Supporting Information for:

Non-Faradaic Current Suppression in DNA Based Electrochemical Assays with a Differential Potentiostat

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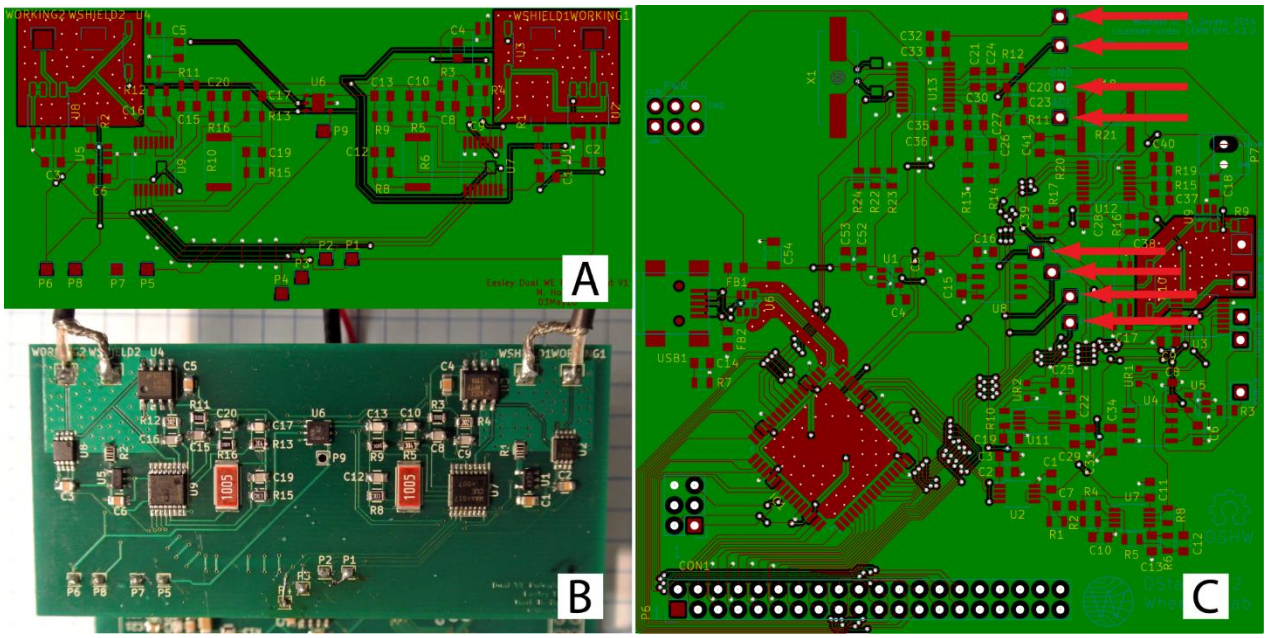

Figure S-1. (A) The PCB artwork for the differential potentiostat and **(B)** the ready-to-use, populated PCB. **(C)** Modification to the ConStat PCB [1] was required to interface with the DiffStat board. Red arrows indicate where the DiffStat board's communication and power connects via header pins to the ConStat PCB. When constructing the DiffStat, some components must be left unpopulated in the ConStat board, as listed in **Table S-1**.

Capacitors	C17, C28, C37, C38, C39, C40, C41
Resistors	R15, R16, R17, R18, R19, R20, R21
Integrated Circuits	U9, U10, U12

Table S-1. List of components that should be left unpopulated on the ConStat PCB in order to interface with the DiffStat add-on board.

Name	DNA sequence 5' to 3'
Thiolated DNA	**/5ThioMC6-D/**GCA TGG TGA CAT TTT TCG TTC GTT AGG GTT CAA ATC CGC G
CTR-DNA	CGC GGA TTT GAA CCC TAA CGA ACG AAA AAT GTC ACC ATG C
MB-DNA	CGC GGA TTT GAA CCC TAA CGA ACG AAA AAT GTC ACC ATG C**/MB-C7/**
MB-DNA2	CCA CCC TCC TCC TTT TCC TAT CTC TCC CTC GTC ACC ATG C**/MB-C7/**
Com-33	TTG AAC CCT AAC GAA CGA AAA ATG TCA CCA TGC

Table S-2. DNA sequences used in this work.

