



Optogenetics: A Bright Future for Voltage Gated Ion Channels

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Presentation Summary

- **Hamamatsu and AXXAM:** FDSS μ CELL 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 μ CELL**: test of reference compounds
- **Comparison with “K⁺ protocol” and patch-clamp** data
- **Conclusions** and future perspectives



FDSS μ CELL DEMO @ AXXAM

Ca²⁺ assay (fluorescent dyes, luminescent photoprotein)

- CCKAR (GPCR)
- GLP1R (GPCR)
- ADORA1 (GPCR)
- DRD1-DRD2 (GPCR)
- Enzymatic assay

Glow Luminescence assay:

- PPAR α , PPAR δ (NHR)
- Promoter assay

Genetically encoded sensor

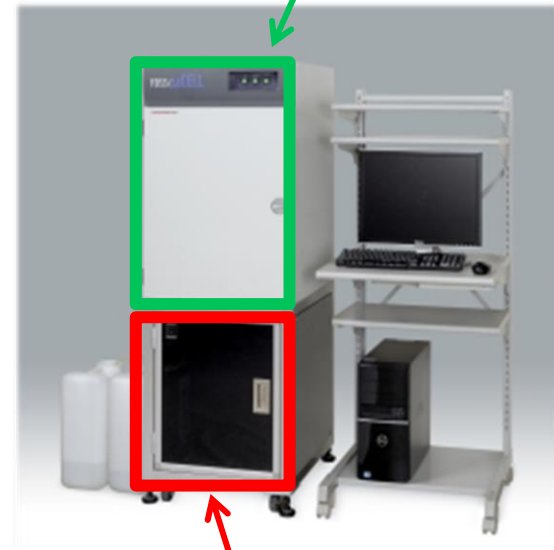
- TMEM16A (EYFP)

Optogenetics

- Cav1.3 (ChR2)



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



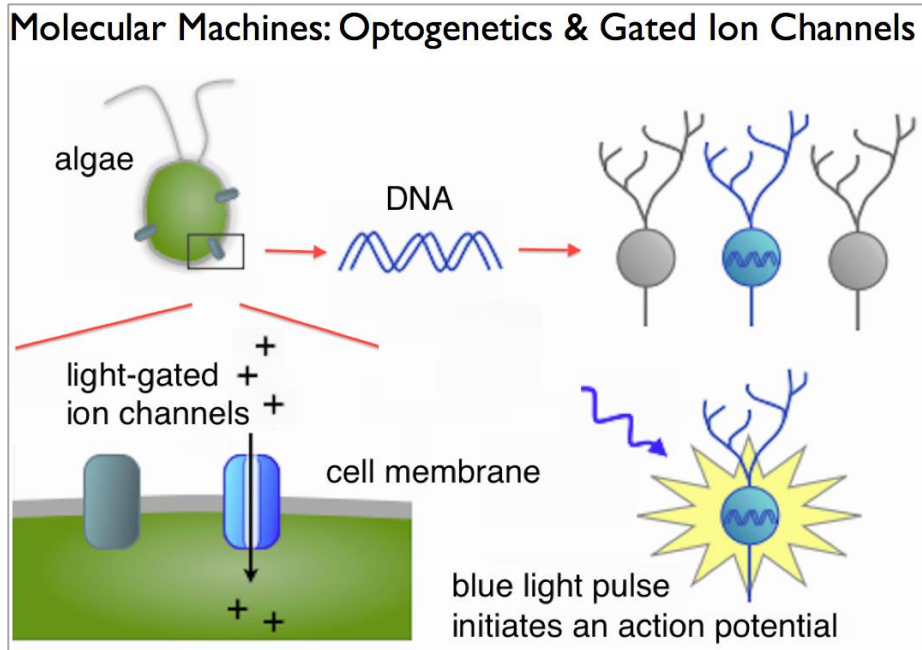
Hybrid Camera
(Fluo & Lumi)



