Voltage imaging with genetically encoded indicators

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Membrane voltages are ubiquitous throughout cell biology. Voltage is most commonly associated with excitable cells such as neurons and cardiomyocytes, although many other cell types and organelles also support electrical signaling. Voltage imaging *in vivo* would offer unique capabilities in reporting the spatial pattern and temporal dynamics of electrical signaling at the cellular and circuit levels. Voltage is not directly visible, and so a longstanding challenge has been to develop genetically encoded fluorescent voltage indicator proteins. Recent advances have led to a profusion of new voltage indicators, based on different scaffolds and with different tradeoffs between voltage sensitivity, speed, brightness, and spectrum. In this review, we describe recent advances in design and applications of genetically-encoded voltage indicators (GEVIs). We also highlight the protein engineering strategies employed to improve the dynamic range and kinetics of GEVIs and opportunities for future advances.

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Introduction

Membrane voltage is an important signal that affects many fundamental aspects of cellular physiology. The transmembrane electric field perturbs the energy landscape of biomolecules embedded in the lipid membrane, including ion channels, G protein-coupled receptors and membrane-associated enzymes [1,2], and is affected in turn by the dynamics of voltage- and ligand-gated ion channels, as well as electrogenic transporters and pumps.

While bioelectrical signaling is most commonly associated with neurons and cardiomyocytes, membrane voltage also forms the basis for signaling in other cell types including bacteria [3••,4], fungi [5] and plants [6]. Compared with electrode-based techniques such as patch clamp electrophysiology, optical measurements offer spatial resolution, non-invasiveness, ease of operation, and high measurement throughput. Much effort has gone toward development of fluorescent genetically encoded voltage indicators (GEVIs), though current-generation GEVIs are still far from optimal. Here we review recent advances in GEVI designs and their applications in voltage imaging of bioelectric phenomena. We hope that this article will help the reader select the right GEVI for his or her application, and will inspire novel ideas for better GEVIs.
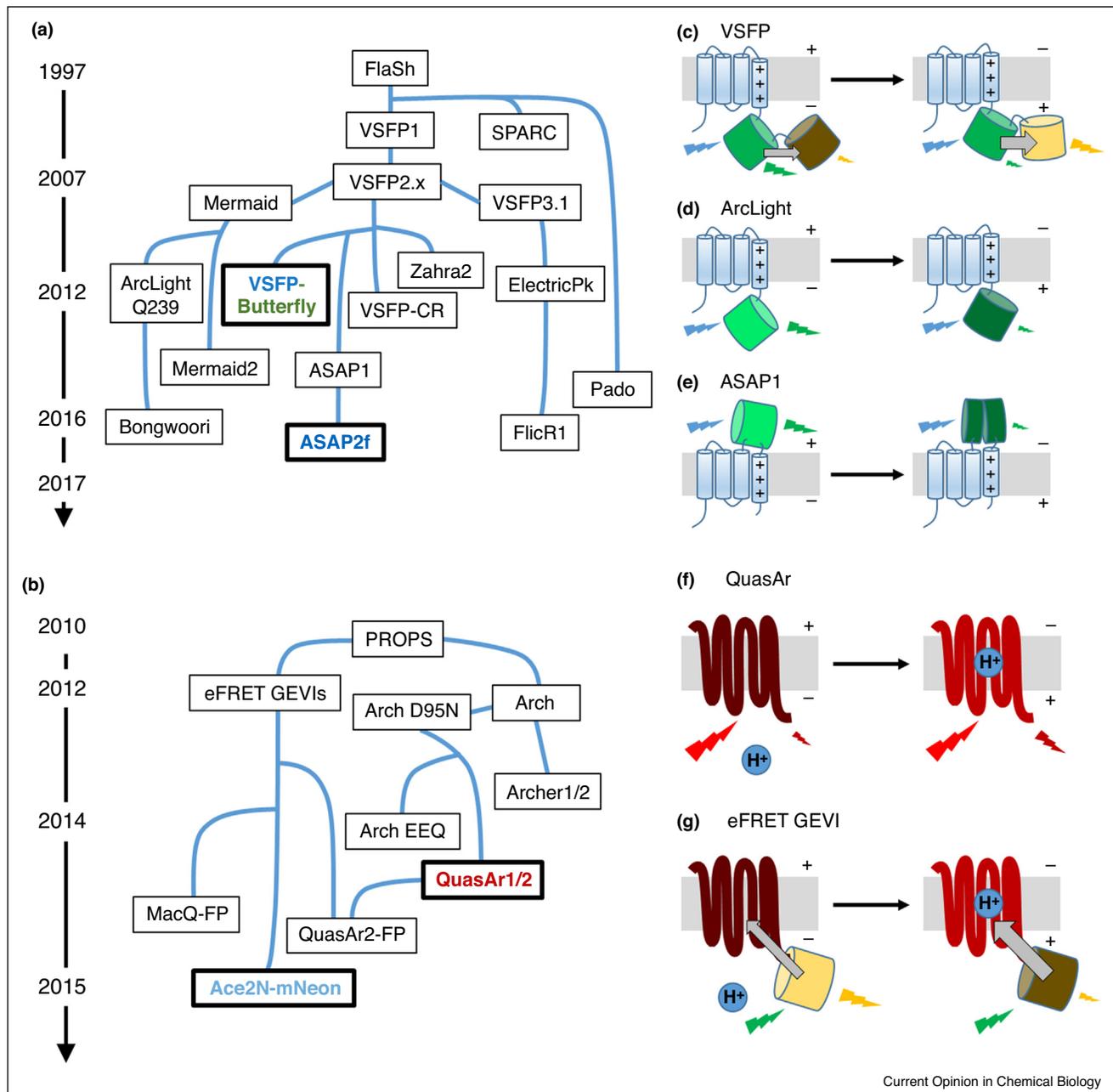
The family of GEVIs
Fluorescent protein-based GEVIs
The history of GEVI development is marked by occasional introduction of qualitatively new scaffold designs, followed by periods of iterative optimization and refinement (Figure 1). The earliest GEVI designs comprised naturally occurring ion channel voltage sensor domains fused to fluorescent proteins (FPs) (FlaSh [7], SPARC [8]) or Förster resonance energy transfer (FRET) FP pairs (VSFP1 [9]). GEVIs based on ion channel scaffolds typically exhibited modest voltage sensitivity ($<5\%$ $\Delta F/F$ per 100 mV) and slow kinetics (typically 10–200 ms), and many suffered from poor membrane trafficking [10].

