Supporting Information for:
A Photostable Silicon Rhodamine Platform for Optical Voltage Sensing

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1. General method for chemical synthesis and characterization

Chemical reagents and solvents (dry) were purchased from commercial suppliers and used without further purification. All reactions were carried out in oven-dried flasks under N_2. Thin layer chromatography (TLC) (Silicycle, F254, 250 μm) and preparative thin layer chromatography (PTLC) (Silicycle, F254, 1000 μm) were performed on glass backed plates pre-coated with silica gel were visualized by fluorescence quenching under UV light. Flash column chromatography was performed on Silicycle Silica Flash F60 (230–400 Mesh) using a forced flow of air at 0.5–1.0 bar. NMR spectra were measured on Bruker AVB-400 MHz, 100 MHz, AVQ-400 MHz, 100 MHz, Bruker AV-600 MHz, 150 MHz. NMR spectra measured on Bruker AVII-900 MHz, 225 MHz, equipped with a TCI cryoprobe accessory, were performed by Dr. Jeffrey Pelton (QB3). Chemical shifts are expressed in parts per million (ppm) and are referenced to $CDCl_3$ 7.26 ppm, 77.0 ppm. Coupling constants are reported as Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; sep, septet dd, doublet of doublet; ddd, doublet of double of doublet; dt, doublet of triplet; td, triplet of doublet; m, multiplet. High-resolution mass spectra (ESI EI) were measured by the QB3/Chemistry mass spectrometry service at University of California, Berkeley. High performance liquid chromatography (HPLC) and low resolution ESI Mass Spectrometry were performed on an Agilent Infinity 1200 analytical instrument coupled to an Advion CMS-L ESI mass spectrometer.. Columns used for the analytical HPLC was Phenomenex Luna C18(2) (4.6 mm I.D. × 150 mm) with flow rates 1.0 mL/min. The mobile phase were MQ-H_2O with 0.05% formic acid (eluent A) and HPLC grade acetonitrile with 0.05% formic acid (eluent B). Signals were monitored at 254 and 650 nm in 20 min with gradient 10-100% eluent B.

2. Spectroscopic Studies

Stock solutions of BR and BeRST 1 were prepared in DMSO (1.0–10 mM) and diluted with TBS (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl) solution containing 0.10 % (w/w) SDS (1:100 –1:1000 dilution). UV-Vis absorbance and fluorescence spectra were recorded using a Shimadzu 2501 Spectrophotometer (Shimadzu) and a Quantamaster Master 4 L-format scanning spectrofluorometer (Photon Technologies International). The fluorometer is equipped with an LPS-220B 75-W xenon lamp and power supply, A-1010B lamp housing with integrated igniter, switchable 814 photon-counting/analog photomultiplier detection unit, and MD5020 motor driver. Samples were measured in 1-cm path length quartz cuvettes (Starna Cells).

3. Cell culture

All animal procedures were approved by the UC Berkeley Animal Care and Use Committees and conformed to the NIH Guide for the Care and Use and Laboratory Animals and the Public Health Policy.

Human embryonic kidney 293T (HEK) cells were passaged and plated onto 12 mm glass coverslips pre-coated with Poly-D-Lysine (PDL; 1 mg/ml; Sigma-Aldrich) to provide a confluency of ~15% and 50% for electrophysiology and imaging, respectively. HEK cells were plated and maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 4.5 g/L D-glucose, 10% FBS and 1% Glutamax.

Hippocampi were dissected from embryonic day 18 Sprague Dawley rats (Charles River Laboratory) in cold sterile HBSS (zero Ca2+, zero Mg2+). All dissection products were supplied by Invitrogen, unless otherwise stated. Hippocampal tissue was treated with trypsin (2.5%) for 15 min at 37 °C. The tissue was triturated using fire polished Pasteur pipettes, in minimum essential media (MEM) supplemented with 5% fetal bovine serum (FBS; Thermo Scientific), 2% B-27, 2% 1M D-glucose (Fisher Scientific) and 1% glutamax. The dissociated cells were plated onto 12 mm diameter coverslips (Fisher Scientific) pre-treated with PDL (as above) at a density of 30-40,000 cells per coverslip in MEM supplemented media (as above). Neurons were maintained at 37 °C in a humidified incubator with 5 % CO2. At 3 days in vitro (DIV) half of the MEM supplemented media was removed and replaced with Neurobasal media containing 2% B-27 supplement and 1% glutamax. Transfection of genetic tools was carried out using Lipofectamine 3000 at 7 DIV. Functional imaging was performed on mature neurons 13-20 DIV, except electrophysiological experiments which were performed on 12-15 DIV neurons.

Unless stated otherwise, for loading of HEK cells and hippocampal neurons, BeRST 1 was diluted in DMSO to 1 mM, and then diluted 1:1000 in HBS (in mM) 140 NaCl, 2.5 KCl, 10 HEPES, 10 D-glucose 1.3 $MgCl_2$ and 2 $CaCl_2$; pH 7.3 and 290 mOsmol. All imaging experiments were performed in HBS.

4. DNA constructs

Channelrhodopsin-2-YFP (ChR2-YFP) was a gift from Karl Deisseroth (Addgene plasmid # 20945) and drives neuronal expression of channelrhodopsin-2 using the synapsin promoter. Puro-CAG-ASAP1 was a gift from Michael Lin (Addgene plasmid # 52519) and its expression is driven by the chicken beta-actin promoter. GCaMP6s was a gift from Douglas Kim (Addgene plasmid # 40753) and was driven in mammalian cells by the cytomegalovirus promoter.

5. Imaging parameters

Epifluorescence imaging was performed on an AxioExaminer Z-1 (Zeiss) equipped with a Spectra-X Light engine LED light (Lumencor), controlled with Slidebook (v6, Intelligent Imaging Innovations). Co-incident excitation with multiple LEDs was controlled by Lumencor software triggered through a Digidata 1332A digitizer and pCLAMP 10 software (Molecular Devices). Images were acquired with either a W-Plan-Apo 20x/1.0 water objective (20x; Zeiss) or a W-Plan-Apo 63x/1.0 water objective (63x: Zeiss). Images were focused onto either an OrcaFlash4.0 sCMOS camera (sCMOS; Hamamatsu) or an eVolve 128 EMCCD camera (EMCCD; Photometrix). More detailed imaging information for each experimental application is expanded below.

5a. Multicolor imaging of BeRST 1 in HEK cells and Photostability

HEK cells were incubated with a HBSS solution (Gibco) containing BeRST 1 (1.0 μM), Hoechst 33342 (1.0 μM, Molecular Probes) and Rhodamine 123 (5.0 μM, Sigma-Aldrich) at 37°C for 15 min. Microscopic images were acquired with a W-Plan-Apo 63x/1.0 objective (Zeiss) and OracFlash4.0 sCMOS camera (Hamamatsu). For BeRST 1 images, the excitation light was delivered from a LED (67 W/cm^2; 100 ms exposure time) at 631/28 (bandpass) nm and emission was collected with a quadruple emission filter (430/32, 508/14, 586/30, 708/98 nm) after passing through a quadruple dichroic mirror (432/38, 509/22, 586/40, 654 nm LP). For Hoechst 33342 images, the excitation light was delivered from a LED (33 W/cm^2; 100 ms exposure time) at 390/22 nm and emission was collected with an emission filter (540/50 nm) after passing through a dichroic mirror (510 nm LP). For Rhodamine 123 images, the excitation light was delivered from a LED (57 W/cm^2; 100 ms exposure time) at 475/34 nm and emission was collected with an emission filter (540/50 nm) after passing through a dichroic mirror (510 nm LP).

HEK cells were incubated separately with BeRST 1 (0.20 μM) and VF2.1.Cl (0.20 μM) in HBSS at 37°C for 15 min. Data were acquired with a W-Plan-Apo 63x/1.0 objective (Zeiss) and Evolve 128 emCCD camera (Photometrics). Images (pixel size 0.38 μm × 0.38 μm) were taken every 20 sec for 10 min with constant illumination of LED (162 W/cm^2; 100 ms exposure time). For BeRST 1 images, the excitation light was delivered at 631/28 nm and emission was collected with a quadruple emission filter (430/32, 508/14, 586/30, 708/98 nm) after passing through a quadruple dichroic mirror (432/38, 509/22, 586/40, 654 nm LP). For VF2.1.Cl images, the excitation light was delivered at 475/34 nm and emission was collected with an emission filter (540/50 nm) after passing through a dichroic mirror (510 nm LP). The obtained fluorescence curves (background subtracted) were normalized with the fluorescence intensity at t = 0 and averaged (six different cells of each dye).

5b. Extracellular stimulation experiments.

