Supporting Information for:

Photoactivation of an Acid-Sensitive Ion Channel Associated with Vision and Painl

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1 Experimental Methods

1.1 General Procedures

Samples of **1** for study were all prepared using the following methods. A stock solution was prepared by dissolving a known amount of **1** (variable amounts given nature of experiment) in minimal 18 M-Ω purified water (Barnsted E-Pure). The stock solution was then diluted to the desired concentrations for further experiments. For experiments where a pH change was required, **1** was diluted (*ca.* 100 to 1000 times) into ND96 salts (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$) and the pH was adjusted to the indicated pH using a pH meter (VWR sympHony, SB70P). pH switching

studies were performed with **1** dissolved in ND96 salts using the pH meter to measure the pH before and after irradiation by a 455 nm LED (Thor Labs, M455L1). UV-visible studies were carried out using a HP 8453 spectrometer, using a 1 cm pathlength quartz cell cuvettes. Extinction coefficients were calculated by dissolution of a known amount of **1** into pure water, followed by dilution at various concentration into the mixed buffer system at both pH 4.0 and pH 8.0. Electrophysiology experiments were carried out with the OpusXpress (Molecular Devices) using established methods for GLIC expression and whole-cell voltage clamp protocols.[1] All chemicals were obtained from Aldrich, and used without further purification. **3, 4**, 3-formyl-4-hydroxybenzenesulfonate and 3-(2,3,3-trimethyl-3H-indol-1-ium-1-yl)propane-1-sulfonate were synthesized according to literature methods.[2,3,4] All data processing occurred using MATLAB R2013b (Mathworks, Inc.).

1.2 Synthesis of (E)-3-(2-(3,3-dimethyl-1-(3-sulfonatopropyl)-3H-indol-1-ium-2-yl)vinyl)-4-hydroxybenzenesulfonate (1)

1 was prepared by a modification of literature procedures.[3] Briefly, 3-formyl-4-hydroxybenzenesulfonate (419 mg, 2.02 mmol) and 3-(2,3,3-trimethyl-3H-indol-1-ium-1-yl)propane-1-sulfonate (501 mg, 1.84 mmol) were added dry ethanol (5 mL). The resulting dark purple solution was stirred vigorously and refluxed overnight under argon. The reaction was then filtered to yield an orange solid, which was then washed with excess cold ethanol, and dried overnight, giving **1**. ^{1}H NMR (300 MHz,$(CD_3)_2SO$) 11.45 (s, 1H), 8.53 (d, J = 16.3 Hz, 1H), 8.28 (d, J = 2.1 Hz, 1H), 8.12-8.02 (m, 1H), 7.98-7.81 (m, 2H), 7.75-7.54 (m, 3H), 6.98 (d, J = 8.6 Hz, 1H), 4.76 (t, J = 7.8 Hz, 2H), 2.63 (t, J = 6.8 Hz, 2H), 2.17 (m, J = 7.6 Hz, 2H), 1.79 (s, 6H). ESI-MS (Negative Mode) Calculated for $C_{21}H_{22}NO_7S_2^-$ 464.1, found 464.2.

1.3 UV-Visible Kinetics Studies

1 was dissolved as described above, and diluted such that A_{455} <0.4. The kinetics were followed for two hours, collecting a spectrum every 30 seconds, using the kinetics software package for the spectrometer. The first two points were baseline of **1**, followed by 30 seconds of irradiation with a 455 nm LED (Thor Labs, M455L1), and the remaining time in the dark. It is important to note that complete conversion of **1** to **2** occurs in a few seconds, however for the kinetics traces irradiation for the entire dead-time between spectrum collection was needed for a valid baseline.

1.4 Transient-Absorption Spectroscopy

Samples of **1, 3, 4** were prepared as above, and bromocresol green was prepared by dissolution of BCG into ethanol, followed by 1:1000 dilution into ND96 salts at pH 6.0. The concentrations of each compound used were 10 μM for the dye and 20 μM for BCG. These were then placed in a quartz cuvette open to air, and the laser experiments conducted. Excitation using the third harmonic from a Spectra-Physics Q-switched Nd:YAG laser (Spectra-Physics, Quanta-Ray PRO Series) provided 355 nm pulses, 8 ns, at 10 Hz. This pulse was then used to pump an optical parametric oscillator (Spectra-Physics Quanta-Ray MOPO-700) to provide 455 nm laser pulses. Single-wavelength transient absorption experiments were conducted with a 10 mW He-Ne Laser passed through the sample colinnearly with the excitation pulse. The probe wavelength of 632 nm was selected using a double monochromator (Instruments SA DH-10), with appropriate short-pass and long-pass filters to remove stray light, as well as a neutral density filter to regulate intensity. Light was detected by a photomultiplier tube (Hamamatsu R928), and amplified using a custom

built voltage amplifier. Around 15 shots were collected for each wavelength, and the data were log-compressed, then fitted, in MATLAB using custom scripts.