The transmembrane voltage-sensing domain (VSD) of the ascidian *Ciona intestinalis* voltage-sensing phosphatase (Ci-VSP) [11], turned out to be a good transducer for many GEVIs (VSFP2.x [12,13], Mermaid [14,15], Butterfly [16], VSFP-CR [17], ArcLight [18], ASAP [19••,20•], Bongwoori [21], FlicR1 [22]). GEVIs based on Ci-VSP had much improved membrane trafficking, presumably due to the monomeric nature of the VSD. Ci-VSP VSDs fused with FRET pairs showed good voltage sensitivity ($\Delta R/R$ approaching 48% per 100 mV for Mermaid2 [14,15]) and have been applied to report large-area membrane voltage fluctuations *in vivo* (optical EEG with Butterfly 1.2 [16]). While the ratiometric nature of FRET signals is useful for canceling motion and blood flow artifacts, the broad spectrum of FRET GEVIs often precludes multiplex imaging with other sensors. Surprisingly, a VSD fused with a single FP also reported membrane voltage (VSFP3.1), suggesting that a mechanism other than FRET was responsible for some of the

Figure 1

The family of GEVIs. (a) and (b) Approximate lineages of the major classes of GEVIs. The GEVIs highlighted in bold have shown the greatest sensitivity for use *in vivo* and are colored with their approximate excitation wavelengths. (a) GEVIs based on voltage-sensor domains. (b) GEVIs based on microbial rhodopsins. (c)–(g) Voltage sensing mechanisms in the major classes of GEVIs.

observed fluorescence change [23]. One drawback of these monochromic GEVIs was their limited sensitivity.

A breakthrough came in 2012, when Jin *et al*. discovered that an unintended mutation (A227D) in the FP boosted voltage sensitivity 14-fold, and further engineering through mutagenesis and linker optimization resulted

in ArcLight Q239 with voltage sensitivity 35% ΔF/F per 100 mV [18]. However, the slow temporal response of ArcLight (10 ms for its fast component) hindered action potential detection (3.2% ΔF/F in cultured neurons). Replacing the Ci-VSP VSD with homologues from chicken and zebrafish VSPs resulted in faster kinetics, but at the cost of reduced sensitivity [24•]. Sequence

alignment analysis and a cassette mutagenesis screen led to Bongwoori, a triple mutant of ArcLight with truncated linker, which resolved neuronal action potentials at 60 Hz [21]. Recently, the Ci-VSP VSD was fused to NanoLuc luciferase and Venus to create a bioluminescent GEVI called LOTUS-V. This GEVI had ΔR/R of 21% in response to 100 mV membrane voltage change, but its response was dominated by a ∼200 ms time constant and the GEVI required an exogenous substrate to luminesce [25].