Extracellular field stimulation was delivered by Grass Stimulator connected to a recording chamber containing two platinum electrodes (Warner), with triggering provided through a Digidata 1332A digitizer and pCLAMP 10 software (Molecular Devices). Action potentials were triggered by 1 ms 80 V field potentials delivered at 5 Hz. To prevent recurrent activity the HBS bath solution was supplemented with synaptic blockers 10 μM 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; Santa Cruz Biotechnology) and 25 μM DL-2-Amino-5-phosphonopentanoic acid (APV; Sigma-Aldrich). Functional imaging of BeRST 1 was performed using the EMCCD camera and a 63x objective. BeRST 1 was excited with a 631 nm light (LED; 631nm, 28 nm bandpass) with an intensity of 127 W/cm^2. Emission from BeRST 1 was collected with a 680/10 nm bandpass emission filter (after passing through a quadruple dichroic mirror (432/38 nm, 509/22 nm, 586/40 nm, 654 nm LP) or for GCaMP6s and ASAP experiments a QUAD filter (quadruple emission filter (430/32 nm, 508/14 nm, 586/30 nm, 708/98 nm) after passing through the quadruple dichroic mirror. Optical sampling rate of BeRST 1 imaging was 0.5-0.53 kHz, except when imaging with ASAP1 (1.25 kHz). Green spectrum genetic tools (GFP, GCaMP6s and ASAP1) were illuminated using 475 nm light (LED; 475 nm, 34 nm bandpass). For functional imaging an intensity of 33 W/cm^2 for GCaMP6s and 200 W/cm^2 for the more weakly fluorescent ASAP1 was used, which is the maximum 475 nm LED intensity for our system. Emission was collected with a 540/50 nm bandpass filter after passing through a 510 nm longpass dichroic. Functional imaging of GCaMP6s and ASAP was performed at 40 Hz and 1.25 kHz, respectively. Images showing cellular morphology were performed by switching to a lower magnification 20x objective in conjunction with either the EMCCD (GCaMP6s/ASAP1) or sCMOS (GFP) camera.

5c. Voltage sensitivity in HEK cells.

Functional imaging of BeRST 1 was performed using a 20x objective paired with image capture from the EMCCD camera at a sampling rate of 0.5kHz. BeRST 1 was excited using the 633 nm LED with an intensity of 6.7 W/cm^2. For initial voltage characterization (Figure 2) emission was collection with the QUAD filter and dichroic (see above). For investigation into voltage sensitivity during excitation cross-talk (SI Fig. 5) BeRST 1 was excited by a 633 nm LED as above and 390 nm light (violet LED, 20 nm bandpass) with an intensity of 3.3 W/cm^2 and emission was collected with a 680/10 nm bandpass emission filter.

5d. Imaging groups of cells

Imaging experiments looking a functional responses from many >5 neurons (Figure 4 and Figure 8) required a larger field of view which were obtained using the sCMOS camera with a 20x objective. BeRST 1 was excited using a 633 nm LED with an intensity of 20 W/cm^2 and emission was collected with a 680/10 nm bandpass emission filter. Images were binned 4x4 to allow sampling rates of 0.5 kHz. GFP was excited by the 475 nm LED and emission was collected with a 540/50 nm bandpass filter after passing through a 510 nm longpass dichroic. ChR2 positive neurons were identified by the YFP fusion by excitation with 510 nm light (LED; 510 nm, 25 nm bandpass) and emission was collected via a triple emission filter (473/22 nm, 543/19 nm, 648/98 nm) after passing through a triple dichroic mirror (475/30 nm, 540/25 nm, 642/96 nm. ChR2 was activated by 80 mW/cm^2, 5 ms pulses of 475 nm LED light at a frequency of 5 Hz.

5e. Imaging of BeRST 1 in patch-clamped hippocampal neurons.

Functional imaging of patched neurons was performed using an EMCCD camera and a 20x objective. This objective has a larger working distance and allowed for positioning of the patch electrode. BeRST 1 was excited using a 633 nm LED with an intensity of

10 W/cm^2 and emission was collected with a 680/10 nm bandpass emission filter. For optical assessment of the action potential waveform (Figure 3) the sampling rate was increased to 1.8 kHz. For all optical electrophysiology (Figure 7 and SI Fig 7) the sampling rate was increased to 1 kHz. YFP images were collected as above. ChR2 was stimulated by 80m W/cm^2, 5 ms pulses of 475 nm LED light at a frequency of 5 Hz.

5f. Cross-excitation

Cross-talk was assessed by exciting hippocampal neurons loaded with (1 μM) BeRST 1 with 390 nm, 475 nm and 633 nm LEDs all illuminating with an intensity of 9.7 W/cm^2. Emission was collected with a 680/10 nm bandpass emission filter.

6. Image Analysis

Analysis of voltage sensitivity in HEK cells was performed using custom Matlab routines. Briefly, a region of interest (ROI) was selected automatically based on fluorescence intensity and applied as a mask to all image frames. Fluorescence intensity values were calculated at known baseline and voltage step epochs.

For analysis of BeRST 1 voltage responses in neurons, regions of interest encompassing cell bodies were drawn in ImageJ and the mean fluorescence intensity for each frame extracted. ΔF/F values were calculated by first subtracting a mean background value from all raw fluorescence frames, bypassing the noise amplification which arises from subtracting background for each frame, to give a background subtracted trace (bkgsub). A baseline fluorescence value (F_{base}) is calculated either from the first several (10-20) frames of the experiment for evoked activity, or from the median for spontaneous activity, and was subtracted from each timepoint of the bkgsub trace to yield a ΔF trace. The ΔF was then divided by F_{base} to give ΔF/F traces. The photostability of BeRST 1 means that over short (<3 seconds) timeseries no bleach correction was required. For longer experiments, (Figures 4 and 8, main text) changes in baseline fluorescence was minimally corrected using the manual baseline adjustment in Clampfit 10 software for cosmetic purposes. Example traces before and after baseline correction can be found in SI Figure 9. No averaging has been applied to any voltage traces.

For comparisons of action potentials recorded electrophysiologically and optically, imaging traces were analyzed using spike detection algorithms in the Clampfit 10 software (Molecular Devices).

For cross-excitation quantification images were background subtracted. ROIs were made by thresholding to the 25% of the maximum pixel intensity to remove non-stained areas. Mean intensity values for stained neurons were calculated.

7. Electrophysiology

For electrophysiological experiments, pipettes were pulled from borosilicate glass (Sutter Instruments, BF150-86-10), with a resistance of 5–8 MΩ, and were filled with an internal solution; (in mM) 115 potassium gluconate, 10 BAPTA tetrapotassium salt, 10 HEPES, 5 NaCl, 10 KCl, 2 ATP disodium salt, 0.3 GTP trisodium salt (pH 7.25, 275 mOsm). Recordings were obtained with an Axopatch 200B amplifier (Molecular Devices) at room temperature. The signals were digitized with Digidata 1332A, sampled at 50kHz and recorded with pCLAMP 10 software (Molecular Devices) on a PC. Fast capacitance was compensated in the on-cell configuration. For all electrophysiology experiments, recordings were only pursued if series resistance in voltage clamp was less than 30 MΩ.

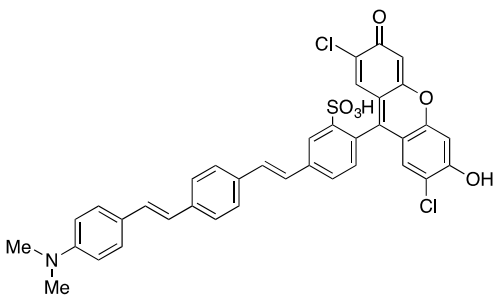
For whole-cell, voltage clamp recordings in HEK 293T cells, cells were held at -60 mV and 100 ms hyper- and de-polarizing steps applied from -100 to +100 mV in 20 mV increments.

For whole-cell, current clamp recordings in hippocampal neurons, following membrane rupture, resting membrane potential was assessed and recorded at I = 0 and monitored during the data acquisition. Neurons were switched to current clamp mode if they displayed series resistance in voltage clamp <30 MΩ. Pipette tip resistance was corrected by performing a bridge balance compensation.

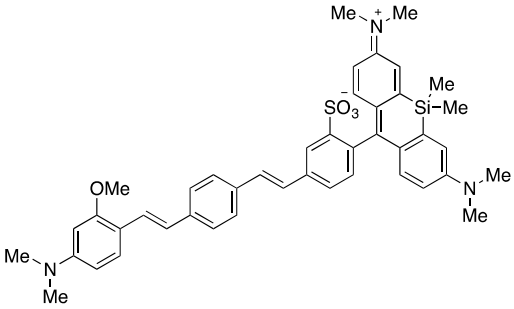
To test if loading of BeRST 1 onto the membrane of neurons has any effect on their action potential firing, ten 500 ms current steps were injected into neurons, in increments of 0.05 pA. The first action potential from each sweep was analysed in Clampfit 10 software (Molecular Devices) to give amplitude and kinetic data. The cell capacitance determined by the Clampex software during recording. To evoke single action potentials for electrophysiological and imaging comparisons, short (10 ms) current injections were applied which were 2x the threshold required to evoke a single action potential. Analysis of action potentials was performed using spike detection algorithms in the Clampfit 10 software.

