Supporting Information

A reagentless and reusable electrochemical DNA sensor based on target hybridization-induced stem-loop probe formation

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MATERIALS AND METHODS

Materials and Reagents. A thiolated DNA probe modified with three methylene blue (MB) (**3MB-P**) was used as the surface-immobilized signaling probe. A 25-base thiolated DNA probe was used as the target capturing probe (**T8-P**). Both probes were purchased from Biosearch Technologies Inc. (Novato, CA). Five DNA targets were obtained from Integrated DNA Technologies (Coralville, IA) and used as received. The sequence information of the probes and targets are shown in the following. The single underlined portion of **3MB-P** forms a 10-base duplex with the italicized portion of **T8-P**, whereas the double underlined portion of **T8-P** hybridizes to the target DNA. The mismatches in the targets are highlighted in bold.

3MB-P: 5'SS $(CH_2)_6$ CCG TTA CGC CAC CAG <u>CTC CAA ACG G</u>-T(MB)-T(MB)-T(MB) 3'

T8-P: 3'SS $(CH_2)_6$ <u>TTT TTT TTA TCG ATG *GAG GTT TGC C*</u> 5'

PM-25: 5' AAA AAA AAT AGC TAC CTC CAA ACG G 3'

PM-21: 5' AAA ATA GCT ACC TCC AAA CGG 3'

PM-17: 5' TAG CTA CCT CCA AAC GG 3'

1-base Mismatch target (17-1M): 5' TAG CTA CCT CCA AA**G** GG 3'

2-base Mismatch target (17-2M): 5' TAG CTA CCT C**G**A AA**G** GG 3'

The reagents 6-mercapto-1-octanol (C6-OH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and iron fortified bovine calf serum were used as received (Sigma-Adrich, St. Louis, MO). All other chemicals were of analytical grade. All the solutions

were made with deionized (DI) water purified through a Millipore system (18.2 MΩ·cm, Millipore, Billerica, MA). The sensors were interrogated either in a phosphate buffer (PBS) containing 10 mM sodium phosphate and 300 mM sodium chloride (pH 7.4) or 1:1 bovine calf serum/PBS.

E-DNA Sensor Preparation. Prior to sensor fabrication, gold disk electrodes with geometric area of 0.0314 cm^2 (CH Instruments, Austin, TX) were polished with a 0.1 μm diamond slurry (Buehler, Lake Bluff, IL), rinsed with deionized water and sonicated in a low power sonicator 5 minutes to remove bound particulates. They were then electrochemically cleaned by a series of oxidation and reduction cycles in 0.5 M H_2SO_4. The real area of the electrode was determined from the charge associated with the gold oxide stripping peak obtained after the cleaning process. The average surface roughness factor (f_r), which is the ratio between the real surface area and the geometric surface area, was found to be 1.13±0.11 (n=3). Fabrication of the E-DNA sensor involved several steps. First, 1.5 μL of 200 μM **T8-P** was added to 1 μL of 200 μM **3MB-P**, then 2.5 μL of 10 mM TCEP was added to reduce the disulfide bond of these two probes. This solution was subsequently diluted with Phys2 buffer (10 mM Tris, 70 mM NaCl, 2.5 mM KCl, 0.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$, pH 7.4) to achieve a probe concentration of 5 μM (3 μM **T8-P** and 2 μM **3MB-P**). Both **T8-P** and **3MB-P** were simultaneously immobilized onto the surfaces of gold electrodes by incubating the clean electrodes in this 5 μM diluted probe DNA solution for 1 h. The modified electrodes were then rinsed with PBS and further passivated with 2 mM C6-OH in PBS overnight to displace non-specifically bound oligonucleotides.

Electrochemical Measurements. Electrochemical measurements were performed at room temperature using a CHI 1040A Electrochemical Workstation (CH Instruments, Austin, TX). The E-DNA sensors were characterized by alternating current voltammetry (ACV) over a wide range of frequencies (1-5000 Hz) using an amplitude of 25 mV. Higher AC frequencies were used with sensors interrogated in pure buffer. Owing to the small MB peak current observed at high frequencies, only low frequencies were used with sensors interrogated in 50% serum. Cyclic voltammetry was also used to determine the electron transfer rates. DNA probe-modified gold disk electrodes were used as working electrodes. A platinum wire electrode was used as the counter electrode and a

Ag/AgCl (3.0 M KCl) electrode served as the reference electrode (CH Instruments, Austin, TX).

