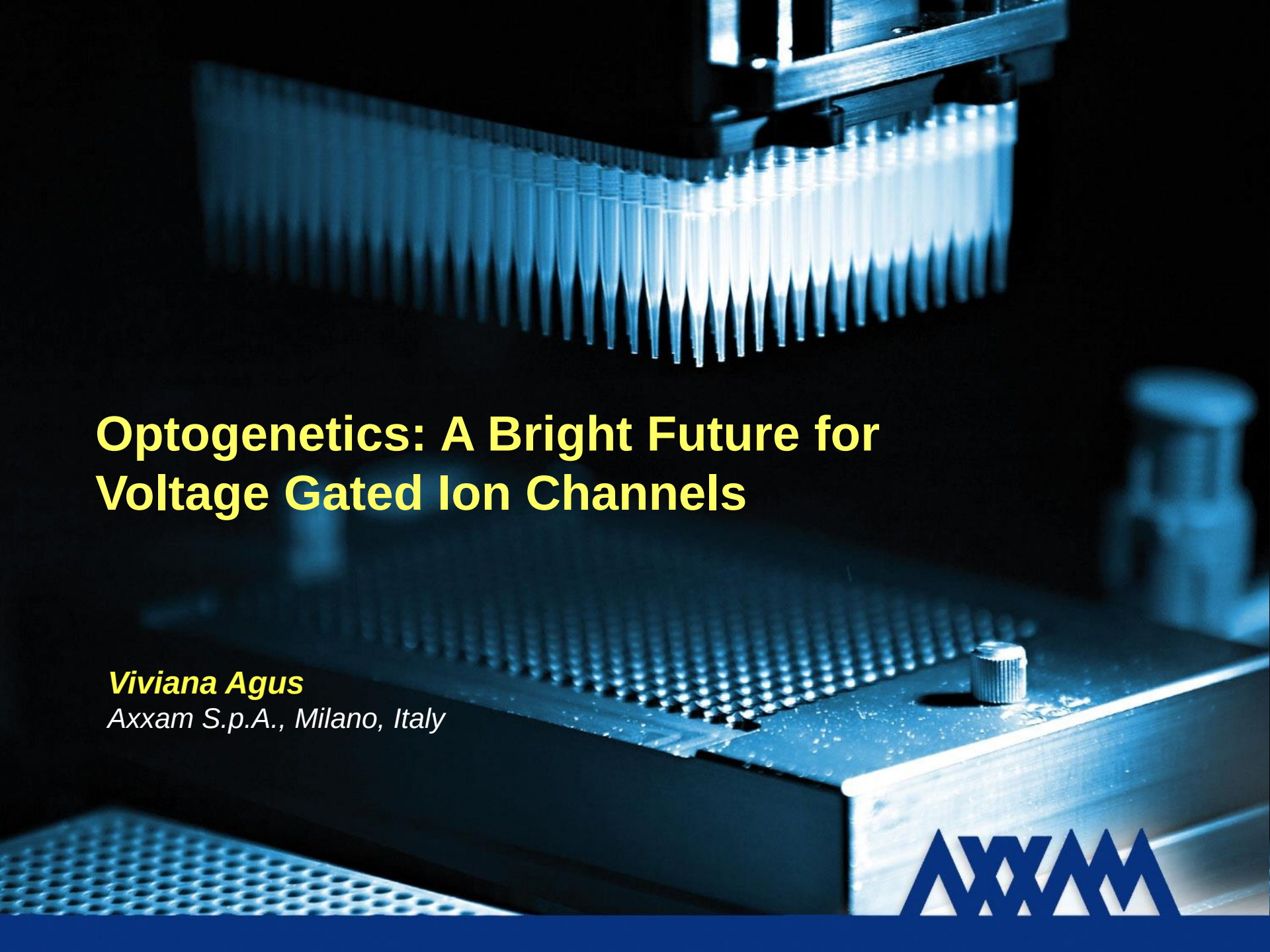

Optogenetics: A Bright Future for Voltage Gated Ion Channels

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Presentation Summary

- **Hamamatsu and AXXAM:** FDSS µCELL demo period

Cav1.3 and ChR2 assay

- **Optogenetics**: overview and advantages
- **Channelrhodopsin2** to modulate cell membrane voltage
- **Activation of Cav1.3 by ChR2**: recombinant assay setup
- Validation of **"light protocol" at FDSS µCELL**: test of reference compounds
- **Comparison with "K^+ protocol" and patch-clamp** data
- **Conclusions** and future perspectives

FDSS µCELL DEMO @ AXXAM

Ca^{2+} assay (fluorescent dyes, luminescent photoprotein)
- CCKAR (GPCR)
- GLP1R (GPCR)
- ADORA1 (GPCR)
- DRD1-DRD2 (GPCR)
- Enzymatic assay

Glow Luminescence assay:
- PPARα, PPARδ (NHR)
- Promoter assay

Genetically encoded sensor
- TMEM16A (EYFP)

Optogenetics
- Cav1.3 (ChR2)

Fluo8 LED (blue light)
MPdye LED (green light)

Hybrid Camera (Fluo & Lumi)

