Supporting Information

An Ionophore-Based Persistent Luminescent 'Glow' Sensor for Sodium Detection

Mark S. Ferris,[a] Madeline R. Behr,[a] Kevin J. Cash*[a]

[a] *Department of Chemical and Biological Engineering, Colorado School of Mines, Golden, CO 80401, USA*
* *Corresponding author*
E-mail: kcash@mines.edu

Materials

Poly(vinyl chloride), high molecular weight (PVC), bis(2-ethyl- hexyl) sebacate (BEHS), tetrahydrofuran (THF), dichloro- methane (DCM), sodium tetrakis[3,5-bis(trifluoromethyl) phenyl]borate (NaBARF; Selectophore™), 4-tert-Butylcalix[4] arene-tetraacetic acid tetraethyl ester (sodium ionophore X (NaI X); Selectophore™), sodium chloride (NaCl), and Trimethoxy(octyl)silane were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-[4-(2-Hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES; Molecular Biology grade), 2-amino-2-hydroxymethylpropane-1,3-diol (TRIS; 2 M), hydrochloric acid concentrate (HCl; 10 N, ACS certified), and sodium hydroxide concentrate (NaOH; 10 N, ACS certified) were purchased from Fisher Scientific (Waltham, MA, USA). Blueberry-C6-ester-652 (Blueberry dye) was purchased from Berry & Associates, Inc (Dexter, MI,USA). The persistent luminescent microparticles used in this work are the Coated Ultra Green V10 Glow in the Dark Powder (15-35μm) from Glow Inc. (Severn, MD, USA).

Glow Sensor Synthesis

To create glow sensor spots, an optode cocktail containing all sensing components except the phosphorescent microparticles was dissolved in organic solvent. To do this, 15 mg PVC was mixed with 30 mg BEHS and separately 3 mg NaI X, 0.5mg NaBARF, and 4 mg blueberry dye are combined in 500 μL THF. The latter solution is added to the PVC/BEHS suspension and immediately vortexed until all PVC particles are dissolved. This solution is referred to as an optode cocktail. Then, 50 μL of optode cocktail solution is added to 12.5 mg of phosphorescent microparticles. Sensor spots are created by vigorously mixing the optode cocktail/phosphorescent microparticle suspension and then quickly pipetting a 2 μL spot on a silane-modified glass-bottomed petri dish. The petri-dish was silane-modified by spreading a small amount of trimethoxy(octyl)silane on the glass surface and allowing it to dry over several hours. The spotting process is usually repeated to create at least four spots for analysis.

Glow Sensor Data Collection with Modified Fluorescence Microscope

Glow sensors were analyzed with an Olympus IX81 fluorescence microscope, using the following settings for all experiments: integration time 50 ms, gain 50, binning 4, resolution 16-

bit, excitation filter 475 nm, and emission filter 525 nm. Before analysis, the sensor spots were conditioned by submerging in 2 mL of HEPES/TRIS buffer (pH=7.2), followed by 0.1 N NaOH, and then 2 mL of 0.1 N HCl for 30 mins. each before changing back to 2 mL HEPES/TRIS and allowing the samples to sit overnight. The sensor spots were washed 3 x with millipore H_2O in between each solution change. The next day, 2 mL of the desired test solution was added to the petri dish and allowed to equilibrate for 30 mins. followed by data collection with the microscope before switching to the next test solution. During data collection, cellSens (Shinjuku, Tokyo, Japan) was used to control a shutter program to open and close a shutter blocking the excitation light source while HCImage (Sewickley, PA, USA) was used to operate the microscope. Data collection began before beginning the shutter program and ended immediately following the completion of the shutter program. For all experiments except for response time and phosphorescence lifetime, the shutter program consisting of the following: Open for 60 s (collecting fluorescence and phosphorescence), closed for 10 s (collecting only phosphorescence), open for 30 s (F&P), closed for 10 s (only P), open for 30 s (F&P), closed for 10 s (only P). This data collection process was followed for all test conditions, as well as the acid and base conditions during optode conditioning for each experiment. To test the background signal of the system, this same program was used to analyze a glass-bottomed petri dish containing 2 mL HEPES/TRIS and no Glow Sensor spots. For the initial dose/response curve regeneration, four spots were cycled through test solutions of 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 M NaCl, before washing 3 x with millipore H_2O and storing in 2 mL HEPES/TRIS. Several days later, the solution was changed to 100 mM Na and a modified shutter program was used to analyze response time (open for 60 s, closed for 10 s, and then 15 cycles of open for 30 s and closed for 10 s). For reversibility, the test solution was alternated between 0 mM and 100 mM Na for 5 total cycles, washing the petri dish 3x with millipore H_2O in between 100 mM and 0 mM readings. For selectivity, the sensor spots were tested by cycling through solutions of 10^{-7}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 M NaCl on day 1, before washing 3x with millipore H_2O and storing in 2 mL of HEPES/TRIS overnight. On day 2, the spots were tested in solutions of 10^{-7}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 M KCl before washing 3 x with millipore H_2O and storing in 2 mL of HEPES/TRIS overnight. On day 3, the spots were tested in solutions of 10^{-7}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 M LiCl. For stability, the sensors were tested in 10^{-7}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1M NaCl on days 1, 2, 3, 7, and 14. After data collection, the images were analyzed for mean intensity using ImageJ. The sensor signal for each test condition was determined by integrating the average of the three phosphorescence decay curves from 1 – 2s for each test condition. All luminescence values in the first and last 0.2s of each decay curve were excluded to account for the time it takes for the shutter to completely shut and open.

