

Supporting information for the article:

“A Reagentless Signal-on Architecture for Electronic, Aptamer-Based Sensors via Target-Induced Strand Displacement”

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Experimental conditions for the preparation of E-AB sensor.

Labeled DNA oligonucleotides were synthesized by BioSource, Int. (Foster City, CA), and purified via C18 HPLC and PAGE, and confirmed by mass spectroscopy. The sequences of these three oligomers employed are given below:

(1): 5'-HS-(CH₂)₆-CCATCTCCACTTGGTTGGTGTGGTTGG-3'

(2): 5'-MB-(CH₂)₂-CCAACCTTTTAAGTGGAGATGG-3'

(3) 5'-HS-(CH₂)₆-CCATCTCCACTTGGTGGTGGTTGTGGT-3'

(4) 5'-MB-(CH₂)₂-ACCACTTTTAAGTGGAGATGG-3'

MB was conjugated to the 3' end of these probes via succinimide ester coupling (MB-NHS obtained from EMP Biotech, Germany).

The human alpha-thrombin used in this study was purchased from Haematologic Technologies Inc. (Essex Junction, VT, specific activity: 3545 units/mg), and diluted with sterile water as appropriate. Bovine serum albumin (BSA) was purchased from Sigma-Aldrich, Inc. (USA) and used without further processing.

The E-AB sensor was fabricated using polycrystalline gold disk electrodes (1.6 mm diameter, BAS, West Lafayette, IN). The electrodes were prepared by polishing with diamond and alumina (BAS), sonicating in water, and electrochemically cleaning (a series of oxidation and reduction cycling in 0.5 M NaOH; 0.5 M H₂SO₄; 0.01 M KCl / 0.1 M H₂SO₄; and 0.05 M H₂SO₄) before being modified with the thiolated DNA. The clean gold surface was interacted with a solution of thiolated thrombin aptamer DNA (1), 0.8 μM including 8 μM TCEP (tris-(2-carboxyethyl) phosphine hydrochloride, which is included to reduce disulfide bonded oligos) in 200 mM Tris-HCl buffer, pH 7.4, for 16 hrs. The surface was then rinsed with deionized water and subsequently passivated with 6-mercaptohexanol (1 mM in 10 mM Tris-HCl buffer, pH 7.4) for 1 hrs. The electrodes were then rinsed again with 10 mM Tris-HCl buffer, pH 7.4. The resulting monolayer-functionalized surface was treated with the MB-tagged complementary DNA (2), 1.0 μM, in Perfect HybTM plus hybridization buffer

(Sigma) (1×), for 3 hours to yield the ds-DNA-aptamer assembly on the surface. Control electrodes modified with oligonucleotides (3) and (4) were prepared in an identical fashion.

E-AB sensor measurements were conducted using alternating current voltammetry (ACV) with a CHI 603 potentiostat (CH Instruments, Austin, TX) in a standard cell with a platinum counter electrode and a Ag/AgCl reference electrode.

All measurements were conducted by monitoring the electrode in 20 mM Tris-HCl, pH 7.4 with 140 mM NaCl, 20 mM MgCl and 20 mM KCl. For all thrombin detection measurements, electrodes were incubated in each sample in 20 mM Tris-HCl, pH 7.4 with 140 mM NaCl, 20 mM MgCl and 20 mM KCl for 3 hrs at 37 °C.

