Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Development of a "Signal-on" Electrochemical DNA Sensor with an Oligo-thymine Spacer for Point Mutation Detection

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MATERIALS AND METHODS

Probe and Target DNA Sequences

A thiol and methylene blue (MB)-modified linear oligonucleotide were used as the probe DNA (Biosearch Technologies, Inc., Novato, CA). The DNA probe was modified at the 5'-terminus with a C6-disulfide linker (HO-$(CH_2)_6$-S-S-$(CH_2)_6$-5'-DNA) and at the 3'-end with a MB redox label. The probe has two target binding domains (underlined), including an 8-base region at the 5'-end and a 9-base region at the 3'-end.

DNA Probe (MB-P): 5'- HS-C6-TTAGCTCCAAttttttttttTACGCCACC T-MB-3'

DNA Probe (MB-P-A10): 5'- HS-C6-TTAGCTCCAAaaaaaaaaaTACGCCACC T-MB-3'

13 target DNA sequences purchased from Integrated DNA Technologies (Coralville, IA) were used without further purification. The mid-sequence inserted bases are underlined and the mismatch bases are double underlined.

The sequences are as follows:

DNA Targets:

17-base target without adenine (A) or thymine (T) insertion (T-17):

5'-TTGGAGCTGGTGGCGTA-3'

18-base with 1A insertion (MA_1):

5'-TTGGAGCTAGGTGGCGTA-3'

18-base with 1T insertion (MT_1):

5'-TTGGAGCTTGGTGGCGTA-3'

19-base with 2A insertion (MA_2):

5'-TTGGAGCTAAGGTGGCGTA-3'

19-base with 2T insertion (MT_2):

5'-TTGGAGCTTTGGTGGCGTA-3'

20-base with 3A insertion (MA_3):

5'-TTGGAGCTAAAGGTGGCGTA-3'

20-base with 3T insertion (MT_3):

5'-TTGGAGCTTTTGGTGGCGTA-3'

21-base with 4A insertion (MA_4):

5'-TTGGAGCTAAAAGGTGGCGTA-3'

21-base with 4T insertion (MT_4):

5'-TTGGAGCTTTTTGGTGGCGTA-3'

22-base with 5A insertion (MA_5):

5'-TTGGAGCTAAAAAGGTGGCGTA-3'

22-base with 5T insertion (MT_5):

5'-TTGGAGCTTTTTTGGTGGCGTA-3'

Signal mismatch target (MA_2-1M):

5'-TTGGACCTAAGGTGGCGTA-3'

2-bases mismatch target (MA_2-2M):

5'-TTGGAGCTAAGCTGCCGTA-3'

Reagents

6-mercapto-1-hexanol (C6-OH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 3H_2O$), 8 M guanidine hydrochloride, and bovine calf serum were used as received (Sigma-Aldrich, St. Louis, MO). Synthetic stimulated human parotid saliva was purchased from US Biocontract Inc (San Diego, CA). All other chemicals were of analytical grade. All of the solutions were made with deionized water (DI) purified through a Synergy Ultrapure Water System (18.2 MΩ· cm, Millipore, Billerica, MA). The physiological buffer solution (Phys2) consisted of 20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ (pH 7.4).

E-DNA Sensor Fabrication

Prior to sensor fabrication, gold disk electrodes with a 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 DI water, and sonicated in a low-power sonicator for approximately 5 min 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 surface area of each electrode was estimated on the basis of the amount of charge consumed during the reduction of the gold surface oxide monolayer in 0.05 M H_2SO_4, and a reported value of 400 μC cm^{-2} was used for the calculation.[1]