Prior to sensor interrogation, the modified electrodes were allowed to equilibrate in PBS or 50% serum. 1 μM (or 50 nM) of the target DNA was then added to the electrochemical cell for sensor interrogation. AC voltammograms were collected at different time intervals until a stable peak current was obtained. Calibration curve results were obtained by sequential addition of the target DNA at an interval of 90 min. Concentrations used to obtain the calibration curves were 10 pM, 100 pM, 1 nM, 10 nM, 50 nM, 100 nM, 500 nM, 1 μM, and 2 μM. Sensor regeneration was achieved by rinsing with room temperature DI water for 30 sec for sensors interrogated in PBS or by incubating in 4 M guanidine-HCl (GHCl) for 4 min, followed by rinsing with DI water for 30 sec for sensors interrogated in 50% bovine calf serum. Mechanical stirring was not used in any of the hybridization experiments. All experiments were performed at room temperature. Unless mentioned otherwise, all experimental results presented in this study are averaged from three different sensors.

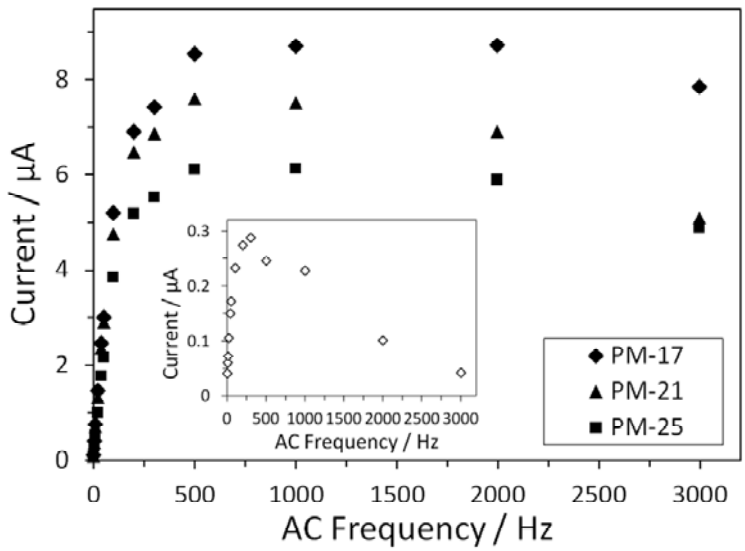

Fig. S1. AC frequency-dependent change in MB current upon hybridization to 1.0 µM **PM-25**, **PM-21**, and **PM-17**. Inset: change in MB current with frequency in absence of the target DNA.

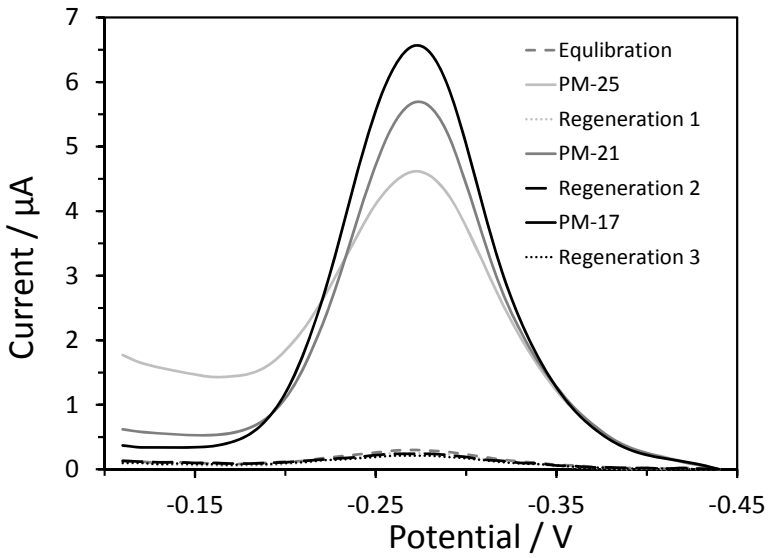

Fig. S2. Shown are AC voltammograms of the sensor before and after hybridization with 1.0 µM **PM-25**, **PM-21**, and **PM-17** in PBS. Also shown are the sensor regeneration scans collected after the 30-sec room temperature DI water rinse. All scans were collected at 2000 Hz.

