Supplementary Information

A Generalized Platform for Antibody Detection using the Antibody Catalyzed Water Oxidation Pathway

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Equipment

Quartz crystal microbalance (QCM) measurements were performed using a Model PM-740 plating monitor (Maxtek, Inc., Torrance, CA), and data were recorded using a custom LabWindows program. Stanford Research Systems (Sunnyvale, CA) and Tangidyne Corp. (Greenville, SC) were the sources for 5 MHz AT-cut quartz crystals, 1 inch diameter with Ti/Au. Square wave voltammetry (SWV) and cyclic voltammetry (CV) were performed using a Model 900 potentiostat from CH Instruments (Austin, TX) or an epsilon potentiostat (Bioanalytical Systems Inc., West Lafayette, IN). In some cases, such as the electropolymerization and DNP analysis, CVs were obtained using a Princeton Applied Research Model 173 Potentiostat-Galvanostat, a Model 175 Universal Programmer, and Model 176 Current Follower, and data were recorded using a custom LabVIEW program. For irradiating the samples, two UV sources were used. One was a UVEXS (Sunnyvale, CA) Model SCU-110B spot-curing system lamp, broad wavelength emission with a peaks near 365 and 440 nm, with the optical fiber at 2 cm above the sample. Radiant power at the sample surface was 16 mW/cm^2, as measured with an EG&G Electrooptics Model 450-1 Radiometer Photometer. The other source was a Model M-20V transilluminator (UVP LLC, Upland, CA) with emission in the midrange, 302 nm. Radiant power at the sample was 0.7 mW/cm^2. Overall, we found that the transilluminator yielded the best results, and these results are reported in the main text. Confidence intervals are reported to within 90 % confidence.

Materials

Allyl-2-bromo-2-methylpropionate, chlorodimethyl- hydrosilane, Pt on activated carbon (10 wt %), triethylamine, anhydrous toluene, 2,2′-bipyridine, 11-mercapto-1-undecanol, 2-bromo-2-methylpropionyl bromide, anhydrous pyridine, copper (I) bromide (99.999%), copper (II) bromide(99.999%), copper (I) chloride (99.999%), poly(propylene glycol) methacrylate (POEGMA), N, N-diisopropyl-carbodiimide

(DIPC), dinitrophenol-ε-amino-*n*-caproic acid, dimethyl formamide (DMF), horseradish peroxidase (Type 1) (HRP), Amplex Red reagent, albumin from bovine serum (BSA) were purchased from Aldrich and used without further purification. Phosphate-buffered saline solution (PBS) was prepared from 0.1 M sodium phosphate buffer solution with 0.15 M sodium chloride and the pH adjusted to 6.0 or 7.2 using hydrochloric acid or sodium hydroxide. 4-(dimethylamino)pyridinium 4-toluene sulfonate (DPTS) was prepared from dimethyl amino pyridine and sodium p-toluene sulfonate following a literature procedure.[1] Deionized water (18.2 MΩ•cm at 25°C) from a Millipore Milli-Q Synthesis A10 system was used to clean glassware and prepare aqueous solutions. All other solvents for rinsing and cleaning were purchased from Fisher Scientific.

Synthesis and Immobilization of Surface Initiators

Gold and silicon surface initiators were synthesized according to published procedures.[2] Gold and silicon substrates were both cleaned using a Harrick Plasma Cleaner for 10 minutes, rinsed with ethanol, and blown dry with nitrogen gas. The gold wafers were immersed in 1 mM initiator solution in anhydrous hexanes overnight under nitrogen. The silicon substrates were placed in a vacuum oven to ensure removal of all water particles before being placed in a 1 % (v/v) solution of the silane initiator in anhydrous toluene containing catalytic amounts of triethylamine for about 12 h at room temperature. After immobilization, both substrates were rinsed with anhydrous ethanol and dried under nitrogen.