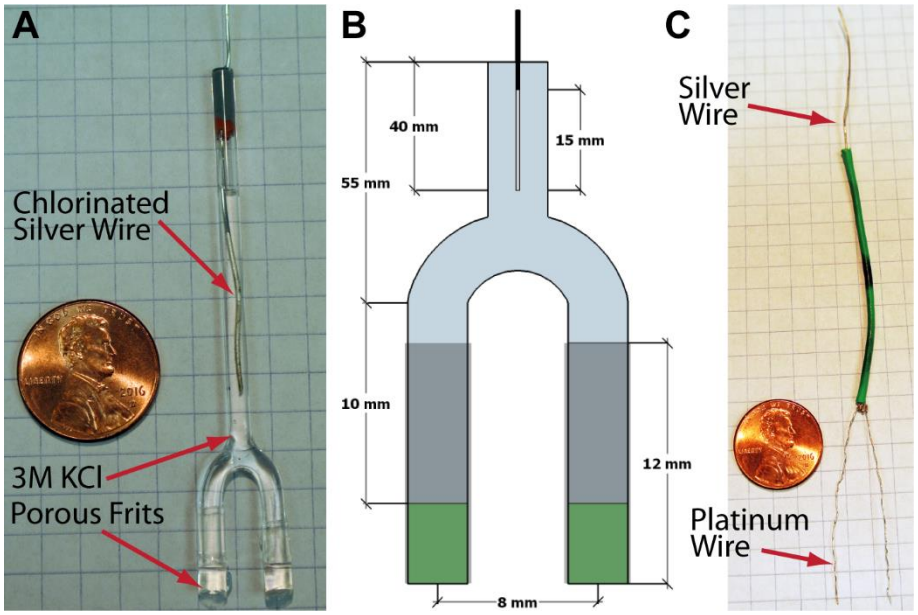

Figure S-2: **(A)** For DiffStat use, a split reference electrode was custom made from 3 mm OD glass tubing, two CoralPor glass frits (BASi West Lafayette, IN), and 0.5 mm silver wire (Alfa Aesar), and it was filled with 3 M KCl. A 65-mm length of silver wire was cut from stock, and a 3-mm diameter rubber plug was cut from a rubber stopper with a 3-mm biopsy punch. The plug was fitted onto the silver wire ~30 mm from the end. The lower 15 mm was chlorinated by immersion in 3 M KCl solution with application of 9 V (dc) for ~10 seconds, where the wire was positive with respect to ground, and the return electrode was a platinum electrode. This procedure produced a gray/white AgCl coating on the wire. **(B)** The wire was then inserted ~40 mm into the electrode top, creating a small cavity at the top of the electrode to accept epoxy (Loctite EA 445). The electrode was then filled with 3 M KCl, ensuring no air remained, and porous frits were affixed with Teflon heat-shrink tubing. **(C)** The counter electrode was created from an 8-cm length of 0.25 mm diameter platinum wire bent in half. At the halfway point, 0.5 mm diameter silver wire was wrapped 8 times around the platinum wire for electrical contact and to avoid short circuits.

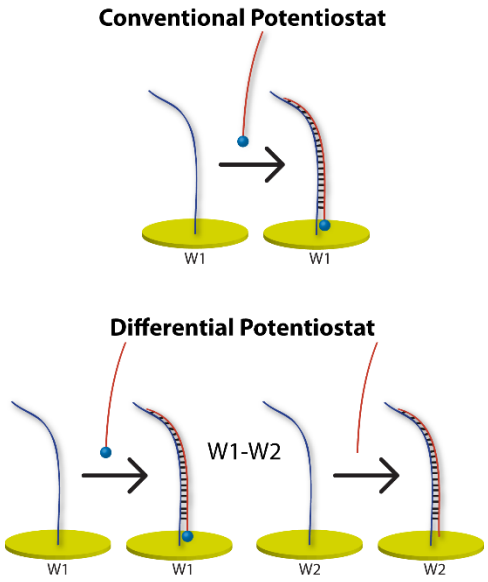

Figure S-3. Schematic of electrochemical measurements of DNA hybridization used in all experiments unless noted. For the conventional potentiostat (ConStat), a thiolated-DNA strand was immobilized on a gold working electrode surface (W1), and a complimentary methylene blue labeled DNA strand (MB-DNA) was introduced which hybridized with the immobilized thiolated DNA (top schematic). This hybridization brought the redox active molecule (MB) close to the surface, allowing electron transfer and a signal when interrogated by CA, CV, or SWV. The differential potentiostat (DiffStat) system was similar (bottom schematic), except that a second gold working electrode (W2) was utilized. In one approach, a complimentary DNA strand was introduced without a redox tag (CTR-DNA, unlabled red strand), allowing correction of non-faradaic baseline through W2.