The protocol used to fabricate the sensor on a gold-plated screen-printed carbon electrode (GPE) was similar to that shown in our previously published work.[2] Briefly, a gold film was electrodeposited on a screen-printed carbon electrode by holding the electrode (Pine Instrument, Grove City, PA) at -0.40 V vs. Ag/AgCl (3 M KCl) in a stirred gold solution (1.2 mg mL^{-1} $HAuCl_4$, 1.5 wt. % HCl and 0.1 M NaCl) for 20 min. The GPE was electrochemically cleaned by a series of oxidation and reduction cycles in 0.5 M H_2SO_4 and in 0.05 M H_2SO_4. The real area of the electrode was determined using the aforementioned method.[1]

For both sensor substrates (gold disk electrode and GPE), fabrication of the E-DNA sensor involved several steps. First, 1 μL of the 200 μM DNA probe solution was mixed with 1 μL of 10 mM TCEP; this solution was left at room temperature (~23^o C) for 1 hr to reduce the disulfide bond. The solution was diluted to 1 mL with a 10 mM phosphate buffer supplemented with 0.1 M NaCl (pH 7.4). The diluted probe solution (0.2 μM) was drop cast onto freshly cleaned gold electrode surfaces for 1 hr. The electrodes were then rinsed with water and subsequently passivated with 2 mM C6-OH overnight to displace nonspecifically bound oligonucleotides.

The density of electroactive DNA probes on the electrode surface, Γ^*, was determined by integration of charges under the reduction peak in the cyclic voltammetric (CV) scans collected at slow scan rates (equation 1).

$$\Gamma^* = Q/nFA \quad (1)$$

where Q is the integration of charges under the reduction peak in the CV scans, n is the number of electrons transferred per redox event (n = 2, MB), F is the Faraday current, and the A is the gold electrode area. Γ^* is given by the average values obtained at different scan rates (v = 20, 50 and 100 mV/s).

To calculate the electron transfer rate constant (k_s) for MB, a series of CV scans were collected. Increasing values of CV peak separation ($\Delta E_p = E_{p,a}-E_{p,c}$) as a function of increasing scan rate (v) reflects control of the voltammetry by the rate of heterogeneous electron transfer reactions of the MB labels in the monolayer. When $\Delta E_p > 200/n$ mV, a graph of ΔE_p versus log v yields a straight line which is in accordance with the Laviron equation (equation 2).[3]

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/nFv) - \alpha(1-\alpha)nF\Delta E_p/2.3RT \quad (2)$$

where k_s is the electron transfer rate constant (s^{-1}), α is the electron transfer coefficient, v is the CV scan rate (V/s), and ΔE_p is the difference between the anodic and cathodic potentials (V). α can be determined from the slope of the straight line, and k_s can be calculated with the help of the intercept.

Electron transfer kinetics was also determined using a previously developed AC method.[4] To obtain electron transfer rates for the sensor before and after target hybridization, the following fitting parameters were utilized: C_{dl} = 2.0 X 10^{-6} F cm^{-2}; solution resistivity = 5 Ω cm; temperature = 296 K; electrode area = 0.0314 cm^2.

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 (1-2000 Hz) using an amplitude of 25 mV. CV was also used to determine the surface probe coverage and electron transfer rate. 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 M KCl) electrode served as the reference electrode (CH Instruments, Austin, TX). Prior to

sensor interrogation, the modified electrodes were allowed to equilibrate in Phys2, 50% synthetic human saliva (with 50% Phy2) or 25% fetal bovine serum (with 75 % Phys2) for at least 20 min. After the addition of the target DNA, AC voltammograms were collected every 5 min until a stable peak current (i.e., signal saturation) was observed. Unless mentioned otherwise, the target concentration was1.0 μM in all the experiments in this study.

The ratio between the MB peak current in presence and absence of the target DNA was used to calculate the % signal enhancement (SE) (equation 3).

$$\text{Signal Enhancement (\%)} = [(I - I_0)/I_0] * 100 \quad (3)$$

where I is the baseline-subtracted peak current obtained in presence of the target, and I_0 is the baseline-subtracted peak current in the target-free solution.

