Supporting information for the article:

Fabrication of Aptamer-Modified Paper Electrochemical Devices for On-site Biosensing

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EXPERIMENTAL SECTION

Materials. Gold (III) chloride trihydrate, trisodium citrate dihydrate, Triton X-100, sodium chloride, potassium chloride, silver nitrate, magnesium chloride, L-ascorbic acid, tris(hydroxymethyl)aminomethane (Tris base and HCl), Tris(2-carboxyethyl) phosphine (TCEP), 6-mercapto-1-hexanol, monosodium hydrogen phosphate, sodium dihydrogen phosphate, methylene blue, potassium hexacyanoferrate (III), potassium ferrocyanide, cocaine hydrochloride, sulfuric acid (95-98%), nitric acid (70%), hydrochloric acid (36.5-38%), and calf serum were purchased from Sigma-Aldrich. 3,4-methylenedioxypyrovalerone (MDPV) was purchased from Cayman Chemicals. Mixed cellulose ester filter papers (pore size: 100 nm, diameter: 47 mm, thickness: 105 μm) were purchased from Millipore. Polyethylene terephthalate film (laminating sheets made by AmazonBasics, thickness = 76.2 μm) was purchased from Amazon. Bare gold slides (Au/Ti-coated microscope slides, Au layer thickness 50±5 nm) were purchased from Ted Pella. Bemis Parafilm was purchased from Thermo Scientific. Pure single-walled carbon nanotube (SWCNT) solutions (0.25 mg/mL) (diameter range: 1.2-1.7 nm, length range: 0.3 to 5 microns) were purchased from NanoIntegris. All oligonucleotides were synthesized by Biosearch Technologies and dissolved in PCR-grade water. Aptamer sequences used in this work are listed below (SH-C_6 = 6-carbon thiol linker, MB = methylene blue):
Cocaine aptamer: 5'-SH-C_6-AGACAAGGAAAATCCTTCAATGAAGTGGGTCT-MB-3'
MDPV aptamer: 5'-SH-C_6- ACCTTAAGTGGGGTTCGGGTGGAGTTTATGGGGT-MB-3'

Instrumentation. DNA concentrations were determined using a NanoDrop 2000 (Thermo Scientific). All stencils were created using the Silhouette Portrait 2 Electronic Cutting Tool (Silhouette America) purchased from Amazon. Stencil lamination was performed using an AmazonBasics Thermal Laminator Machine purchased from Amazon. Electrochemical measurements were performed on a CHI 760D series electrochemical system. Transmission electron microscopy images were obtained using a Philips CM200 TEM. Photographs and videos were taken using a Nikon D750 camera.

Synthesis of metallic nanoparticles. Before the synthesis of any metal nanoparticles, all glassware were incubated with aqua regia solution (HNO_3:HCl at a 1:3 molar ratio) overnight and then washed with deionized water to remove the acids.

A. Gold nanoparticles (AuNPs): AuNPs were synthesized following a modified Turkevich method.[1] 50 mL of 0.01% $HAuCl_4$ solution (w/v) was heated until boiling with a reflux setup. Then, 5 mL of 1% sodium citrate solution (w/v) was added quickly with vigorous stirring. The solution was boiled for 30 min and then cooled slowly to room temperature. The prepared AuNPs were filtered through a 0.22-μm syringe filter (Millipore).

B. Silver nanoparticles (AgNPs): AgNPs were synthesized following a previously reported protocol.[2] 47.5 mL of distilled water was heated to boiling with a reflux setup. Then 50 μL of 1.76% L-ascorbic acid (w/v) was added. Afterwards, 2 mL of 1% sodium citrate solution (w/v) and 0.5 mL of 1% $AgNO_3$ (w/v) were mixed with 2.5 mL of distilled water drop-by-drop and the mixture was incubated for 4 min at room temperature. 2.5 mL of the mixture was then added into the boiling L-ascorbic acid solution quickly using a 5 mL syringe. The color of the solution slowly turned from colorless to grayish yellow, indicating the formation of AgNPs. The mixture was boiled for another 60 min. After cooling to room temperature, the particle solution was filtered using a 0.22 μm syringe filter (Millipore).