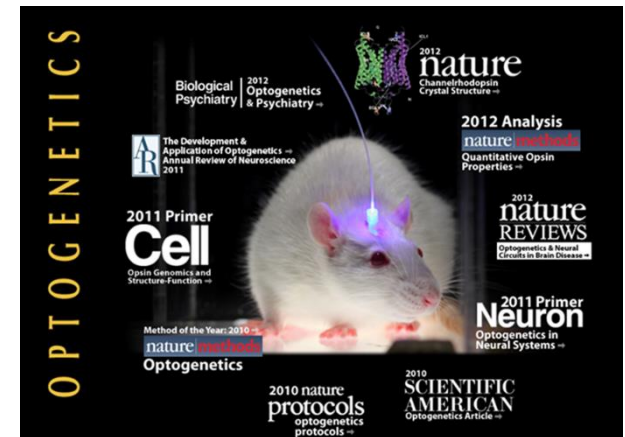
Optogenetics: overview

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

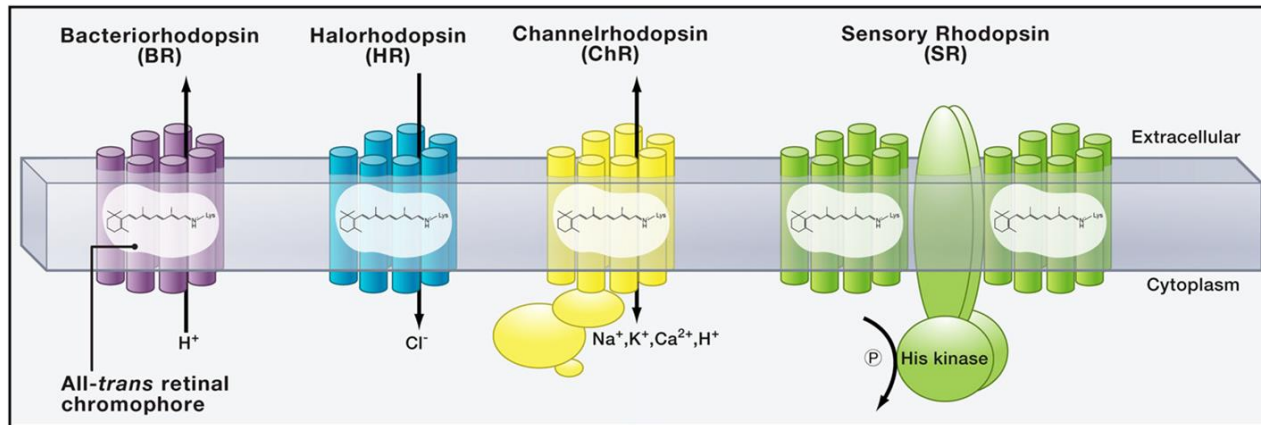


Method of the year 2010

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

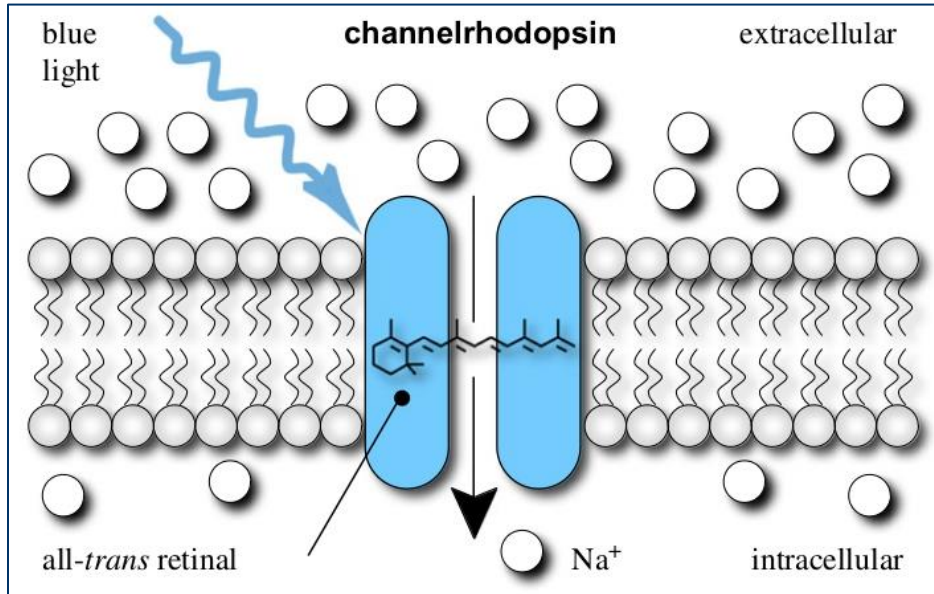
Structural simplicity, fast kinetics ► attractive tool for a **rapid control of specific cellular processes**, such as, for example, modulation of membrane voltage and neuronal action potentials propagation