Sensor regeneration was achieved by rinsing with DI water for 30 sec for sensors interrogated in Phys2 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 25% bovine calf serum or 50% synthetic human saliva.

Calibration curve results were obtained by sequential addition of the target DNA (i.e., MT_2 and MA_2) at an interval of 120 min. Concentrations used to obtain the calibration curves were 200 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM, and 1 μM. Both 600 and 800 Hz were used as the interrogation frequencies. Experiments were performed to determine the "noise" level in this system, which is the MB signal change within 120 min in the absence of the target. The %SE recorded when the sensor was with interrogated with 200 fM MT_2 or MA_2 was at least 3 times larger than that observed without the target within the same time period (120 min); 200 fM can thus be considered as the limit of detection. This criterion was met for both interrogation frequencies. Mechanical stirring was not used in any of the hybridization experiments. All experiments were performed at room temperature (~23^{o}C).

References

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[2] W. Yang, J. Y. Gerasimov, R. Y. Lai, *Chem. Commun.* **2009**, *20*, 2902.

[3] E. Laviron, *J. Electroanal. Chem.* **1979**, *101*, 19.

[4] S. E. Creager and T. T. Wooster, *Anal. Chem.* **1998**, *70*, 4257.

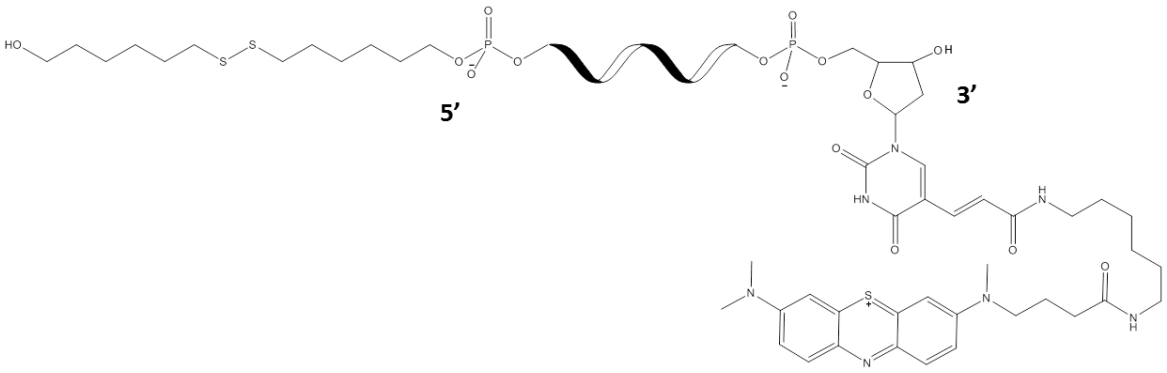

Figure S1. Structure of the dual-labeled DNA probes used in this study. The MB label is directly conjugated to an added thymine base.

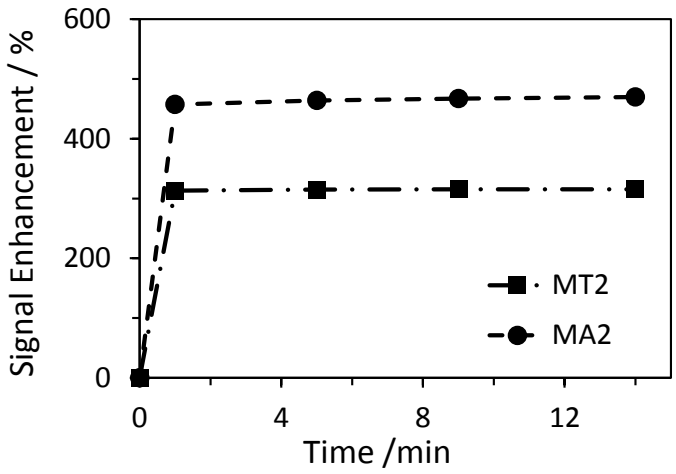

Figure S2. Binding kinetics data collected for both MT_2 and MA_2 at 600 Hz when MB-P-A10 was used. Independent of the probe, the A-series targets generally showed higher %SE when compared to the T-series targets.