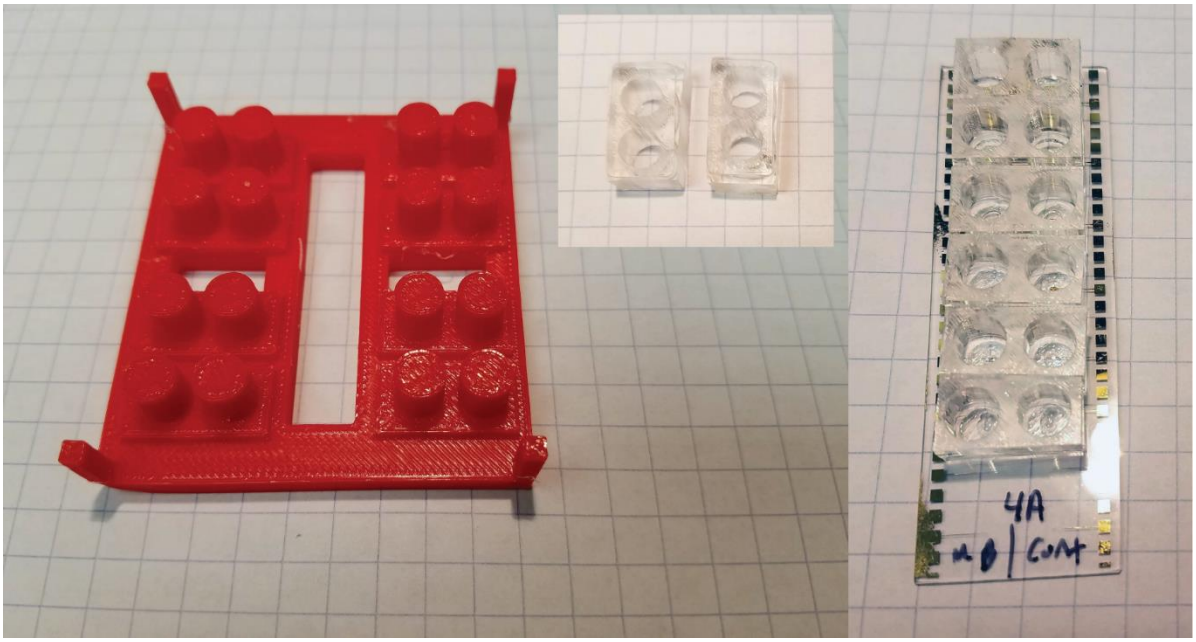

Figure S-4. 3D-printed mold used for making polydimethylsiloxane (PDMS) electrochemical cells. This electrochemical cell was plasma oxidized and bonded with a GoG slide. Seven differently sized electrodes were fabricated as duplicate electrodes in a single GoG slide (right).

EXPERIMENTAL DESIGN

Reagents and materials. All solutions were prepared with deionized and ultrapure water (Thermo Scientific). The following reagents were used as received: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.5 M solution pH 7.5 (Alpha Aesar), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), mercaptohexanol (MCH), standard gold and chromium etchants (Sigma-Aldrich, St.Louis, MO). Glass microscope slides (1" x 3") coated with chromium (5 nm) and gold (100 nm), were obtained from Deposition Research Labs, Inc. (St. Charles, MO). AZ-40XT positive photoresist and AZ-300 MIF developer was purchased from Integrated Micro Materials (Argyle, TX). Photomasks were designed in Adobe Illustrator and printed at 50k dpi by Fine Line Imaging (Colorado Spings, CO). Dimethoxy sulfoxide (DMSO) was from Anachemia (Montreal, QC, Canada). Polydimethylsiloxane (PDMS) polymer GE Sylgard 184 was purchased from Ellsworth Adhesives (Germantown, WI). Concentrated sulfuric acid and 30 % hydrogen peroxide were obtained from Avantor (Center Valley, PA). Normal human serum (pathogen screened), was obtained from BioIVT (Westbury, NY). HPLC-purified methylene-blue tagged DNA (MB-DNA) was obtained from Biosearch Technologies (Novato, CA). All other DNAs were bought from Integrated DNA Technologies (IDT; Coralville, Iowa) with purity and yield confirmed by mass spectroscopy and HPLC. All of the DNA sequences used in this work are listed in **Table S-2**.