To record ChR2-evoked action potentials electrophysiologically, neurons were held in cell-attached mode (seals 0.5-1 G Ω) or following membrane rupture, data was acquired at I = 0.

8. Supplemental Figures and Table



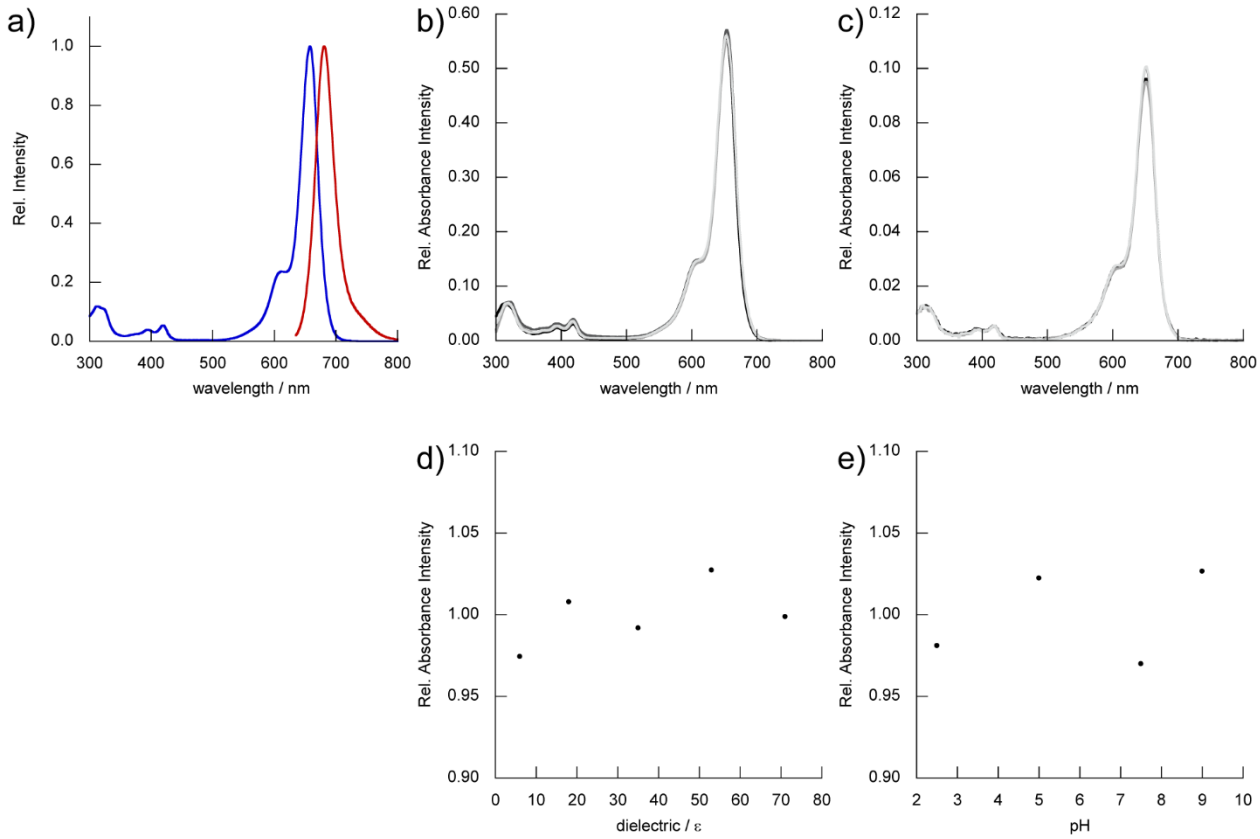
VF2.1.Cl



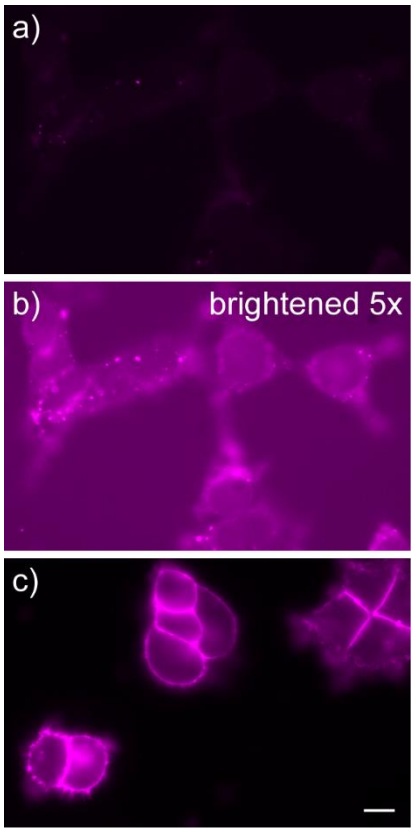
BeRST 1

λ_{abs}/ $\lambda_{em, max}$ (nm)	522/535[a]	658/683[b]
ε_{max} (M^{-1} cm^{-1})	98,000[a]	150,000[b]
Φ	0.057[a]	0.017[b,d]/0.022[c,d]
ΔF/F per 100 mV	27% ± 1%[e]	24% ± 5%[f]
bleaching half-time	< 60 sec[g]	5 min[h]

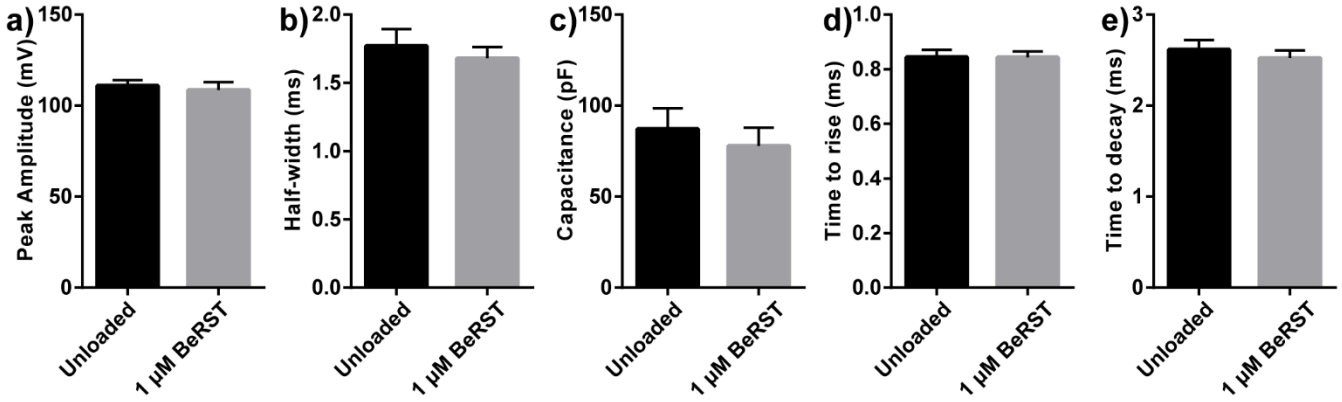
SI Table 1. Properties of VoltageFluor **VF2.1.Cl** and **BeRST 1**. Measurement were conducted in a) in 5 mM sodium phosphate, pH 9.0, 0.10% Triton X-100. (Values are from reference 13.) b) in TBS (50 mM Tris-HCl, 0.15 M NaCl), pH 7.5, 0.10 % SDS. c) in MeOH. d) referenced to cresyl violet in MeOH. e) HEK cells stained with 200 nM **VF2.1.Cl**. f) HEK cells stained with 1 μM **BeRST 1**. g) HEK cells stained with 200 nM **VF2.1.Cl** with constant illumination of LED (475 nm, 162 W/cm^2). h) HEK cells stained with 200 nM **BeRST 1** with constant illumination of LED (631 nm, 162 W/cm^2).