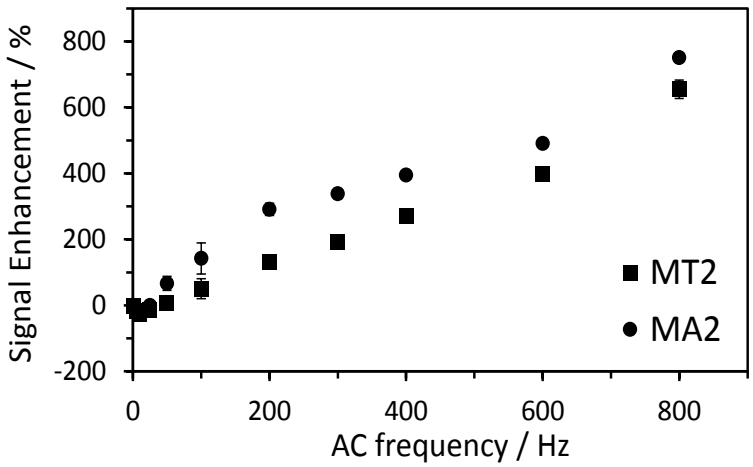

Figure S3. AC frequency-dependent %SE recorded for MT_2 and MA_2. Shown are data averaged from three different sensors.

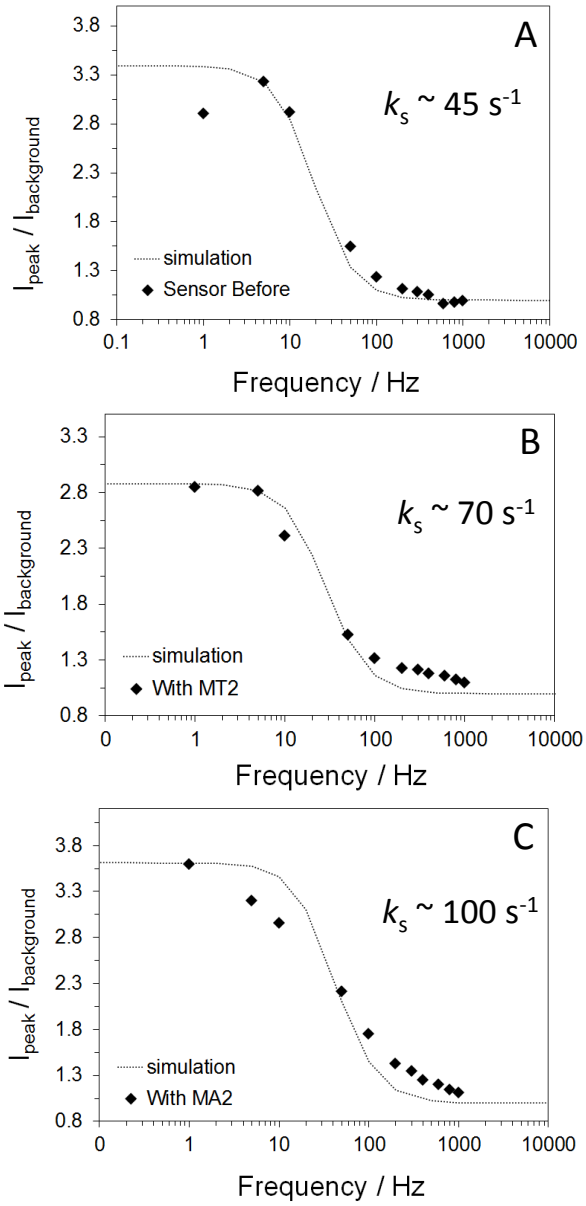

Figure S4. Shown are representative I_{peak} /$I_{background}$ *vs.* log (frequency) plots for the sensor before (A) and after hybridization with 1.0 μM MT_2 (B) and MA_2 (C). While the fit of the data is poor (precluding the determination of precise electron transfer rates), the discrepancy in the inflection points of the three curves indicates that electron transfer is faster in the presence of the target DNA, in particular, MA_2. These values, in general, agree with the results obtained using the Lavion method.