Another way to capitalize on the conformational change in the VSD is to insert a circularly permuted FP (cpFP) on the C-terminus (ElectricPk) [26,27]. Recently, Abdelfattah *et al.* applied this design to a screen of VSD-cpmApple mutants. Through a combination of directed protein evolution and rational design they created a red-shifted GEVI, FlicR1, which faithfully detected action potentials, albeit with modest signal amplitude (∼3% ΔF/F) [22]. In 2014, St-Pierre *et al.* reported a new design, called ASAP1, where a cpGFP was inserted into the extracellular loop connecting the third and fourth transmembrane helices of the Ci-VSP VSD. The combination of high speed (∼2 ms) and high sensitivity (18–29% ΔF/F per 100 mV) enabled detection of action potential waveforms up to 200 Hz [19••]. Further mutagenesis of the linker connecting the VSD and cpGFP led to ASAP2f, which produced larger fluorescence changes *in vivo* than ASAP1 and was applied in two-photon voltage imaging of *Drosophila* visual system [20•].

Microbial rhodopsin-based GEVIs

Microbial rhodopsin proteins were initially used for optogenetic control of membrane potential, but in 2011 these proteins were shown to function as fluorescent voltage reporters too [3••]. The endogenous retinal chromophore shows weak near infrared fluorescence, but only when a proton resides on the Schiff base linking the retinal to the protein core. Changes in membrane potential shift the local electrochemical potential of protons, and thereby tune fluorescence-determining acid–base equilibrium. A green-absorbing proteorhodopsin mutant (PROPS) revealed that bacteria generate spontaneous electrical spikes [3••]. However, PROPS did not traffic to the plasma membrane in eukaryotic cells, and thus was not a useful mammalian voltage sensor.

Archaerhodopsin 3 (Arch) from *Halorubrum sodomense* expressed and trafficked well in mammalian cells [28]. This protein functioned as a near-infrared GEVI that resolved individual action potentials in mammalian neurons *in vitro* [29•]. Arch has submillisecond response kinetics and exhibits large voltage sensitivity (∼35% ΔF/F per 100 mV). Directed evolution identified mutations that improved brightness, kinetics, and voltage sensitivity, while eliminating the proton-pumping photocurrent (Archers [30,31] and QuasArs [32••]).

Surprisingly, some of these mutations are far away from the retinal chromophore, suggesting subtle influences on the rhodopsin conformation and photocycle dynamics [32••]. Despite their broad utility, Arch-derived GEVIs suffer from weak fluorescence, typically 30–80-fold dimmer than GFP. Voltage imaging with microbial rhodopsin endogenous fluorescence requires intense red laser illumination, typically 200–1000 W/cm^2 [29•,32••]. Efforts to evolve Arch variants that accept unnatural chromophores have increased the quantum yield, but have not yet produced usable voltage sensors [33].

Electrochromic FRET (eFRET) provides a means to improve the brightness of microbial rhodopsin-derived GEVIs while maintaining their fast kinetics. In eFRET, voltage-induced changes in the rhodopsin absorption spectrum alter the degree of non-radiative quenching of an appended FP [34,35]. The first generation eFRET GEVIs used either QuasAr2 or a proton-pumping rhodopsin derived from *Leptosphaeria maculans* (Mac). Microbial rhodopsin absorption spectra are very broad, so FPs with different colors can be used as eFRET donors, leading to a palette of GEVIs spanning much of the visible spectrum [34]. While these sensors are sufficiently bright to detect action potentials in cultured neurons their sensitivity is lower than the parent rhodopsin (*e.g.* 13%/100 mV for QuasAr2-Citrine eFRET vs. 90%/100 mV for QuasAr2) [34]. A recently developed eFRET GEVI fused a proton-pumping rhodopsin mutant from a green algae *Acetabularia acetabulum* (Ace) to a bright and photostable FP, mNeonGreen [36••]. This GEVI showed remarkably bright and fast signals, enabling voltage imaging with single-cell and single-spike resolution in mouse and fly brains *in vivo* (Table 1).

Near infrared GEVIs open combinatorial possibilities

Pairing of voltage imaging with patterned optogenetic stimulation greatly facilitates exploration of neural circuits [32••,37–40]. Optogenetic stimulation allows one to explore neural behavior over a wider range of parameters, and in a more systematic fashion, than one can achieve through passive observation or sensory stimulation alone. Knowledge of input–output properties under defined conditions can then shed insight into observations of native function.