Differential potentiostat fabrication. Circuit development and simulation was performed in Tina-TI V9 (DesignSoft, Inc.; Texas Instruments). Schematics and printed circuit boards (PCB) were created in KiCad EDA software. Electronic parts were obtained from Mouser Electronics (Mansfield, TX) and Digi-Key Electronics (Thief River Falls, MN), and PCBs were ordered from Pentalogix Inc. (Tualatin, OR). The DiffStat was designed as a daughterboard fitted to the front end of an open source potentiostat[1]. To verify the functionality of the DiffStat, a "control" potentiostat was constructed as described by Dryden et al. [1] and will be referred to herein as a "conventional potentiostat" (ConStat). Assembly details, images, and design files for the DiffStat are shown in Figure S-1 and Table S-1.

Electrochemical cell preparation.

Gold-on-Glass Electrode Fabrication. Photolithographically patterned gold on glass electrodes (GoG) and PDMS electrochemical cells, which provide low electrode-to-electrode variability, were used by following similar fabrication steps presented in our previous work.[2] Briefly, GoG slides were placed in an oven at 200 °C for a minimum of 15 min then cooled to room temperature (RT). A layer of ~20 μm thickness of AZ40XT photoresist was spin-coated onto the GoG then baked at 126 ^{o}C for 6 min. A photomask was illuminated (5 min) at 365 nm above the photoresist. The resulting pattern was baked (105 °C) and developed in MIF-300 developer (6 min), then it was rinsed in deionized water and dried under nitrogen. To remove the excess gold and chromium, patterned GoGs were then immersed in gold etchant (30 s) followed by chromium etchant (15 s), and later the cured photoresist was dissolved by DMSO (100 °C, 30 min), rinsed with deionized water, and dried under a stream of nitrogen. These electrodes could also be separated into smaller units by scribing with a carbide-tipped pen and fractured by hand.

PDMS Chambers. 3D-printed templates [2,3] were designed in Sketchup (Trimble, Inc.; Sunnyvale, CA) to align with GoG electrodes, and models were sliced in Makerbot Desktop software (New York, NY) then printed on a Makerbot 2 3D printer at high resolution (100 μm layer) with 75% infill. PDMS precursors were mixed (10:1, prepolymer:initiator), degassed, and poured over a silanized 100-mm polished silicon wafer. The 3D-printed mold was placed in the PDMS, and the mold and PDMS were degassed then cured at 60 °C for 24 hours. [3] After demolding, the PDMS was sliced to a proper shape, sonicated in pure methanol for 30 min, dried, and placed in an oven at 100 °C overnight. The GoG electrodes were cleaned with piranha solution (3:1, conc. H_2SO_4:30% H_2O_2) for 1 min and plasma oxidized in an air plasma for 45 s. The PDMS molds were also plasma oxidized, and the GoG was

finally bonded to these 3D-printer templated PDMS molds. The electrochemical cells were then ready for DNA immobilization and follow-up measurements.

DNA immobilization. All dilutions and experiments were done in buffer (10 mM HEPES with 0.5 M $NaClO_4$ at pH 7) unless otherwise mentioned. We followed a similar immobilization protocol explained in our previous work.[2] Briefly, 200 μM thiolated DNA was combined with 10 mM TCEP (reducing agent) at a 1:3 ratio and incubated 1 h at RT in darkness. The reduced thiolated DNA was then diluted to 1.25 μM and introduced into the electrochemical cell. After 1 h incubation at RT in the dark, the solution was removed and rinsed with buffer. The electrodes were incubated in 3 mM MCH for 1 h at RT in darkness, rinsed three times in buffer, then were ready for use. Electrodes could be stored at this condition in buffer for up to 1 week at 4 °C.