Polymerization and Functionalization of POEGMA Brushes

The silicon substrates (1 cm × 2 cm) were placed in a dry Schlenk flask. Poly(propylene glycol) methacrylate (3.0 g, 8 mmol), CuCl (19.5 mg, 0.2 mmol), $CuBr_2$ (4.3 mg, 0.02 mmol), and 2,2′-bipyridine (76.0 mg, 0.49 mmol) were added to another 25 mL Schlenk flask equipped with a magnetic stir bar. Both flasks were evacuated and replaced with nitrogen four times. DI water (5.25 mL) was purged with nitrogen for at least 30 min and then transferred to the Schlenk flask containing the monomer via cannulation. The brown colored solution was stirred under nitrogen for about 10 minutes before being transferred into the flask containing the substrates. Polymerization was carried out at room temperature for 8-12 minutes, after which the substrates were rinsed with water and ethanol, blown dry under nitrogen gas, and characterized by ellipsometry. POEGMA brushes were functionalized by a similar method. A solution of dinitrophenyl-ε-amino-n-caproic acid (DNP) (37.5 mg, 1.12 mmol), DIPC (125 μL, 0.8 mmol), and DPTS (187.5 mg, 0.63 mmol) in anhydrous DMF (10 mL) was cannulated into the flask containing the substrates. The reaction was allowed to continue for 24 hours at 32°C. When finished, the substrates were washed with water and ethanol and dried under nitrogen. Fluorescently labeled DNP-antibodies were used to confirm the functionalization of the DNP groups to the brushes (Figure S1).

Quantification of DNP Surface Coverage

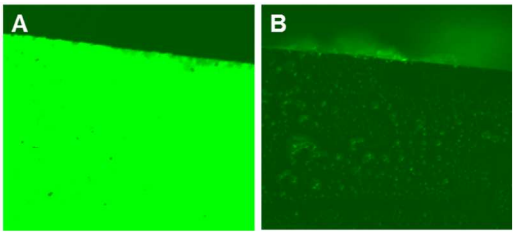

Figure S1. Binding of AlexaFluor 488 (green) labeled anti-DNP IgE to POEGMA brushes functionalized (A), or not (B), with DNP. Brushes with DNP on the surface show green fluorescence (A). No fluorescence above background is observed on surfaces with POEGMA brushes without DNP functionalization under the same conditions of incubation with anti-DNP (B).

The surface coverage, Γ, of DNP was determined using cyclic voltammetry (Figure 3B). Cyclic voltammetry was performed after the square wave voltammetry measurements because DNP groups are irreversibly reduced (near -0.4 V vs. Ag/AgCl) to hydroxylamine groups. The hydroxylamine group (+0.25 V vs. Ag/AgCl) is electrochemically reversible.[3] The surface coverage of DNP was calculated by integrating the area of the anodic peak at +0.28 V vs. Ag/AgCl, assuming that each of the DNP groups is converted to a hydroxylamine group.[4] The area is related to the charge, Q, of an electroactive species, in this case hydroxylamine, attached to an electrode. From Q, the surface coverage of the hydroxylamine is calculated from the following equation:

$$Q = nFA\Gamma \qquad (1)$$

where n is the number of electrons passed per hydroxylamine (n = 4 in this case), A is the area of the electrode (0.43 cm^2, which is one-fourth of the total electroactive area, 1.7 cm^2) and F is Faraday's Constant, 96485 C/mole e^-.

Quantification of Adsorbed Anti-DNP Antibody

After functionalizing a gold-plated QCM crystal with DNP-modified brushes and photosensitizer, it was immersed in pH 7.2 PBS containing 60 mL of 1 mg/mL BSA controlled at 25 ^oC in a water jacket while the solution was stirred with a magnetic stirring bar. Once the frequency stabilized to a constant value, 100 µL of 1 mg/mL rat anti-DNP IgG antibody solution (11 nM resultant concentration) was added to the PBS solution. By measuring the change in frequency, Δf, (Figure 3C), the surface coverage of antibodies adsorbed to the functionalized brushes was calculated using the Sauerbrey equation:

$$\Delta f = -C_f \bullet \Delta m \qquad (2)$$

where C_f is the integral sensitivity factor, 56.6 Hz μg^{-1} cm^2 for a 5 MHz AT-cut quartz crystal and Δm is the change in mass in units of g cm^{-2}.[5]

Hydrogen Peroxide Generation and Detection using SWV

We used square wave voltammetry (SWV) primarily for detecting hydrogen peroxide (H_2O_2). After immobilizing the antibody on the DNP-functionalized brushes, the substrate (i.e., silicon wafer chip or QCM crystal) was placed in 3 mL of PBS, pH 7.2 and then irradiated with UV light for 60 minutes. Then, an aliquot of the irradiated solution was removed and diluted with PBS, pH 6.0 to a final volume of 5 mL in a three-electrode cell, followed by deaeration with N_2. 8.8 µL of 10 µM HRP in PBS and 10.0 µL of 0.1 mM Amplex Red reagent in DMSO were added (resultant concentration of 10 µM and 0.2 units/L, respectively) to the deaerated solution, and SWV was performed at a glassy carbon electrode (3 mm diameter) with an amplitude of 25 mV, step height 5 mV, and frequency 25 Hz. A Ag/AgCl reference electrode in a salt bridge (3% w/v agar with 0.2 M potassium nitrate) and a Pt coiled wire auxiliary electrode were used.