To combine stimulation and measurement, the actuator and reporter must be spectrally orthogonal. While there exist red-shifted channelrhodopsin variants [41–44], none is truly spectrally orthogonal to GFP or its derivatives. All retain 20–30% activation at wavelengths used for GFP excitation [45]. Thus it has not been feasible to pair red-shifted optogenetic actuation with blue-excited GEVIs. Examination of optical action spectra shows that pairing of a blue-shifted channelrhodopsin with a red-excited near infrared (NIR) emitting GEVI provides better protection from optical crosstalk.

Table 1

Recently developed GEVIs appropriate for *in vivo* voltage imaging

GEVI name	Voltage-sensing structure	Fluorescence reporter (emission wavelength)	ΔF/F or ΔR/R (%)		τ_{on} (ms)		τ_{off} (ms)	
			100 mV	AP[b]	Fast	Slow	Fast	Slow
Mermaid2 [15]	Ci-VSP	mUKG (499 nm)/mKOκ (563 nm)	48.5	2.6	0.92	13	10.3	–
VSFP-Butterfly 1.2 [16]	Ci-VSP	mCitrine (529)/mKate2 (633 nm)	∼6	0.3	2	10	∼80	–
ArcLight Q239 [18]	Ci-VSP	s.e. pHluorin A227D[a] (509 nm)	∼−39	−3.2	9	48	17	60
Bongwoori [21]	Ci-VSP/Kv chimera	s.e. pHluorin A227D[a] (509 nm)	∼−16	−1.5	8	NA	7	–
ASAP2f [20•]	chicken VSP	cpGFP (509 nm)	∼−25	∼−8	2.8	135	2.4	155
QuasAr1 [32••]	Archaerhodopsin 3	retinal cofactor (715 nm)	33	21	0.05	3.2	0.07	1.9
QuasAr2 [32••]	Archaerhodopsin 3	retinal cofactor (715 nm)	90	48	1.2	11.8	1	15.9
Ace2N-mNeon [36••]	*Acetabularia* rhodopsin	mNeonGreen (517 nm)	−18	−12	0.36	4.2	0.42	5.2

[a] Super ecliptic pHluorin A227D.

[b] The ΔF/F amplitude associated with an action potential (AP) values reported here are for cultured neurons. This value varies depending upon the efficiency of membrane trafficking and the method of fluorescence extraction (peripheral membrane only vs. whole cell) and the method of background subtraction. The numbers given should be considered approximate.

Hochbaum *et al.* paired a NIR GEVI, QuasAr2, with a blue-shifted channelrhodopsin, CheRiff, to create an all-optical method for simultaneous optogenetic stimulation and voltage imaging [32••]. This 'Optopatch' technique has been used to probe neuronal excitability in cultured neurons, organotypic [32••] and acute [46•] brain slices and mouse somatosensory ganglia *in vivo* [46•]. The high-throughput nature of Optopatch measurements has also been applied to screening for pharmacological modulators of heterologously expressed voltage-gated sodium channels [48•].

NIR GEVIs can also be paired with GFP-based fluorescent reporters, to study the interrelation of voltage and soluble analytes. For example, fusion of QuasArs and GCaMP-series calcium indicators (CaViar) has enabled simultaneous measurement of calcium and voltage signal in zebrafish heart *in vivo* [49] and in human iPSC-derived cardiomyocytes [47]. NIR GEVIs could in principle be paired with a great many other GFP-based fluorescent reporters, for example for vesicle release, neurotransmitters, cAMP, or kinase activity.

There are also technical merits to working with NIR GEVIs. In brain tissue, autofluorescence is 136-fold lower with excitation at 640 nm than at 488 nm [46•]. Thus, despite the fact that Arch-based GEVIs are ∼30–80-fold dimmer than mCitrine, the signal-to-background ratio of QuasAr is similar to that of mCitrine when the two proteins are expressed as a 1:1 fusion and imaged with 1-photon excitation *in vivo*. Photochemical toxicity in cultured cells is >100-fold lower at 640 nm than at 488 nm [50]. Optical scattering lengths are ∼50% longer at 640 nm than at 488 nm, enabling correspondingly deeper imaging [51]. The greatest concern for imaging NIR GEVIs in tissue is photothermal heating. At 640 nm, optical absorption of brain tissue, and hence laser-induced heating, is ∼20-fold lower than at 488 nm, and 3–5-fold lower than in the 800–1000 nm band used for conventional 2P imaging [51]. Optical powers up to 280 mW (λ = 925 nm) [52] have been safely used in 2P imaging *in vivo*, suggesting that similar optical powers are acceptable for imaging NIR GEVIs. Thus development of brighter NIR GEVIs would be an enabling technology for neuroscience.