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

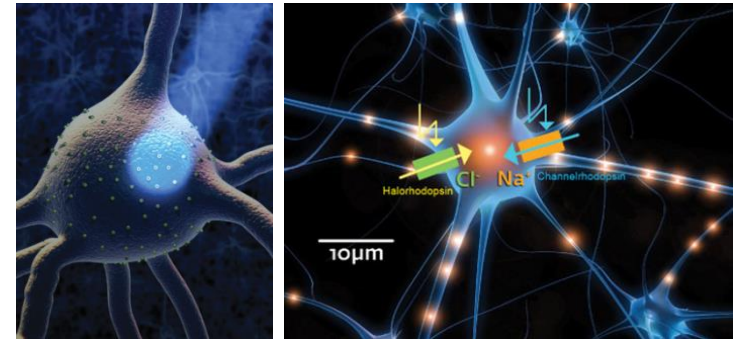
Possibility to target their expression to specific cell types ► **genetically defined** modulation of cellular processes



Channelrhodopsin-2



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

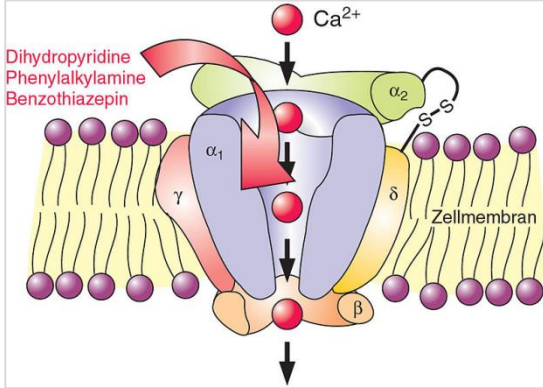


From Ed Boyden Lab.

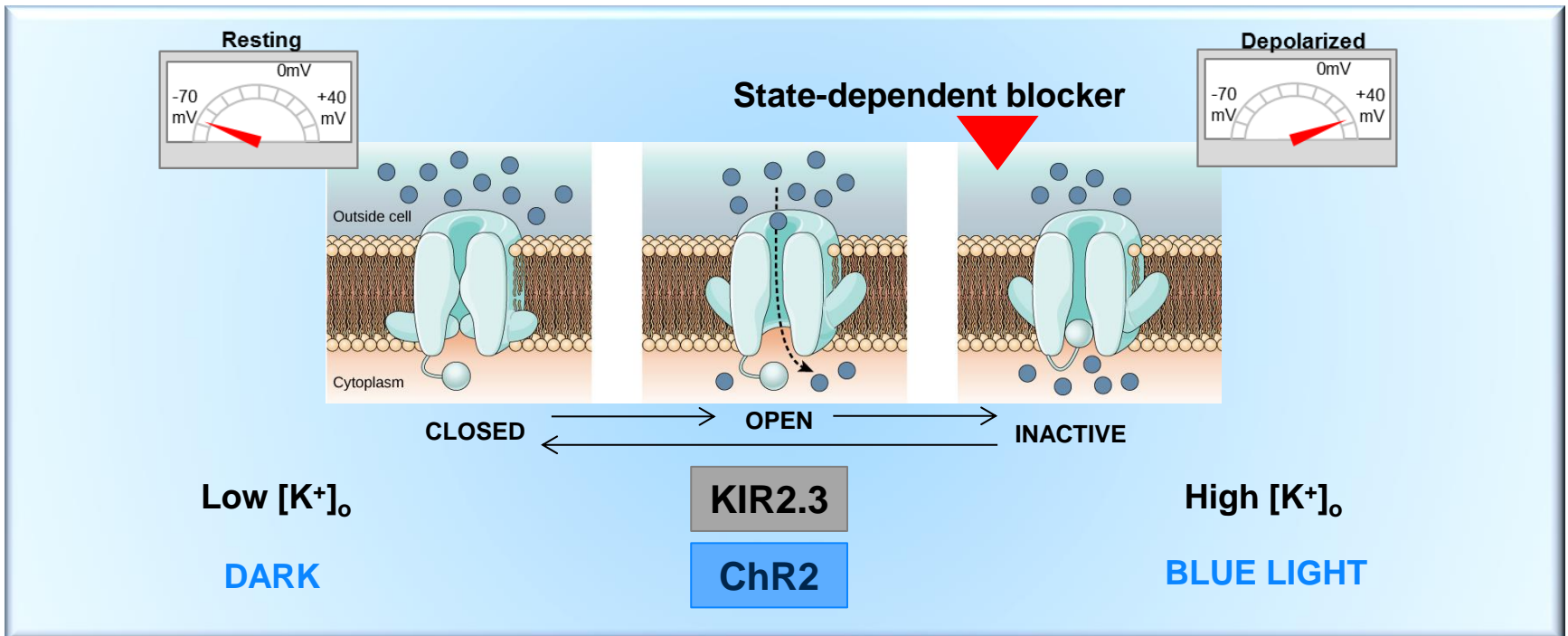
- **Seven transmembrane opsin** (eyespot of unicellular alga *Chlamydomonas reinhardtii*)
- **Activated by blue light (470 nm)**: the light causes a **conformational change** in the light sensitive molecule (retinal), which in turn causes a conformational change and the opening of the channelrhodopsin 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