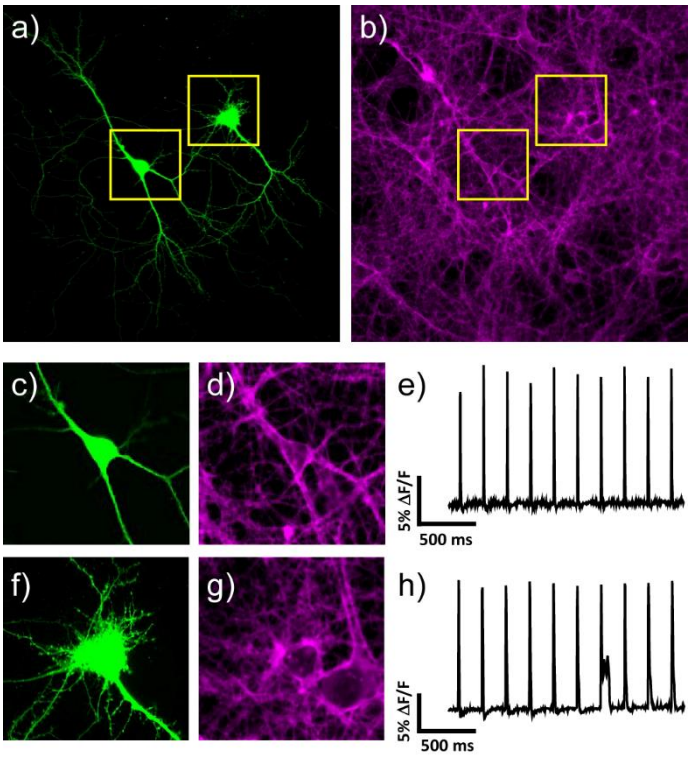
SI Figure 1. Spectroscopic characterization of Berkeley Red. a) Absorbance (blue) and emission (red) spectrum of BR in aqueous buffer. UV-vis spectrum of BR at b) various dielectric constant (achieved by mixtures of dioxane/water ranging from 10 to 90%) or c) pH (ranging from 2.5 to 9). Plot of relative absorbance of BR vs. d) dielectric constant and e) pH.



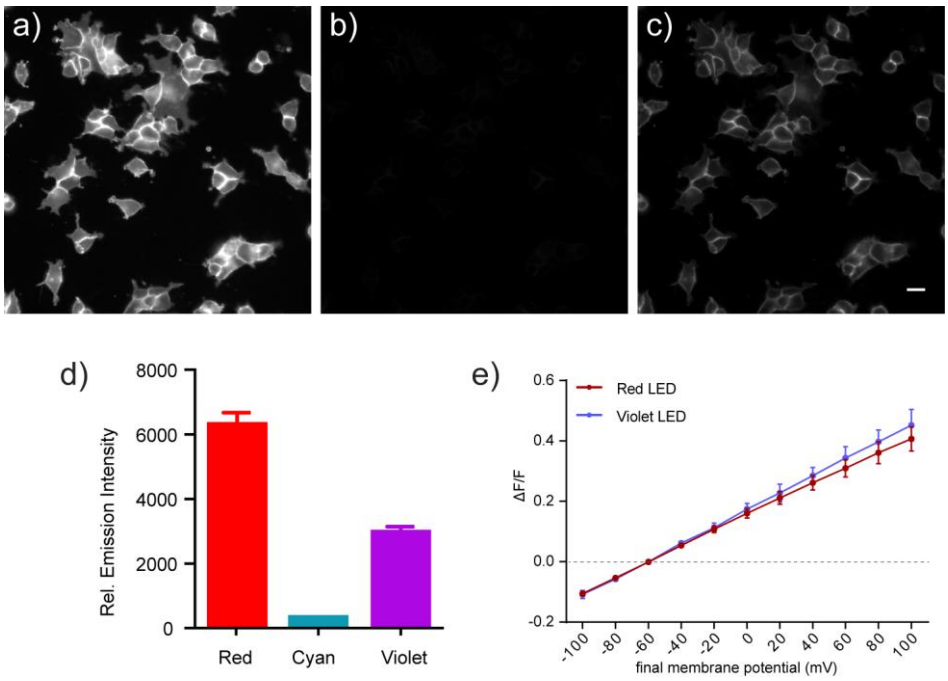
SI Figure 2. Epifluorescence imaging of a,b) Berkeley Red and c) BeRST 1 in HEK cells. a) Cells stained with 1 μM BR and illuminated with 631 nm light (188 W/cm^2) show negligible cellular fluorescence. b) Brightening of panel a) shows a small amount of dye internalization, with very poor contrast between intra- and extracellular space. c) Cells stained with 1 μM BeRST 1 and illuminated at with 631 nm light (80 W/cm^2, 2.4x less light intensity than in panel a). Clear membrane staining is observed. All imaging parameters and display settings are identical for panels a and c, except light intensity, which is 2.4x lower for illumination of BeRST 1. Scale bar is 20 μm.



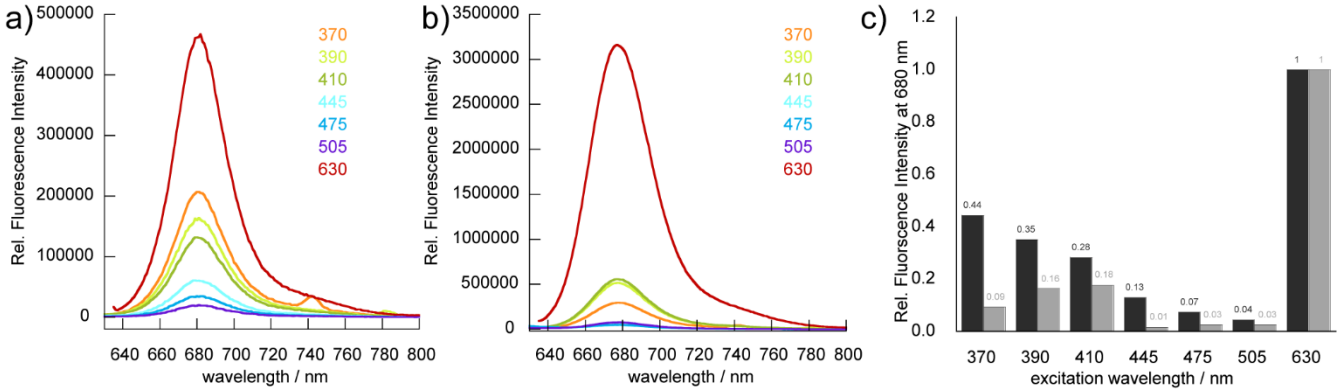
SI Figure 3. Comparison of cellular electrophysiological parameters in rat hippocampal neurons loaded with either 1 μM BeRST 1 or nothing. Evoked action potentials were assessed under dye-loaded and dye-free conditions. Action potentials were averaged across n = 12 and n = 11 cells for unloaded neurons and neurons loaded with 1 μM BeRST 1. No statistically significant differences across a number of electrophysiological parameters, including a) peak action potential amplitude, b) action potential duration as measured by full width at half-max, c) cellular capacitance, d) rise time, or e) decay time. Unpaired t-tests, $p > 0.5$. Error bars are SEM.



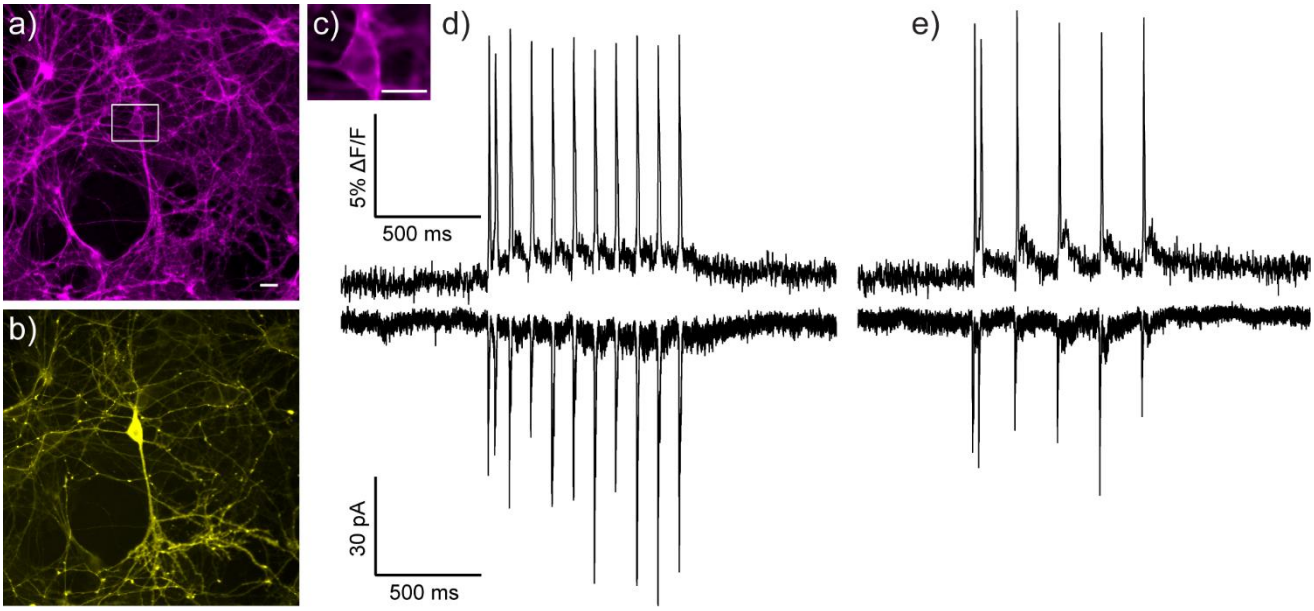
SI Figure 4. Imaging evoked activity in GFP-positive rat hippocampal neurons with BeRST 1. Action potentials from neurons expressing a) GFP and stained with b) BeRST 1 were evoked via field stimulation and recorded (c-h) in the regions of interest indicated in panels a and b. Stimulation was provided at 5 Hz and optically recorded at 500 Hz.