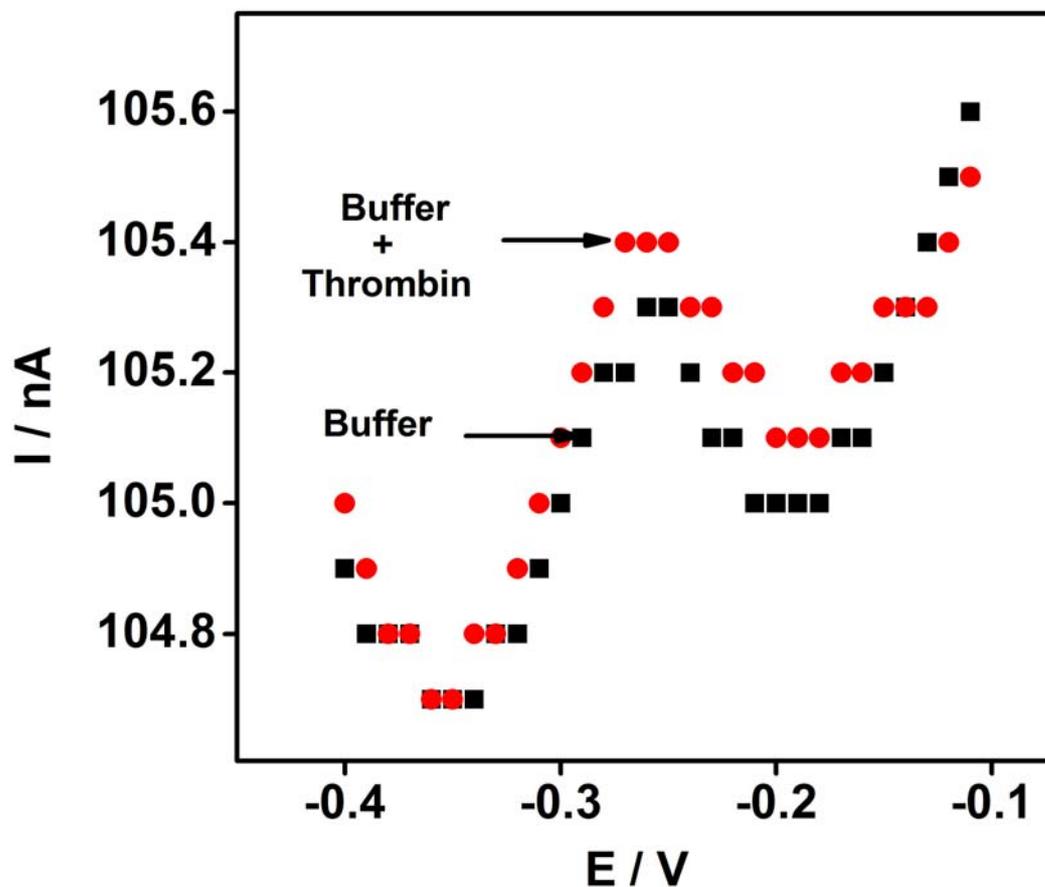


Fig. X: The E-AB sensor made by thiolated control oligonucleotide (3) and its complementary MB-tagged DNA (4) does not exhibit any significant signal change even when challenged with 76 nM of the target protein. Shown is the sensor electrochemical signal arising from thrombin-free buffered saline and from the same buffer doped with 76 nM thrombin.

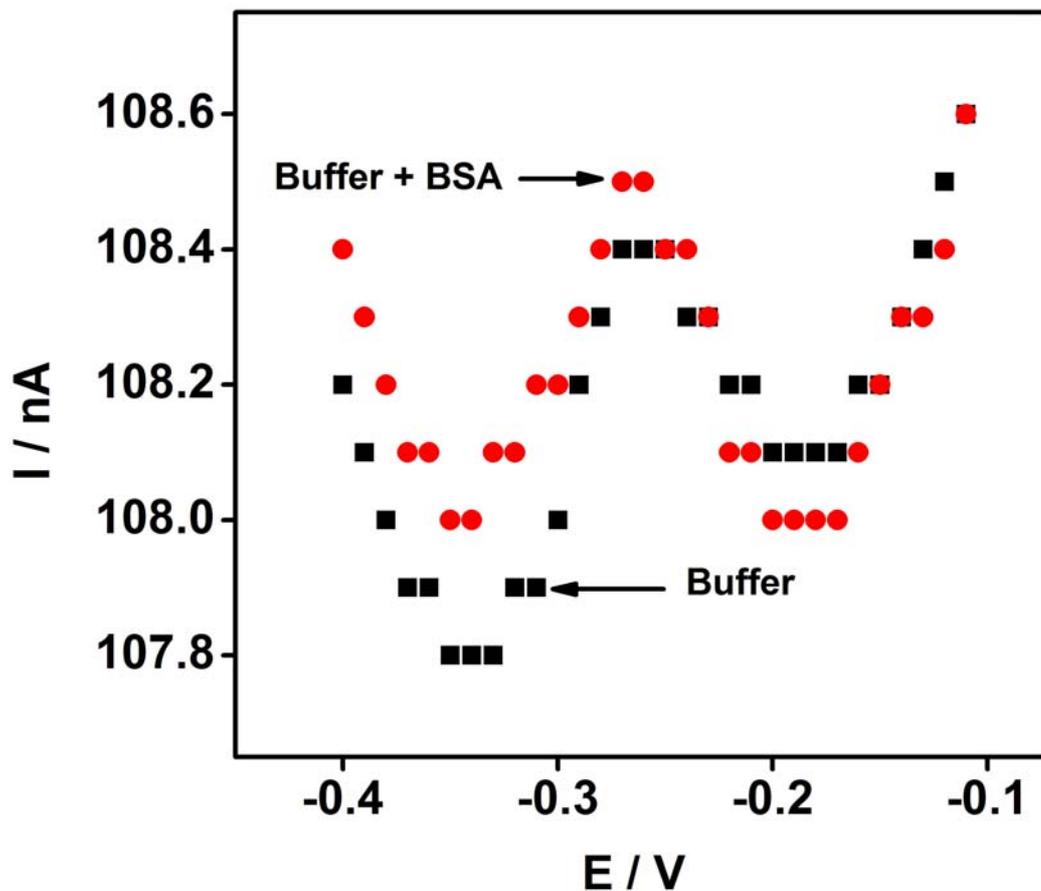


Fig. Y: The E-AB sensor made by thiolated control oligonucleotide (1) and its complementary MB-tagged DNA (2) does not exhibit any measurable signal change when challenged with BSA. Shown is the sensor electrochemical signal arising from thrombin-free buffered saline and from the same buffer doped with 0.3 μ M BSA.