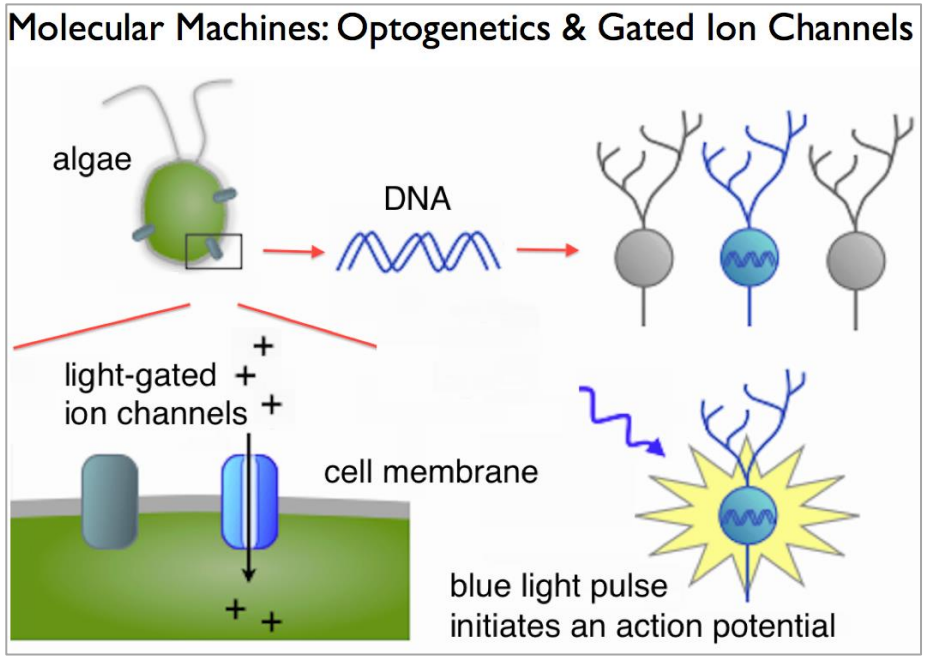
Optogenetics: overview

Optogenetics is a technology that combines:

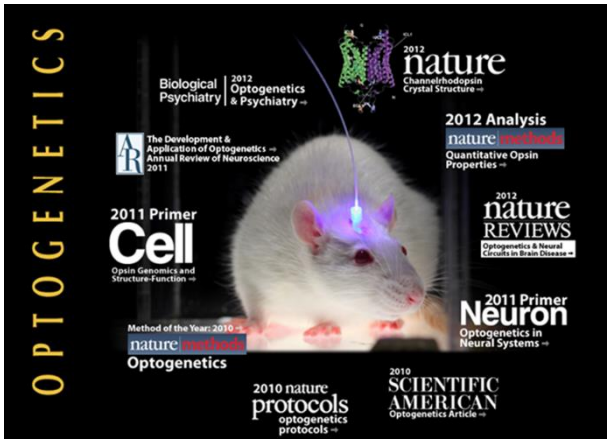
1) A **«genetic»** component, able to target specific neuron types

2) An **«optical»** component, able to interact specifically with the genetic component

to achieve fast control of well-defined events in specific cells of living tissue



The starting point was the idea to have a system available to control the activity of specific neuron types in the brain in a better way

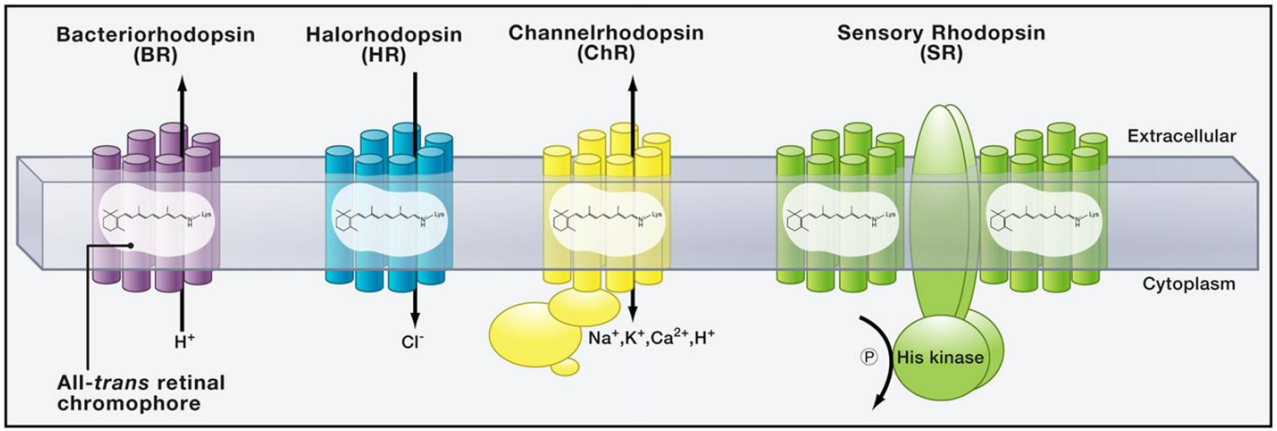


Method of the year 2010

Rhodopsins: Light-gated ion channels

Opsins:

- Seven-transmembrane, **light-responsive** proteins
- **Rapidly translocate specific ions** across the membranes of the cells in which they are expressed
- Contain the Vitamin-A derived chromophore **all-*trans*-retinal** as a light capture molecule
- Studied since the 1970s for their fascinating biophysical properties
- Used by several different life forms that use light as energy source or sensory cue



