Electronic Supplementary Material (ESI) for Lab on a Chip.
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Supporting information

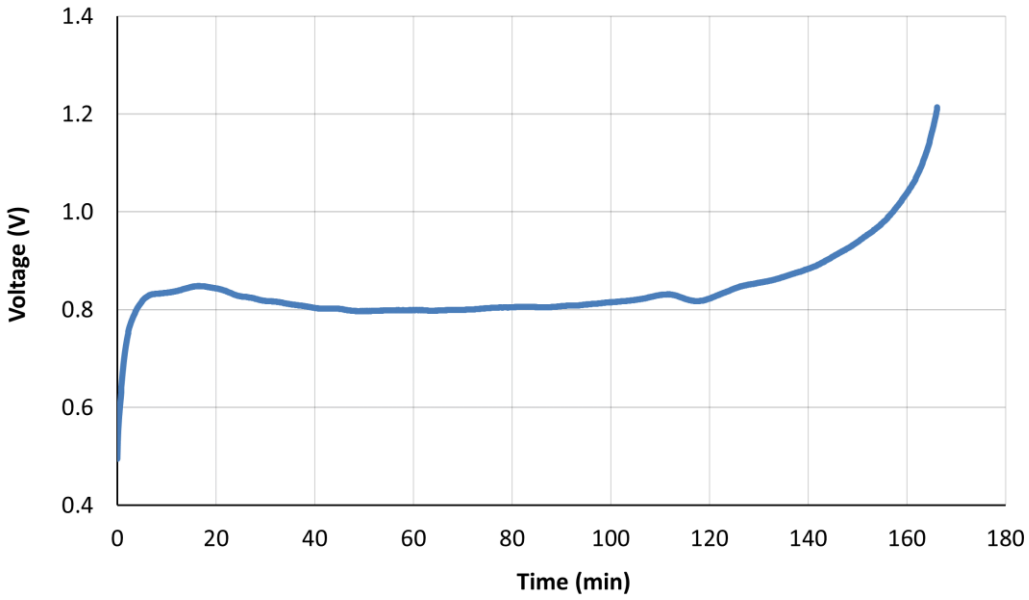

Figure S1. Chronopotentiometry under typical experimental conditions reveals a transition time (τ) in excess of 120 minutes; for experiments of shorter duration, quinone concentrations at the electrode surface will remain sufficient for pH modulation. An ITO electrode was bathed in modulation buffer (3.5 mM MHQ, 3.5 mM MBQ, 0.1x PBS, 0.1 M NaCl) and a fixed 5 µA current was applied until the voltage spiked, indicating water hydrolysis was required to sustain the current.