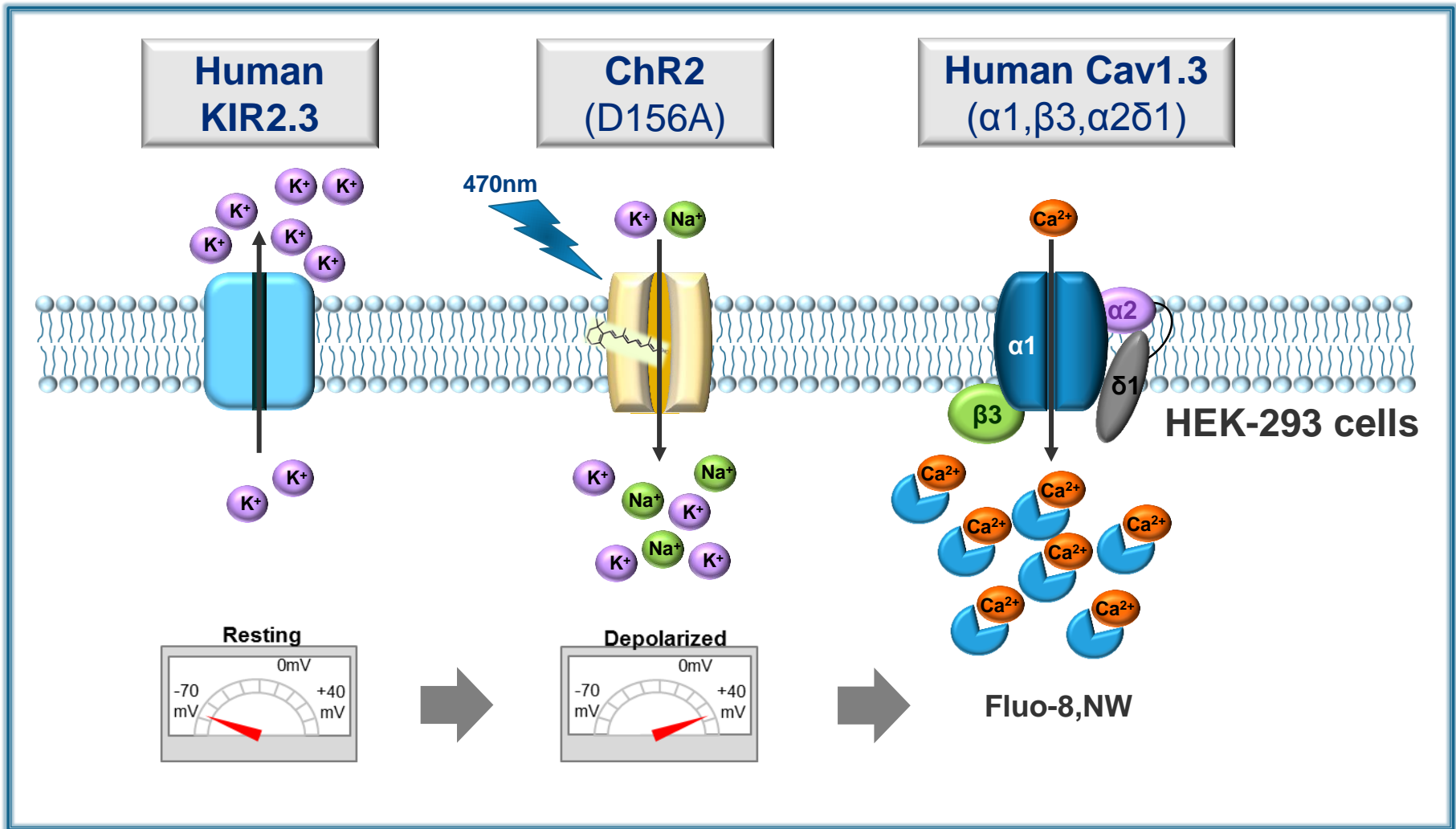
Optogenetic control of Cav1.3



- **L-type calcium channel**
- **High Voltage Activated (HVA)**
- **$\alpha 1$ (pore) + $\alpha 2\delta$, 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 μ CELL 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?

GOAL

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

POTENTIAL ISSUES

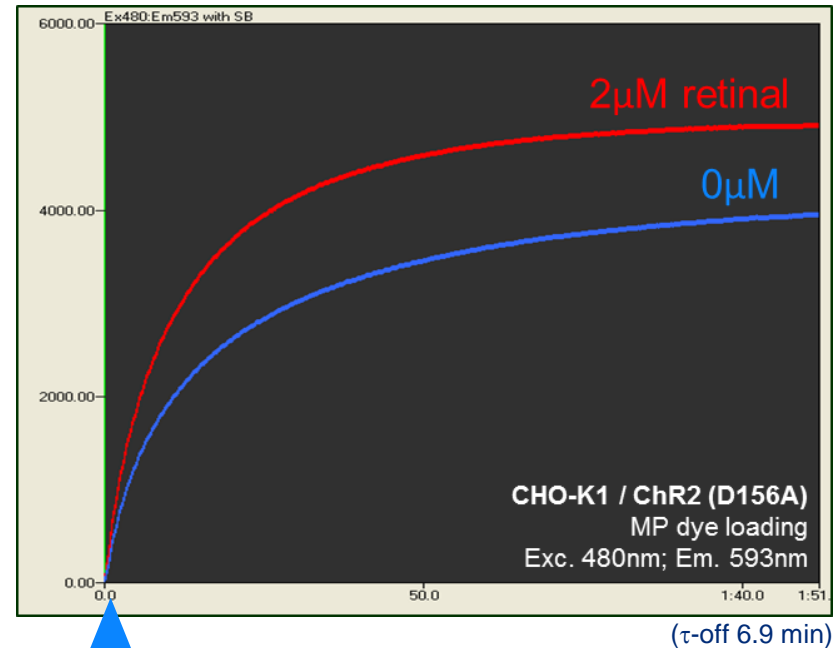