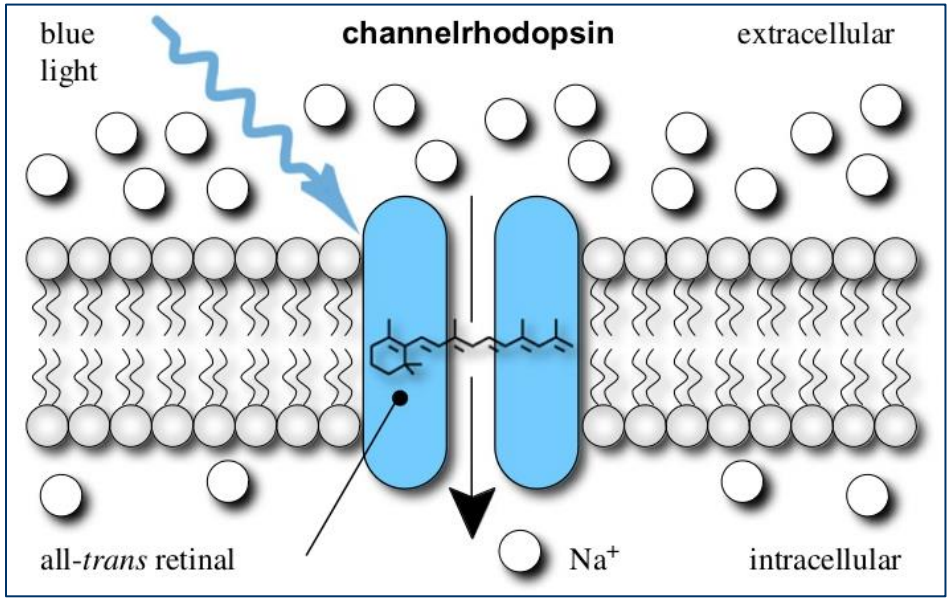
From Zhang F. et al; Cells 147, 2011. 1446-1457

Structural simplicity, fast kinetics ► attractive tool for a **rapid control of specific cellular processes**, such as, for example, modulation of membrane voltage and neuronal action potentials propagation

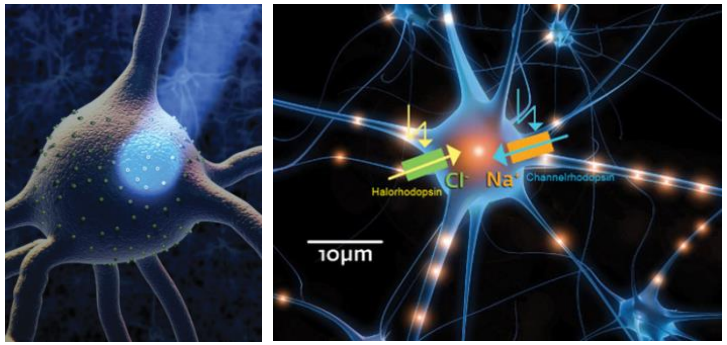
Light as activating stimulus ► **more physiological**, compared to other hyperpolarizing or depolarizing stimulus (for example K^+ injection)

Possibility to target their expression to specific cell types ► **genetically defined** modulation of cellular processes

Channelrhodopsin-2



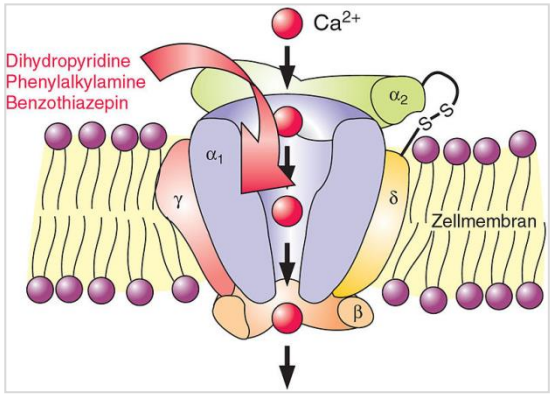
From: Wong J, J Mech Phys Solids 2012 Jun 1; 60(6) 1158-1178



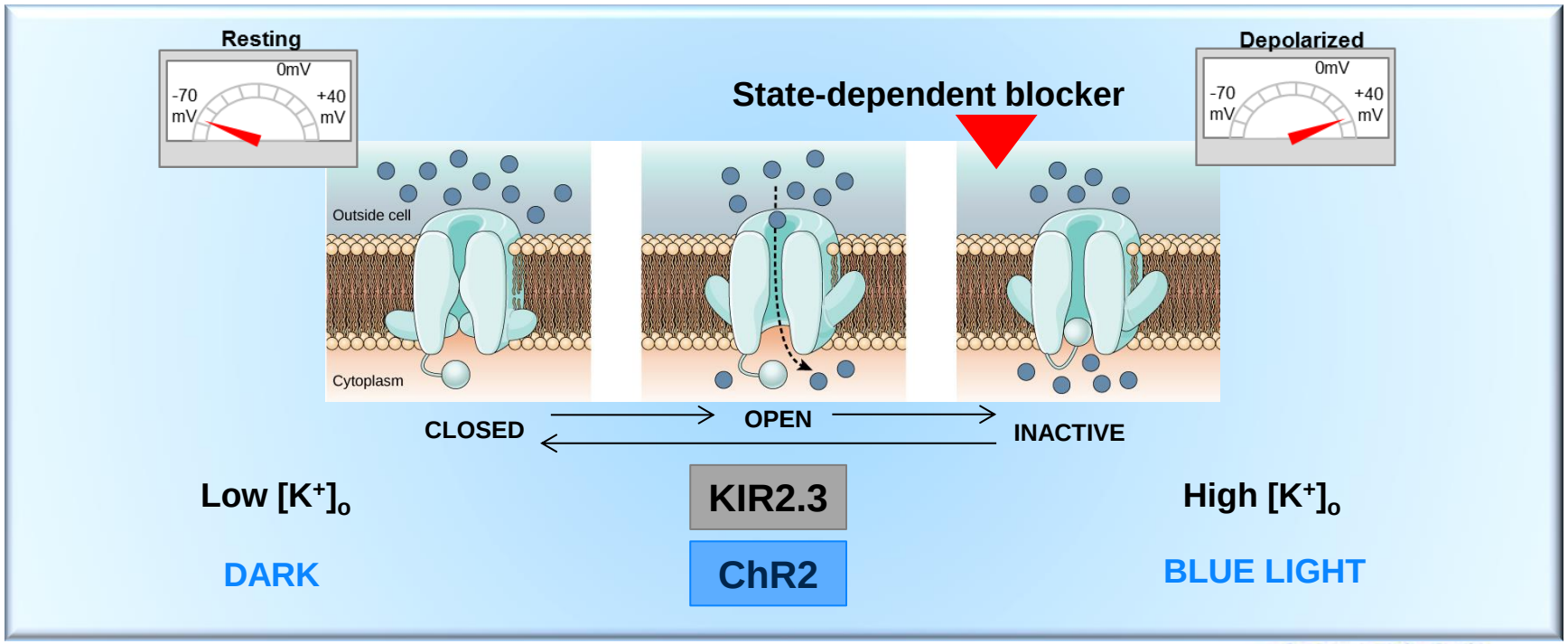
From Ed Boyden Lab.

