Supplementary Information (SI) for Sensors & Diagnostics.
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Supplementary Information

for

A Nucleic Acid-Based Electrochemical Detection Method for Post Hoc Sample Analysis

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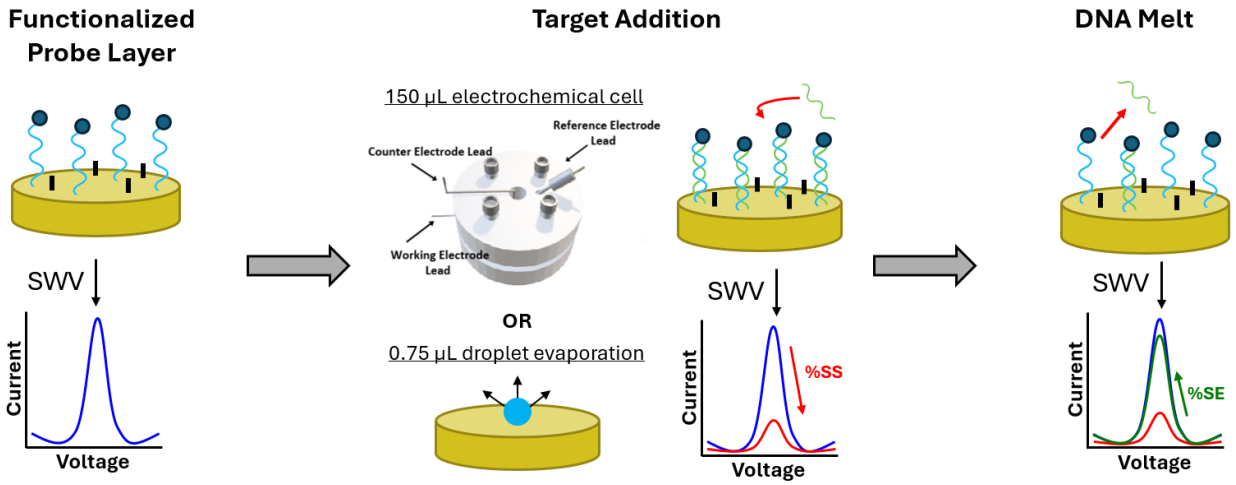

Figure S1. Pictorial representation of the experimental protocol. Nanoporous gold (np-Au) and planar gold (pl-Au) electrodes are cleaned in piranha solution and functionalized with 26-mer probe DNA followed by mercaptohexanol back-fill. A square wave voltammetry (SWV) measurement of the probe DNA layer provides the baseline for the subsequent measurements to determine target DNA presence. Target DNA is added onto the electrode via either a 150 µL solution for a standard electrochemical cell or via a 0.75 µL droplet for evaporation on electrode surface. A SWV measurement after this step produces the signal from the hybridized layer. A resulting percent signal suppression (%SS) can be calculated to quantify the amount of target captured by the immobilized probe DNA layer. Finally, the electrode with the duplex DNA layer is exposed to warm deionized water to melt off the target DNA. The subsequent SWV measurement recovers nearly the entire original baseline signal and thus can be used to quantify the amount of target captured via a new metric, referred to as percent signal enhancement (%SE).

Experimental Details

Materials. Glass coverslips (22 mm x 22 mm x 0.15 mm) from Electron Microscopy Science, USA, were used as the substrates for the gold films. Gold, silver, and chrome targets (99.95% pure) were purchased from Kurt J. Lesker for physical vapor deposition. A homemade 25 mM phosphate buffer (PB) solution was used which consisted of nuclease free water, sodium phosphate monobasic monohydrate, and sodium phosphate dibasic 7-hydrate purchased from Ambion Life Technologies Corporation, USA, Sigma-Aldrich, USA, and JT Baker, USA, respectively. Other functionalization chemicals included Tris(2-chloroethyl)-phosphate (TCEP) 1x phosphate-buffered saline (PBS), and magnesium chloride ($MgCl_2$) from Fisher Scientific, USA, and mercaptohexanol (MCH) from Sigma-Aldrich, USA. Desalting columns with 7 kDa molecular weight cut-off (Zeba Spin #89882) were used for DNA preparation. Fetal bovine serum (FBS) was used for detection in complex media and purchased from Life Technologies, USA. Synthetic oligonucleotides were purchased from Integrated DNA Technologies (IDT) for the probe and target nucleic acids. The probe DNA had a covalently attached MB redox tag at the 3' terminal and the 5' end was modified with a thiol group C6 spacer. The 26-base pair sequences are listed below:

26mer Probe: /5ThioMC6-D/ CG TGT TAT AAA ATG TAA TTT GGA ATT /3MeBlN/

26mer Target: AAT TCC AAA TTA CAT TTT ATA ACA CG

Electrode Fabrication. Planar gold (pl-Au) and nanoporous gold (np-Au) chips were fabricated in a Class 100 clean room. The glass slides were cleaned in piranha solution (1:4 ratio of 30% hydrogen peroxide to 96% sulfuric acid) for ten minutes, rinsed thoroughly with DI water and dried under a nitrogen stream. To prevent delamination, an adhesion layer of chrome was deposited via sputtering (160 nm for np-Au and 50 nm for pl-Au). For pl-Au chips, gold is directly sputtered onto the chrome layer with a target thickness of 250 nm. For np-Au chips, an 80 nm-thick gold intermediate layer is sputtered onto the chrome later followed by a Au-Ag alloy deposition by co-sputtering silver and gold. The final alloy composition was 64% Ag / 36% Au (atomic %) with a target film thickness of 500 nm. The silver atoms are then selectively removed by immersing the sample in 70% nitric acid at 55 °C for 15 minutes. The samples are rinsed once more with deionized water and cut into four 11 mm x 11 mm pieces with a diamond scribe. CAUTION: Piranha solution and nitric acid are highly corrosive and reactive with organic materials and must be handled with extreme care.

SEM Characterization. A scanning electron microscope (SEM; Thermo Fisher Scientific Apreo S LoVac) was used to obtain high magnification images of the film surface. The surfaces were interrogated at 100 kX and 200 kX magnification to assess nanoscale morphological features. The images were analyzed using ImageJ to calculate average pore size and coverage. Our np-Au films had an average pore radius nm with a total pore coverage of 23%.

Probe DNA preparation. The methylene-blue tagged probe DNA arrived from the vendor in the disulfide form. To regenerate the free thiol group, the DNA was incubated with 0.5 M TCEP for 3 hours in an opaque PCR centrifuge tube. The mixture was filtered using a desalting column, centrifuging at 4,000 RPM for 2 minutes.

Surface functionalization with probe DNA. The electrodes were cleaned with piranha solution for 20 seconds followed by plasma cleaning for 30 seconds prior to probe functionalization. The thiolated DNA was diluted to 0.5 μM in a solution consisting of 75 mM $MgCl_2$ and 25 mM PB. Each electrode was treated with 150 μL of the functionalization solution and placed into a sealed petri dish overnight (at least 15 hours) at room temperature. The chips were rinsed with PB to remove non-specifically bound probe molecules and dried in air. The washed electrodes were then treated with 150 μL of 2 mM MCH for 5 hours to passivate the surface not covered by probe DNA and to form a well-ordered DNA monolayer. The electrodes were then rinsed with PB and stored in PB refrigerated to 4 °C until use.

Target DNA hybridization. Probe functionalized chips were challenged with the target DNA using two approaches. In the first approach, the probe functionalized chip was assembled into a homemade Teflon electrochemical cell (shown in Figure S1). To introduce the target DNA molecules onto the chip surface, the electrochemical cell was filled with 150 μL of target-spiked solution and incubated for 45 minutes. In the second approach, the probe functionalized chip was challenged with a 0.75 μl droplet of target-spiked solution and left to evaporate. In both approaches PBS and 10% FBS in PBS were used to compare detection in buffer versus physiologically complex media.