- The light produced by the instrument LED system might not have the adequate intensity for ChR2 activation
- The ion flux through the ChR2 might be not sufficient to induce membrane depolarization
- The membrane depolarization induced might be not sufficient to drive the activation of transfected voltage 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



ChR2 induced membrane depolarization

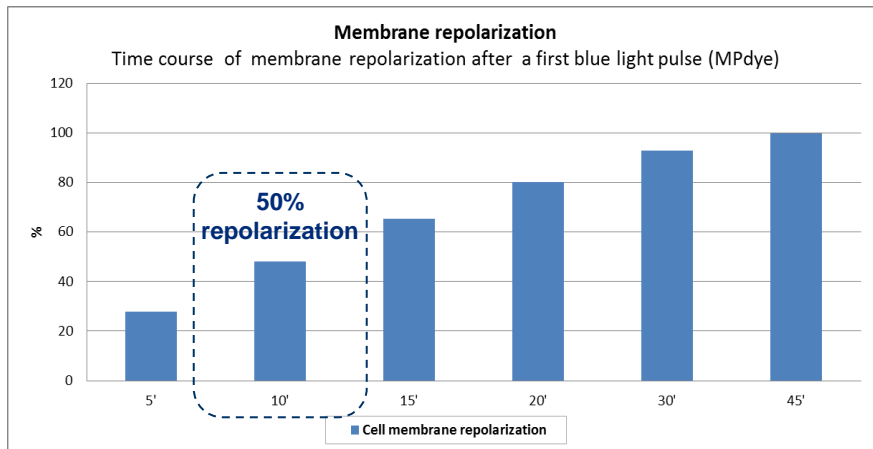
- **FDSS μ CELL LED efficiently activates ChR2_{D156A}**