Preparation of polyethylene terephthalate stencils. Polyethylene terephthalate film was used to prepare the stencils and masks employed in this work based on a procedure from a published work with modification.[3] The film was cut into squares the size of A4 paper. To make bottom stencils, the Silhouette Portrait 2 Electronic Cutting Tool was used to respectively cut the film into the pattern of bottom stencil 1 (**Supporting Information** (**SI**)**, Fig. S1A**) and 2 (**SI, Fig. S1B**). For the top stencil and bottom mask, the rough side of the film was first thermally laminated (130 ˚C, ~2 cm/sec) with one layer of parafilm to facilitate adhesion to the filter paper. The parafilm-laminated film was then cut into the top stencil (**SI, Fig. S1C**) and bottom mask (**SI, Fig. S1D**) patterns.

Preparation and characterization of bare PEDs. The fabricated PED comprised a three-electrode-patterned filter paper between PET-parafilm films (**SI, Fig. S3**). Vacuum filtration was used to fabricate PEDs on mixed cellulose ester filter paper. First, the filter paper was laminated with the top stencil (diameter = 47 mm). The laminated filter paper was then placed onto a glass-fritted Buchner funnel and moisturized using deionized water. 10 mL of 5 μg/mL SWCNT solution dispersed in 1% Triton X-100 was added onto the filter paper under vacuum with a flux of 0.29 mL cm^{-2} min^{-1} to form a thin SWCNT underlay on the filter paper, followed by five washes with 10 mL of distilled water to remove Triton X-100 while still under vacuum. Bottom stencil 1 was then placed underneath the SWCNT-laden filter paper, and 10 mL of 0.35 nM AuNP solution was added onto the filter paper under vacuum with a flux of 0.77 mL cm^{-2} min^{-1} to form a reflective gold film that serves as working and counter electrodes as well as electrical connections. Afterwards, bottom stencil 1 was removed, bottom stencil 2 was applied underneath the filter

paper, and 50 µL of 6.9 nM AgNP solution was pipetted under vacuum to the filter paper area designated for the reference electrode. This step was repeated three times to form the AgNP pseudo-reference electrodes. Four PEDs were fabricated on each filter paper. The paper was dried at room temperature overnight. After complete drying, the bottom mask was laminated onto the filter paper and heated at 65 ℃ in an oven for 5 min to melt the parafilm underneath the top and bottom masks. This effectively sealed any unexposed areas on and within the PED and established a rigid hydrophobic support that protected the electrodes from water to avoid a short circuit. The fabricated device is five-fold thicker (0.506 mm) than the bare filter paper substrate (0.100 mm). **Movie S1** shows the physical attributes and rigidity of our fabricated PEDs. Finally, the four PEDs were cut out from the paper and used for further electrochemical experiments. Characterization of electron transfer rate for the prepared PEDs and bare gold slides was performed with 1 mM $K_3[Fe(CN)_6]$ or 1 mM methylene blue in 0.1 M KCl solution.

Modification of aptamers on PEDs and electrochemical measurements of aptamer-based PEDs. Bare PEDs must be modified with aptamers to achieve detection of specific targets. Prior to modifying the PED with aptamers, the working electrode was electrochemically cleaned in 0.05 M sulfuric acid for 10 cycles of cyclic voltammetry with a range of 0.2 to 1.5 V at a scan rate of 100 mV/s (a commercial platinum counter electrode (CHI) and Ag/AgCl reference electrode (CHI) were used for this step).[4] Afterwards, these electrodes were washed with distilled water and dried using nitrogen gas. Next, 482 pmol of thiolated, methylene blue-modified cocaine aptamer or 343 pmol of thiolated, methylene blue-modified MDPV aptamer were each mixed with 100-fold excess TCEP and incubated for 2 h to reduce the disulfide bonds of the thiolated aptamers. Then, the reduced aptamer was diluted 200-fold with 10 mM phosphate-buffered saline (pH 7.2) containing 1 M NaCl and 1 mM $MgCl_2$. 4 pmol of reduced cocaine aptamer or 2 pmol of MDPV aptamer (20 µL) was applied on the working electrode surface and incubated for 2 h. The working electrodes were washed with distilled water and then backfilled with 20 µL of 3 mM 6-mercapto-1-hexanol at room temperature for 1 h. The modified PEDs were finally washed with distilled water and used to detect cocaine (0–600 µM) or MDPV (0–100 µM) in buffer (10 mM Tris (pH 7.4), 20 mM NaCl, 0.5 mM $MgCl_2$) as well as 50% saliva, serum and urine using square wave voltammetry. Electrochemical measurements were performed by immersing the PEDs in a 200 µL home-made electrochemical cell, such that all three electrodes were completely submerged in the sample.