Spectrometer Phosphorescence Spectra Collection

The glow sensor phosphorescence spectra, as well as the phosphorescence spectra of optode spots made without Blueberry dye, were characterized with an AvaSpec-ULS2048 Starline Versatile fiber-optic spectrometer (Avantes, Apeldoorn, Netherlands). First, 5 mm circular glass slides were adhered to the wells of a 96-well plate using a small dab of vacuum grease. Optode

spots were then placed on the glass slides using the same method described above. 200 µL of test solution were added to each optode and allowed 30 mins. to equilibrate before testing. A 200 µM, 0.22 NA bifurcated fiber-optic cable (ThorLabs, Inc., Newton, New Jersey, United States) was coupled to an RPH-SMA Holder Block for Fiber Optic Probes with SMA Connectors (ThorLabs, Inc., Newton, New Jersey, United States) and taped to the top of a Nunc™ MicroWell™ 96-Well Optical-Bottom Plate with Polymer Base (Nalge Nunc International, Roskilde, Denmark) so that the spots would be excited by an LED through one cable while the luminescence output would be recorded by the spectrometer through the other cable. Optode spots were analyzed by illuminating for two mins. with a 405 nm LED (ThorLabs) at 85 mA, removing the excitation for one second, and finally collecting an emission spectra from the optode with an integration time of one second.

Well Plate Absorbance Collection

The absorbance spectra of the Blueberry dye was analyzed with a Synergy H1 microplate reader using Nunc™ MicroWell™ 96-Well Optical-Bottom Plate with Polymer Base (Nalge Nunc International, Roskilde, Denmark). 5 mm circular glass slides were adhered to the bottom of a well plate using a small dab of vacuum grease. Optode spots were then placed on the glass slides using the same method described above. 200 µL of test solution were added to each optode and allowed 30 mins. to equilibrate before testing.

Glow Sensor Analysis

Upon initial excitation, each spot shows a sharp increase in luminescence intensity for the first 15-20s before leveling off to a consistent signal. For the second and third excitations (at time = 70s and time = 110s), the spots showed a sharp increase in luminescence for only the first 7-8s before reaching a consistent signal. Glow Sensor luminescence takes the form of fluorescence when the shutter is open and phosphorescence when the shutter is closed. For phosphorescence decay plots, time zero is defined as the time when the shutter is closed to stop excitation.

The glow sensor dynamic range was determined by first normalizing the response to the range of sodium concentrations between fully protonated (0.1 N HCl) and fully deprotonated (0.1 N NaOH) test conditions followed by fitting the sodium response to a four-parameter logistic response curve,

$$Y = Bottom + \frac{(Top - Bottom)}{1 + 10^{\left((\alpha_{0.5} - X) * HillSlope\right)}}$$

with GraphPad Prism Software version 7.03, where Top and Bottom represent the maximum and minimum sensor signals, $\alpha_{0.5}$ is the sodium concentration corresponding to half-maximal response, and HillSlope is the slope of the tangent line drawn at the $\alpha_{0.5}$. The linear range was then defined by the x-axis range when a tangent line at $\alpha_{0.5}$ deviates less than 5% from the non-

