## Supporting Information

# A dual-signalling electrochemical DNA sensor based on target hybridization-induced change in DNA probe flexibility

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## MATERIALS AND METHODS

**Materials and Instrumentation.** A thiolated methylene blue (MB)-modified probe (**MB-P**) and a thiolated ferrocene (Fc)-modified probe (**Fc-P**) were used in this study. Both probes (HPLC purified) were purchased from Biosearch Technologies Inc. (Novato, CA) and were used as received. Four 17-base DNA targets (HPLC purified) were obtained from Integrated DNA Technologies (Coralville, IA). The sequence information of the probes and targets are shown in the following. The single underlined portion of **MB-P** hybridizes to the 17-base targets, whereas the double underlined portion of **Fc-P** forms a 7-base duplex with the italicized portion of **MB-P**. The mismatches in the targets are highlighted in bold. The non-complement sequence (**Non**) is a 10-base mismatch sequence.

**MB-P**: 3'SS $(CH_2)_6$ TTT T<u>AA CCT CGA CC*A CCG CAT*</u> 5'MB

**Fc-P**: 5'SS $(CH_2)_6$ TTT TTT TTT TTT TT<u>T GGC GTA</u> T 3'Fc

**Perfect Match target (PM)**: 5' TTG GAG CTG GTG GCG TA 3'

**1-base Mismatch target (1-MM)**: 5' TTG GAG CT**C** GTG GCG TA 3'

**2-base Mismatch target (2-MM)**: 5' TTG **C**AG CT**C** GTG GCG TA 3'

**Non-complement target (Non)**: 5' T**G**G **C**A**T GAA CC**G G**A**G **G**A 3'

The reagents 8-mercapto-1-octanol (C8-OH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and trizma base and iron-supplemented 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

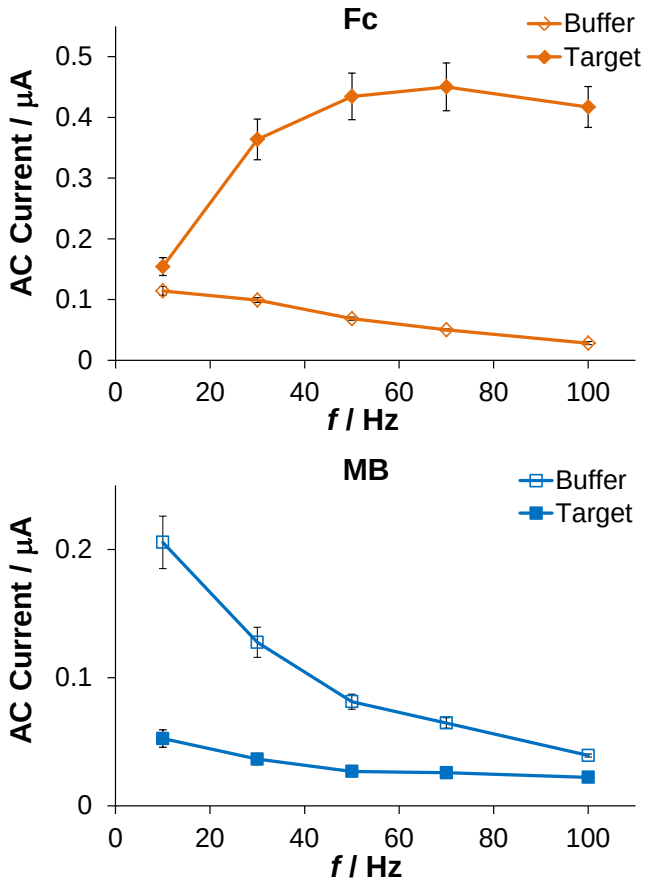
Millipore system (18.2 MΩ·cm, Millipore, Billerica, MA). The interrogation buffer was a physiological buffer supplemented with $NaClO_4$ (Phys/$NaClO_4$ buffer: 10 mM Tris, 70 mM NaCl, 2.5 mM KCl, 0.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$, and 0.5 M $NaClO_4$, pH 7.4).

