Supporting Information for:

Fluorescence-Enabled Electrochemical Microscopy with Dihydroresorufin as a New Fluorogenic Indicator

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**Figure SI-1.** Schematic of a closed bipolar electrode. A conductor is embedded in an insulator substrate that completely separates two solution compartments. A small voltage bias can be applied with two driving electrodes to couple faradaic reactions at the poles of the bipolar electrode, enabling control of the system without direct electrical connection to the bipolar electrode. A bipolar electrode array can be formed with several parallel electrodes in the insulating membrane. Replacing a conventional redox reaction at one of the poles of the bipolar electrode with a fluorogenic redox reaction is the central idea of FEEM. Since the rate of reaction at the two poles will be equal, the progress of a conventional redox reaction at one pole of a bipolar electrode can be reported by optically monitoring a fluorogenic redox reaction at the other pole of the bipolar electrode.
Figure SI-2. Photograph of 100 µM resazurin/resorufin/dihydroresorufin solutions in 0.5 M NaOH with varying concentrations of glucose taken 10 min after adding the glucose. The leftmost solution remains as resazurin (blue) as no glucose is available to reduce it. As the glucose concentration increases, the resazurin is reduced first to resorufin (pink), and then dihydroresorufin (clear) to a greater extent. The portion of the 67 and 100 mM glucose solutions in contact with air remains pink because the oxygen present in air is enough to oxidize dihydroresorufin back to resorufin.
**Figure SI-3.** Forward scan of F-CVs obtained with a 25 µm diameter Au disk electrode in solutions with a total resazurin/resorufin/dihydrosorufin concentration of 100 µM and varying concentrations of ascorbic acid in 0.5 M KCl. The potential was swept from -0.4 to 0.6 V at 200 mV/s. Note that an electron multiplier gain of 300 was used (as compared to a gain of 0 for the varying glucose concentration data in **Figure 3**) to amplify the fluorescence signal.

**Figure SI-3 Discussion:**

At an ascorbic acid concentration of 0.5 mM, the fluorescence signal decreases as the potential is swept in the positive direction. This indicates that the solution is primarily resazurin, with the reduction of resazurin to resorufin at potentials negative of approximately -150 mV responsible for the strong fluorescence signal. At potentials positive of about -150 mV, this reaction no longer occurs to a significant extent and the fluorescence signal decays as resorufin gets reduced by ascorbic acid to the non-fluorescent dihydrosorufin. At an ascorbic acid concentration of 1 mM, the solution is primarily dihydrosorufin, and the onset of the fluorescence wave at ~50 mV indicates the onset of dihydrosorufin oxidation. As the ascorbic acid concentration is increased to 5 mM and above, the fluorescence signal decreases due to the quenching effect of ascorbic acid, which chemically reduces any resorufin produced at the electrode back to the non-fluorescent dihydrosorufin. The positive potential shift in the onset of dihydrosorufin oxidation as the ascorbic acid concentration is increased is due to the decrease in solution pH. As dihydrosorufin oxidation involves the loss of two protons, decreasing the pH makes the oxidation reaction thermodynamically less favorable, thus increasing the onset potential. This also explains the difference in the onset potential of dihydrosorufin oxidation in glucose (~550 mV for 10 mM glucose) and ascorbic acid (±10 mV for 10 mM ascorbic acid).
Figure SI-4. Diagram of a basic FEEM experimental setup. A bipolar electrode is formed by electrically connecting two microelectrodes in series. The cathodic pole of the bipolar electrode is placed in the analyte solution. The anodic pole of the bipolar electrode is placed in a solution of dihydroresorufin, which is positioned on top of an Olympus IX70 inverted microscope for observation. The dihydroresorufin solution is in a small plastic container epoxied (Loctite 0151 Hysol Epoxy) to a microscope slide (VWR). The reactions are driven by applying a potential across two Ag/AgCl reference electrodes (BASi RE-5B). For the experiments reported here, all fluorescence imaging was done through a 20X 0.40NA objective (Olympus LCPlanFl 20X/0.40 Ph1), with an additional 1.5X magnification on the microscope used. The excitation source was a Thorlabs M530L2 Collimated LED, which has a dominant wavelength of 530 nm. The LED was powered by a DC2100 LED Driver running at 1500 mA, which provided about 4.3 mW at the sample as measured by a power meter (Thorlabs PM100D).
Figure SI-5. CV of a 25 μm diameter Au disk electrode in 250 μM ferricyanide obtained at a scan rate of 10 mV/s using a two-electrode setup. The onset potential of ferricyanide reduction is +310 mV vs. Ag/AgCl.
Figure SI-6. (a) Series of fluorescence still-images taken from a video of the FEEM detection of 250 μM hexamine ruthenium(III) at a 25 μm diameter Au disk electrode using a solution of 100 μM dihydroresorufin, 67 mM glucose, and 0.5 M NaOH. Potential was cycled from +1 to 0 V at 200 mV/s as applied to two Ag/AgCl driving electrodes. The last panel shows fluorescence intensity at the electrode over the duration of the scan. (b) CV of a 25 μm diameter Au disk electrode in 2 mM hexamine ruthenium(III) obtained at a scan rate of 100 mV/s using a two-electrode setup.

Figure SI-6 Discussion:

As can be seen, the onset of the fluorescence burst occurs at roughly 390 mV. This corresponds to the predicted onset potential within 10 mV. The predicted onset potential for the coupled reaction can be determined from the difference in the onset potentials of the two individual reactions. The dihydroresorufin oxidation onset potential is -540 mV vs. Ag/AgCl (Figure 2a) and the hexamine ruthenium(III) reduction onset potential is -160 mV vs. Ag/AgCl (as determined from (b)).
Figure SI-7. FEEM-based detection of ferricyanide in ambient and deoxygenated solutions at a 25 µm diameter Au disk electrode using a solution of 100 µM dihydroresorufin, 67 mM glucose, and 0.5 M NaOH. The potential was cycled from +1.2 V to +0.2 V at 200 mV/s as applied to two Ag/AgCl driving electrodes.

Figure SI-7 Discussion:

The solid red trace shows the signal obtained with a 10 µM ferricyanide solution. A strong fluorescence signal is observed, however upon deoxygenating the solution (dashed red trace), no signal is observed. This shows that the coupling of oxygen reduction, rather than ferricyanide reduction, to dihydroresorufin/glucose oxidation is responsible for the fluorescent signal observed. It also shows that a ferricyanide concentration of 10 µM is too low to yield enough current for fluorescence generation, indicating that the ferricyanide reduction is coupling primarily to glucose oxidation. At a higher ferricyanide concentration, such as 200 µM, it is clear that oxygen reduction contributes significantly to the fluorescence signal by comparing the ambient trace (solid black) to the deoxygenated trace (dashed black). However, the deoxygenated solution still gives a strong fluorescence signal, indicating that at a concentration of 200 µM ferricyanide reduction occurs at a rate sufficient enough to couple to both glucose and dihydroresorufin oxidation.