References:

[1] J. Turkevich, P. C. Stevenson, J. Hillier, Discuss. Faraday Soc. 1951, 11, 55–75.
[2] H. Li, H. Xia, D. Wang, X. Tao, Langmuir 2013, 29, 5074–5079.
[3] Y. Lu, Z. Shi, L. Yu, C. M. Li, RSC Adv. 2016, 6, 85468–85472.
[4] Y. Xiao, R. Y. Lai, K. W. Plaxco, Nat. Protoc. 2007, 2, 2875–2880.

Table S1. Limits of detection of aptamer-modified PEDs and gold disk electrodes for detection of MDPV and cocaine in buffer and biosamples.

Target	PED LOD (µM)	Gold disk electrode LOD (µM)	Sample matrix
MDPV	0.1	0.1	Buffer
	1	1	50% Serum
	1	0.5	50% Urine
Cocaine	1	1	Buffer
	5	5	50% Serum
	5	5	50% Saliva

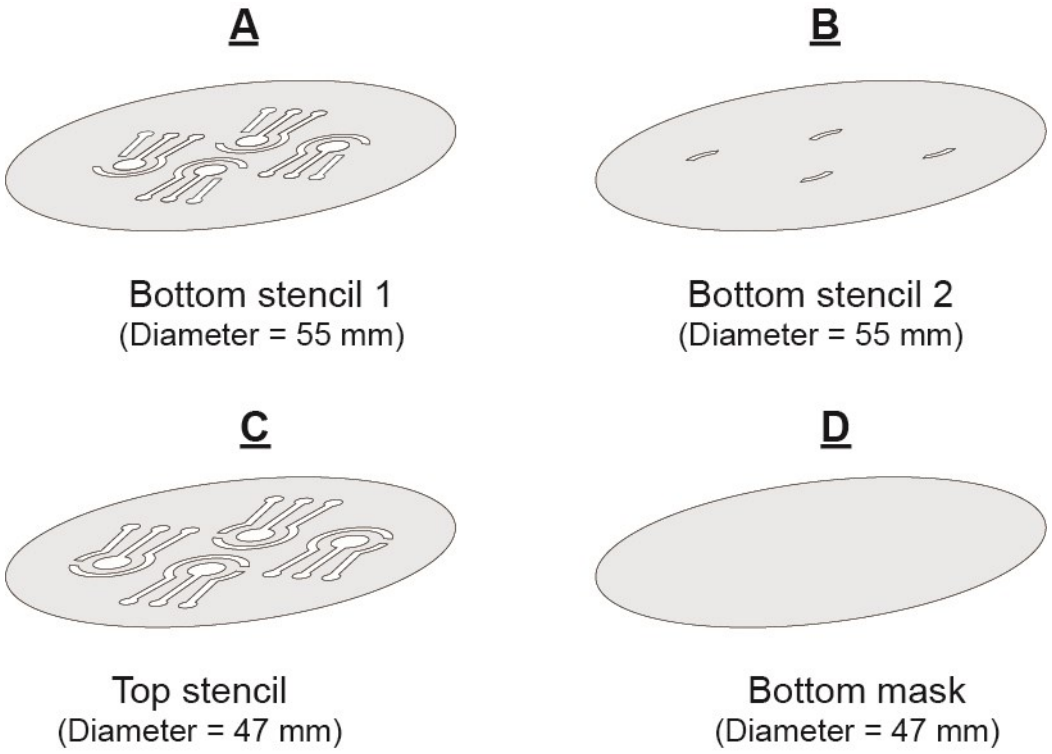

Figure S1. Design and diameter of the stencils and mask used to fabricate the PEDs described in this work: (**A**) bottom stencil 1; (**B**) bottom stencil 2; (**C**) top stencil and (**D**) bottom mask.