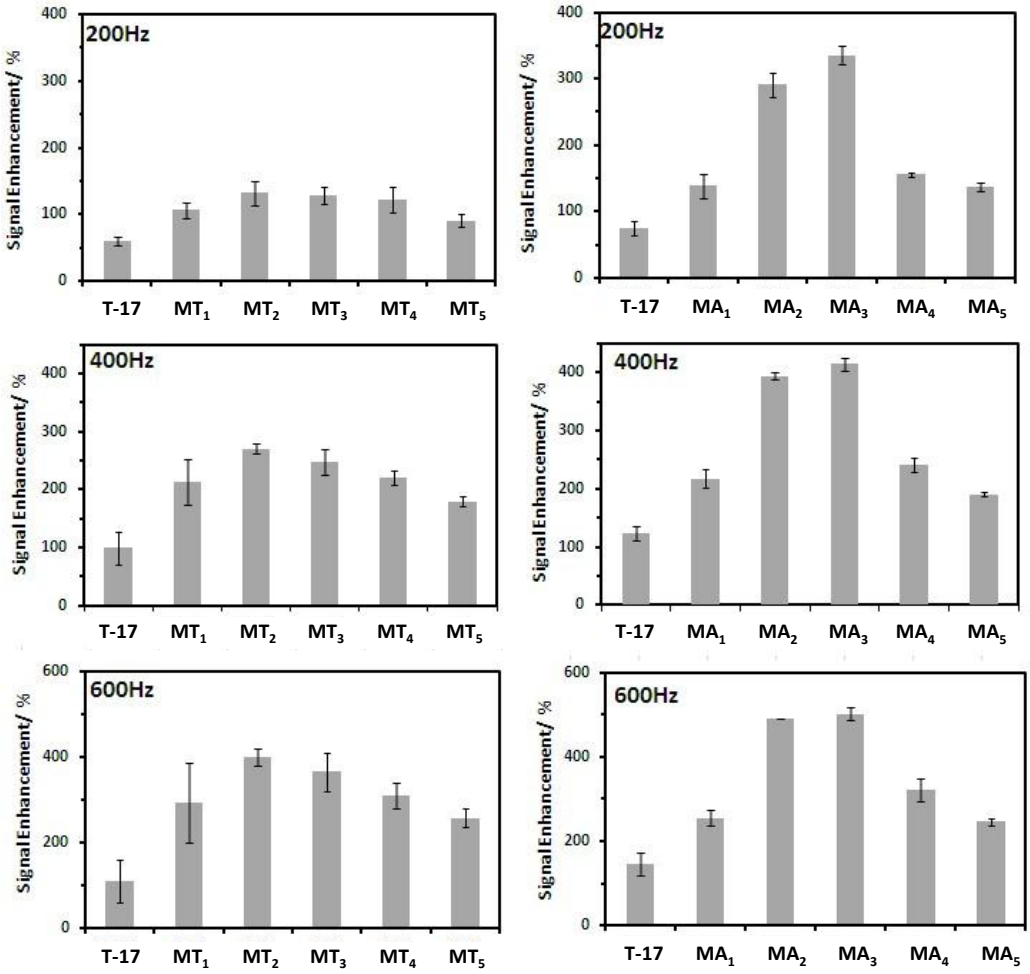

Figure S5. %SE recorded for T-17 and five other targets with varied number of mid-sequence thymine (MT_n) (left) or adenine (MA_n) (right) insertion under 3 different interrogation frequencies. Each sensor was used to interrogate all six targets, including T-17 and the five T- (left) or A-series (right) targets. Shown are data averaged from three different sensors. Among all the targets used in this study, targets with two or three bases (A or T) (i.e., MA_2, MA_3, MT_2, MT_3) inserted between the two binding domains of the probe showed the largest %SE, whereas the target without any nucleotide insertion (T-17) showed the smallest %SE.