linear fit to sodium response. The glow sensor selectivity was determined by the Nicolskii-Eisenman model for a fixed interfering ion,[1, 2]

$$logK_{ij}^{opt} = \alpha_{0.5}^{i} - \alpha_{0.5}^{j}$$

where $\alpha_{0.5}^{i}$ and $\alpha_{0.5}^{j}$ are the $\alpha_{0.5}$ constants for the interfering ion (potassium or lithium) and the target ion (sodium), respectively. The glow sensor response time was determined by first fitting the response to 100mM Na over time to a one-phase decay equation,

$$Y = (Y0 - Plateau) * e^{(-k*X)} + Plateau$$

where Y0 is the initial sensor signal, plateau is the final sensor signal, and k is a rate constant in units of the reciprocal of the X axis units. The response time was then determined to be the time for the curve to decay 95% of the way from the Y0 value to the Plateau value.

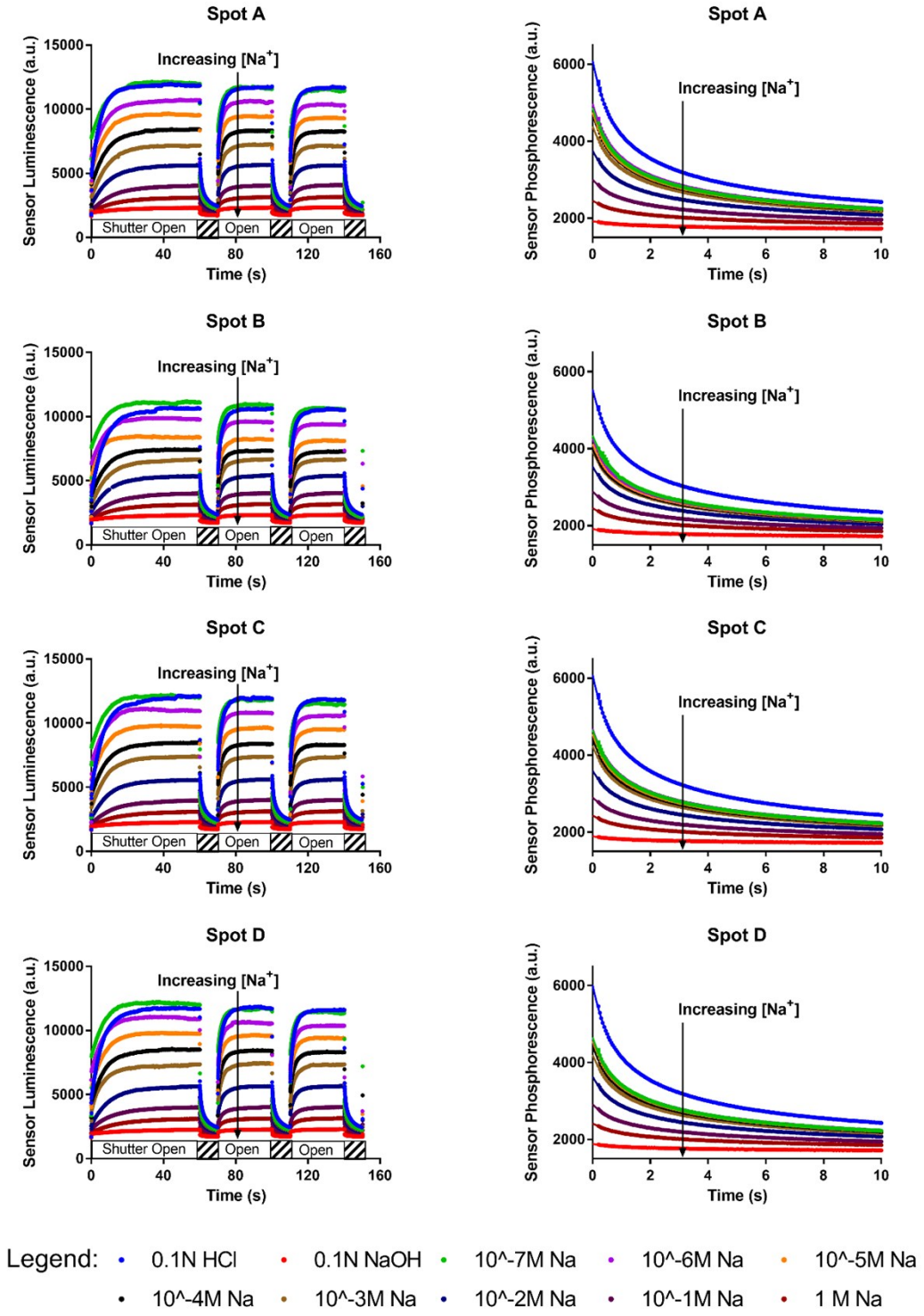