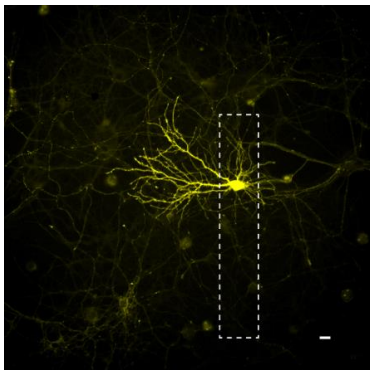
SI Figure 5. Cross excitation of BeRST 1 in rat hippocampal neurons. Panels a-c represent the same field of neurons from a culture loaded with 1 μM BeRST 1 and excited at a) 631 nm, b) 475 nm, or c) 390 nm (9.7 W/cm^2 for all wavelengths). Scale bar is 20 μm. d) The relative emission intensity at 680 nm is quantified for each excitation condition. Error bars are ±SEM for n = 5 separate cells. e) Voltage sensitivity of neurons stained with BeRST 1 when excited at 631 nm (6.7 W/cm^2) or 390 nm (3.3 W/cm^2) light (experimental set-up is identical to data recorded in main text, Fig. 2d). Fractional changes in fluorescence are calculated to be 26% and 28% ΔF/F per 100 mV for 631 nm and 390 nm excitation, respectively (n = 4 separate cells).



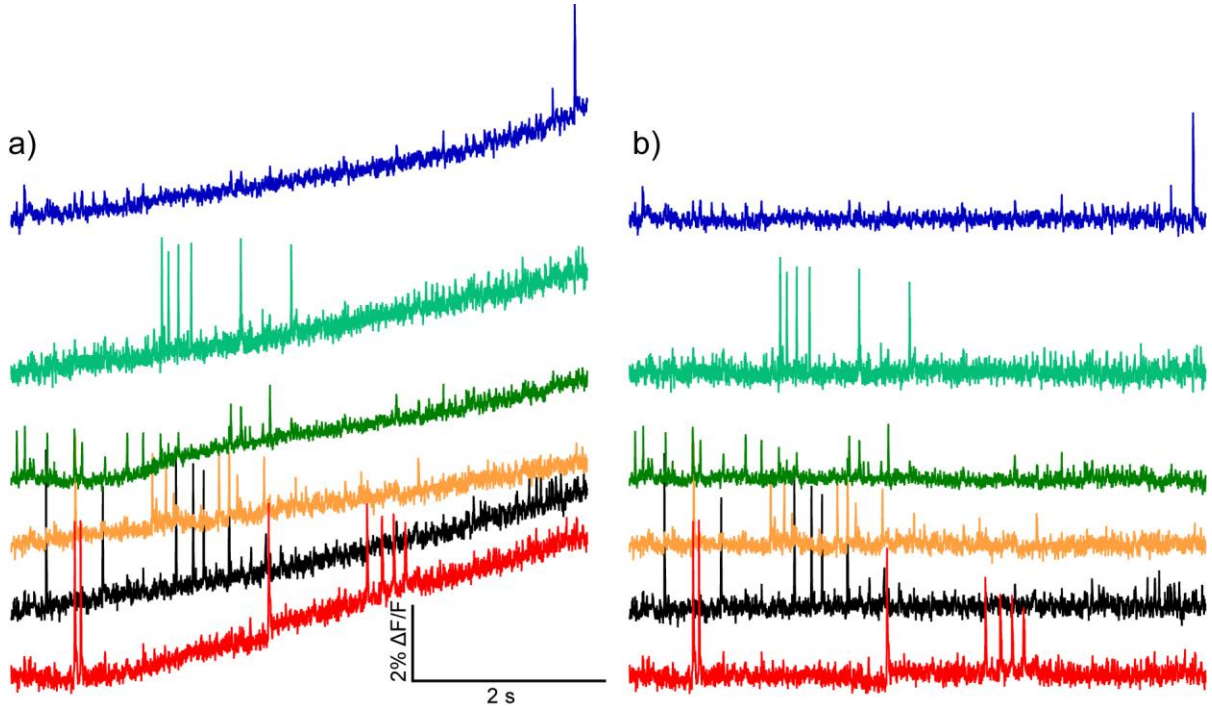
SI Figure 6. *In vitro* cross excitation of BeRST 1 and BR. Emission scans of a) BeRST 1 and b) BR in aqueous buffer (50 mM TBS, pH 7.5, 0.1% SDS) with differing excitation wavelengths. c) Relative fluorescence emission intensity at 680 nm vs. excitation wavelength for BeRST 1 (black bars) and BR (grey bars).



SI Figure 7. All-optical electrophysiology with BeRST 1 and ChR2. Epifluorescence images of rat hippocampal neurons stained with a) BeRST 1 and expressing b) YFP-ChR2. c) Magnified view of white box in panel a. This image is a single frame used to acquire data for panels d and e. Scale bars are 20 μm. Optical (upper) and electrophysiological (lower) responses to optogenetic stimulation of the neuron in panels a-c with 5 ms pulse of cyan light (475 nm, 0.08 W/cm^2) at a rate of d) 10 Hz or e) 5 Hz. Electrophysiological recordings were acquired at a sampling rate of 20 kHz in cell-attached patch clamp configuration. Optical traces were acquired at 1 kHz are were not corrected for bleaching.

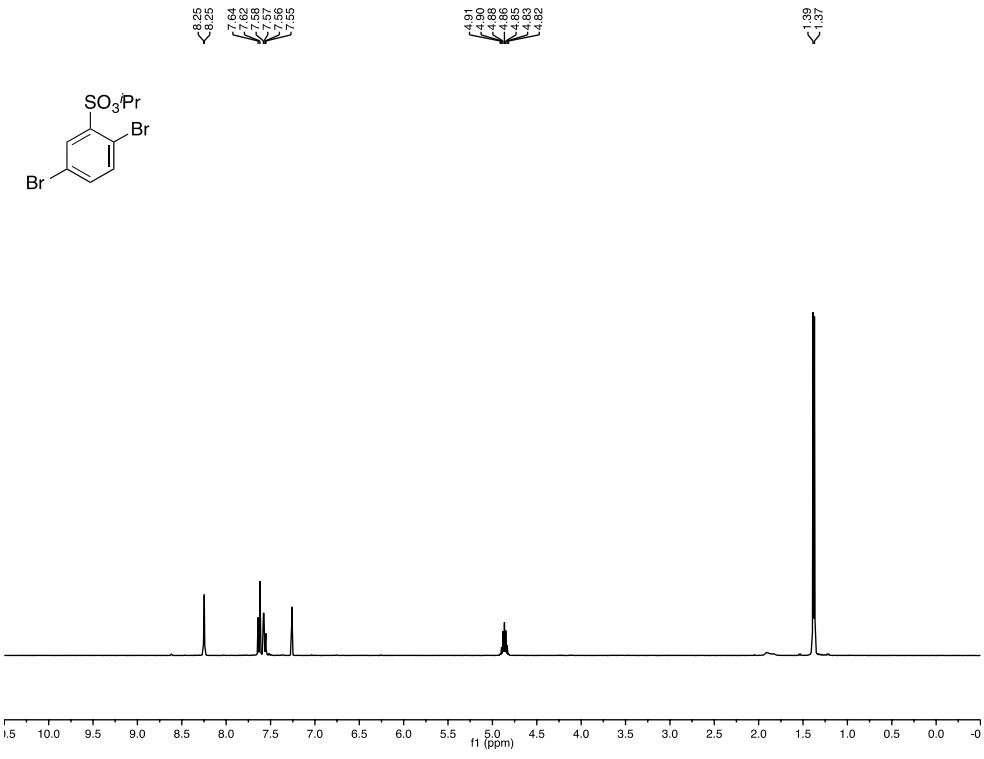


SI Figure 8. Neuron expressing YFP-ChR2. Scale bar is 20 μm. Total image area is 665 × 665 μm. Dashed box indicates approximate imaging area from Figure 8 in the main text.



SI Figure 9. Raw vs. baseline-corrected traces. Fluorescence intensity traces from Fig. 4 in main text, a) before and b) after cosmetic baseline correction in Clampfit 10.