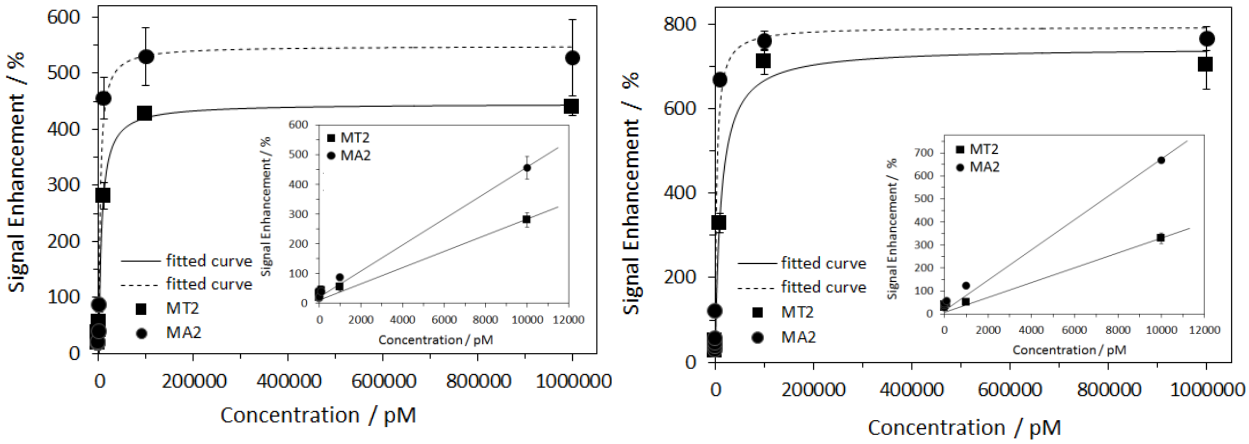

Figure S6. Dose-response curves for MT_2 and MA_2 collected in Phys2 at 600 Hz (left) and 800 Hz (right). Inset figures show the sensor's response in the presence of lower target concentrations. Shown are data averaged from three different sensors.

