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Review

Genetically-encoded voltage indicators

Luxin Peng[a,c,1], Yongxian Xu[b,c,1], Peng Zou[a,b,c,*]

[a] College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
[b] Peking-Tsinghua Center for Life Sciences, Peking University, Beijing 100871, China
[c] Synthetic and Functional Biomolecules Center, Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, PKU-IDG/McGovern Institute for Brain Research, Peking University, Beijing 100871, China

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ABSTRACT

A holy grail in neuroscience is to understand how brain functions arise from neural network-level electrical activities. Voltage imaging allows for the direct visualization of electrical signaling at high spatial and temporal resolutions across a large neuronal population. Central to this technique is a palette of genetically-encoded fluorescent probes with fast and sensitive voltage responses. In this review, we chronicle the development and applications of genetically-encoded voltage indicators (GEVIs) over the past two decades, with a primary focus on the structural design that harness the power of fluctuating transmembrane electric fields. We hope this article will inform chemical biologists and protein engineers of the GEVI history and inspire novel design ideas

Membrane voltage is ubiquitous in cell biology. It arises from the selective charge transport across lipid bilayers and regulates diverse physiological processes, with the most famous being the electrical signaling in neurons and cardiomyocytes [1]. While the classical patch-clamp technique has enabled fast and sensitive tracking of membrane voltage at the single cell level, it is difficult to parallelize this technique for recording from a large cell population. In comparison, optical recording methods readily offer high spatial resolution and measurement throughput. For this reason, much effort has been devoted to the development of fluorescent voltage indicators over the past few decades. In particular, genetically-encoded voltage indicators (GEVIs) emerged as promising tools because they allow for cell-specific targeting of measurement. In this mini-review, we outline the development of GEVI designs and highlight their applications in voltage imaging of bioelectric phenomenon. We hope that this article will inform chemical biologists and protein engineers of the history of GEVI development and inspire ideas for future improvements.

A voltage indicator acts as an electrochromic signal transducer. In many cases, the voltage-sensing moiety initially transduces electrical signal into intramolecular mechanical stress, which drives the conformational change of appended fluorescent protein (FP) reporters. This is best exemplified in GEVIs derived from either ion channels or voltage-sensitive phosphatases (VSP). While the detailed mechanism was not well understood, it is generally accepted that voltage sensitivity arises from voltage-induced movement of the fourth transmembrane helix, due to its multiple positively charged amino acid residues. In other cases, the voltage-sensing domain could utilize the transmembrane electric field to shift the chemical equilibrium betweenprotonated and deprotonated states of a membrane-anchored retinal chromophore, as is shown in the case of rhodopsin-derived GEVIs. The protonated state is more fluorescent than the deprotonated state, due to stronger absorption in the visible spectrum. Fig. 1 outlines the structure of these designs.

The first GEVI, called FlaSh, was constructed almost two decades ago as a chimera of voltage-gated Shaker potassium channel and modified green fluorescent protein (GFP [5]).Subsequent mutations in both GFP and the ion channel resulted in spectral variants with voltage sensitivity ranging between 1%-5% $\Delta F/F$ per 100 mV (Table 1) [6]. However, the response time constants of these GEVIs typically range from 10–200 ms, which are too slow to capture the millisecond-scale neuronal action potentials. With shorter linker between the FP and the ion channel, response time could reach sub-millisecond range (VSFP1) [7]. Meanwhile, Ataka *et al.* sought to improve on the kinetics by using voltage-gated sodium channel as the voltage-sensing domain (SPARC) and achieved response time of 2 ms [8]. Coincidentally,

* Corresponding author at: College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.
E-mail address: zoupeng@pku.edu.cn (P. Zou).
[1] Equal contribution.

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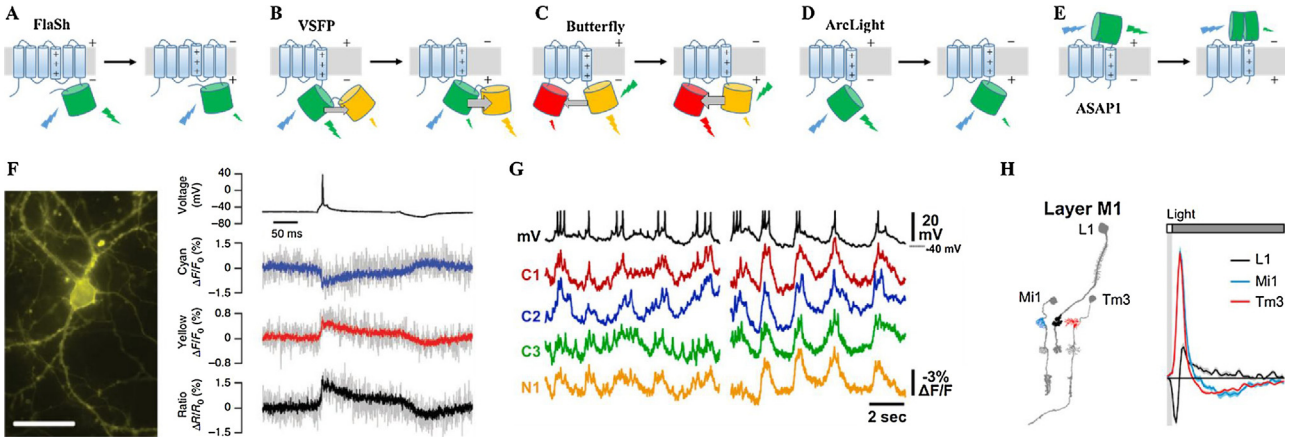