9. Spectra



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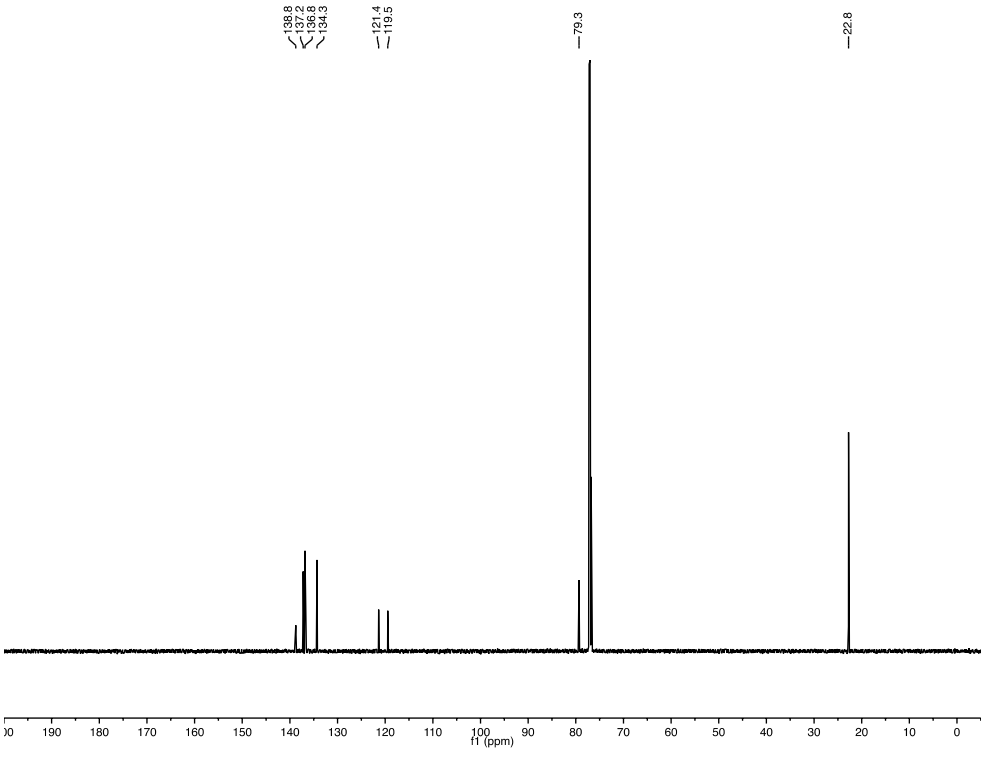

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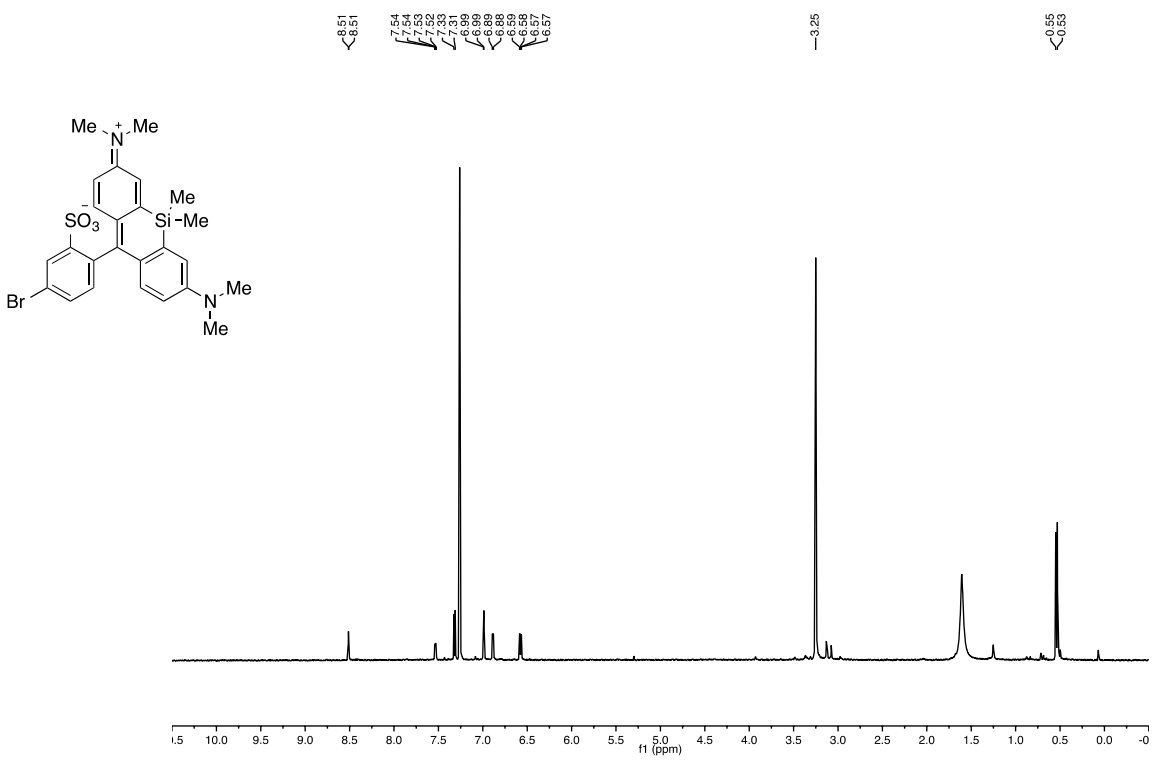

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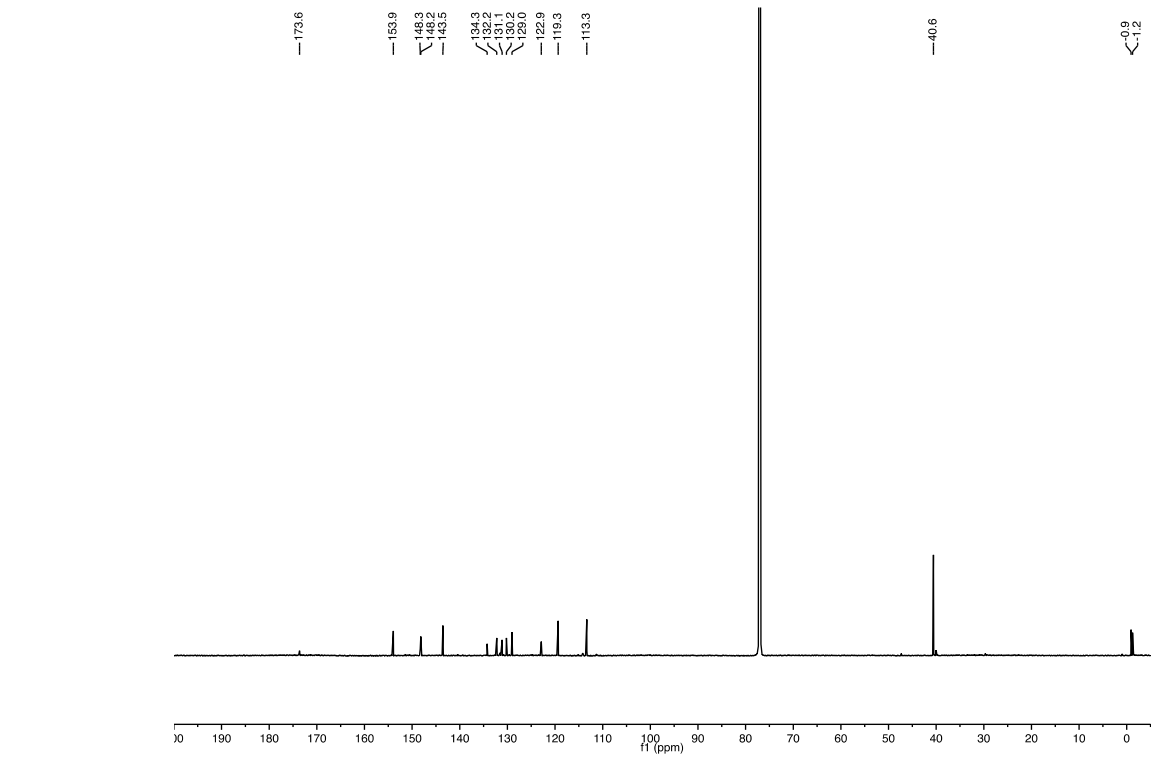