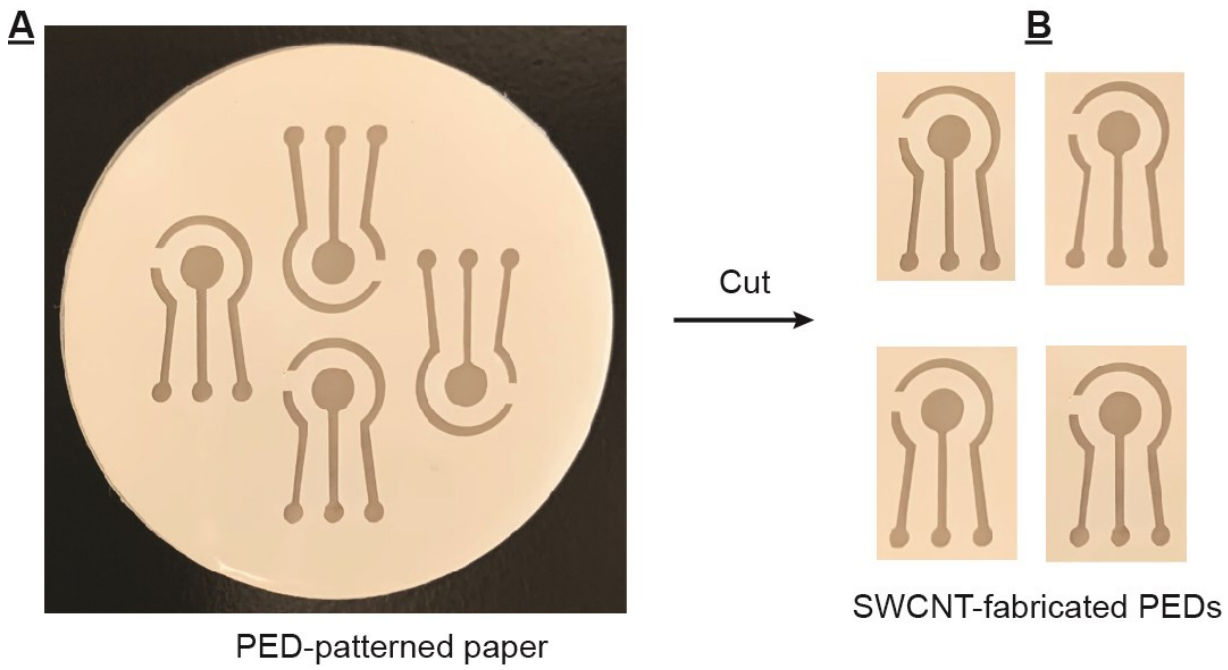

Figure S2. Photograph of (**A**) a filter paper fabricated with single-walled carbon nanotubes (SWCNTs) containing (**B**) four individual PEDs.

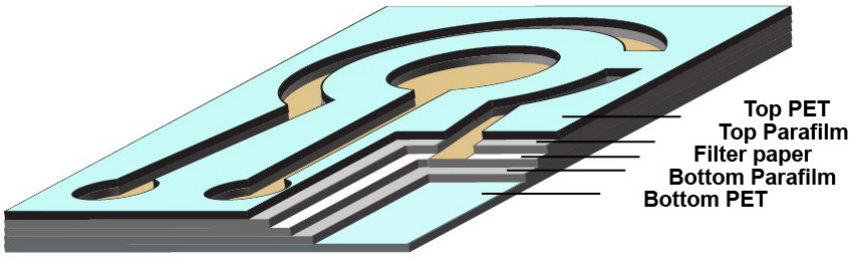

Figure S3. Schematic of the cross-section of an individual PED comprising a three-electrode-patterned filter paper sandwiched between polyethylene terephthalate (PET)-parafilm films.