**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 to remove bound particulates. They were then electrochemically cleaned by a series of oxidation and reduction cycles in 0.5 M $H_2SO_4$. Fabrication of the E-DNA sensor involved several steps. First, 2 µL of 10 mM TCEP was added to 2 µL of 200 µM **MB-P** to reduce the disulfide bond of the probe DNA. This solution was subsequently diluted with Phys/$NaClO_4$ buffer to achieve a probe concentration (**MB-P**) of 16 µM. A16 µM **Fc-P** solution was obtained using the same method. Equal volume of **MB-P** was added to a **Fc-P** solution and was mixed thoroughly. Both **MB-P** and **Fc-P** were simultaneously immobilized onto the gold electrode surface by incubating the electrode in a mixed probe solution containing 8.0 µM **MB-P** and 8.0 µM **Fc-P** for 1 hr. The modified electrode was rinsed with DI water and further passivated via an overnight incubation in 2 mM C8-OH in $H_2O$.

**Electrochemical Measurements**. Electrochemical measurements were performed at room temperature (22±1°C) 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 (10-100 Hz) using an amplitude of 25 mV. 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 **MB-P** and **Fc-P**-modified electrodes were allowed to equilibrate in Phys/$NaClO_4$ buffer. 1 µM of the target DNA (i.e., **PM**, **1-MM**, **2-MM**, or **Non**) 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. Sensor regeneration was achieved by rinsing with DI water for 30 sec for sensors interrogated in Phys/$NaClO_4$ buffer or by incubating in 4 M guanidine-HCl

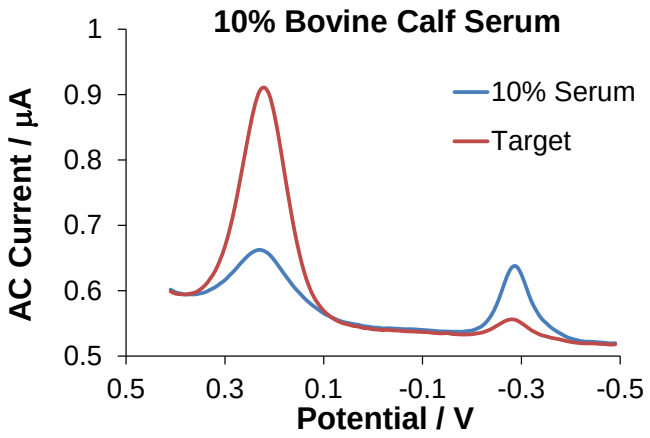
(GHCl) for 4 min, followed by rinsing with DI water for 30 sec for sensors interrogated in 10% bovine calf serum.



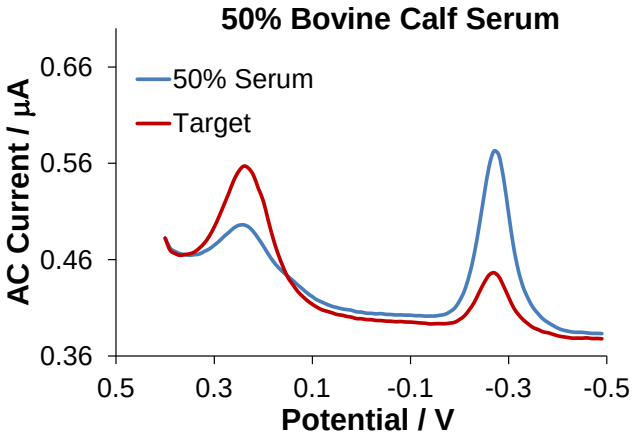

**SI Fig. 1** Shown are AC frequency-dependent currents of **Fc-P** and **MB-P** before and after hybridiztion to 1 µM **PM** target in Phys/$NaClO_4$ buffer. The results presented here are averages from three different sensors.

| | $k_{s,\ Fc}\ (s^{-1})$ | $k_{s,\ MB}\ (s^{-1})$ |
|---|---|---|
| Sensor Before | 33.6±1.3 | 2.71±0.10 |
| Sensor with 1 µM **PM** Target | 136±11 | 0.95±0.09 |
| Sensor with **Fc-P** only | 114±8 | --- |
| Sensor with **MB-P** only | --- | 3.88±0.13 |

**SI Fig. 2** Shown are the electron transfer rate constants of MB and Fc before and after hybridization to 1 µM **PM** in Phys/$NaClO_4$ buffer. Also shown are the electron transfer rate constants for sensors fabricated using either **MB-P** or **Fc-P**. The results presented here are averages from three different sensors.




**SI Fig. 3** AC voltammograms of the E-DNA sensor recorded in 10% bovine calf serum in absence and presence of 1 µM **PM** target. The scans were collected at 30 Hz.




**SI Fig. 4** AC voltammograms of the E-DNA sensor recorded in 50% bovine calf serum in absence and presence of 1 µM **PM** target. The scans were collected at 30 Hz.