Electrochemical measurements. Both the DiffStat and ConStat were used for measurements in this work. The ConStat functioned as a typical potentiostat, with three electrodes: working (W1), counter, and reference. Use of the DiffStat required four electrodes: two working (W1, W2), one counter, and one reference. Customized, split platinum counter electrodes and a split Ag/AgCl (3M KCl) reference electrodes were used in these experiments (**Figure S-2**). CA, CV, and SWV measurements were made with both potentiostats. *CA parameters:* Working electrodes were held at -350 mV, and measurements at 3.75 kHz were made using a two-step approach (step 1: -350 mV, 2 s; step 2: -150 mV, 2 s). *CV parameters:* Working electrode potentials were scanned between -450 and 0 mV at a scan rate of 100 mV s^{-1}. *SWV parameters:* Initial potential was -450 mV, final potential was 0 mV, step size was 1 mV, pulse height was 25 mV, and SWV frequency was 100 Hz. All electrochemical measurements were carried out at least in triplicate, and representative individual traces are shown in the figures in the main text.

Experimental protocols.

DNA hybridization measurements. Once an electrode was ready with immobilized thiolated DNA and MCH, baseline measurements were made with CA and CV. Later, MB-DNA and unlabeled CTR-DNA (same DNA sequences, 40-bp complementary to thiol-DNA) were diluted to working concentrations (10 nM) and added to the wells containing the working electrode(s) (2 mm diameter). For the DiffStat, MB-DNA was added to W1, and CTR-DNA was added to W2. Following 1 h incubation at RT, CA and CV measurements were made again. This experimental system is depicted in Figure S-3.

Capacitance suppression on a range of electrode surface areas. 0.1, 0.25, 0.5, 1, 2, 4 and 6 mm diameter electrodes were fabricated, and thiol-DNA immobilization was done. For the DiffStat, MB-DNA (10 nM) and CTR-DNA (10 nM) were introduced in W1 and W2 respectively and incubated overnight at 4 ° C. Solutions were added to separate working electrodes for the ConStat. SWV measurements were done with the DiffStat and ConStat using six different instrumental ranges between ±15 nA and ±500 μA. Figure S-4 shows the 3D-printed mold and cell design for varying electrode areas.

Real-time hybridization measurement. These measurements were done only with the DiffStat. Thiol-DNA and MCH modified electrodes (2 mm) were prepared, then the MB-DNA was introduced into the well of W1, and SWV measurements were acquired every 5 min. At 30 min, MB-DNA was added to the cell of W2, and measurements were continued for 60 min total.

Signal-OFF to signal-ON. Only the DiffStat was used for these measurements. Thiol-DNA and MCH modified electrodes (4 mm) were prepared then incubated with 100 nM MB-DNA2 overnight; MB-DNA2 hybridized to thiolated DNA with only 10 bp. 100 nM of Com-33, which hybridizes with thiolated DNA and displaces MB-DNA2, was introduced onto W2, and SWV measurements were made at 20 s intervals.

Human serum measurement. Both the ConStat and DiffStat were used in these studies. After thiolated DNA and MCH incubation, the electrode was subjected to buffer and measured for 20 min with CV at 5 min intervals. At 20 min, 10 nM of MB-DNA and CTR-DNA (targets) were introduced into W1 and W2, respectively, and measurements were continued. At 40 min, buffer was reintroduced, and at 60 min the targets were injected again. From 90 min to 180 min, the same protocol was followed, except that both buffer and target solutions contained 50 % human serum.

Data Analysis. *Peak height.* SWV raw data from the ConStat (V_{step} and I_{diff} columns) were transferred to Microsoft Excel. To remove capacitance current, a third-order polynomial baseline was calculated using the "Linest" function; data from -0.445 to -0.370 V and -0.150 to -0.005 V were used in this calculation. Calculated baselines were subtracted from raw I_{diff} data, and the maximum current from this graph was used as the peak height. When using the DiffStat, the capacitance current was automatically corrected by the instrument. The average baseline current was taken as simply the average I_{diff} between -100 and -110 mV and subtracted from maximum of the raw data to get the peak height. The baseline noise was calculated as the standard deviation of baseline current between -15 mV and -30 mV. To calculate capacitance current for CV, the current output between -400 and -380 mV of the reverse curve was subtracted from that of the forward curve. For CV faradaic current, the difference of the forward and reverse current at -210 mV was used.

References:

(1) Dryden, M. D.; Wheeler, A. R. *PloS one* **2015**, *10*, e0140349.

(2) Somasundaram, S.; Holtan, M. D.; Easley, C. J. *Analytical chemistry* **2018**, *90*, 3584.

(3) Brooks, J. C.; Ford, K. I.; Holder, D. H.; Holtan, M. D.; Easley, C. J. *The Analyst* **2016**, *141*, 5714.