Device Fabrication

A checkerboard patterned device, consisting of 35 rows of sixteen 300 µm x 300 µm silicon oxide squares bordered by a gold lattice, was fabricated for signal amplification purposes. The silicon islands are surrounded by a continuous gold grid with lines 150 µm wide and enclosed by a 500 µm wide border (Figure 3A). Conventional UV photolithographic methods were employed to pattern the electrodes used in this work. Silicon wafers were spun coated with lift-off resist (LOR 5A) and photoresist (SPR 220-3) and baked at 180°C for 3 min and 115°C for 90 sec, respectively. Soft contact exposure was performed on an EV620 contact aligner for 10 sec and a post-exposure bake was carried out at 115 °C for 90 sec. The wafers were developed by a Hamatech-Steag wafer processor using a double puddle process and then descumed for 4 minutes in a Branson P2000 barrel etcher. A CVC SC4500 e-beam evaporator was used to deposit 10 nm of Ti (adhesion layer) and 90 nm of Au. After metal deposition, the LOR/photoresist was removed by soaking the wafers in a solution of Remover 1165 for a few hours.

QCM devices were also fabricated by conventional methods (Figure 3B). Photoresist (SPR220-3) was spin coated on individual QCM devices and baked at 115°C for 90 sec. The samples were exposed for 10 seconds by an ABM Contact Aligner. After a post exposure bake at 115°C for 90 seconds, the devices were

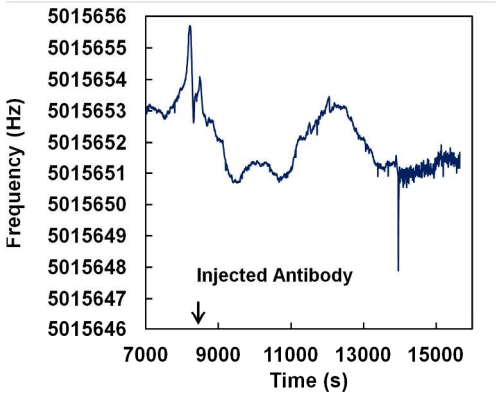

Figure S2. QCM frequency response to the addition of 100 µL of 1 mg/mL non-specific sheep IgG antibody (resultant concentration, 11 nM) to a 60 mL solution of PBS, pH 7.2 containing 1 mg/mL BSA solution at 25°C. The solution was stirred throughout the measurement.

developed for 90 seconds in MIF 726 and then rinsed with water and dried under nitrogen. Using a Branson P2000 barrel etcher, the samples were descummed for 4 minutes. An ATRP initiator with a thiol functional group was used to attach to the gold surface.[6] The QCM substrates were kept in 1 mM initiator solution in anhydrous hexanes overnight under nitrogen. After immobilization, the remaining photoresist was removed by soaking the substrates in ethanol for 10 minutes and then rinsed with acetone and dried under nitrogen. The photosensitizer was then electrochemically immobilized on the newly exposed regions of the QCM gold surface (*vide infra*) and lastly polymer brushes was grown from the ATRP initiator sites.

Non-specific Antibody Adsorption

To test for non-specific adsorption, the DNP-polymer brush system on a QCM crystal was exposed to a solution of 10 nM non-specific sheep IgG antibody in 1 mg/mL BSA in PBS, pH 7.2. The frequency did not change significantly (Figure S2), indicating that the non-specific antibody did not bind to the DNP groups. Furthermore, SWV measurements (Figure S3) indicated that no additional H_2O_2 was generated after exposing the patterned QCM crystal to the non-specific antibodies.

Electrochemical Polymerization of Photosensitizer

The silicon wafer chip or QCM crystal containing polymer brush initiator was immersed in a 0.5 mM solution of photosensitizer, $[Ru(v\text{-}bpy)_3](PF_6)_2$, where v-bpy is 4-vinyl, 4'-methyl bipyridine, in 0.1 M tetrabutylammonium perchlorate in acetonitrile, and the solution was deaerated using nitrogen for 15 minutes. A coiled platinum wire and a silver wire were used as the auxiliary and reference electrodes, respectively. Potentials were calibrated using ferrocene, with $E_{1/2}$ +0.342 V vs. SCE.[7] The potential was