Figure S1: (Left Column) Glow Sensor luminescence (fluorescence and phosphorescence when the shutter is open, phosphorescence only when the shutter is closed) during shutter program for four spots: A, B, C, and D. (Right Column) The average of the three phosphorescent decay curves collected while the shutter is closed for spots A, B, C, and D.

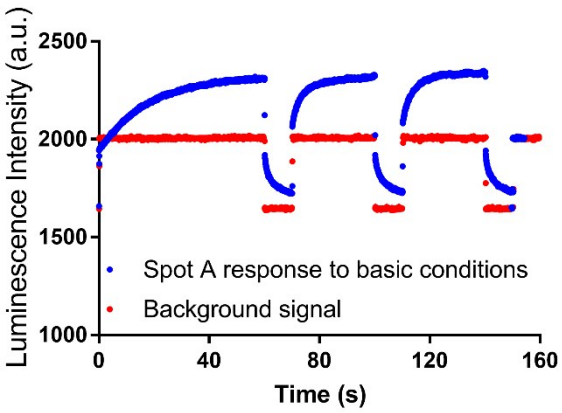

Figure S2: Spot A under basic conditions compared to background (no sensor) signal. This shows that the phosphorescence is predominantly quenched by full deprotonation of the blueberry dye, although some residual signal remains.

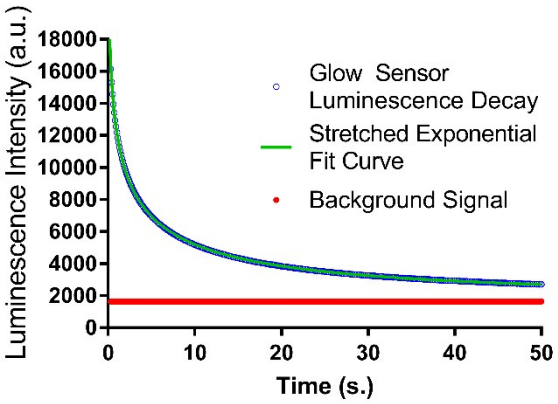

Figure S3: Luminescent signal from the Glow Sensor compared to background noise over time after ending excitation. The trend is described by a stretched exponential fit (supported by related work[3,4]) with a luminescent lifetime of 0.635 +/- 0.005 s and stretching exponent of 0.2979 +/- 0.0005.