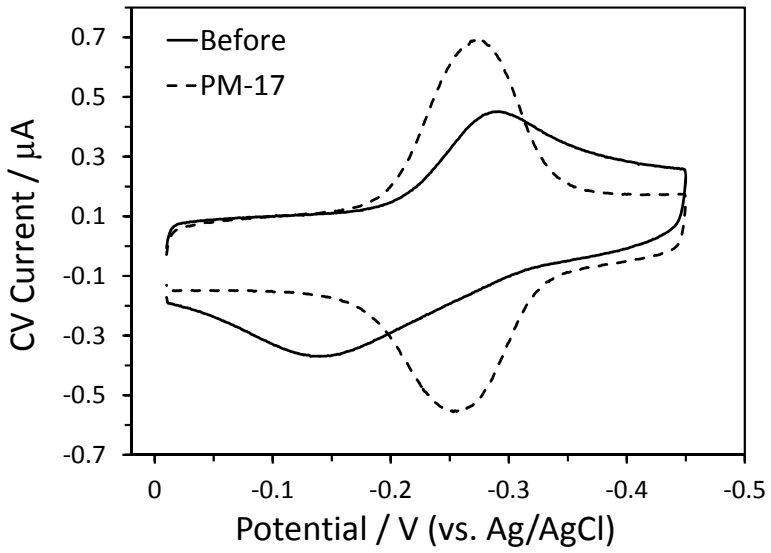

Fig. S3. Shown are cyclic voltammograms of the sensor before and after hybridization to 1.0 µM **PM-17** at a scan rate of 0.5 V/s.

	$k_s(s^{-1})$
Sensor Before	1.92(±0.1)
PM-17	311(±16)
PM-21	294(±18)
PM-25	272(±12)
MB-P only	219(±0.1)

Fig. S4. Shown are the electron transfer rate constants for MB before and after hybridization to 1.0 μM **PM-17**, **PM-21**, and **PM-25** in PBS. Also shown is the electron transfer rate constant for MB from a sensor modified with **3MB-P** only (i.e., without **T8-P**). The data presented here are averaged from three different sensors.

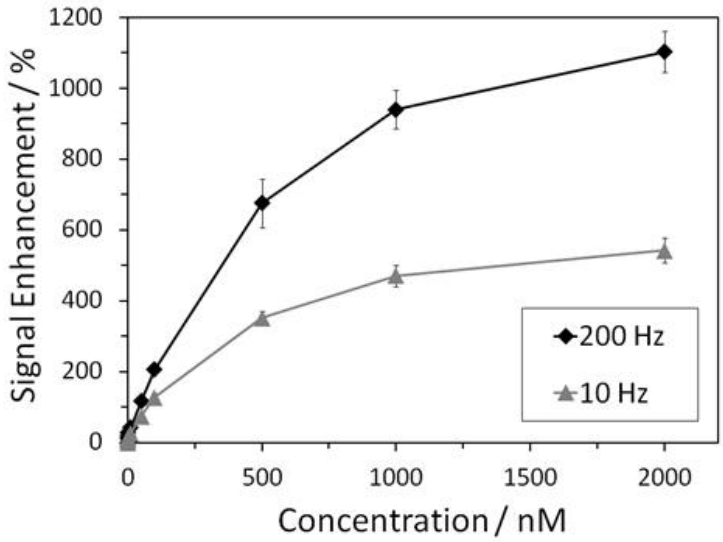

Fig. S5. Dose-response curves of the sensor collected in 50% serum at 10 Hz and 200 Hz. Target concentrations used in this experiment were 10 pM, 100 pM, 1 nM, 10 nM, 50 nM, 100 nM, 500 nM, 1 μM, and 2 μM. Shown are data averaged from three different sensors.