A JEDI Toolbox for Imaging Cell Membrane Potential Dynamics

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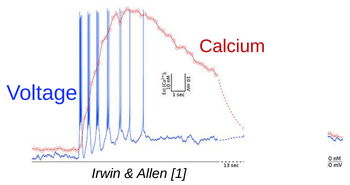
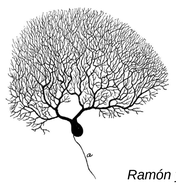
Abstract

Genetically Encoded Voltage Indicators (GEVIs) are a promising new technology for monitoring electrical activity in the brain noninvasively with high spatiotemporal resolution. GEVIs fundamentally work by changing their brightness in response to changes in membrane potential. As a relatively new technology, thus there is room for further optimization of GEVI to expand their versatility and applications. We present here a newly designed GEVI called **Jellyfish-derived Electricity-reporting Designer Indicator (JEDI)** with significantly improved brightness and responsivity as compared to other commonly used GEVIs. JEDIs can be readily expressed in several different host systems through plasmid or virus delivery, enabling a broad range of applications *in vivo*.

GEVI Design goals

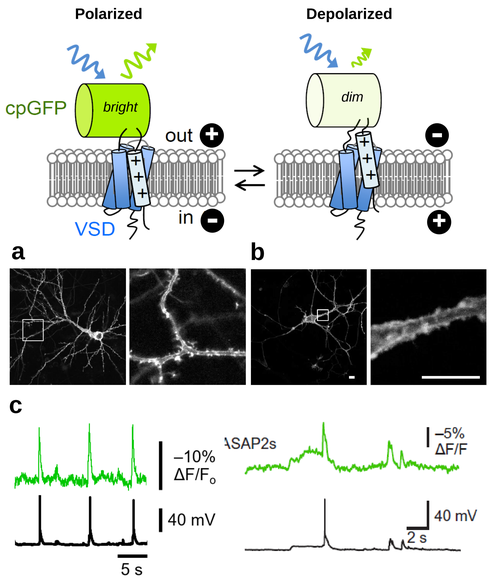




High temporal resolution to capture the full richness of voltage dynamics

High spatial resolution to monitor voltage dynamics with subcellular resolution

First generation GEVIs



In ASAP sensors, voltage-induced movement of a positively charged transmembrane helix of a voltage sensing domain (VSD) is hypothesized to perturb the protonation state of a circularly permuted GFP, resulting in changes in fluorescent intensity. . Image adapted from [1,2]

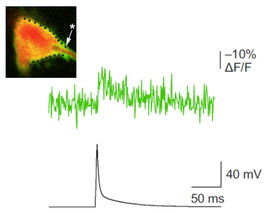
Reference

[1] Irwin RP and Allen CN. J Vis Exp. 2013.

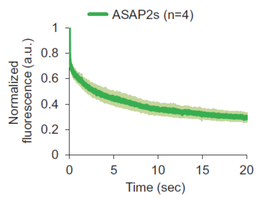
[2] Chamberland S*, Yang H*, et al. eLife. 2017