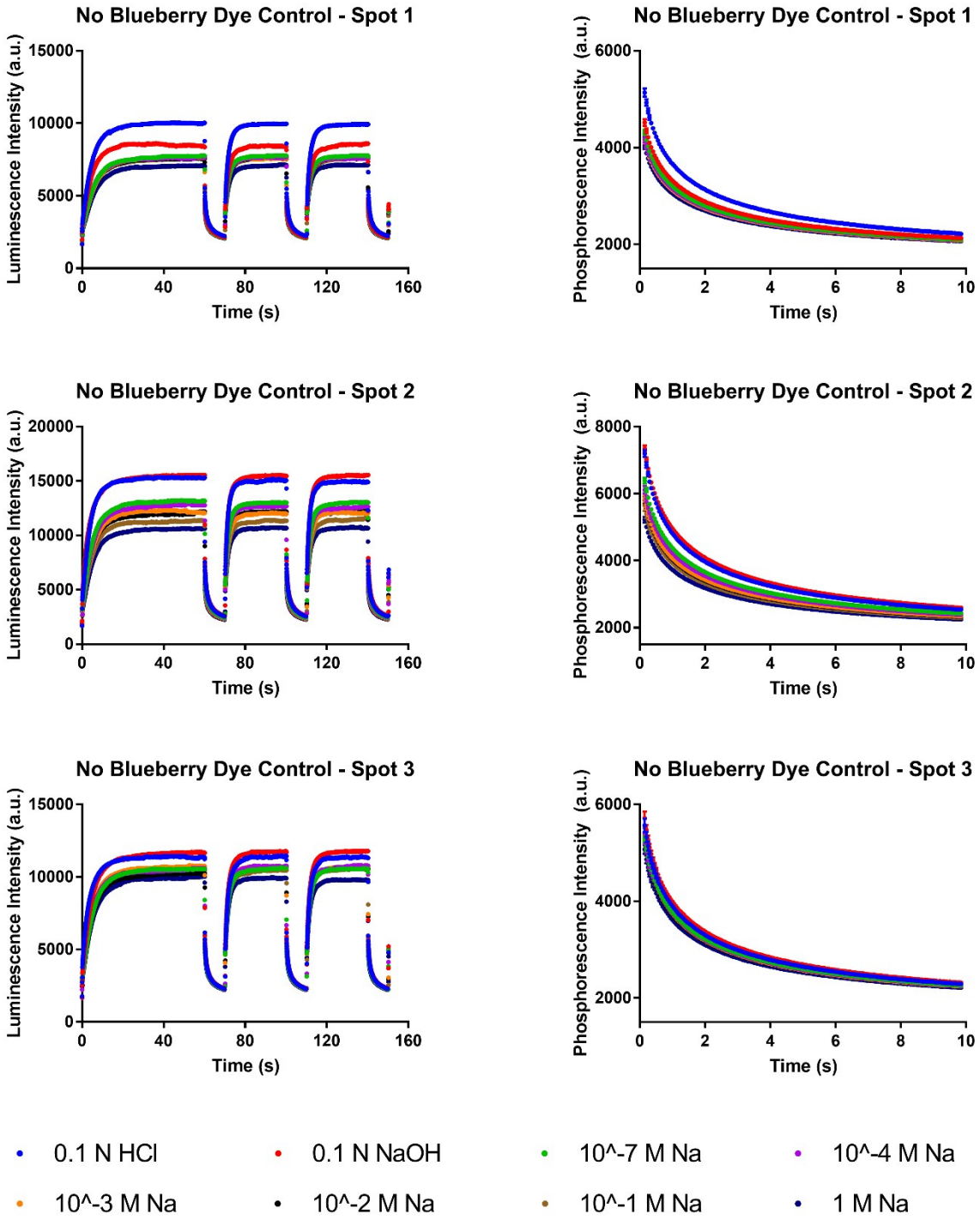

Figure S4: (Left column) Luminescence during shutter program for Glow Sensor spots without Blueberry dye. (Right Column) Averaged phosphorescent decay curves for Glow Sensor spots without Blueberry dye.

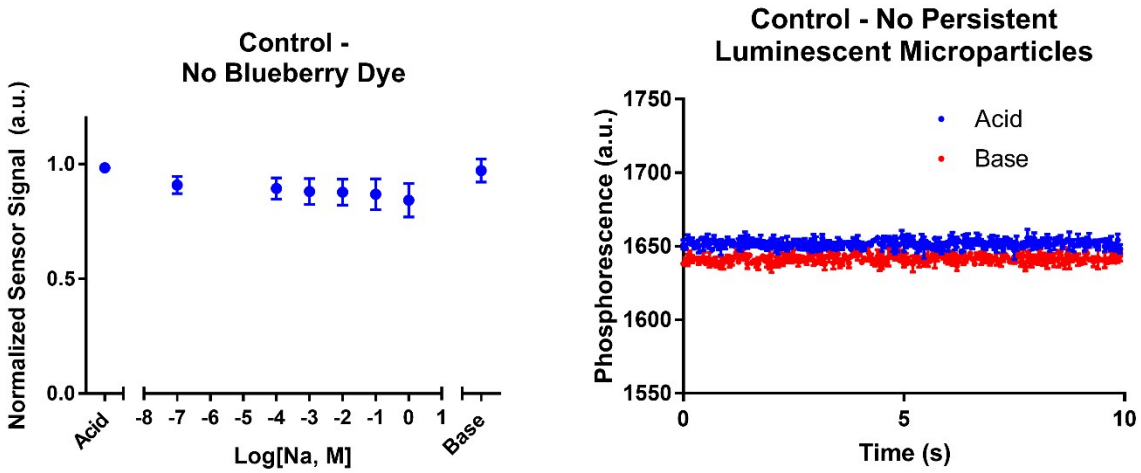

Figure S5: (Left) Dose/Response curve for Glow Sensor spots made without Blueberry Dye. Minimal response to Na^+ is observed. (Right) Glow Sensor spots made without persistent luminescence microparticles show no phosphorescence under acid and base conditions (see Fig S2 red curve for comparison of background signal). Time zero in this panel is the time when the shutter is closed and the excitation source blocked from the sample.