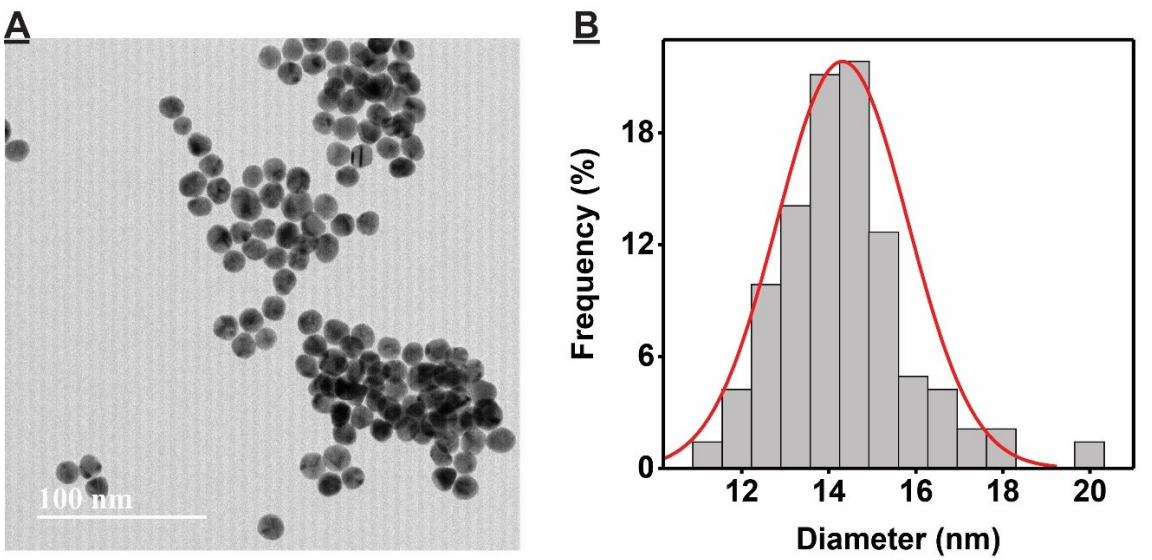

Figure S4. Characterization of gold nanoparticles (AuNPs). (**A**) Transmission electron microscopy (TEM) image of 15-nm AuNPs and (**B**) the distribution of particle diameter obtained using ImageJ software.

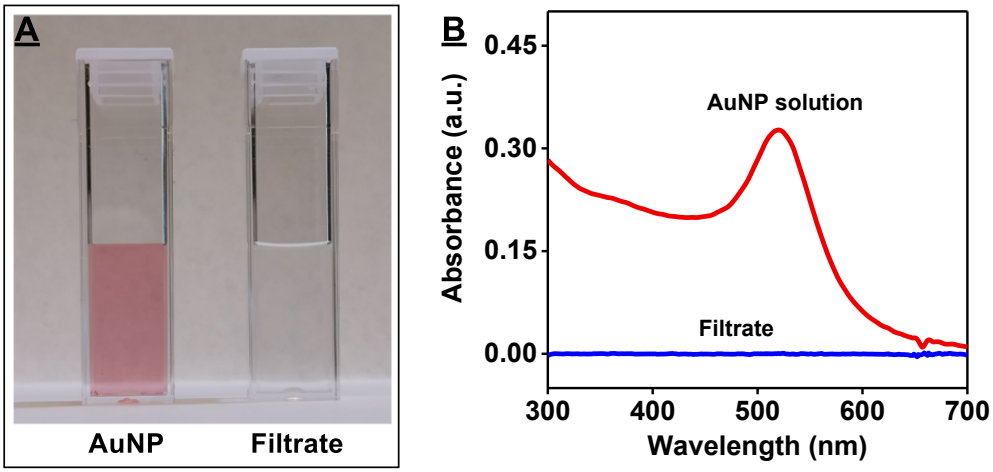

Figure S5. Characterization of filtrate collected after vacuum filtration based on (**A**) a photographic image, and (**B**) UV-vis spectra of the AuNP solution employed for PED fabrication and the filtrate.

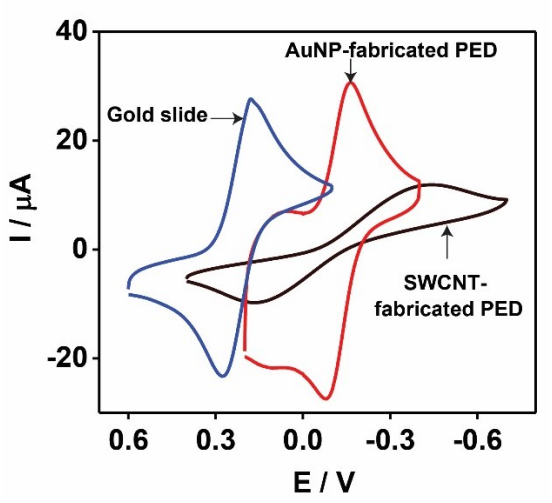

Figure S6. Cyclic voltammograms of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl produced using a commercial gold slide (blue), all-gold PED (red), and all-SWNCT PED (black). A standard Ag/AgCl reference electrode and platinum counter electrode were used with the gold slide working electrode. For the all-gold PED, the reference, working, and counter electrodes are made of AuNPs. For the all-SWCNT PED, the reference, working, and counter electrodes are made of SWCNTs. (Scan rate = 100 mV/s)