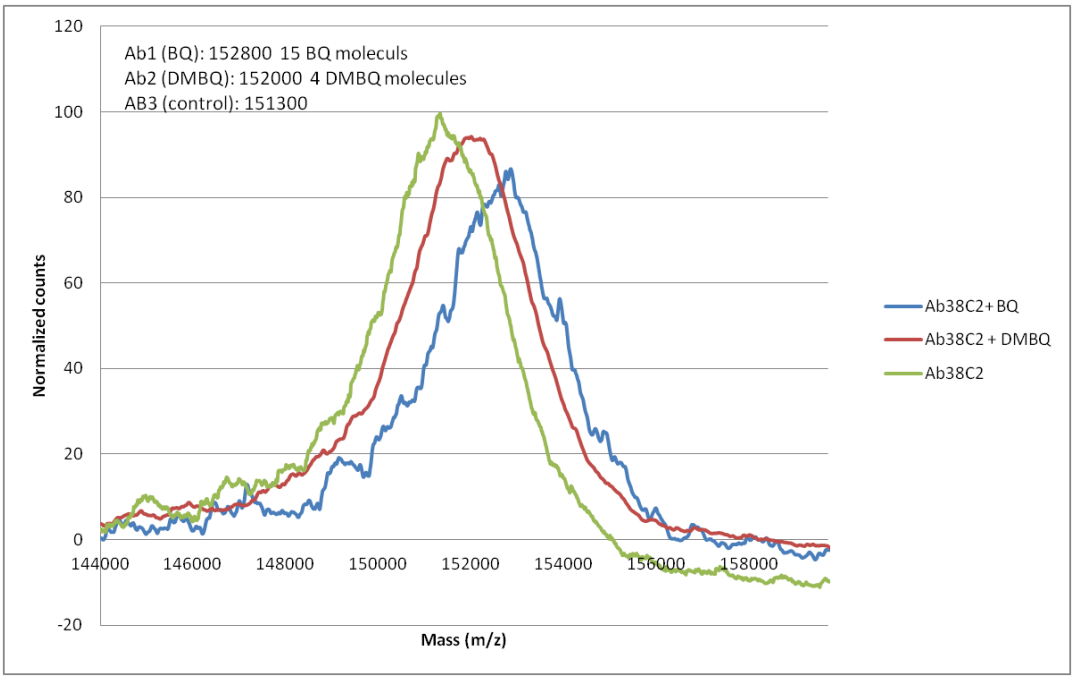

Figure S2. Mass spectra of Ab38C2 after incubation in 1x PBS pH 7.4 with 5 mM BQ (blue trace), 5 mM DMBQ (red trace), and a control sample containing no electroactive agent (green trace). The shift in the mass peak indicates covalent attachment of benzoquinone molecules to the antibody. The number of benzoquinone molecules covalently attached to the antibody (upper left corner) is calculated by subtracting the mass of the control sample (green trace) from the samples incubated with each quinone and dividing by the molecular weight of the corresponding quinone. Fewer DMBQ molecules indicates impeded ability of substituted quinone to undergo Michael addition to nucleophiles compared to unsubstituted benzoquinone.

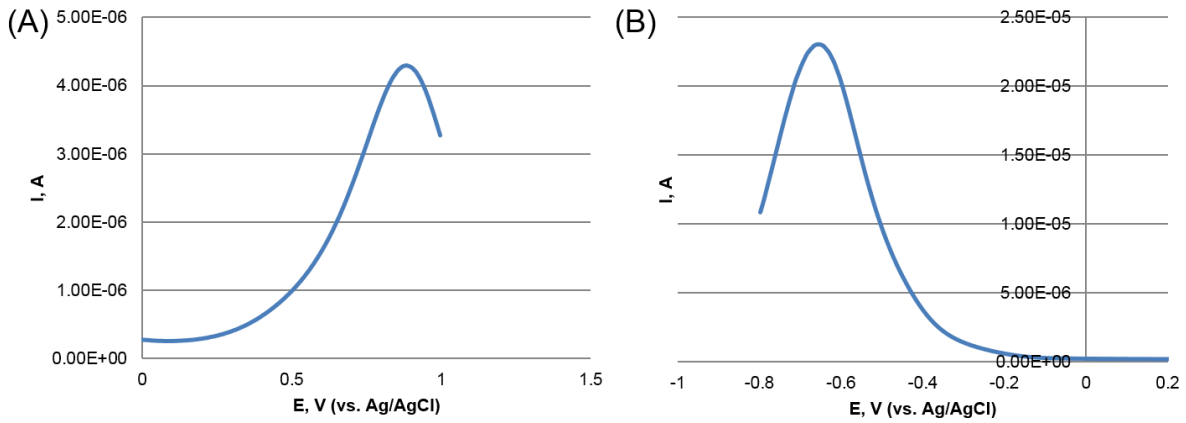

Figure S3. Square wave voltammograms of DMHQ (a) and DMBQ (b) in 0.1x PBS with 100 mM NaCl, pH 7.4. [DMHQ]=[DMBQ]=0.5 mM. Voltammogram parameters: amplitude 25 mV, frequency 15 Hz, scan rate 15 V/s.The oxidation and reduction potentials of the DMHQ and DMBQ lay within the acceptable potential window for ITO electrode (from -0.7 V to +1.0 V vs. Ag/AgCl)[36].