Electrochemical Measurements. A BioLogic SP-300 potentiosat and EC-lab software was used for electrochemical measurements. Standard square wave voltammetry techniques were used to investigate the reduction of MB which indicates DNA immobilization or hybridization. The SWV parameters were as follows:

- E_i = 0 V vs ref for t_i = 30 s (set initial voltage to 0 and equilibrium time to 0)
- Scan E_{we} from E_i to E_f = 0.5 vs. ref.
- Pulse height P_h = 40 mV
- Pulse width P_w = 16.6 ms (60 Hz for pl-Au) or 55.56 ms (18 Hz for np-Au)
- Step height = 4 mV
- Average over last 0% of each step
- Reverse scan for E_f = 0 V (captures both the oxidation and reduction peak for MB)

DNA melting. Probe functionalized chips that have already been challenged with the target DNA were assembled into the custom Teflon electrochemical cell. 150 μL of DI water heated to 70 °C was added to the electrochemical cell and incubated for 15 minutes. If using np-Au chips, melted target DNA are likely ‘trapped’ inside the pores. Electrically ‘push’ the target out of the pores by applying a -0.5 V potential to the working electrode for 10 minutes. Then the cell was thoroughly washed with PBS to remove all cleaved DNA.

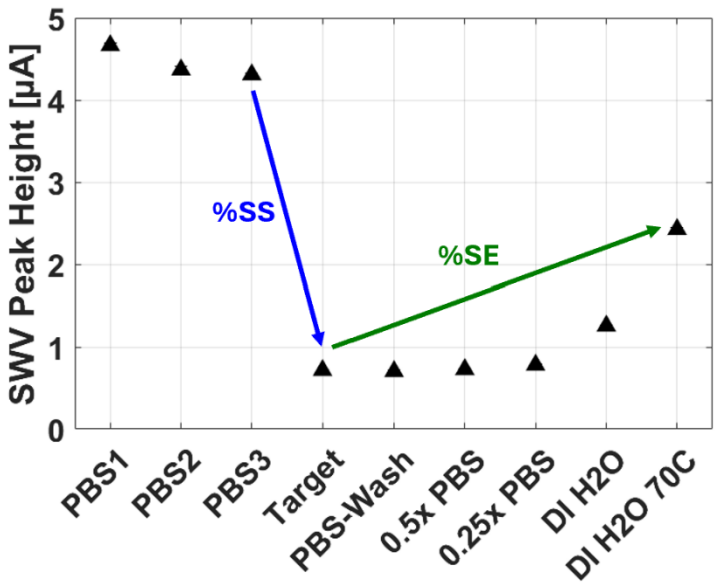

Figure S2. Representative peak SWV current of a probe DNA-functionalized pl-Au electrode undergoing various treatments. The first three steps represent three iterations of SWV measurements of the probe layer in PBS (PBS1, PBS2, PBS3). Then, an 83% signal suppression is observed after treating the electrode with 2 µM target DNA suspended in PBS for 45 minutes (Target condition). After hybridization, the electrode is successively treated with solutions of progressively lower ionic strength (i.e., reduced concentrations of PBS) to promote DNA melting (PBS-Wash, 0.5x PBS, 0.25x PBS, and DI H_2O). The SWV peak current increases once the ionic concentration is zero (i.e., DI H_2O) and the addition of heat (warm water) facilitates DNA melting (DI H_2O 70°C). A 70% signal enhancement is observed for this representative experiment.

