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: \[3'\text{SS (CH}_2\text{)}_6 \text{TTT TAA CCT CGA CCA CCG CAT}5'\text{MB}\]

Fc-P: \[5'\text{SS (CH}_2\text{)}_6 \text{TTT TTT TTT TTT GGC GTA T}3'\text{Fc}\]

Perfect Match target (PM): \[5' \text{TTG GAG CTG GTG GCG TA 3'}\]

1-base Mismatch target (1-MM): \[5' \text{TTG GAG CTC GTG GCG TA 3'}\]

2-base Mismatch target (2-MM): \[5' \text{TTG CAG CTC GTG GCG TA 3'}\]

Non-complement target (Non): \[5' \text{TGG CAT GAA CCG GAG GA 3'}\]

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 NaClO₄ (Phys/NaClO₄ buffer: 10 mM Tris, 70 mM NaCl, 2.5 mM KCl, 0.5 mM MgCl₂ and 0.5 mM CaCl₂, and 0.5 M NaClO₄, pH 7.4).

**E-DNA Sensor Preparation.** Prior to sensor fabrication, gold disk electrodes with geometric area of 0.0314 cm² (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 H₂SO₄. 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/NaClO₄ buffer to achieve a probe concentration (MB-P) of 16 µM. A 16 µM Fe-P solution was obtained using the same method. Equal volume of MB-P was added to a Fe-P solution and was mixed thoroughly. Both MB-P and Fe-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 Fe-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 H₂O.

**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 Fe-P-modified electrodes were allowed to equilibrate in Phys/NaClO₄ 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/NaClO₄ buffer or by incubating in 4 M guanidine-HCl. 

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(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 hybridization to 1 µM PM target in Phys/NaClO₄ buffer. The results presented here are averages from three different sensors.
<table>
<thead>
<tr>
<th>Sensor</th>
<th>( k_x, \text{Fe}, (s^{-1}) )</th>
<th>( k_x, \text{MB}, (s^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensor Before</td>
<td>33.6±1.3</td>
<td>2.71±0.10</td>
</tr>
<tr>
<td>Sensor with 1 µM PM Target</td>
<td>136±11</td>
<td>0.95±0.09</td>
</tr>
<tr>
<td>Sensor with Fe-P only</td>
<td>114±8</td>
<td>---</td>
</tr>
<tr>
<td>Sensor with MB-P only</td>
<td>---</td>
<td>3.88±0.13</td>
</tr>
</tbody>
</table>

**SI Fig. 2** Shown are the electron transfer rate constants of MB and Fe before and after hybridization to 1 µM PM in Phys/NaClO₄ buffer. Also shown are the electron transfer rate constants for sensors fabricated using either MB-P or Fe-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.