Limitations of existing GEVIs

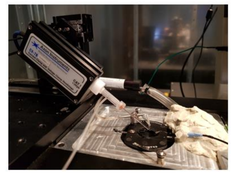
Low SNR



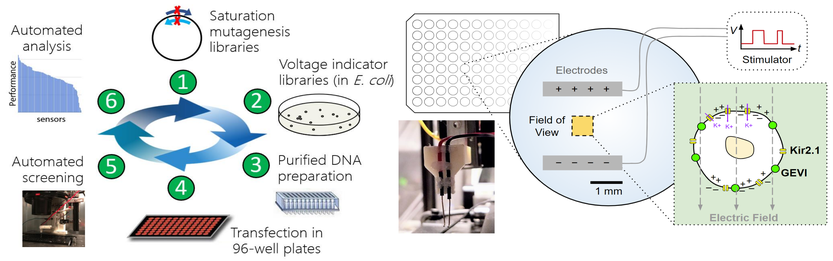
Poor photostability



Slow indicator development

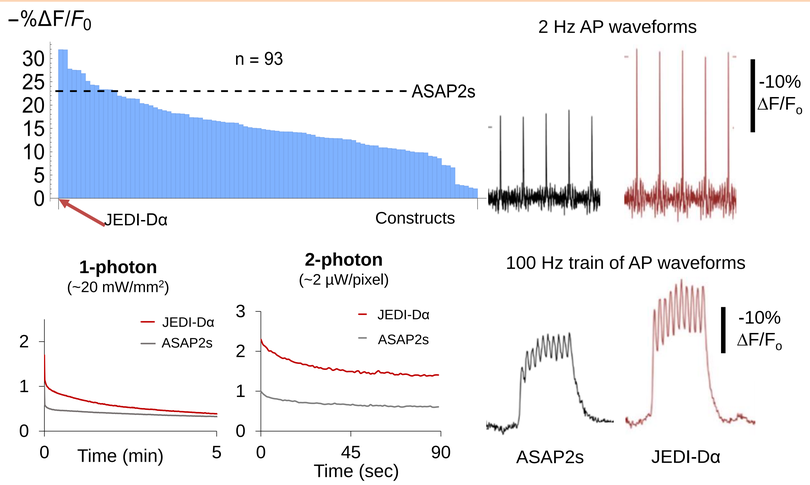


High-throughput engineering of new voltage indicators



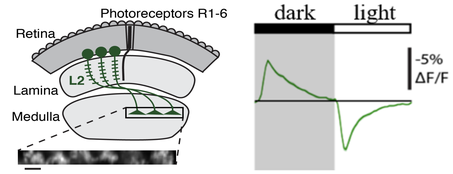
Automated GEVI screening: We have built an efficient workflow for automated high throughput voltage indicator screening by generating an external electrical field in Kir2.1 cells

New sensors from the St-Pierre lab: Jellyfish-derived Electricity-reporting Designer Indicators (JEDIs)

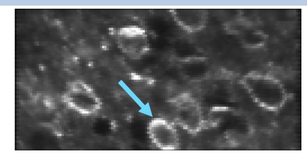


Easy to identify better variants: We are able to identify better GEVIs from a large population of variants, confirmed by electrophysiology testing in HEK293 cells.

JEDI and other GEVIs for high spatiotemporal *in vivo* imaging



ASAP2s reported voltage responses to visual stimuli in fruit flies.



JEDI-Da indicators express well in mice neurons *in vivo,* allowing imaging of voltage in neurons 160 µm from the surface of the cortex

Example	Soma enrichment	Condition expression	Frame rate (Hz)
Measuring firing rate of V1 inhibitory neurons	Yes	Introducing cre expression in V1 inhibitory neurons	>=1000
Measuring subthreshold oscillation at 30Hz	Yes	Optional	>=250
Measuring depolarization at dendrites and axons	No	Optional	>=1000

Dissemination resources of JEDI for *in vivo* applications

AAV expression system for rodent	
AAV Serotype	Promoter
AAV.PHP.eB AAV2/1 AAV2/2 AAV2/5 AAV2/9 DJ8	CAG CaMKII EF1a hSyn

Plasmids for non-rodent systems	
Animal	Promoter
C. elegans	myo3
Zebrafish	Huc
Drosophila	UAS

Subcellular Localization tags can focus signal detection		
Conditional expression system	Pros	• Genetically targeted cell type expression • Reduction of cytotoxicity of long-term expression of transgene • Sparse labeling for better cell separation
	Cons	• Requirement of additional transgenic animal or reagents
Soma localization tag	Pros	• Increase of signal to noise ratio at soma • Reduction of signal mixture of neurites
	Cons	• Removal of the capability to measure voltage in neurites

How would you use GEVIs?

If you are interested in using fluorescent indicators or have any experience in using them, please help us by filling out the survey. Your feedback will be useful for future sensor development and dissemination: https://forms.gle/PvqAtNDDZ8rML7JD9

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