(minimum light intensity required for wild-type ChR2 activation: 1mW/mm²; *Aravanis, 2007*)



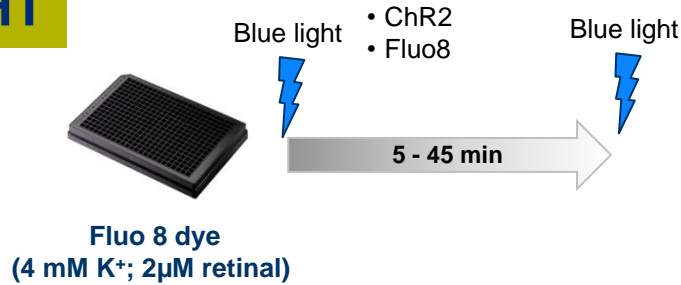
Blue light
($\lambda = 480 \text{ nm}$; 0.013 mW/mm^2)

- **Membrane depolarization half-recovered after $\approx 10 \text{ min}$**

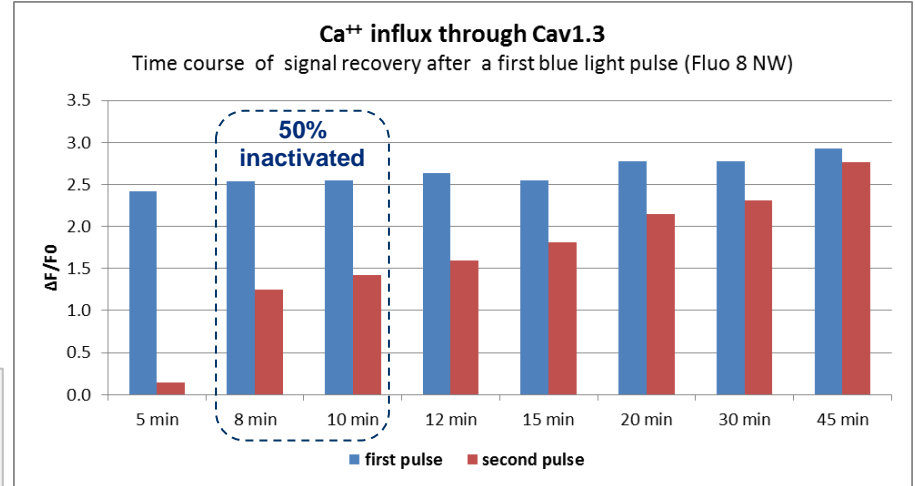


Cav1.3 half-inactivation protocol

LIGHT



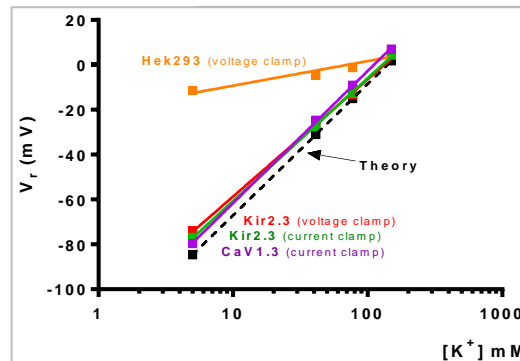
- Cav1.3 efficiently activated by ChR2
- 50% recovery from inactivation after 10 min