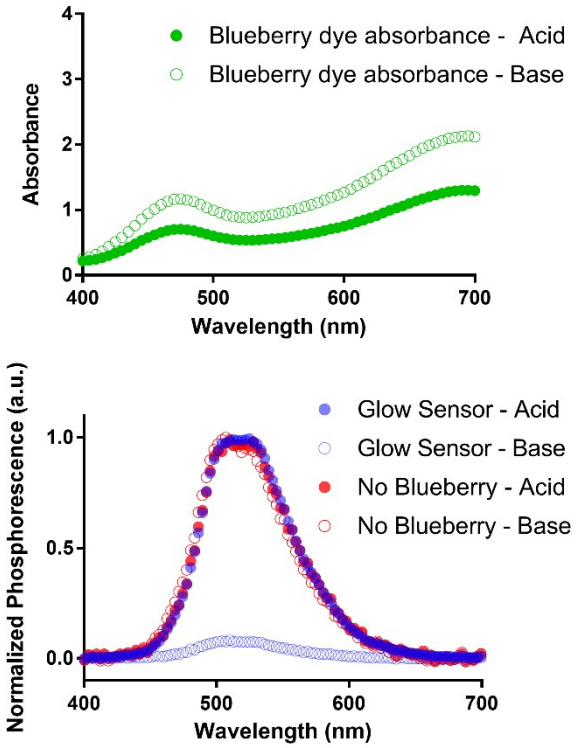

Figure S6: (Top) Absorbance of an optode spot made without phosphorescence microparticles under acidic and basic conditions, showing a change in absorption over a wide range. (Bottom) Phosphorescent spectra of Glow Sensor (Blue) and no-blueberry dye control spot made without blueberry dye (Red) under acidic (solid circles) and basic (hollow circles) conditions. This demonstrates that the glow sensor phosphorescence is greatly reduced in basic conditions, which corresponds with a rise in absorbance from the blueberry dye at the same range of wavelengths. Without blueberry dye, however, the Glow Sensor does not change its phosphorescence between acidic and basic conditions. The energy coupling between the blueberry dye and phosphorescent microparticles is likely either due to the inner filter effect or from resonance energy transfer, both of which would require a rise in absorbance in the blueberry dye to correspond to a decrease in phosphorescence from the phosphorescent microparticles.

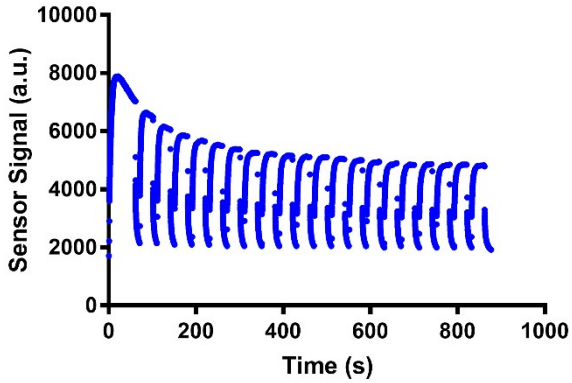

Figure S7: Luminescence during extended shutter program for Glow Sensor spots during response time experiment.

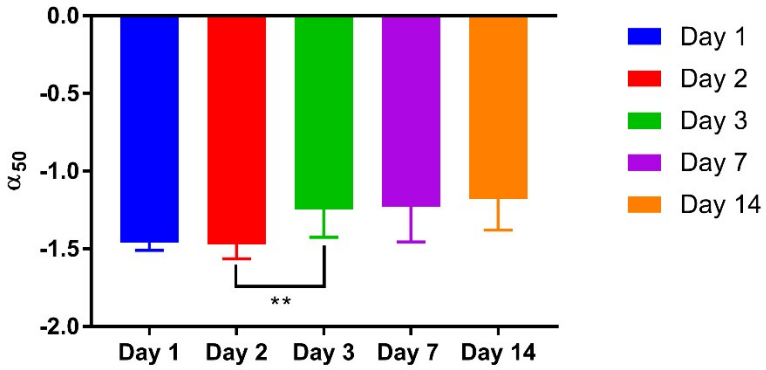

Figure S8: Drift of Glow Sensor α_{50} over the course of the experiment. ** represents a significant difference in α_{50} with $p < 0.01$.