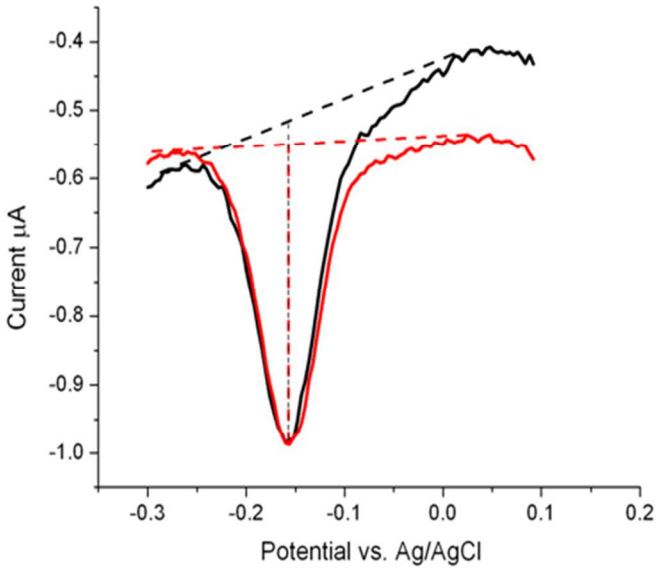

Figure S3. Square wave voltammetry in the presence of a non-specific antibody, measuring H_2O_2 production for two representative cases: (black) QCM with DNP-functionalized polymer brushes and photosensitizer not exposed to antibodies (red) the functionalized QCM after exposure to the non-specific antibody. Experimental conditions were similar to those in Figures 3 and S6.

cycled for 10 cycles at a scan rate of 100 mV/s, as shown by the solid lines in Figure 3C. The current increased as the number of cycles increased, showing that an electroactive layer formed upon reduction. After electropolymerization, the electrode was removed from the photosensitizer solution, rinsed with acetone and then acetonitrile, and then immersed in fresh electrolyte. The surface coverage, Γ, of $[Ru(v\text{-}bpy)_3]^{+2}$ was determined using the charge passed by the $Ru^{2+/3+}$ couple, shown by the dashed lines in Figure 3C, and using a similar procedure as for DNP, assuming that n =1 in this case. The electroactive area used was 1.3 cm^2, corresponding to three-fourths of the total exposed area, 1.7 cm^2. A typical value of Γ was 1.5 (± 0.5) x 10^{-9} mol/cm^2, which corresponds to a thickness of ca. 26 nm, assuming that each monolayer is 8.3 x 10^{-11} mol/cm^2 and is 1.42 nm thick.[8]

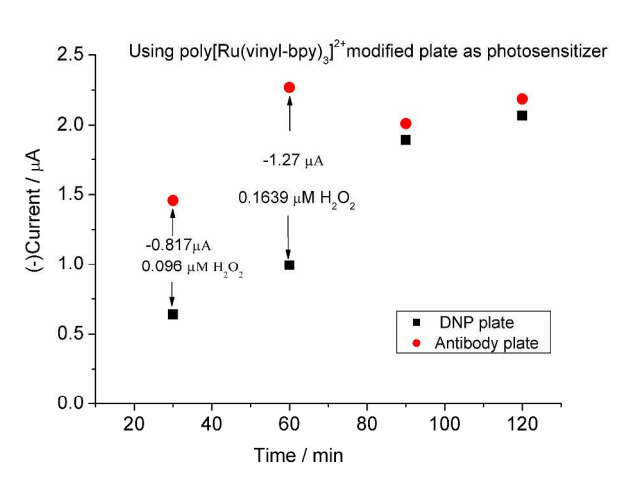

Figure S4. Effect of antibody presence on the DNP-functionalized plate next to a photosensitizer plate exposed to UV light in a solution of Amplex Red and HRP over time.

Optimization of Exposure Time

As the H_2O_2 production is plotted over time antibody catalysis reaches a limit at a time around 90 minutes exposure to UV light. This could be due to the antibody damage upon the long exposures to UV light or possibly to product inhibition of the catalytic activity.[9] A Si wafer with DNP bound brushes was incubated in 3 mL PBS buffer with 60μL (1mg/mL) antibody solution for 2 hours. The substrate was then rinsed to remove any physically bound antibodies, placed into 3 mL PBS buffer, and illuminated with UV light for 1 hour. After, the brush substrate was removed, 6 μL Amplex red (5mM) and 5.28 μL HRP (113U/mL) was added to the PBS solution for electrochemical measurement.