- **Seven transmembrane opsin** (eyespot of unicellular alga *Chlamydomonas reinhardtii*)
- **Activated by blue light (470 nm)**: the light causes a **conformational change** in the light sensitive molecule (retinal), which in turn causes a conformational change and the opening of the channelrhodopsin protein
- **Non-selective cation channel (Na^+, K^+, Ca^{2+}, H^+):** the **flow of ions** changes the electrical potential across the cell membrane which might, if sufficiently large, cause the neuron to fire
- **Widely used to depolarize neurons** and generate action potential firing: very good expression in different cell hosts

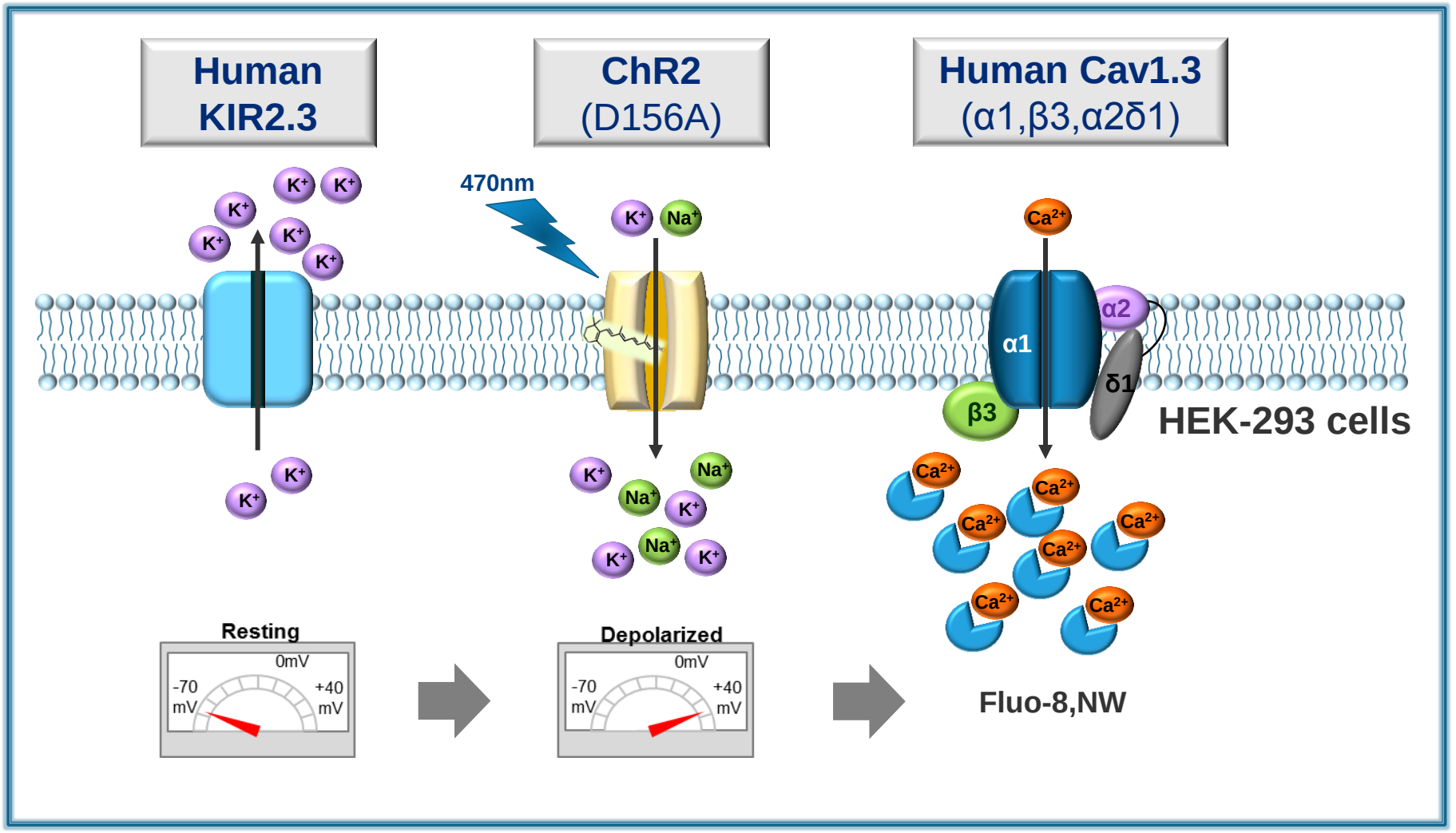
Optogenetic control of Cav1.3



- **L-type calcium channel**
- **High Voltage Activated (HVA)**
- **α1** (pore) **+ α2δ, β, γ** (accessory) **subunits**
- **Therapeutic target:** Cardiovascular, hormone secretion, CNS (Parkinson's, Alzheimer's disease)
- **Drug need:** Cav1.2 selectivity; state-dependent