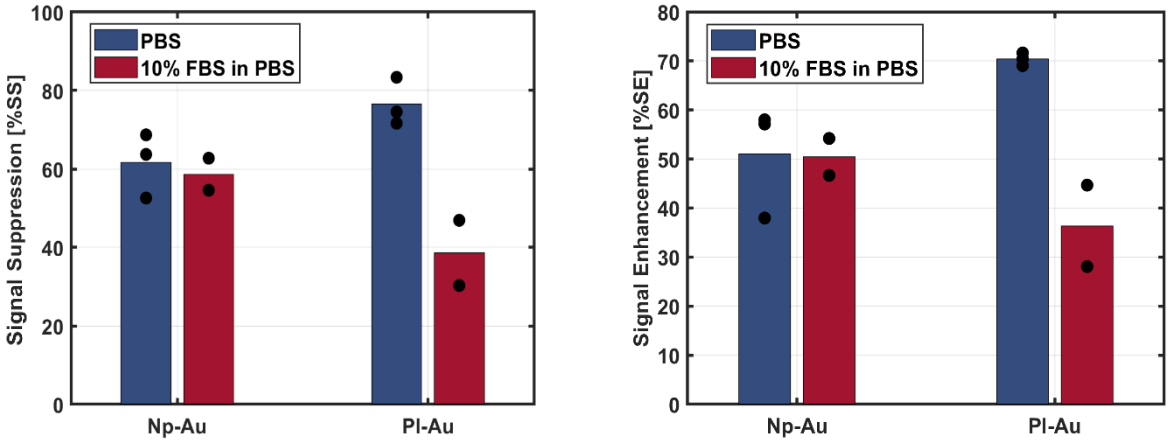

Figure S3. Comparison of np-Au and pl-Au electrode performance using the paired %SS and %SE metrics from the same experiments. These results support the utility of %SE metric and suggest that the np-Au coating with its biofouling resilience is a suitable candidate for electrochemical detection in complex biological media. Moreover, this comparison suggests that the traditional %SS metric is representative of how the sensor would perform with the new %SE metric, since the foundational biomolecular capture mechanism is the same.

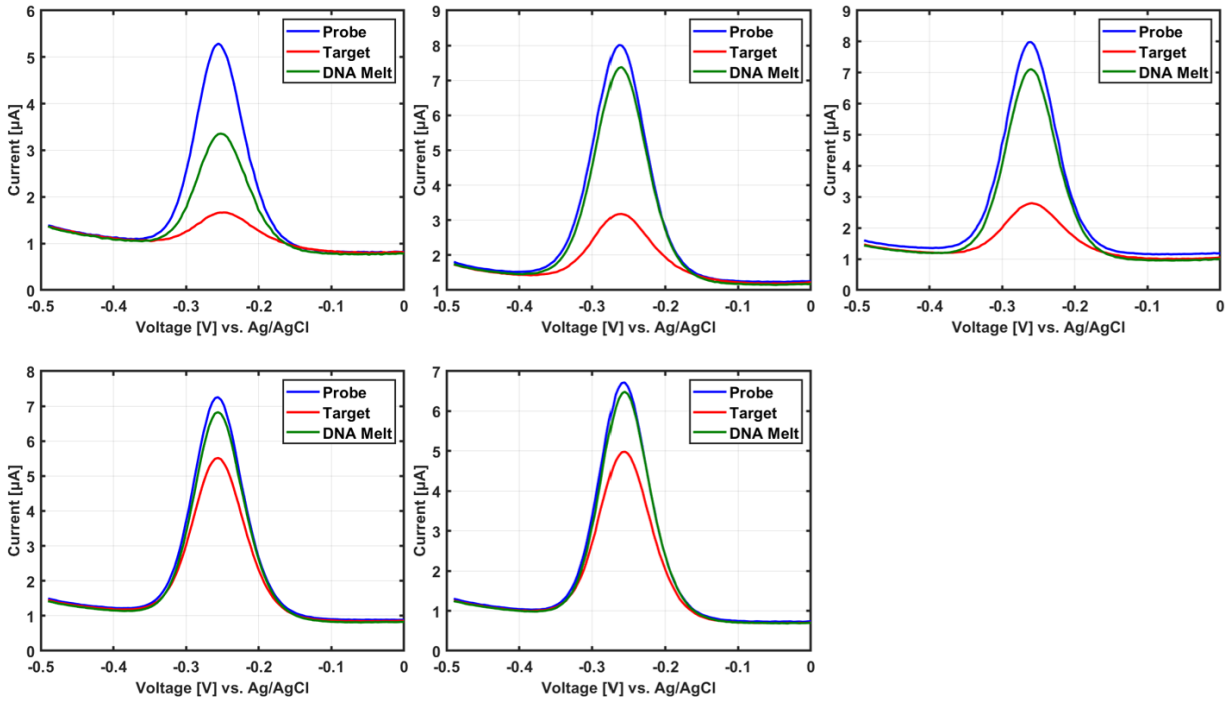

Figure S4. Raw square wave voltammograms from data in Figure 2 in main manuscript. The blue curves represent a SWV measurement on the probe layer of a pI-Au electrode. The red curves represent the measurement after 45-minute incubation in PBS with 2 µM (top row) or 100 nM (bottom row) target DNA concentration. The green curve represents the successive measurement after the DNA melting procedure is complete.