Biological applications of GEVIs

GEVIs have been applied to study electrical dynamics across spatial scales, from single dendritic branches to macroscopic brain regions (Figure 2). On the smallest scale, Optopatch-based measurements in cultured neurons probed the microsecond-timescale dynamics of action potential initiation and propagation [32••]. On an intermediate scale, two-photon imaging of the ASAP2f GEVI in *Drosophila* revealed that the ON and OFF selectivity to visual stimuli arose in the intracellular transformation of voltage to Ca^{2+} responses [20•]. Imaging of single neurons expressing Ace2N-mNeon in mouse visual cortex probed propagation delays associated with dendritic activation [36••]. On the largest scale, wide-field imaging of VSFP Butterfly 1.2 in mouse cortex showed changes in large-scale dynamics associated with waking from anesthesia [53•].

Despite these early results, biological application of GEVIs remains in its infancy. Improvements in GEVI sensitivity could enable a host of new applications (for an in-depth discussion see Ref. [54•]). Voltage imaging could enable (1) to measure subthreshold post-synaptic potentials as a direct probe of synaptic strength. In combination with targeted optogenetic stimulation, such a technique could be used for functional circuit mapping; (2) to resolve subcellular details of voltage

Figure 2
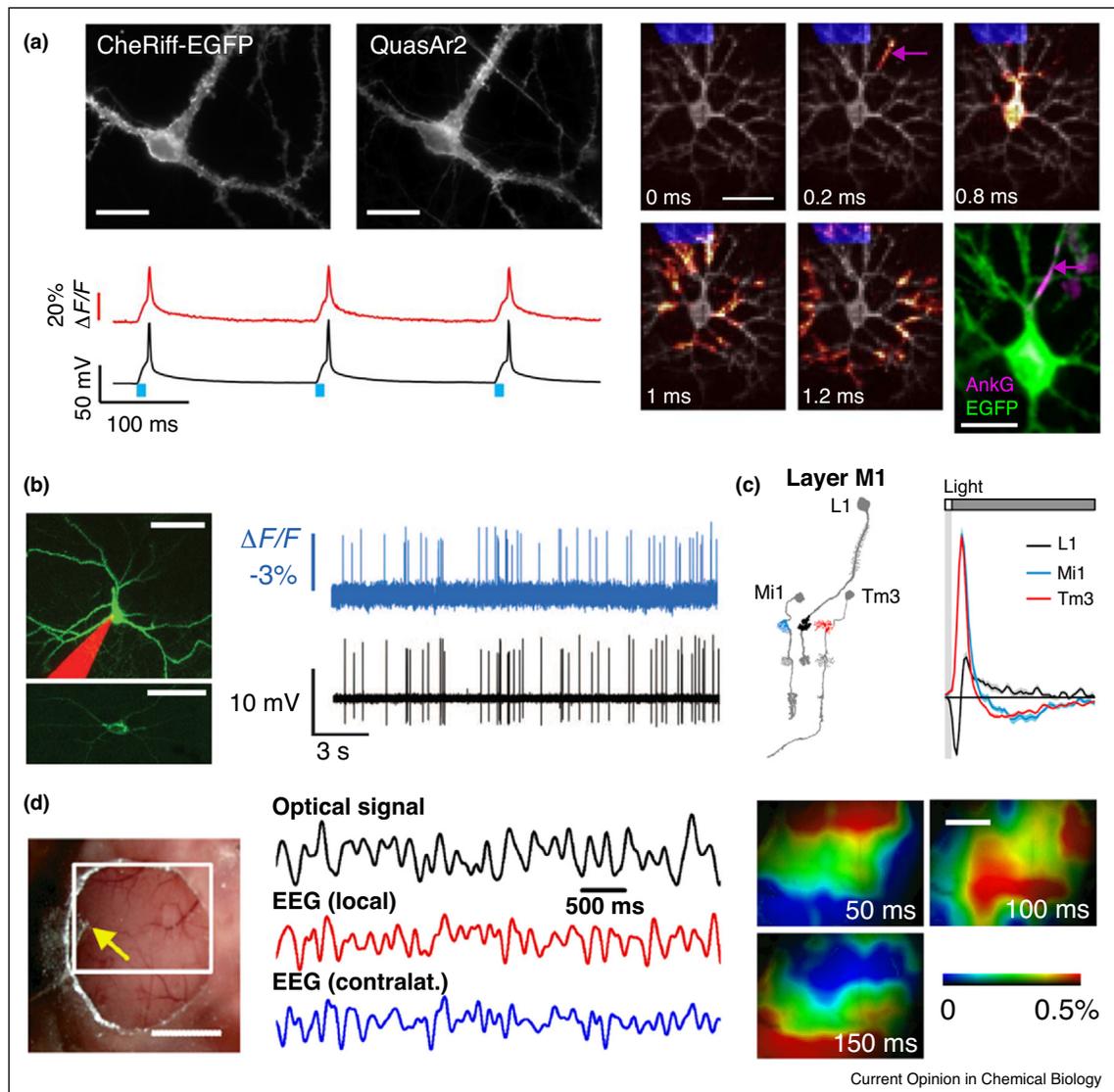
Applications of GEVI imaging. **(a)** All-optical electrophysiology ('Optopatch') in neurons co-expressing a blue-shifted channelrhodopsin, CheRiff, and a red-shifted NIR GEVI, QuasAr2. Left: Optical stimuli evoke electrical spikes and closely corresponding fluorescence transients. Right: Patterned optogenetic stimulation on a dendritic region evokes action potentials whose subcellular propagation initiates at the axon initial segment, marked by an Ankyrin G immunostain. Figures from Ref. [32••]. **(b)** Ace2N-mNeon reports neuronal spikes in mouse visual cortex *in vivo*. Figure from Ref. [36••]. **(c)** Two-photon imaging of ASAP2f in *Drosophila* visual neurons reports subcellular details of stimulus-evoked electrical responses. Figure from Ref. [20•]. **(d)** Wide-field optical voltage mapping in mouse cortex *in vivo*. The optical signal correlates with the EEG from the ipsilateral but not the contralateral hemisphere. Figure from Ref. [16].