Fig. 1. Design and applications of GEVIs based on voltage-dependent conformational changes. A) The first generation voltage indicator, FlaSh, was built upon voltage-gated potassium channel. B) VSFP2 series are ratiometric reporters consisting of a FRET pair fused to VSD. C) VSFP Butterfly has FPs fused separately to the two termini. D) ArcLight is a monochromic GEVI with sensitive voltage response. E) ASAP1 couples the conformational changes in VSD to cpGFP. F) VSFP2.3 reports membrane voltage transients in hippocampal pyramidal neurons through differential two-color fluorescence imaging. Reprinted with permission from Ref. [2]. Copyright 2010, Nature Publishing Group. G) Simultaneous recording of multiple neurons with ArcLight in Drosophila brain. Reprinted with permission from Ref. [3]. Copyright 2013, Elsevier Inc. H) Voltage imaging with ASAP in Drosophila visual system reveals the transformation of voltage responses between pre-synaptic axons and post-synaptic dendrites.Reprinted with permission from Ref [4]. Copyright 2016, Elsevier Inc.

Table 1
Summary of GEVIs.

GEVI name	Voltage-sensing structure	Fluorescence reporter	ΔF/F (%)	$<\tau_{on}>$ (ms)[a]	$<\tau_{off}>$ (ms)[a]	References
GEVIs based on ion channels						
FlaSh	Voltage-gated K^+ channel	GFP	~−5.1	85	160	[5]
SPARC	Voltage-gated Na^+ channel	GFP	~−0.5	0.8	N/A	[8]
VSFP1	Voltage-gated K^+ channel	CFP/YFP	1.8	0.74	0.74	[7]
Pado	Voltage-gated H^+ channel	Super ecliptic pHluorinA227D	~−5	~90	~9	[9]
GEVIs based on VSD-FRET pairs						
VSFP2.1	Ci-VSP	CFP/YFP	8.6	15	75	[12]
VSFP2.3	Ci-VSP	CFP/YFP	13.3	10.9	~80	[16,18]
VSFP2.4	Ci-VSP	mCitrine/mKate2	12.4	9.6	~75	[16]
Mermaid	Ci-VSP	mUKG/mKOk	~28	11.8	~70	[16,17]
VSFP-CR	Ci-VSP	Clover/mRuby2	12.7	5.4	59.5	[18]
VSFP-Butterfly 1.2	Ci-VSP	mCitrine/mKate2	~6	N/A	N/A	[13]
Mermaid2	Ci-VSP	mUKG/mKOκ	48.5	3.5	10.3	[14]
Zahra2	Zebrafish VSP	CFP/YFP	~1.8	3.5	3.5	[19]
Monochromic GEVIs based on VSD						
VSFP3.1	Ci-VSP	CFP	~−0.6	1.3	N/A	[15]
ArcLight Q239	Ci-VSP	Super ecliptic pHluorin A227D	~−39	28.5	26.0	[23]
Bongwoori	Ci-VSP/Kv chimera	Super ecliptic pHluorin A227D	~−16	10	7	[25]
ElectricPk	Ci-VSP	Circularly permuted GFP	−1.2	2.24	2.09	[27]
FlicR1	Ci-VSP	Circularly permuted mKate	6.6	3.4	3.7	[28]
ASAP1	Chicken VSP	Circularly permuted GFP	−17.5	29.7	29.5	[29]
ASAP2f	Chicken VSP	Circularly permuted GFP	~−25	27.9	46.6	[4]
GEVIs based on rhodopsins						
Arch	Rhodopsin	Archaerhodopsin	40	0.6	0.8	[30,31]
Arch D95N	Rhodopsin	Archaerhodopsin	60	~85	~33	[30]
Arch EEQ	Rhodopsin	Archaerhodopsin	60	~5–15	~5–15	[32]
QuasAr1	Rhodopsin	Archaerhodopsin	32	0.24	0.29	[31]
QuasAr2	Rhodopsin	Archaerhodopsin	90	4.6	4.0	[31]
Archer1	Rhodopsin	Archaerhodopsin	85	N/A	N/A	[33]
eFRET-mOrange2	Rhodopsin	mOrange2	−10	13.1	16.2	[34]
eFRET-Citrine	Rhodopsin	Citrine	−13.1	9.9	14.8	[34]
MacQ-mOrange2	Rhodopsin	mOrange2	−20	7.4	6.9	[35]
MacQ-mCitrine	Rhodopsin	mCitrine	−20	20.5	19.6	[35,36]
Ace2N-mNeon	Rhodopsin	mNeongreen	−18	1.4	2.1	[36]

[a] Response time constants $<\tau_{on}>$ and $<\tau_{off}>$ were calculated as $<\tau> = \alpha_{fast}\tau_{fast} + \alpha_{slow}\tau_{slow}$ at room temperature, or estimated from original literature (with"~").

these faster sensors have reduced voltage sensitivity, less than 2% ΔF/F per 100 mV. A more recent study based on *in silico* search identified voltage-gated proton channel from fluke *Clonorchissinesis* to construct a novel GEVI called Pado [9]. Unfortunately, all of these sensors exhibited modest voltage sensitivity, and many suffered from poor membrane trafficking in mammalian cells [10]. The transmembrane voltage-sensing domain (VSD) of the ascidian *Cionaintestinalis* voltage-sensing phosphatase (Ci-VSP) represents

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