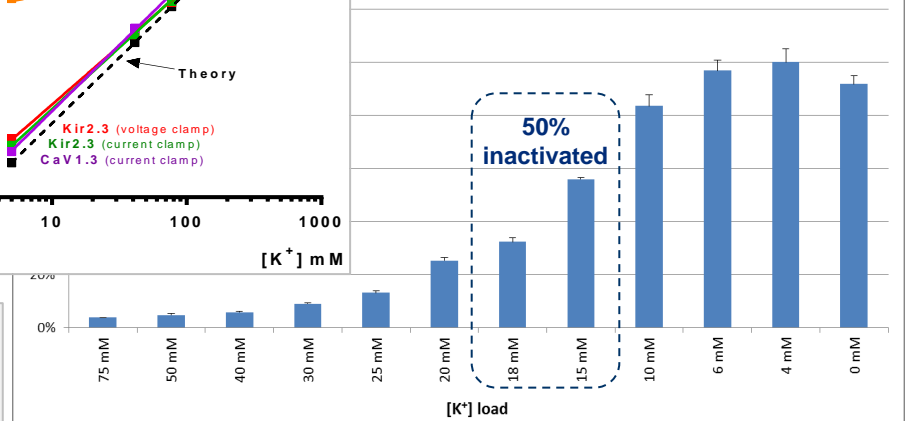
INACTIVE

RESTING

K⁺



Ca⁺⁺ influx through Cav1.3
in different K⁺ concentration (Fluo 8 NW)

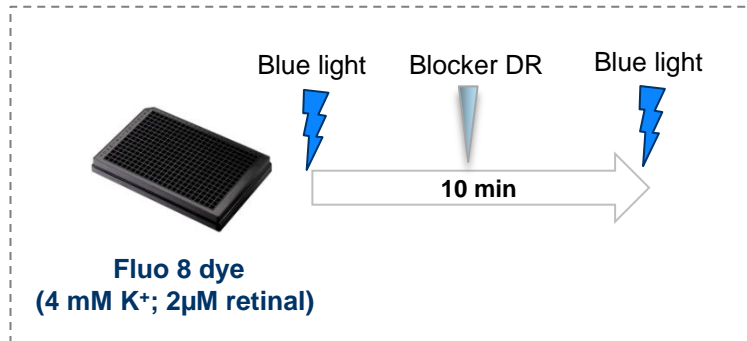


- Cav1.3 efficiently activated by K⁺
- 50% Cav1.3 inactivation in 16mM K⁺

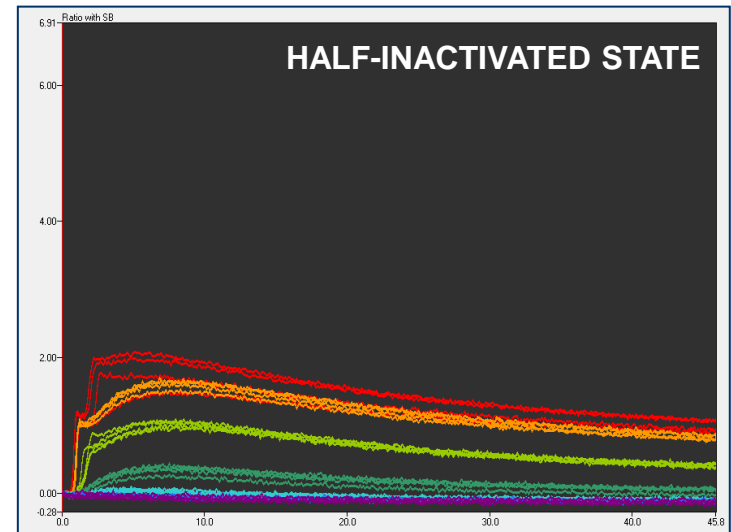
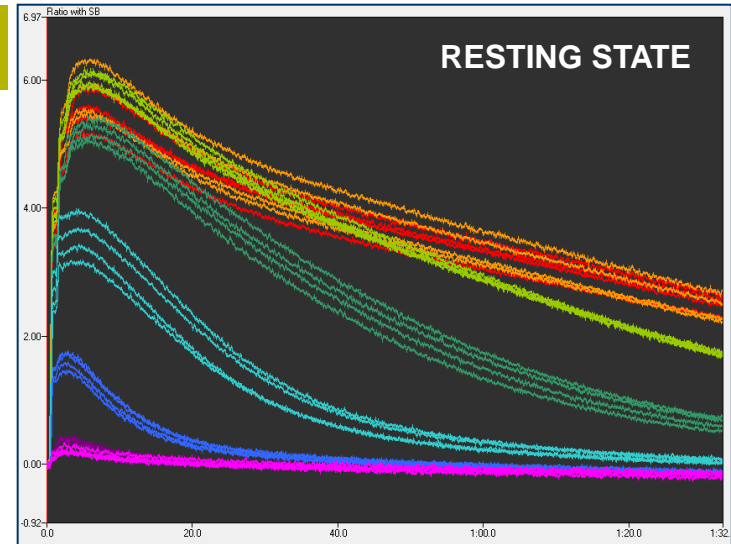