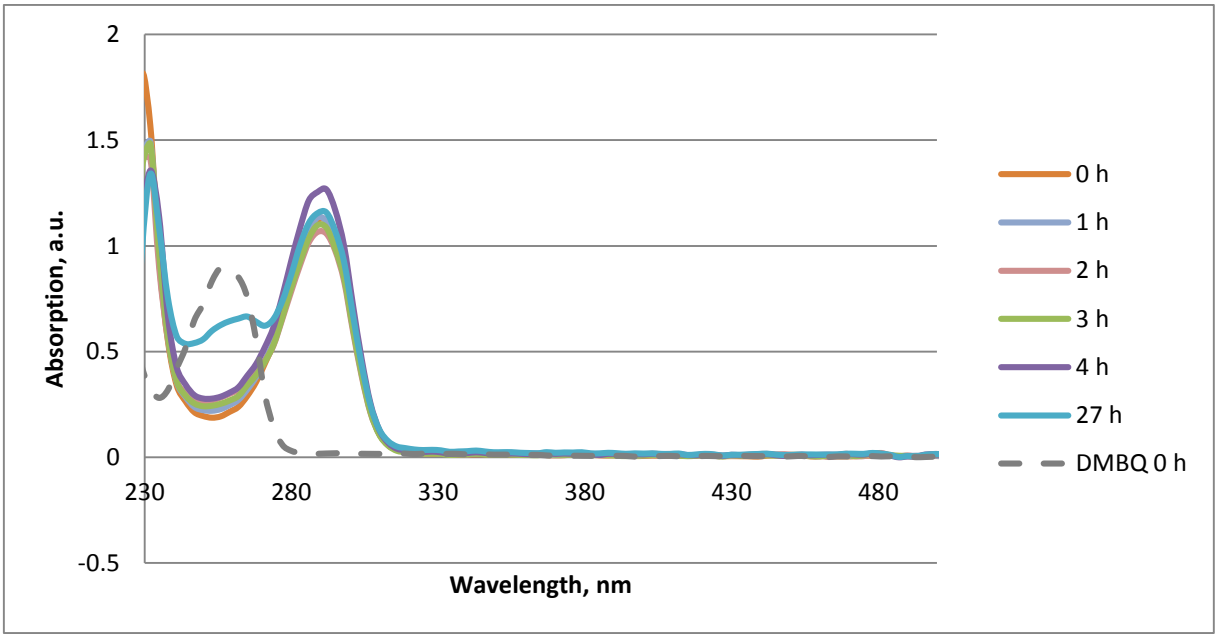

Figure S4. Absorption spectrum of DMHQ solution in 0.1x PBS pH 7.4 in air over time. A shoulder at 259 nm becomes apparent after 27 hours and corresponds to DMBQ generated via autooxidation. A spectrum of DMBQ is provided for reference.