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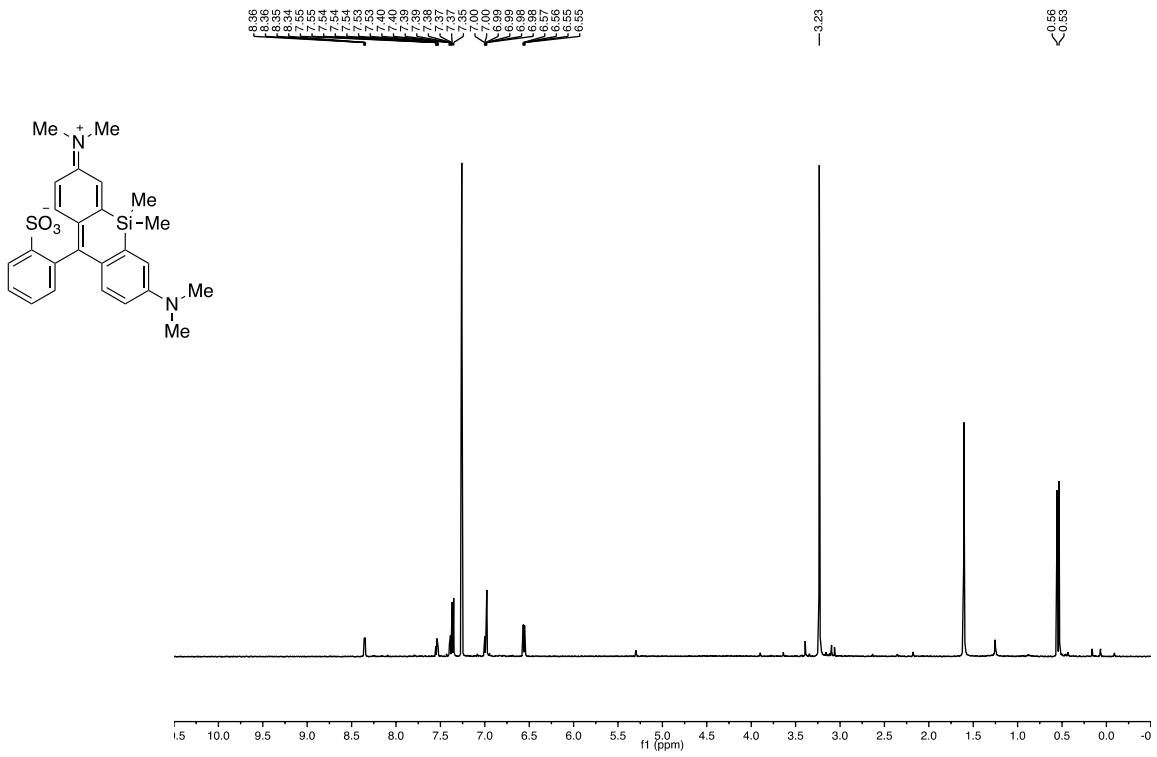
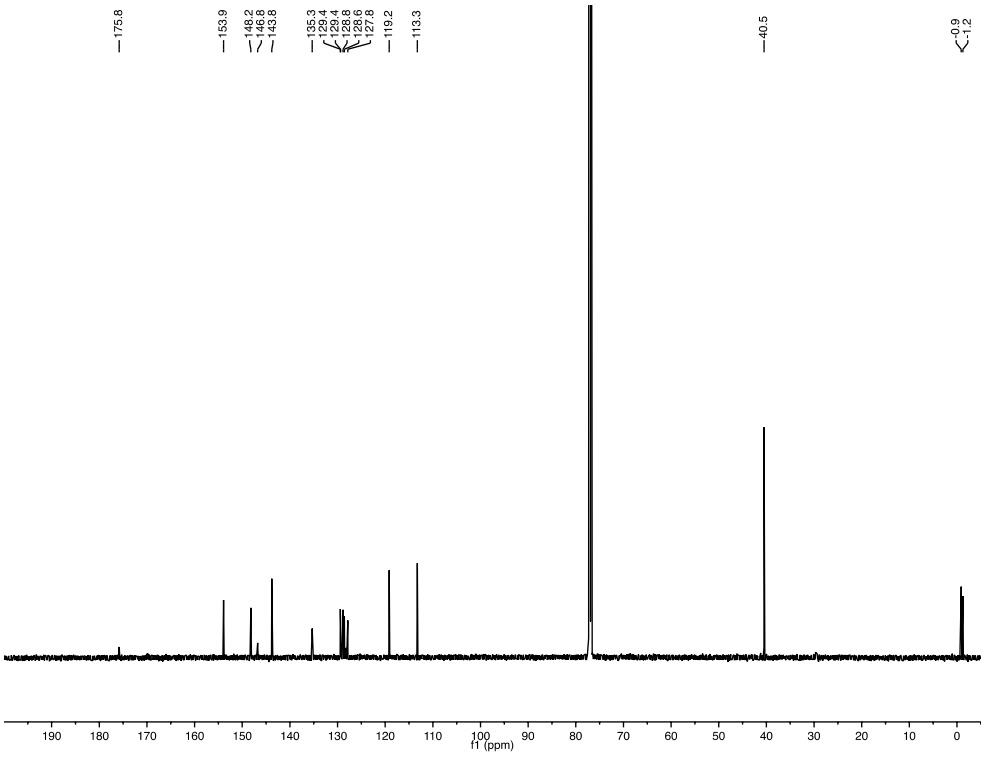
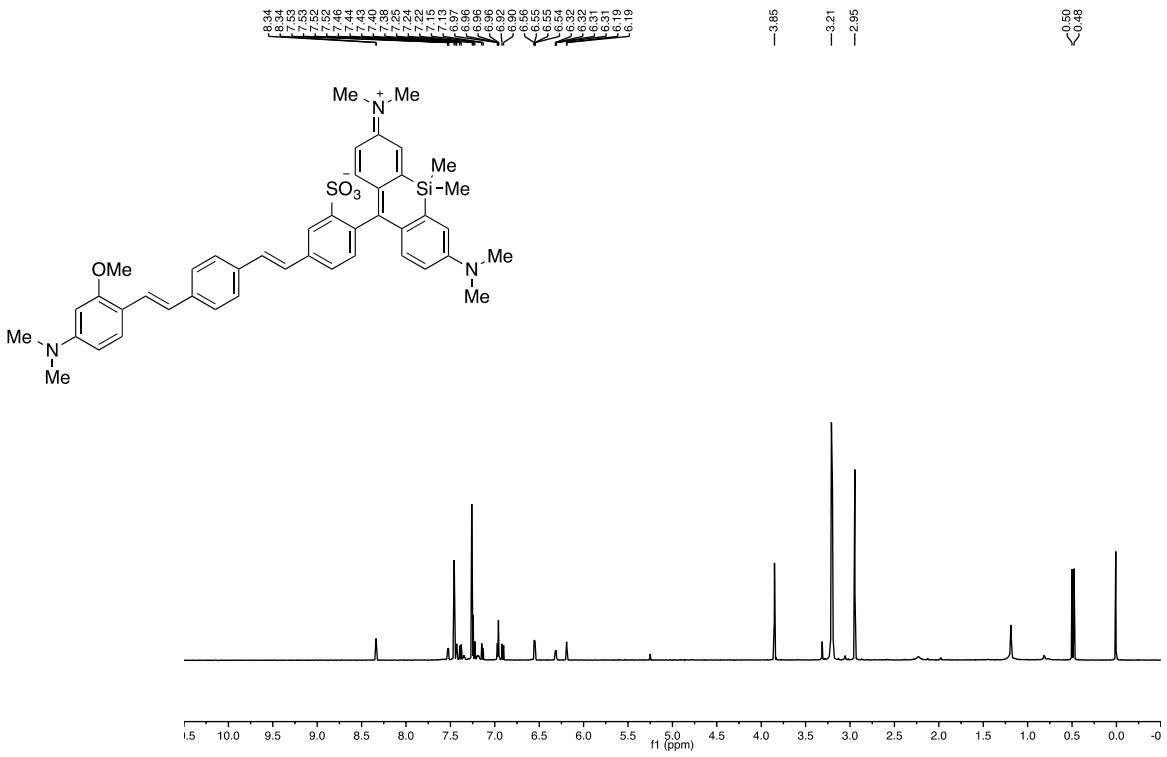