State-dependent blockers with «Light protocol»

ISRADIPINE dose-response @ μ CELL



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

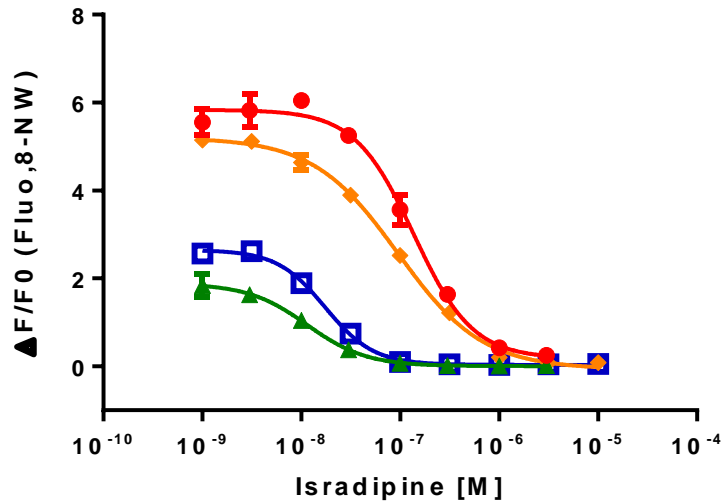


Read interval 0.1s
Exp.: 0.03s; Sens.: 3



Light protocol vs. K⁺ protocol vs. qPatch

Cav1.3 state-dependent blocker pharmacology
Light vs. K⁺ protocol



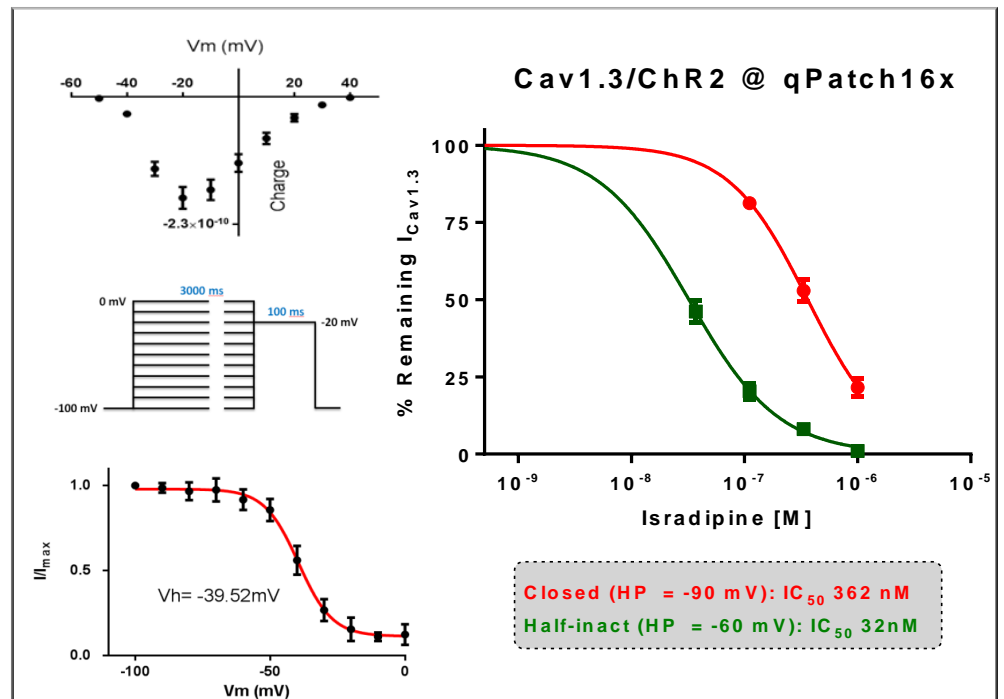
Resting (dark): IC₅₀ 137nM

Resting (4mM K⁺): IC₅₀ 95nM

Half-inact (Blue light 10 min): IC₅₀ 12nM

Half-inact (16mM K⁺): IC₅₀ 14nM

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



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 μ CELL **optics** is well suitable for ChR2 activation
- A “**Light protocol**” was set up at the FDSS μ CELL 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

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Katharina Montag clonings

Loredana Redaelli cell biology head

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