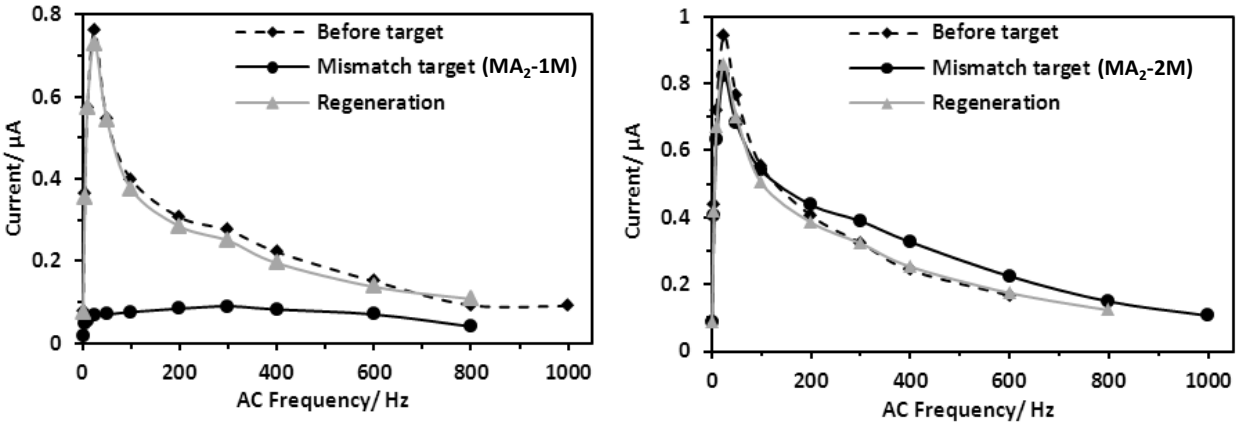

Figure S7. AC frequency-dependent MB peak current profiles collected before, after hybridization to 1.0 µM MA_2-1M (left) and MA_2-2M (right), and after sensor regeneration. Hybridization to MA_2-1M, a single-base mismatch target, resulted in a "single-off" behavior under most interrogation frequencies. The sensor showed minimal change in MB current after hybridization to MA_2-2M, a two-base mismatch target.

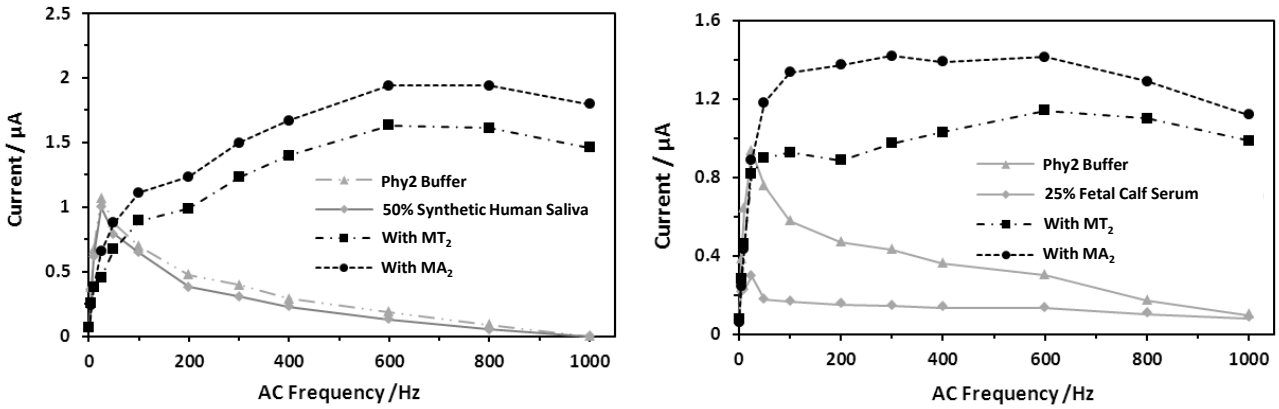

Figure S8. AC frequency-dependent MB peak current profiles collected in 50% synthetic human saliva (left) and 25% fetal calf serum (right) in presence of 1.0 µM MA_2 and MT_2. For comparison, frequency-dependent profiles collected in Phys2 are also included. As observed, the profile collected in 50% salva is similar to that observed in Phy2; whereas the profile obtained in 25% serum is different. The pre-hybridization MB current was significantly lower when serum was used, which could be attributed to the viscosity of the serum and the differences in the ionic strength. Despite this difference, the sensor is fully functional in both media, as evidenced by the similarity in the post-hybridization frequency-dependent profiles to those obtained in a pure buffer.

