Automated Multimodal Screening of Fluorescent Biosensors of Membrane Potential

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***Short Abstract* — A quantitative understanding of neuronal computations can be achieved by monitoring membrane potential reported by genetically-encoded voltage indicators (GEVIs). The fluorescent biosensors enable recordings of cellular electrical activity *in vivo* with subcellular resolution and cell type specificity. However, current indicators are not photostable and bright enough for long-term recording, and their sensitivity and kinetics are not satisfactory for detection of fast voltage dynamics. We report a high-throughput platform to screen mutagenesis libraries of GEVIs by analyzing microscopy videos of HEK293 cells during electric field stimulation. The platform quantitatively ranks candidate GEVIs based on performance scores across multimodal imaging methods. We anticipate that the approach can be extended to the screening of other fluorescent biosensors.**

***Keywords* — Genetically-encoded voltage indicator (GEVI), High-throughput screening.**

I. Purpose

GENETICALLY encoded voltage indicator (GEVI) provides a new scheme of probing neuron circuits by reporting membrane potential changes with fluorescence. Compared to electrophysiological methods, it enables readout of neuronal electrical activity with subcellular resolution. Its ability of achieving cell-type specificity facilitates targeting and differentiating neurons *in vivo*. However, the first generation of GEVIs still suffer from low brightness and/or photostability [1], which prevent their wider applications for prolonged recordings. Moreover, the sensitivity and kinetics of GEVIs still have ample room of improvement to match that of the traditional electrode-based recording [2].

We have previously developed a voltage indicator, ASAP1, by coupling a green fluorescent protein (GFP) to the voltage sensitive domain (VSD) of a voltage sensitive phosphatase [3]. Further improvements of the sensor were achieved by rationally introducing mutations or deletions to the sensitive domains of the protein [4, 5]. This approach is considered low-throughput because each variant must be tested by labor-intensive electrophysiological methods, so we seek faster ways of discovering new GEVIs at a rate of at least thousand constructs per day.

II. Result

We report here the development of a high-throughput platform to screen mutagenesis libraries in a 96-well format. Our platform automatically acquires and analyzes multi-channel high speed microscopy videos of GEVI-expressing cells during electric field stimulation, and ranks variants quantitatively based on multiple performance parameters, such as sensitivity, kinetics, photostability, for both one-photon and two-photon excitations. We create and adapt HEK293 cell lines to better simulate the electrophysiological properties of neurons, while avoiding the excessive costs and labor from culturing neuron. The selected new GEVI candidates are further verified by gold standard voltage clamping.

III. Conclusion

We anticipate that this new directed evolution screening platform will accelerate development of indicators optimized for accurate and sensitive detection of voltage dynamics *in vivo*, laying the foundation of optical investigation of population of cells. The resulting GEVIs and fluorescent biosensors in general should be of broad utility for elucidating complex interactions among neurons that underlie behavior.

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