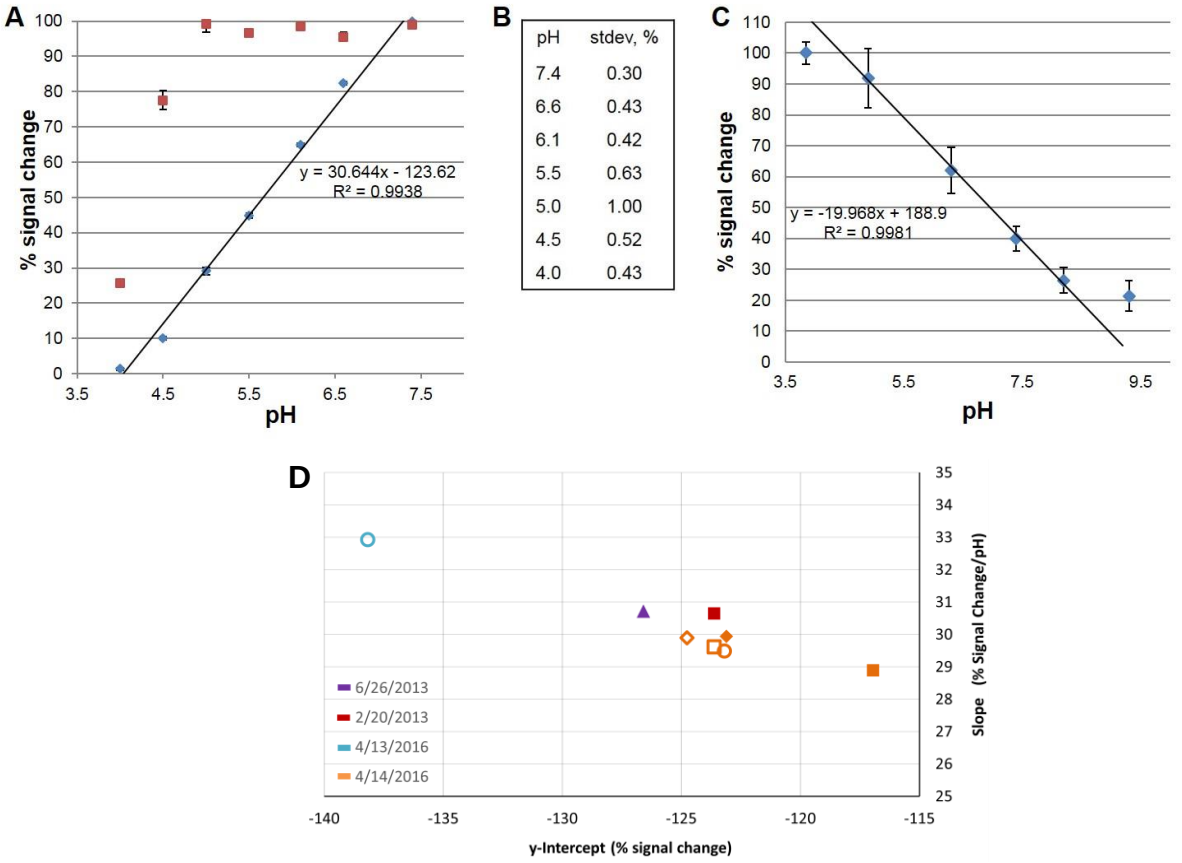

Figure S5. Calibration curves for pH indicators eGFP and pHrodo.

A) eGFP shows a positive correlation between pH and fluorescence (blue diamonds). For each pH, eGFP response was quantified in three sequential experiments: (1) an initial fluorescence measurement in 0.1x PBS, pH 7.4 (2) a pH response measurement in 0.1x PBS, adjusted to the target pH with 100mM HCl, (3) a fluorescence recovery measurement in 0.1x PBS, pH 7.4. The pH response (blue diamonds) and recovery (red squares) are expressed as a percentage of experiment (1). Irreversible signal loss is evident upon exposure to pH < 5, in accordance with literature reports[52].

B) Standard deviations for the eGFP calibration curve (error bars, blue trace) are quantified for clarity; each value was derived from 6 spots distributed across three electrodes.

C) pHrodo avidin shows a negative correlation between pH and fluorescence (M. Ogawa, N. Kosaka, C. A. Regino, M. Mitsunaga, P. L. Choyke, H. Kobayashi, *Mol. Biosyst.,* **2010**, *6*, 888-893). Higher variability in the pH response disqualified pHrodo avidin for quantitative use in subsequent experiments, but it is sufficient to provide qualitative evidence of pH modulation in Figure 3 (main text).