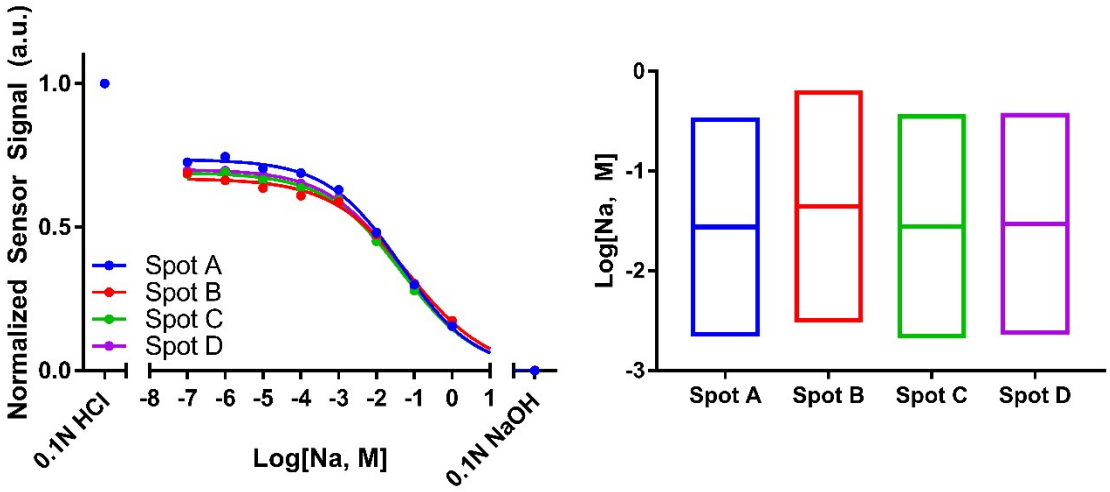

Figure S9: (Left) Dose/Response curve and (Right) dynamic range for spots A, B, C, and D.

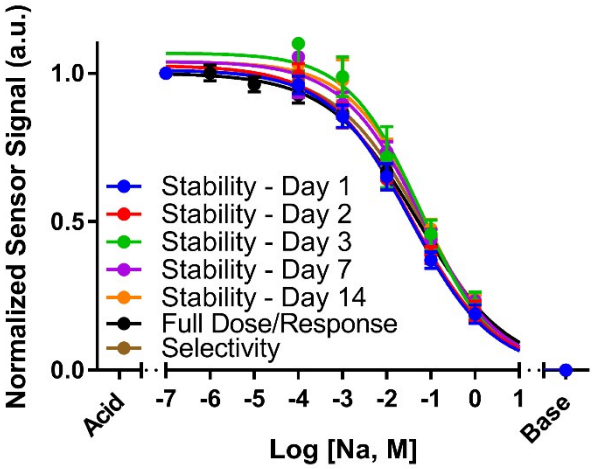

Figure S10: Compilation of all Glow Sensor dose/response curves demonstrating excellent reproducibility between sensor batches.

References

1. Bakker, E.; Simon, W., Selectivity of Ion-Sensitive Bulk Optodes. *Anal. Chem.* **1992,** *64*, 1805-1812.
2. Seiler, K.; Simon, W., Theoretical aspects of bulk optode membranes. *Anal. Chim. Acta* **1992,** *266*, 73-87.
3. Chen, R., Apparent stretched-exponential luminescence decay in crystalline solids. *Journal of Luminescence* **2003,** *102*, 510-518.
4. Lee, K. C. B.; Siegel, J.; Webb, S. E. D.; Leveque-Fort, S.; Cole, M. J.; Jones, R.; Dowling, K.; Lever, M. J.; French, P. M. W., Application of the stretched exponential function to fluorescence lifetime imaging. *Biophysical Journal* **2001,** *81* (3), 1265-1274.