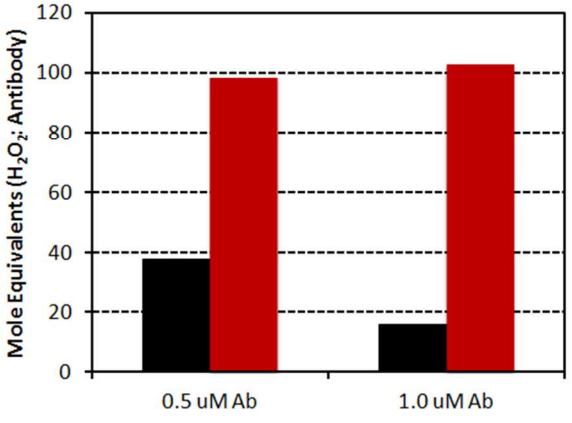

Figure S5. Comparison of the number of mole equivalents of H_2O_2 generated for every mole of antibody as measured using fluorescence (black) and SWV (red) measurements.

SWV Comparison to Fluorescence Measurements

The biochemical assay was based on previously established method with modifications.[4] Solutions of 0.5 and 1.0 μM polyclonal sheep antibody were made in buffer (10 mM KH_2PO_4, 160 mM NaCl, pH 7.4) containing photosensitizer (100 μM tris(2,2'-bipyridine) ruthenium II) (Fig. S4). Samples were placed in acrylic cuvettes (Sarstedt) and irradiated with an 8W ultraviolet (302 nm) transilluminator (UVP). After one hour, aliquots for fluorescence and SWV were taken from the same solutions. For fluorescence measurements, a 50 μL aliquot of the irradiated solution was diluted to a final volume of 300 μl in buffer (50 mM sodium phosphate (monobasic), pH7.4) containing Amplex Red (Invitrogen) (10 μM) and HRP (Sigma) (0.2 U/ml). Samples were then incubated at room temperature, protected from light for 30 minutes. Fluorescence of Amplex Red was measured on a plate reader (Tecan) using λ_{abs}= 540 nm and λ_{em}=590 nm filters. Standard solutions of H_2O_2 were processed concurrently with samples for quantification purposes. For the SWV measurements, 50 μL of the irradiated solution was diluted to a total volume of 5 mL, and the solution was analyzed using the same procedure as for the antibodies adsorbed to a patterned QCM crystal (*vide supra*). The concentration of H_2O_2 was determined using the calibration curve in the main text (Figure 5). The number of mole equivalents (mole H_2O_2 / mole antibody) measured using fluorescence and SWV is plotted in Figure S5. In comparison to Figure 5, these data suggest nonlinearity in the fluorescence assay as well as inhibition of the ACWOP by the H_2O_2 product[4] under the conditions of this experiment.

Temperature Measurements

We evaluated possible increases in temperature during exposure to the UV lamp. Using a thermocouple, the temperature of PBS was measured as it was irradiated with the transilluminator. A typical increase in temperature was about 15°C during irradiation for 60 minutes, and the maximum temperature was attained before 60 minutes.

Hydrogen Peroxide Generation on a Silicon Chip

Figure S6 shows the current response of DNP

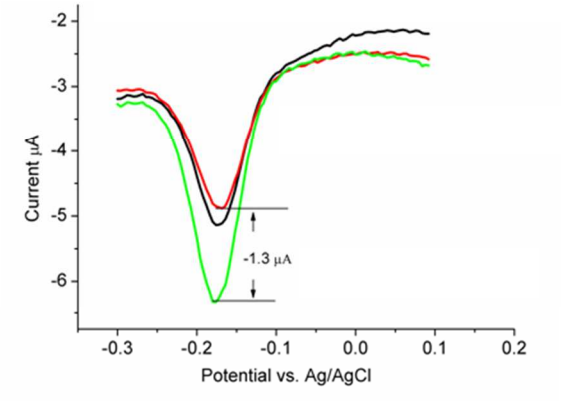

Figure S6. SWVs for resorufin that measures H_2O_2 production for three representative cases: patterned silicon chip with DNP-brushes and polymerized photosensitizer without antibody (red); patterned silicon chip with DNP-brushes with antibodies absorbed but without photosensitizer (black); patterned silicon chip with DNP-brushes with antibodies absorbed and also polymerized photosensitizer. All the samples were exposed under UV light for 1 hour and contained HRP and Amplex Red.

polymer brushes on a silicon chip with specific antibody and without polymerized photosensitizer, without antibody and with photosensitizer, and with both components. Missing either of these components results in a similar background signal. The significant increase in the presence of both components confirms that the ACWOP is catalyzing the H_2O_2 production. A Si wafer with DNP bound brushes was incubated in 3 mL PBS buffer with 60μL (1mg/mL) antibody solution for 2 hours. The substrate was then rinsed to remove any physically bound antibodies, placed into 3 mL PBS buffer, and illuminated with UV light for 1 hour. After, the brush substrate was removed, 6 μL Amplex red (5mM) and 5.28 μL HRP (113U/mL) was added to the PBS solution for electrochemical measurement.

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