Cav1.3 optogenetic assay



Channelrhodopsin-2 and cell based assays

KEY QUESTIONS

- Is it possible to adapt the assay to the FDSS µCELL optics for use in HTS?
- Can ChR2 be used to depolarize cells, such as HEK293, avoiding the artificial depolarization protocols such as KCl injection?
- Does the exposure of the cells to blue light of adequate intensity induce a ChR2 dependent cellular depolarization with subsequent activation of the transfected target?
- Does the ion flux through ChR2 alter the detection of the transfected target?

POTENTIAL ISSUES

- The light produced by the instrument LED system might not have the adequate intensity for ChR2 activation
- The ion flux through the ChR2 might be not sufficient to induce membrane depolarization
- The membrane depolarization induced might be not sufficient to drive the activation of transfected voltage gated channels.
- ChR2 is not permeable to Ca^{2+} in the presence of extracellular Na^{+}; therefore Cav channels are ideal targets to be modulated with optogenetics, since their activity can be monitored by the use of a Ca^{2+} sensitive dye

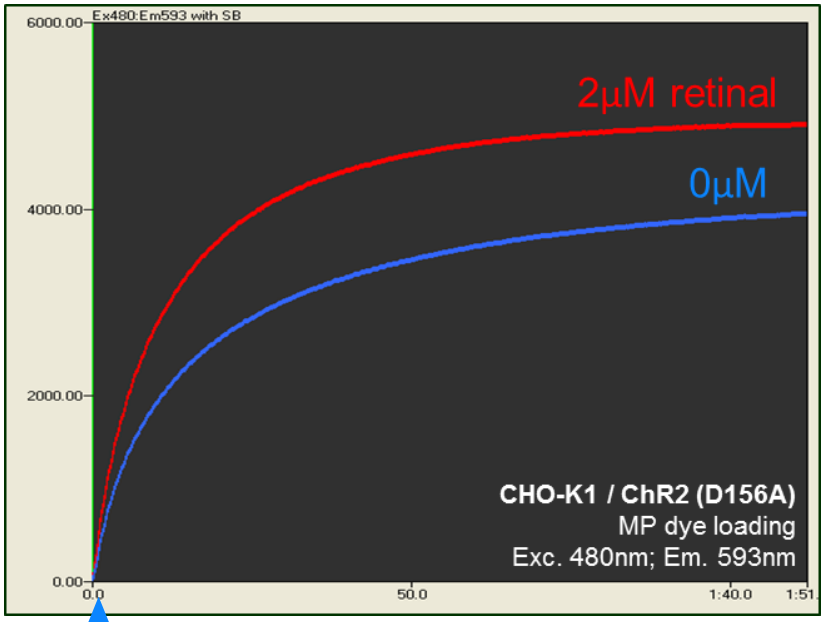
GOAL

Generate stable cell lines co-expressing a Voltage Gated ion channel of interest and ChR2 without altering the ion channel pharmacology

ChR2 induced membrane depolarization

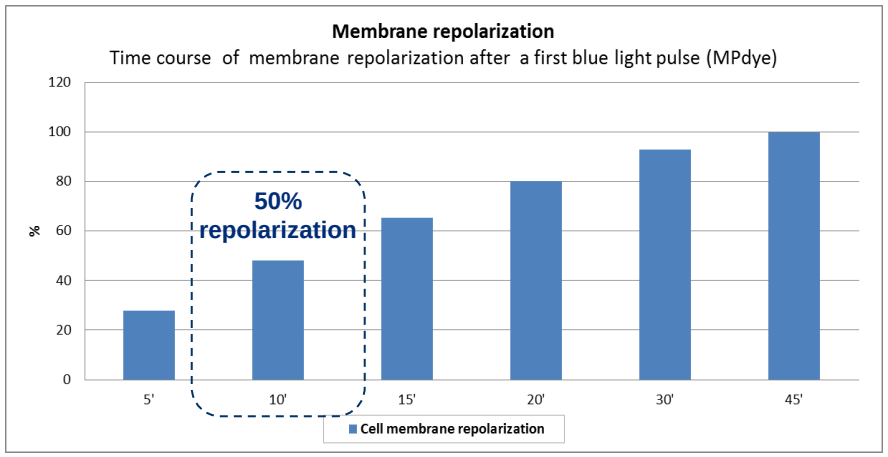
- **FDSS µCELL LED efficiently activates ChR2 $_{D156A}$**

(minimum light intensity required for wild-type ChR2 activation: 1mW/mm^2; *Aravanis, 2007*)



(τ-off 6.9 min)

Blue light
(λ = 480 nm; 0.013 mW/mm^2)