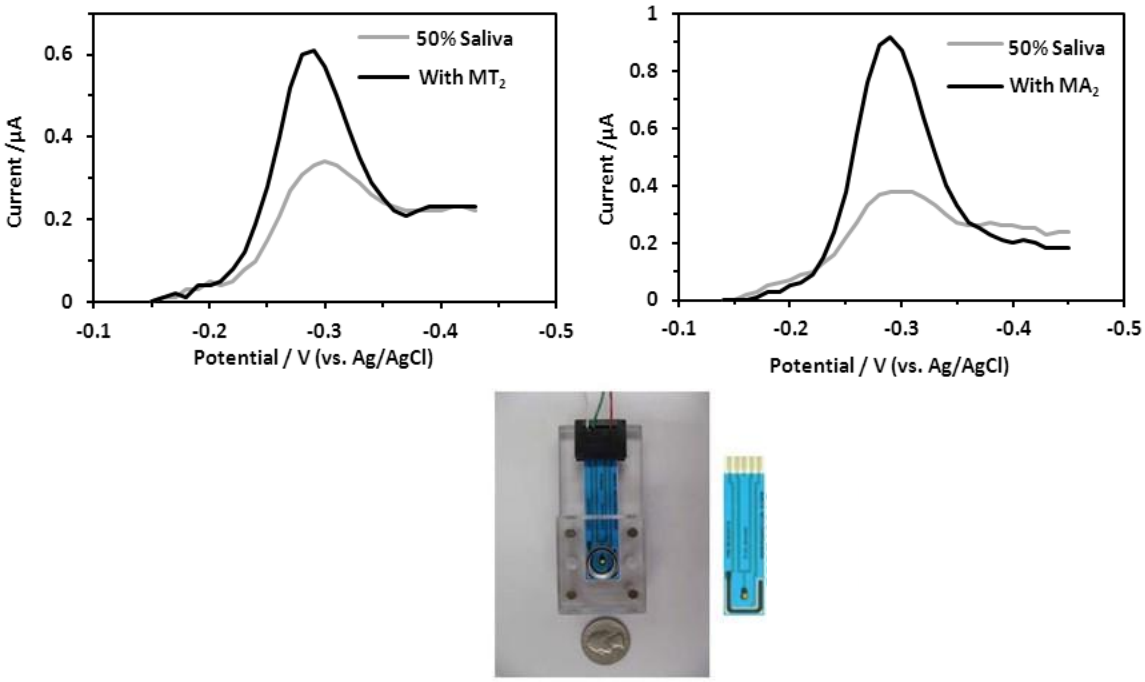

Figure S9. This DNA sensor can also be used with our previously developed gold-plated screen-printed carbon electrodes. Shown on the left are the AC voltammograms of the sensor before and after hybridization with 1.0 µM MT_2. Shown on the right are the voltammograms obtained with 1.0 µM MA_2 under the same experimental condition. The scans were collected at 300 Hz in 50% synthetic human saliva. Also included are the pictures of the disposable strip electrode and the homebuilt electrochemical cell used in this experiment.

MT_n (n = 1-5)	$k_s(s^{-1})$	**MA_n (n= 1-5)**	$k_s(s^{-1})$
Sensor Before	32.9 (±2.5)	Sensor Before	32.9 (±2.5)
T-17	28.2 (±1.4)	T-17	28.2 (±1.4)
MT_1	45.6 (±1.1)	MA_1	75.5 (±3.4)
MT_2	74.6 (±1.8)	MA_2	119 (±0.3)
MT_3	74.6 (±0.6)	MA_3	145 (±1.6)
MT_4	66.0 (±1.1)	MA_4	147 (±2.8)
MT_5	59.7 (±1.1)	MA_5	114 (±3.2)

Table S1. Shown are the electron transfer rate constants (k_s) for MB before and after hybridization to 1.0 µM T-17, MT_n and MA_n (n = 1 -5) in Phys2. The k_s value for T-17 is lower than that observed without target is because at lower AC frequencies, hybridization to T-17 actually resulted in a "signal-off" sensor. Signal enhancement was only observed at higher frequencies, such as those used to obtain the results in Figure S3. It is likely that some T-17 targets bind to either the 8-base region at the 5'-end or the 9-base region at the 3'-end of the probe due to steric hindrance, which could result in either the lack of signal change or "signal-off" sensor behaviour. In particular, binding to the 9-base region at the 3'-end hinders collisional electron transfer to MB, thereby contributing to the slight decrease in the calculated k_s.