1.5 Electrophysiology

In general, electrophysiology experiments were conducted as previously published[1,5] . Briefly, Xenopus laevis oocytes were injected with 5 ng of wt-rASIC2a or 50 ng of wt-GLIC in vitro transcribed mRNA. Following a 24 hour incubation electrophysiology was performed using the OpusXpress. Cells were whole-cell voltage-clamped at -60 mV, and experiments were performed. Expression was tested using low-pH buffers (ND96 salts + MES buffer). After expression was verified, tests with 1 began. To test the ability of **1** to open ASIC2a or GLIC, 3 rounds of pH 5.5 or 4.5 buffer (ASIC2a or GLIC, respectively) was applied to oocytes. Following the third round, **1** (ca. 500 ?M), in ND96 salts, was applied. Solution transfer paused for 180 seconds, during which after a short incubation period (ca. 25 seconds) each oocyte was individually irradiated by a 455 nm LED for ten seconds. Following the irradiation, the solution was exchanged and two more pH 5.5 or 4.5 buffer (ASIC2a or GLIC, respectively) doses were applied to monitor ASIC2a or GLIC expression. Experiments with 1 dissolved in buffer were performed in a similar manner, with 10 mM or 5 mM MES buffer (ASIC2a or GLIC, respectively) present in the salts solution. All data was then processed using MATLAB with custom scripts.

1.6 UV-Visible pH Titration

Assignment of the visible optical bands was determined by pH titration of **1**, and monitoring of the change in the optical spectrum using UV-visible spectroscopy. A stock solution of **1** was prepared as mentioned above. A mixed buffer solution was then prepared with ND96 salts supplemented with a buffer mixture (2.5 mM MES, 2.5 mM NaOAc, and 5 mM Tris) known to support a pH range of 4.0 - 9.0.[6] Solutions were prepared from pH 4.0 to 8.9 by 0.1 steps in pH, using HCl or NaOH to adjust the pH. Then, a 1:1000 dilution of **1** stock solution in each of the above pH buffer solutions occurred and the optical spectrum recorded. The data was then processed using MATLAB with custom scripts.

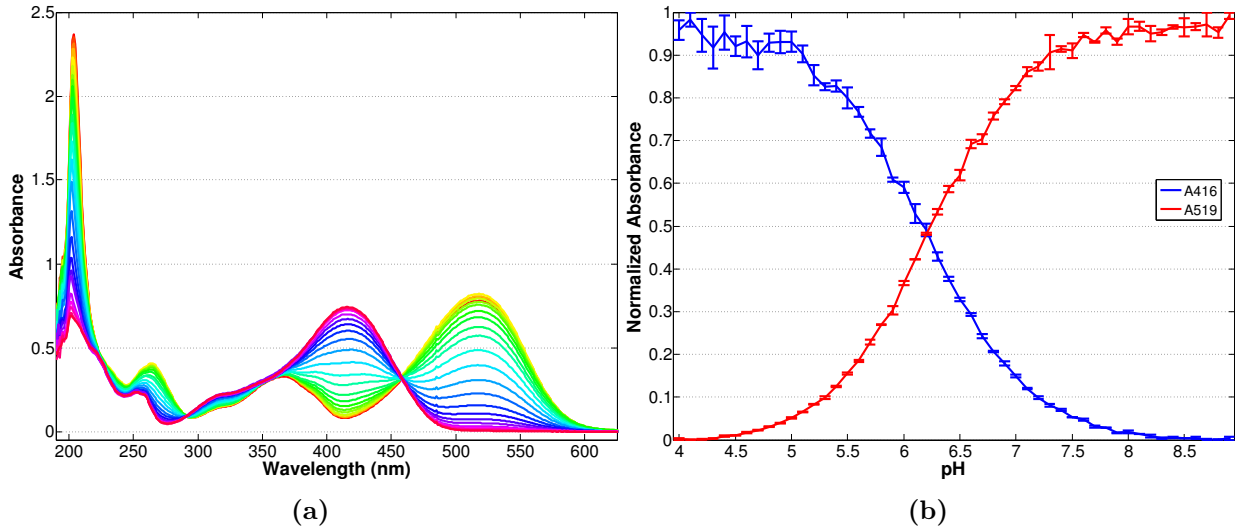

Figure 1: (a) Raw UV-visible optical spectrum of pH titration of **1** from pH 4.0 (red) to pH 8.8 (yellow) by 0.2 units. (b) Plotted normalized absorption at 416 nm (blue) and 519 nm (red) of **1** at indicated pH. (a) displays a clean isosbestic point at 459 nm. When the protonated (416 nm) peak and deprotonated (519 nm) peak are normalized and plotted, the intersection pH indicated the ground-state pKa of the compound, *ca.* 6.2