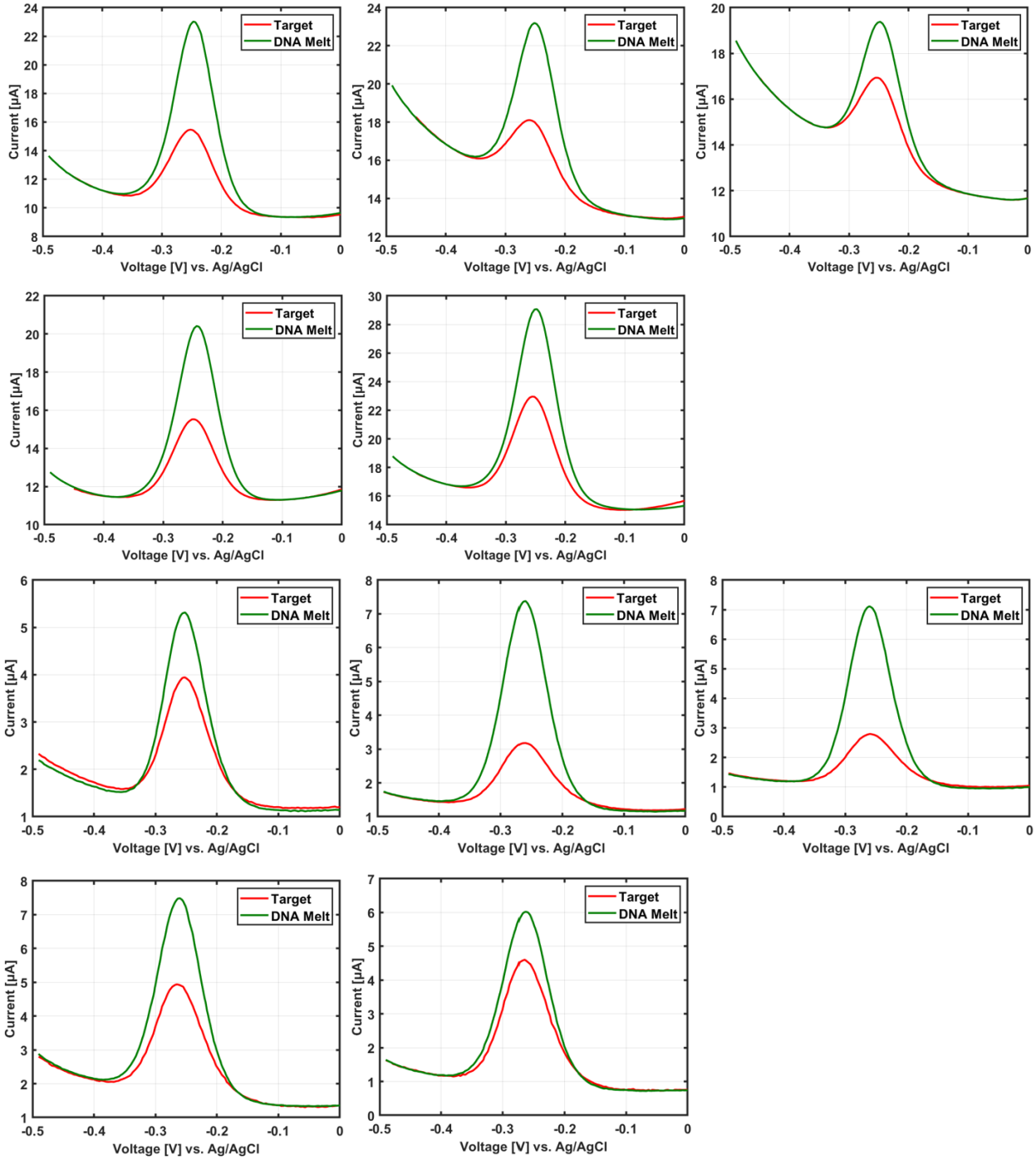

Figure S5. Raw square wave voltammograms from data in Figure 3 in main manuscript. The red curves represent the SWV measurement after 45-minute incubation in media (PBS or 10% FBS) with 2 µM target DNA concentration. The green curve represents the successive measurement after the DNA melting procedure is complete. Row 1 represents np-Au electrodes in PBS, Row 2 represents np-Au electrodes in 10% FBS, Row 3 represents pl-Au electrodes in PBS, and row 4 represents pl-Au electrodes in 10% FBS.