- **Membrane depolarization half-recovered after ≈ 10 min**

Cav1.3 half-inactivation protocol

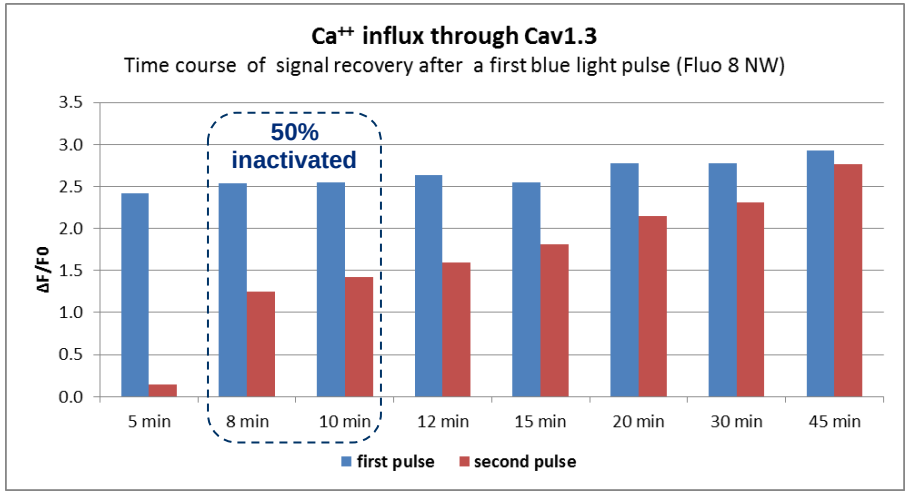
LIGHT

Blue light • ChR2 • Fluo8

Blue light

5 - 45 min

Fluo 8 dye
(4 mM K^+; 2µM retinal)



- **Cav1.3 efficiently activated by ChR2**
- **50% recovery from inactivation after 10 min**

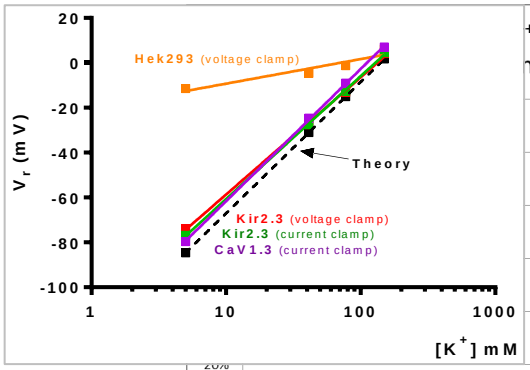
INACTIVE ⟷ RESTING

K^+

Fluo 8 dye

0mM - 75 mM K^+

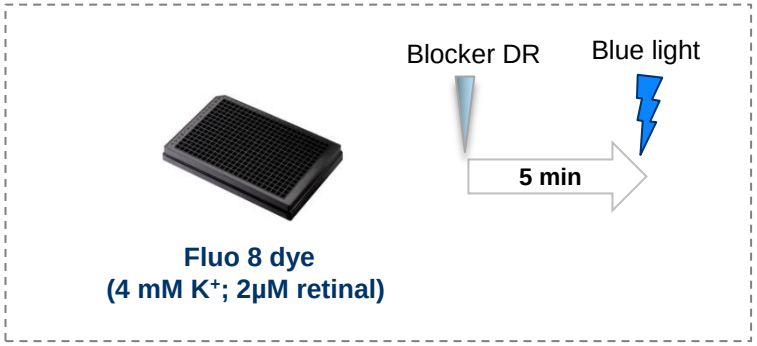
75mM K^+

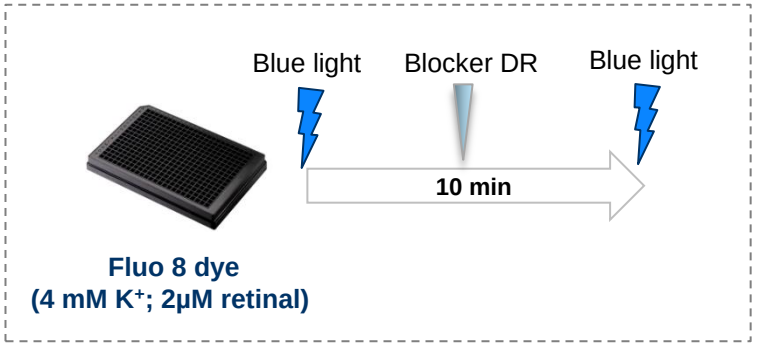


- **Cav1.3 efficiently activated by K^+**
- **50% Cav1.3 inactivation in 16mM K^+**

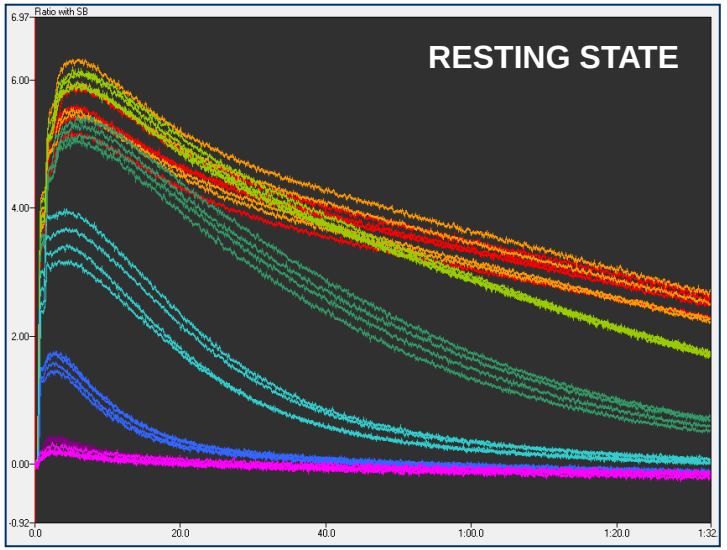
State-dependent blockers with «Light protocol»