propagation in intact tissue. These measurements could probe the ways in which excitatory inputs, inhibitory inputs, and action potentials propagate through the dendritic tree; and how these signals interact with each other when they overlap in space and time; and (3) to make precise measurements of spike timing and action potential waveform in genetically specified cells to probe circuit mechanisms that are masked by the lower temporal resolution of Ca^{2+} imaging. For instance, in fast-spiking interneurons, Ca^{2+} levels do not follow the individual spike dynamics [55], so spike timing is best determined by measuring voltage.

The future of GEVIs: brighter, more sensitive, redder

To achieve these goals will require dramatic improvements in GEVI performance. An ideal GEVI should show large voltage-induced changes in fluorescence over a physiological range, have submillisecond response kinetics, traffic efficiently to the neuronal plasma membrane, be bright and photostable, and fluoresce in the near

infrared part of the spectrum. No such GEVI exists. In engineering new GEVIs, it is important to understand how tradeoffs among these different parameters affect overall GEVI performance.

Ultimately, the ability to record neural activity is governed by the signal-to-noise ratio (SNR) of fluorescent voltage measurements. The shot noise-limited SNR of any fluorescent reporter is:

$$SNR = \frac{\Delta F}{\sqrt{F + B}}, \tag{1}$$

where ΔF is the change in fluorescence associated with the electrical event of interest, F is the basal fluorescence of the indicator, and B is the background fluorescence due to improperly trafficked GEVI molecules, tissue autofluorescence, and out-of-focus fluorescence. All fluorescence values are measured in photon counts. Eq. (1) provides guidance on how tradeoffs in brightness (affects F and ΔF proportionally), trafficking (affects B), speed (affects ΔF), and voltage sensitivity (affects ΔF) impact GEVI performance.

Iterative improvement in GEVIs has historically been slow and labor intensive, due to the multiple parameters governing GEVI performance. In recent years a hierarchical approach has been employed to screen mutant libraries first in bacteria and then in mammalian cells (Figure 3; Archers [30,31] and QuasArs [32••]). While bacteria offer large library size and can be efficiently used to optimize F, trafficking in bacteria is not predictive of trafficking in mammalian cells, nor can one readily perturb membrane voltage in bacteria.

The hardest part of GEVI screening is to measure voltage response kinetics and sensitivity, which together determine ΔF. Mammalian HEK cell lines have been engineered which can produce either spontaneous [56] or optogenetically triggered [48•,57] electrical spikes, analogous to cardiac action potentials. These spiking HEK cells provide a robust platform for evaluating trafficking, brightness, voltage sensitivity, and speed in a high-throughput format. An alternate technique is to apply bulk electric fields which induce oppositely-directed transmembrane potentials on opposite sides of each cell. This technique of induced transient voltage (ITV) does not require cell lines expressing exogenous ion channels, but is less precise and lower throughput than measurements in spiking HEK cells [58]. Whole-cell voltage clamp provides the highest precision and accuracy, but is slow and laborious. Membrane trafficking can differ dramatically between stable cell lines, primary cultured neurons, and neurons *in vivo*. Thus it is essential to validate every promising GEVI in cultured neurons and ultimately in acute brain slice.