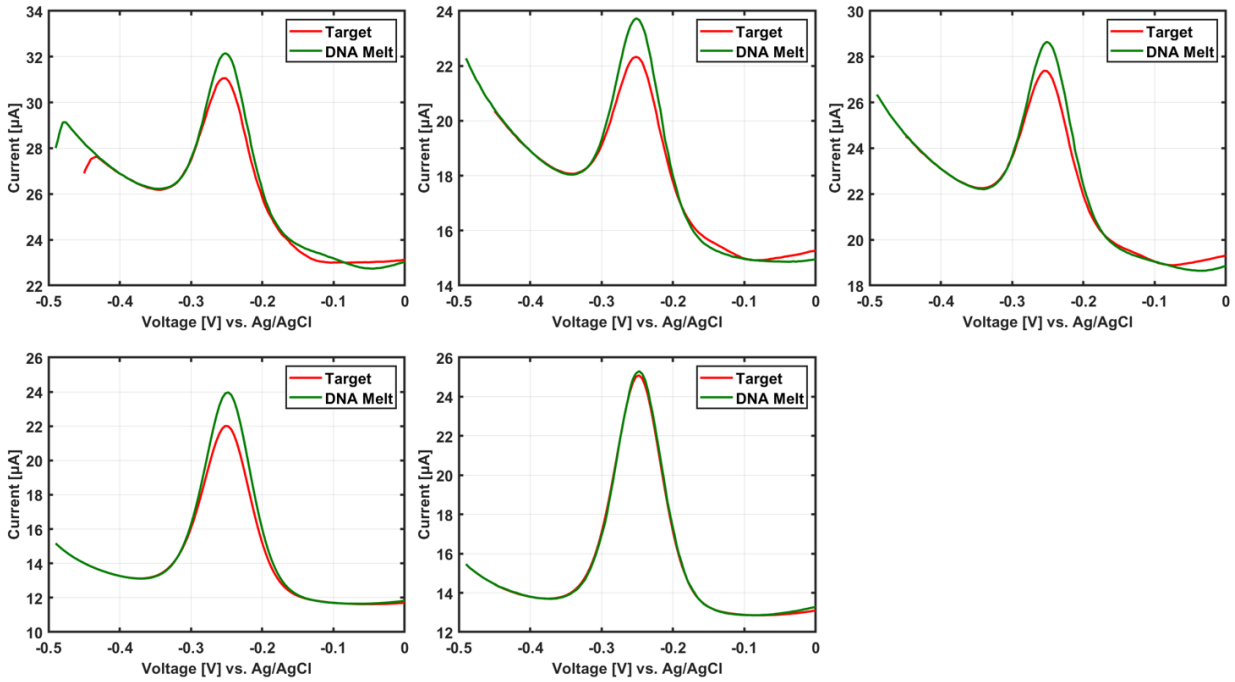

Figure S6. Raw square wave voltammograms from data in Figure 4 in main manuscript. The red curves represent the SWV measurement after a 0.75 μL droplet in media (PBS or 10% FBS) with 2 μM target DNA concentration was evaporated on the substrate surface. The green curve represents the successive measurement after the DNA melting procedure is complete. Row 1 represents np-Au electrodes in PBS, Row 2 represents np-Au electrodes in 10% FBS.