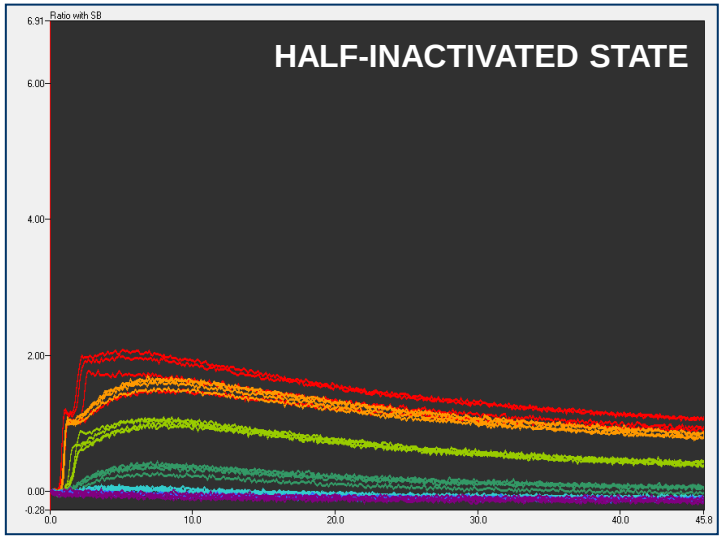
ISRADIPINE dose-response @ µCELL





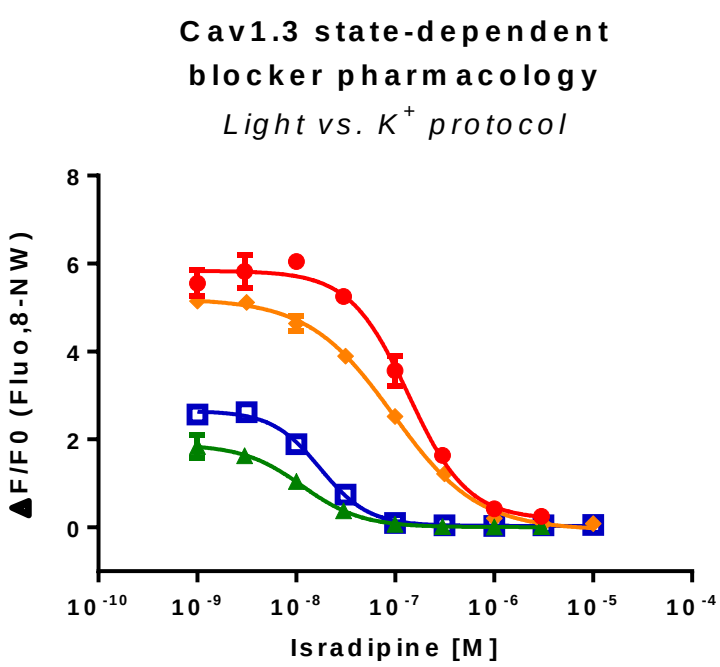
- **Very nice Cav1.3 activation by ChR2**
- **State dependency well detected by Light inactivation protocol**





Read interval 0.1s
Exp.: 0.03s; Sens.: 3

Light protocol vs. K+ protocol vs. qPatch



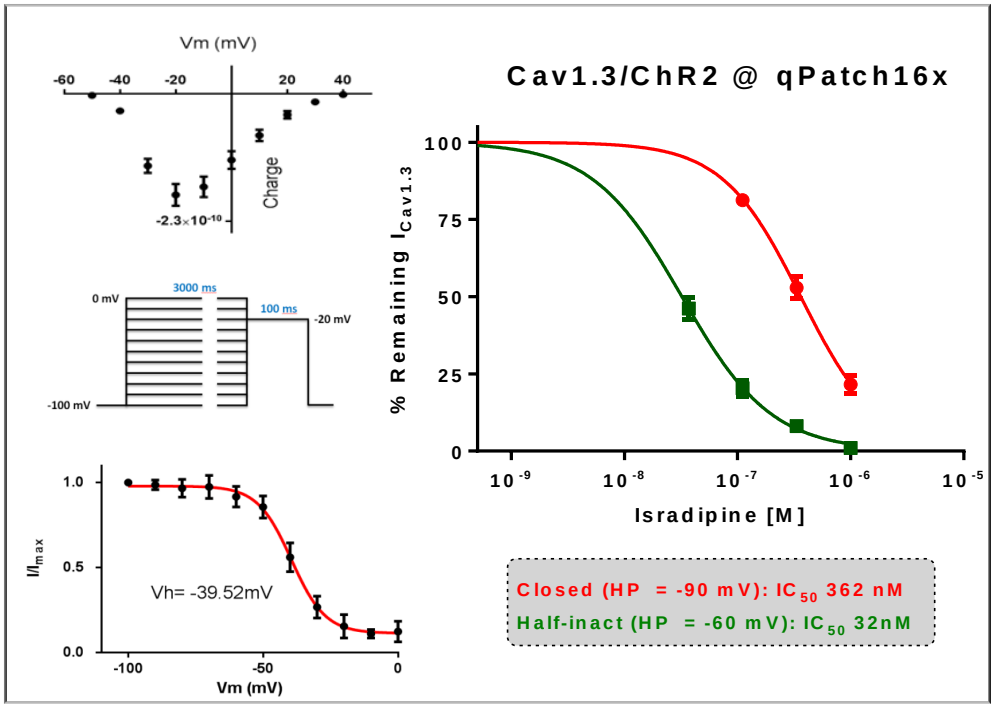
Resting (dark): IC_{50} 137nM

Resting (4mM K^+): IC_{50} 95nM

Half-inact (Blue light ➔ 10 min): IC_{50} 12nM

Half-inact (16mM K^+): IC_{50} 14nM

- "Light protocol" well suitable for state-dependent blockers studies
- Good correlation with classical "K^+ protocol" (less physiological)
- Good correlation with patch-clamp