D) Consistency of the eGFP calibration curve is characterized under various conditions. Eight calibration curves were obtained from six different devices over a span of 3 years and fitted to a linear function. The results are represented in terms of slopes and y-intercepts. Each color represents a different acquisition date (enumerated at bottom left). For orange markers, each shape represents a unique device. Closed markers represent calibration curves obtained on freshly-spotted eGFP, while open markers were obtained on spots that had been exposed to electrochemical pH modulation for 1-2 hours. The aggregate data converges on an average slope of 30.2 ±1.2 (% signal change/pH). The error in slope yields a maximum of ~0.1 pH standard deviation across our dynamic range of 3 pH units (i.e. pH 4.5 may be reported from 4.4 -> 4.6). Variability in the y-intercept is broader (-125.0 ±6.0), but is corrected for each measurement by a 1-point calibration at pH 7.4 that is acquired prior to the modulation experiment (see pH quantification methods below). Given the relatively minor variation in slope, we chose to simply apply the same reference curve in quantifying all of our data.

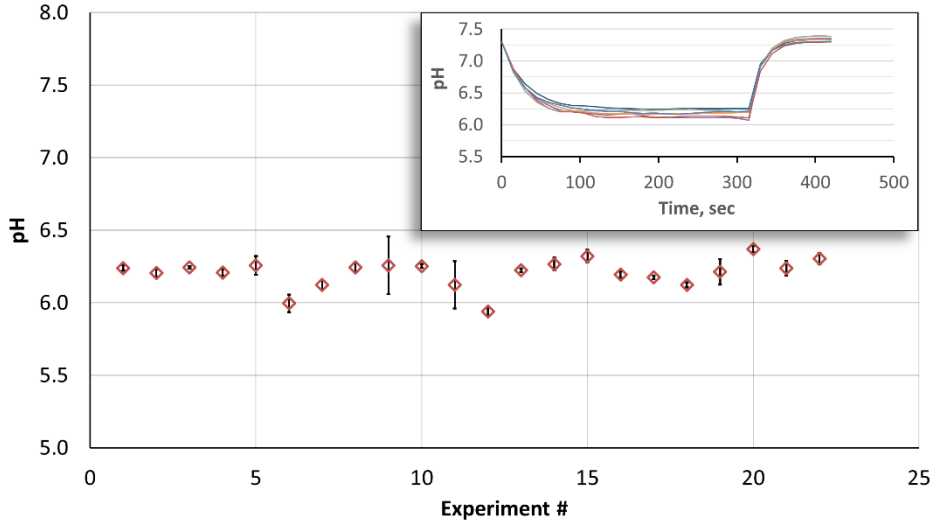

Figure S6. Inter-substrate reproducibility of pH 6.2 current waveform was demonstrated on 22 electrodes spread across 4 separate devices, and yielded an average pH value of 6.21 ±0.098 pH. Experimental conditions from Figure 6 (main text) were reproduced on additional substrates, with excitation time reduced to 5 minutes. Average pH was calculated using the last 3 minutes of excitation. Error bars indicate standard deviation of pH during the last 3 minutes of each experiment. Inset: traces from 10 electrodes overlaid.

pH quantification using eGFP:

Any experiment that reports a quantified pH was preceded by a 1-point calibration measurement performed in bulk buffer at the base pH (typically, pH 7.4). For electrochemical pH modulation experiments, this buffer also contains our electroactive agents. Individual spots were quantified using their entire area, adjusted on a per-spot basis to compensate for local background, and then averaged to yield a single value. This fluorescence value was considered to represent 100% signal, and was used to calibrate out variations in eGFP spotting density, eGFP degradation during storage, and other global perturbations. All subsequent measurements were referenced against this value. For these subsequent measurements, quantification is a three-step process.

First, an image was acquired such that several spots were visible on the working electrode (pH modulated) and an adjacent, non-stimulated electrode. Individual spots were quantified using their entire area, and then adjusted on a per-spot basis to compensate for their local background. Second, the values from each electrode were averaged separately to yield single values for the working electrode and non-stimulated electrode; the working electrode value was then adjusted for signal loss on the non-stimulated electrode to account for photobleaching, yielding the final fluorescence intensity value. Third, this final value was expressed as a percentage of the pre-modulation fluorescence. That percentage was indexed against a single reference curve generated with bulk pH buffers (Figure S5a) to derive the reported value.