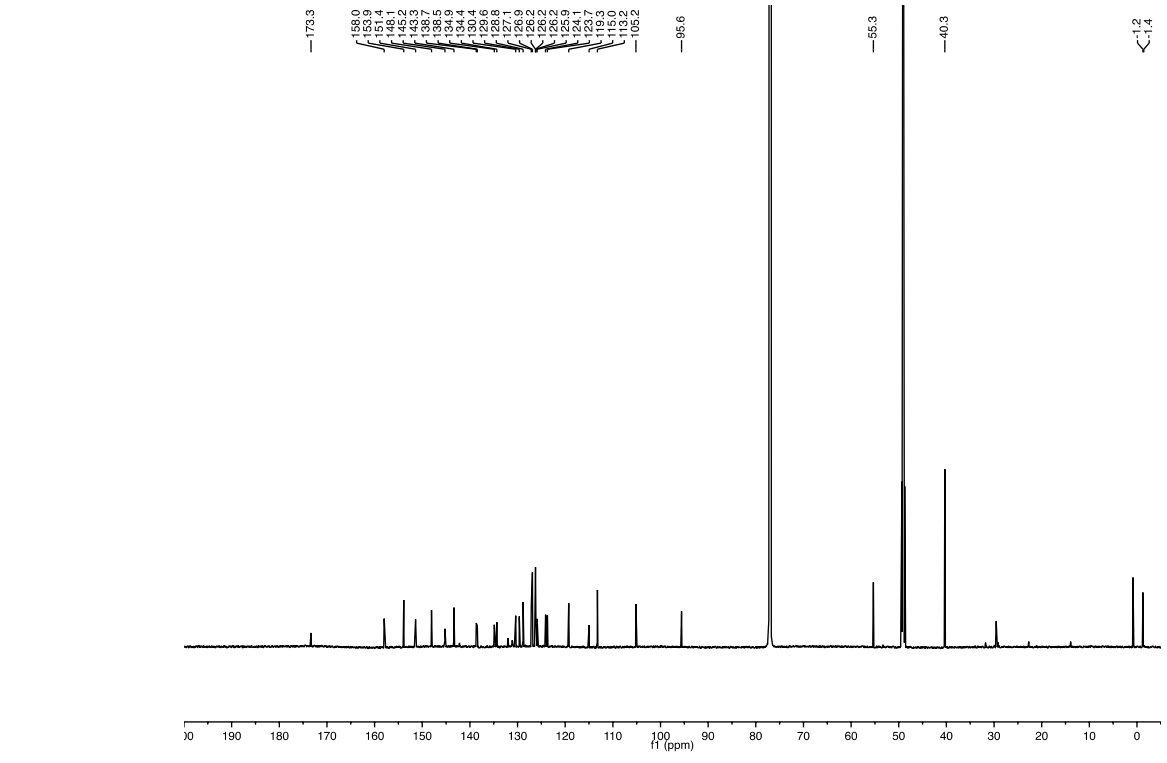
SI Spectrum 5. ^{1}H NMR of BR.



SI Spectrum 6. ^{13}C NMR of BR.

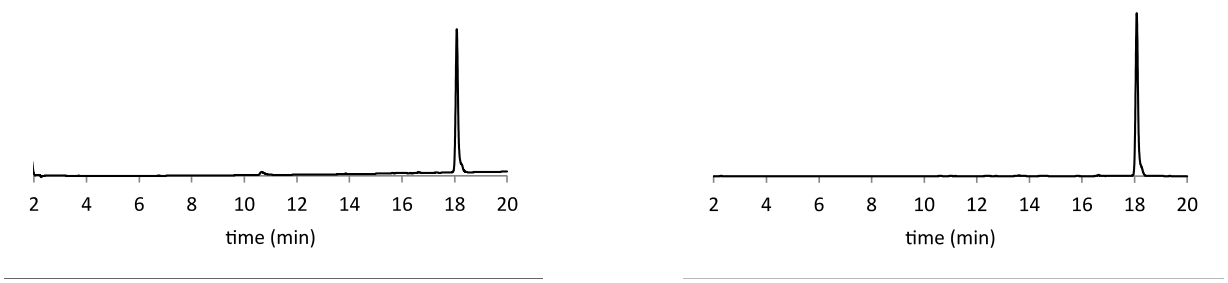


SI Spectrum 7. ^{1}H NMR of BeRST 1.

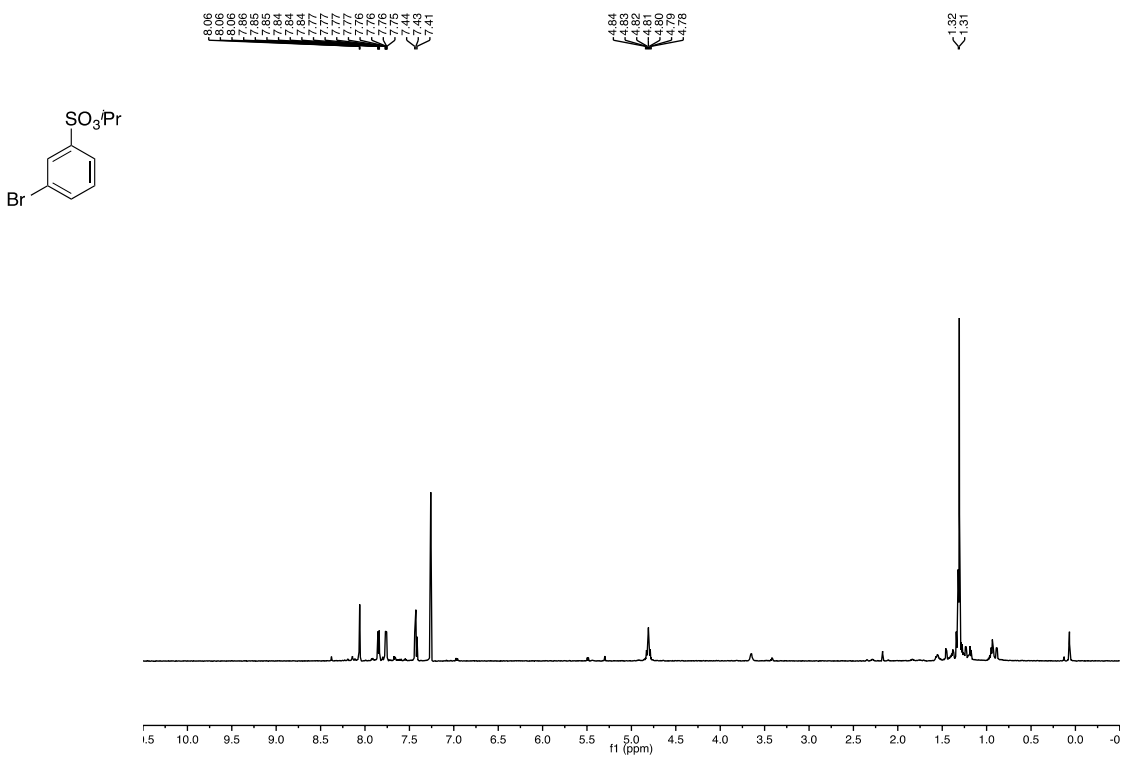


SI Spectrum 8. ^{13}C NMR of BeRST 1.

254 nm **650 nm**



SI Spectrum 9. HPLC traces of BeRST 1 at 254 and 650 nm.



SI Spectrum 10. ^{1}H NMR spectrum organolithium reagent derived from 2, following quenching with H^{+} (crude reaction mixture).

10. Movie Captions

SI Movie 1. Voltage sensitivity of BeRST 1 in patch-clamped HEK cells (see Fig. 2 in main text). HEK cells were loaded with 1 μM BeRST 1 and subjected to whole-cell patch clamp electrophysiology. Under voltage clamp conditions, HEK cells were held at -60 mV and then stepped sequentially through potentials ranging from +100 mV to -100 mV. This movie shows the raw fluorescence response of a single patched HEK cell subjected to 11 such hyper- and depolarizing steps (±100 mV in 20 mV increments). Movie is slowed down 10x from realtime (50 fps, data acquired at 500 Hz).

SI Movie 2. ΔF of BeRST 1 signals during all-optical electrophysiology in rat hippocampal neurons with BeRST 1 and ChR2 (see Fig. 8e in main text)—first optical stimulation train. Cultured rat hippocampal neurons transfected with YFP-ChR2 and stained with BeRST 1 were stimulated with 475 nm light (80 mW/cm^2, 5 ms, 5 Hz, cyan bars) during two separate 1.5 second periods to evoke activity in the YFP-ChR2-expressing cell. This pseudo-colored movie represents the ΔF vs time for the first recording epoch. Optical traces were acquired at 500 Hz and replayed here at 0.1x speed (50 fps). Cyan square indicates approximate time of optical stimulation.

SI Movie 3. ΔF of BeRST 1 signals during all-optical electrophysiology in rat hippocampal neurons with BeRST 1 and ChR2 (see Fig. 8e in main text)—second optical stimulation train. Cultured rat hippocampal neurons transfected with YFP-ChR2 and stained with BeRST 1 were stimulated with 475 nm light (80 mW/cm^2, 5 ms, 5 Hz, cyan bars) during two separate 1.5 second periods to evoke activity in the YFP-ChR2-expressing cell. This pseudo-colored movie represents the ΔF vs time for the second recording epoch. Optical traces were acquired at 500 Hz and replayed here at 0.1x speed (50 fps). Cyan square indicates approximate time of optical stimulation.