Closed (HP = -90 mV): IC_{50} 362 nM

Half-inact (HP = -60 mV): IC_{50} 32nM

Summary and conclusions

Isradipine IC_{50}	"K^+ protocol"	"Light protocol"	qPatch 16x	Literature
Resting	95 nM	137 nM	362 nM	300 nM (-90mV)
Half-inactivated	14 nM	12 nM	32 nM	30 nM (-50mV)
RATIO	6.8	11.4	11.3	10

MAIN ACHIEVEMENTS:

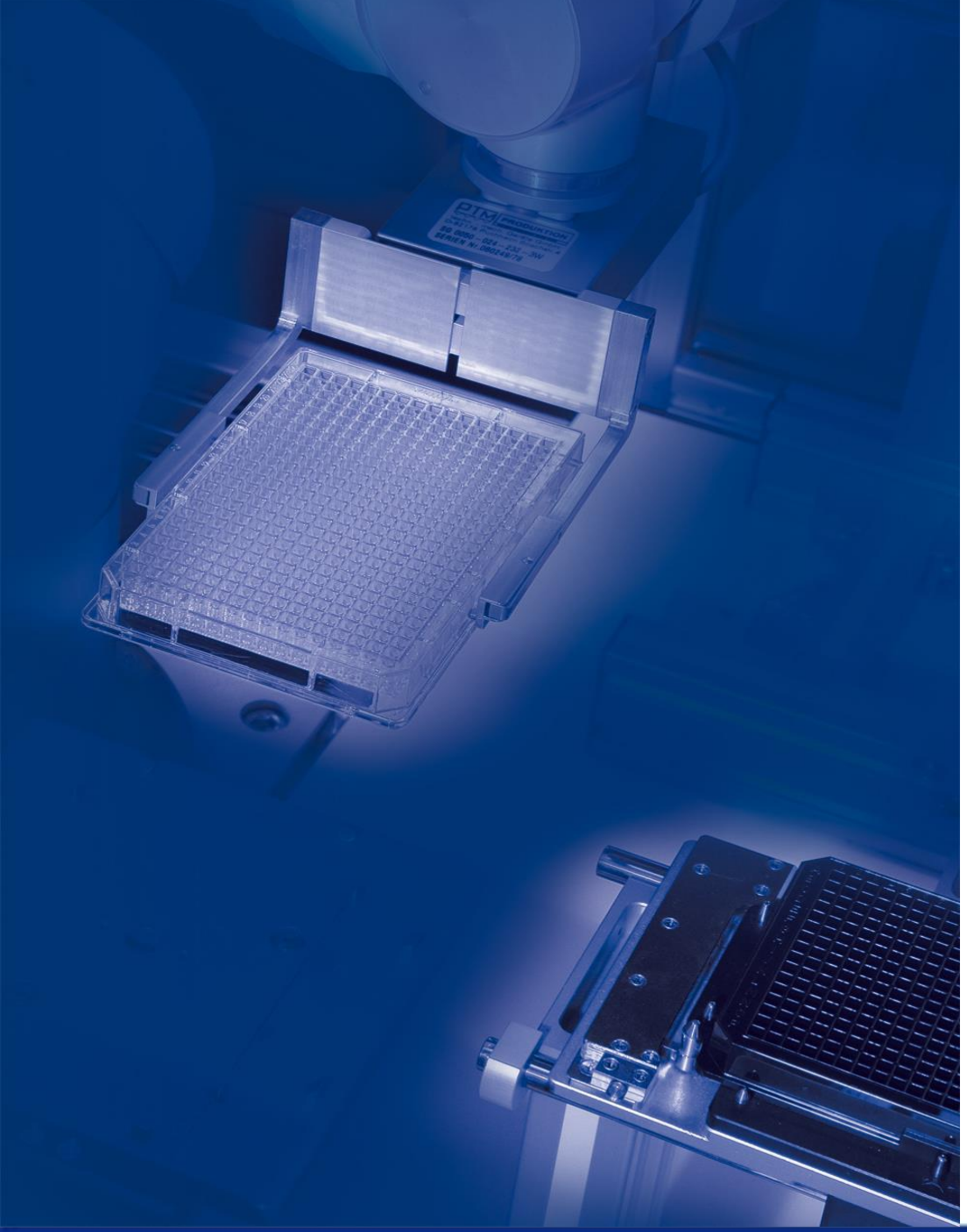
- FDSS µCELL **optics** is well suitable for ChR2 activation
- A "**Light protocol**" was set up at the FDSS µCELL to study the Cav1.3 channel either in resting or inactivated state
- The **pharmacology** of known state dependent blockers has been successfully validated, showing a good agreement with the classical "K^+ protocol", patch clamp experiments and literature data

HIGHLIGHTS:

- **First time ChR2 used for optical control of recombinant voltage-gated calcium channel assay**
- Physiological, robust, precise activation of Cav1.3 channel

FUTURE PERSPECTIVES:

- Light modulation of **other voltage-gated ion channel** target is ongoing



Aknowledgments

AXXAM:

Alberto di Silvio cell line generation
Sara Tremolada cell line validation
Jean-Francois Rolland patch-clamp
Katharina Montag clonings
Loredana Redaelli cell biology head
Lia Scarabottolo discovery services director
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