New forms of molecular logic

A parallel thread is to engineer GEVIs to implement new forms of molecular logic which may enable alternate approaches to studying neural activity. In every GEVI, optical excitation transfers the molecule to a short-lived (ps – ns) electronic excited state. In microbial rhodopsin-based GEVIs, illumination can further drive transitions among distinct retinal isomerization states and protein conformations in a complex multi-state photocycle [59•]. One can use the interaction of light-driven and voltage-driven dynamics to implement several novel forms of voltage sensing.

The numerical values, in millivolts, of the resting potential and action potential height are important biological parameters, but most GEVIs only report relative changes, not the numerical value, of the voltage. Even with FRET-based ratiometric sensors it is challenging to determine absolute voltage because the two FPs may have different rates of folding, maturation, and photobleaching; and it is challenging to maintain accurately calibrated illumination intensities and detection sensitivities at multiple wavelengths.

In some GEVIs, changes in membrane voltage modulate the electronic excited state lifetime, which can be measured by illuminating the GEVI with a pulsed source and performing time-correlated single-photon counting for detection. Time can be measured with absolute accuracy, so lifetime imaging reports absolute voltage. This strategy has been employed with ASAP1 and an eFRET-based GEVI called CAESR [60]. Voltage also affects the kinetics of transitions within the Arch photocycle, and mutants of Arch have been engineered in which optical measurements of these kinetics report absolute voltage [61].

The short duration of neuronal action potentials poses a severe challenge for GEVI imaging: real-time measurements require a frame rate of ∼1 kHz. It has not yet been technically feasible to attain this frame rate over a large field of view *in vivo*. The complex photocycles of Arch-based GEVIs provide a potential solution to this challenge too [62]. Some Arch mutants undergo a photochemical conversion into a metastable fluorescent state, only in the simultaneous presence of illumination and a depolarizing voltage. By flood-illuminating a neural circuit during a user-selected 'write' interval, one can form a photochemical imprint within each cell of the amount of electrical activity during the write interval. Cells that fire more during this interval produce more fluorescent product. This fluorescence can then be probed at a later time using a slow imaging technique. A key merit of this 'Flash Memory' approach is that the light used for the write interval does not need to follow a straight-line path through the tissue. The light can propagate diffusively, enabling penetration deep into the tissue. The Flash Memory scheme has only been demonstrated in cultured

Figure 3
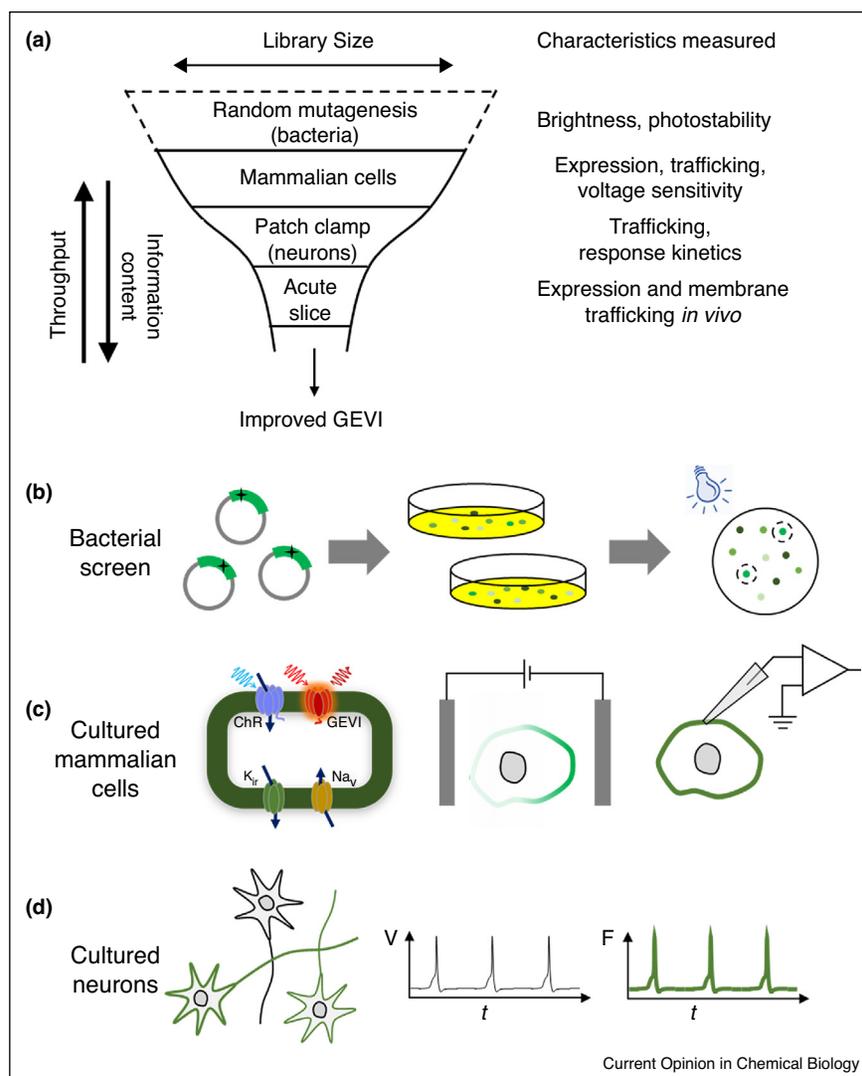
GEVI screening pipeline. **(a)** A hierarchical approach screens large libraries for the most easily measured parameters (brightness), and then characterizes hits for voltage sensitivity and speed. Trafficking must ultimately be tested by GEVI expression *in vivo* and imaging in acute slice. Figure modified from Ref. [56]. **(b)** Bacterial screens for brightness can be performed in a pooled library assay where highly fluorescent colonies are manually selected for propagation. To distinguish brightness from colony size, it is important to have a spectrally distinct reference fluorophore expressed at a constant level. **(c)** Tests for voltage sensitivity and kinetics can be performed in cultured mammalian cells, using either (left) spiking HEK cells, (middle) induced transmembrane voltage, or (right) manual patch-clamp electrophysiology. **(d)** Tests in cultured neurons probe trafficking and high-speed kinetics. Neural activity can be induced either optogenetically or via field-stimulation electrodes.