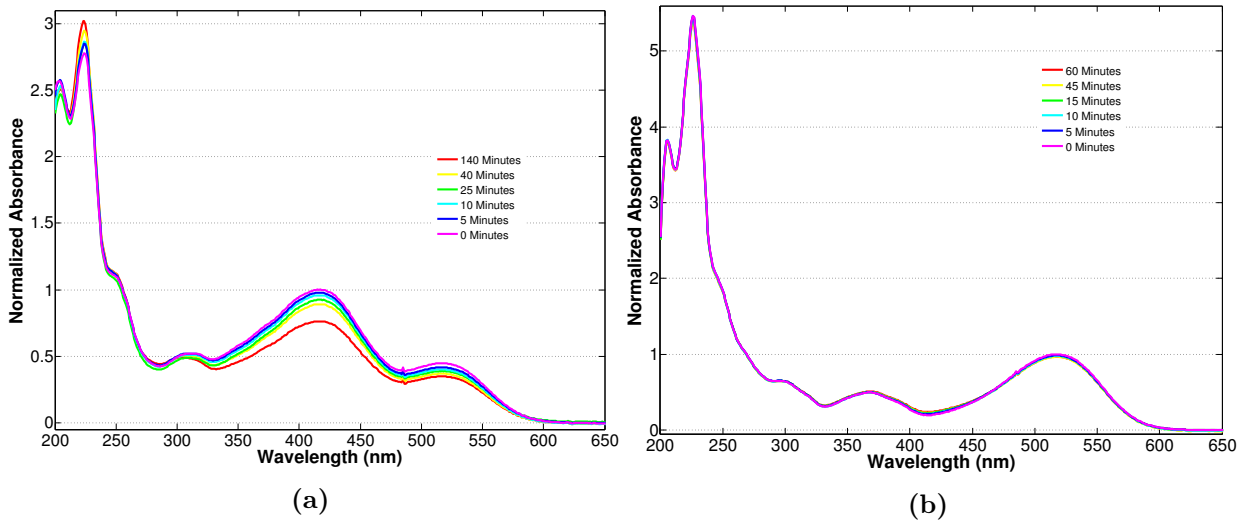

Figure 2: UV-Visible spectrum of **1** upon irradiation using a HeNe laser at pH 6.0 (a) and pH 8.0 (b). The spectra are normalized to the initial starting absorbance at 416 nm (a) or 519 nm (b). Minimal conversion upon irradiation is observed in (a); complete conversion of the spectrum occurs within a few seconds upon 455 nm irradiation. No conversion is observed for (b), again noting complete conversion is observed under 455 nm irradiation conditions.