neurons [63]. Improvements in sensitivity are needed before this can be applied in tissue or *in vivo*.

Conclusions: better GEVIs are only part of the solution

The ideal GEVI has not yet been found. Three protein scaffolds have been explored in depth, with distinct voltage-sensing mechanisms: ion channels and VSPs that undergo voltage-induced conformational changes, and microbial rhodopsins that have voltage-dependent chromophore protonation. Engineering of these scaffolds has led to improvements, but progress is often incremental.

Many potential novel scaffolds remain to be tested. Of the many thousands of microbial rhodopsins identified via metagenomic sequence analysis [64], only a handful have been tested as putative voltage sensors. Mitochondria and chloroplasts have molecular machinery for transmembrane electron transfer. These proteins have endogenous cytochrome chromophores, which might show voltage-dependent spectral properties. Recently developed electrically spiking HEK cell lines should greatly facilitate testing of new GEVI concepts. There remains great scope for enterprising researchers to explore new GEVI scaffolds.

Even with best GEVI imaginable, voltage imaging in the brain will remain technically challenging, far harder than Ca^{2+} imaging. Action potentials are 100–1000-fold faster than Ca^{2+} transients, demanding correspondingly faster imaging speeds. Even if brightness and sensitivity were matched between voltage and Ca^{2+} indicators, the higher imaging speed for voltage would require proportionally higher illumination intensity to maintain comparable SNR, leading to higher rates of photobleaching and phototoxicity. Furthermore, voltage signals in cells come exclusively from the 2-D plasma membrane, while Ca^{2+} reporters reside in the bulk cytoplasm. Thus voltage signals sources are sparsely and irregularly distributed in intact tissue. Light-gated voltage integrators and related concepts open the possibility to relax the stringent requirements on imaging speed, possibly allowing new types of microscopy to be applied to GEVI imaging.

Another difference between voltage and Ca^{2+} imaging comes from the differing surface-to-volume ratios in soma and neuropil. In CNS neurons, the ratio of soma volume to axo-dendritic volume is ~3:2, so most Ca^{2+} signals come from the somata. The ratio of soma surface area to axo-dendritic surface area is ~1:20 [65,66], so most voltage signals come from optically unresolvable neuropil. Signals from soma membranes can be overwhelmed by surrounding neuropil, unless the GEVI is either expressed in a sparse subset of neurons or is constrained to reside in the soma membrane only.

Despite these technical challenges, recent progress in GEVIs has brought us closer to the dream of sensitively mapping electric fluctuations in the brain with high spatial and temporal resolution. Successful application of GEVIs *in vivo* will require advances not just in GEVI scaffolds, but also in techniques for gene delivery and subcellular control of protein trafficking; in microscopes for high-speed imaging in scattering tissues; and in software for analyzing the torrents of data that emerge. This progress will require close collaboration of protein engineers, microscope developers and neuroscientists.

Acknowledgments

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