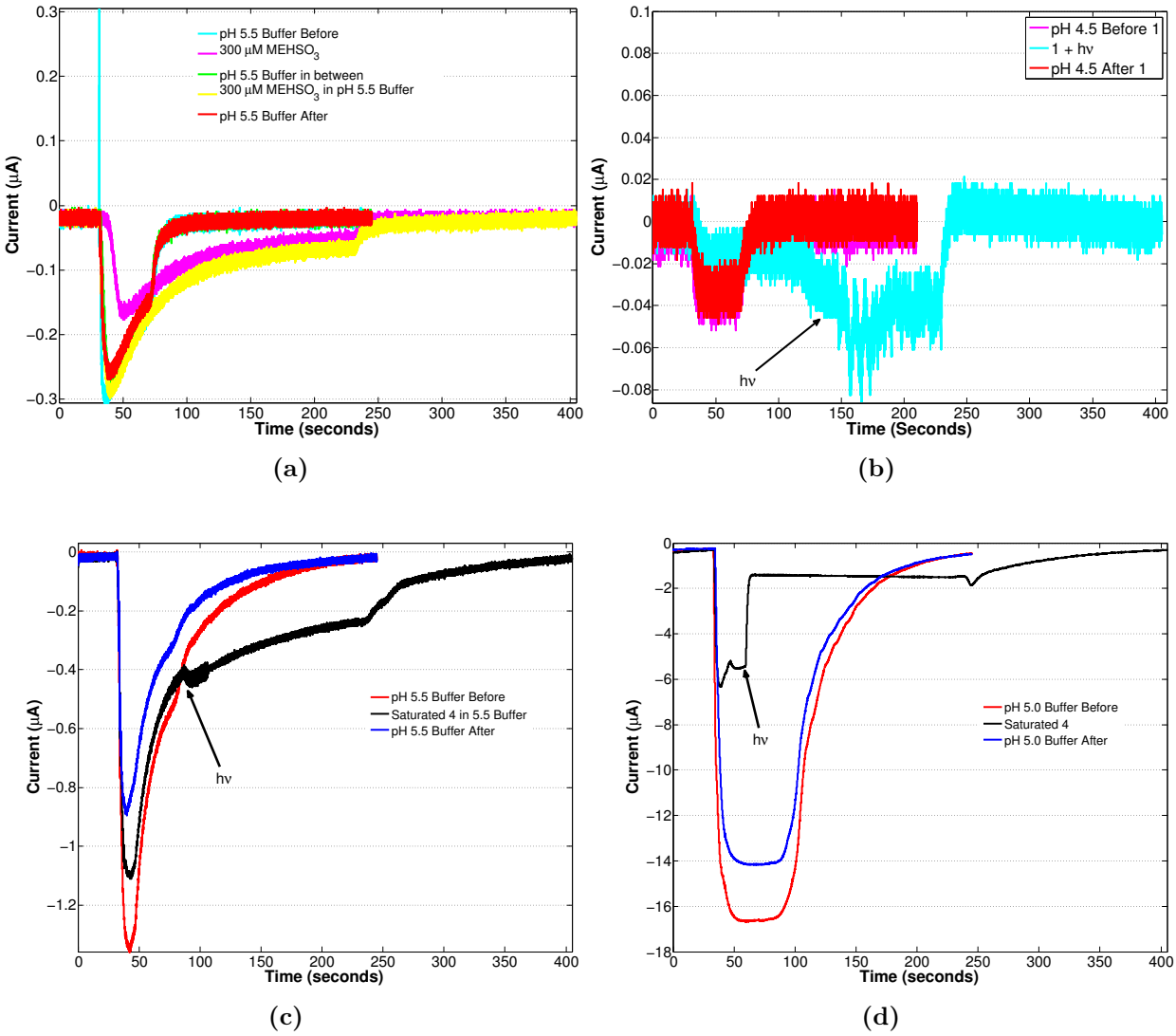

Figure 3: (a) Exposure to **1** (500 uM) without irradiation (cyan trace) does not show activation of GLIC-expressing oocytes. A minimal current change is observed upon application (*ca.* 50 seconds), seen across all samples (Figure 2, pre-irradiation, for example) and is due to the minimal, but still present, bulk activation of GLIC at pH 6.0. (b) Uninjected oocytes are exposed to **1**, and irradiated, as in Figure 1. However, upon irradiation no current change is observed, indicative that native oocyte channels are not activated by 500 uM **1**. (c) Irradiation of saturated **4** in pH 5.5 buffer applied to ASIC2a expressing oocytes (black trace). The pH 5.5 ASIC2a response is measured before and after exposure to **4** (red and blue lines respectively). An attenuation of the pH 5.5 response is observed after exposure to **4**. (d) GLIC-expressing oocytes exposed to saturated **4** in pH 5.0 buffer, and under irradiation (black line). The pH 5.0 GLIC responses were measured before and after exposure to **4** (red and blue lines respectively). Severe attenuation of the pH 5.0 response is observed with exposure to **4**, and under irradiation of **4**.

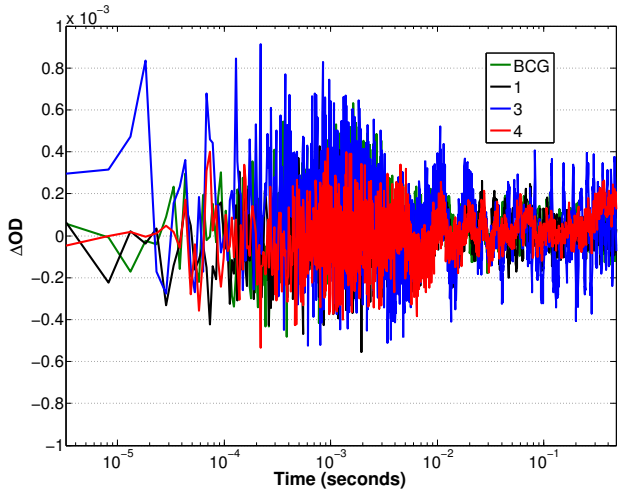

Figure 4: Transient-absorption profiles at 632 nm of **1**, **2**, **2**, and BCG (black, red, blue, and green traces respectively). No change in absorbance is observed for any of the species, individually, at 632 nm upon excitation with a 455 nm laser pulse.

2 References

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