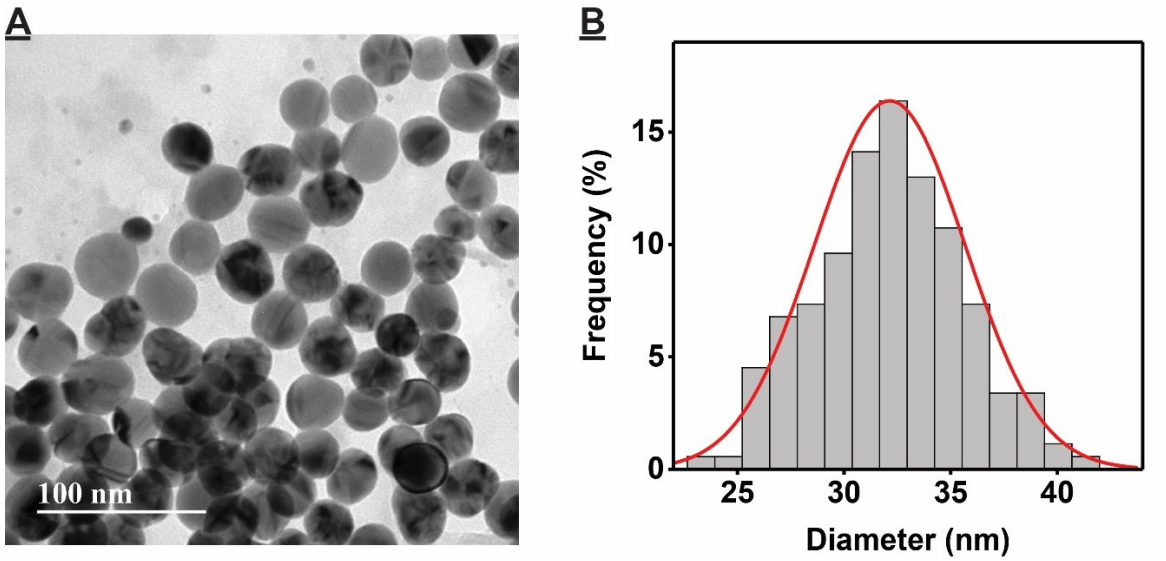

Figure S7. Characterization of silver nanoparticles (AgNPs). (**A**) TEM image of 32-nm AgNPs and (**B**) the distribution of particle diameter obtained using ImageJ software.

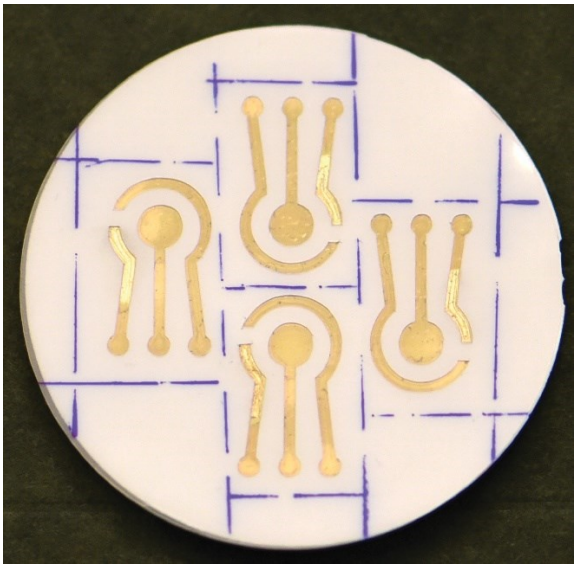

Figure S8. Schematic depicting how four devices are cut from a single filter paper.

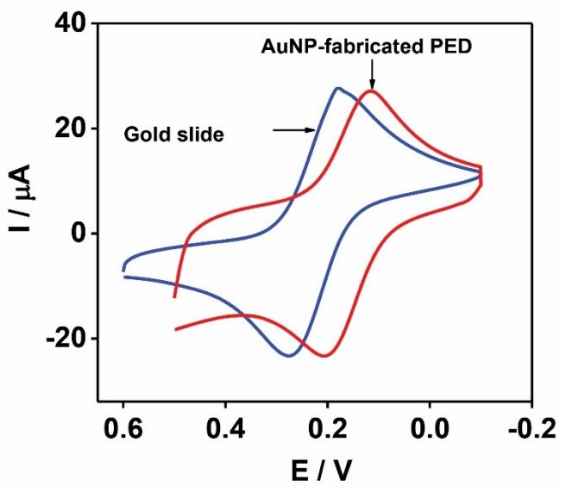

Figure S9. Cyclic voltammograms of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl produced using a commercial gold slide with a standard Ag/AgCl reference electrode and a platinum wire counter electrode (blue), and a PED with AuNP working and counter electrodes and a AgNP pseudo-reference electrode (red). (scan rate = 100 mV/s)

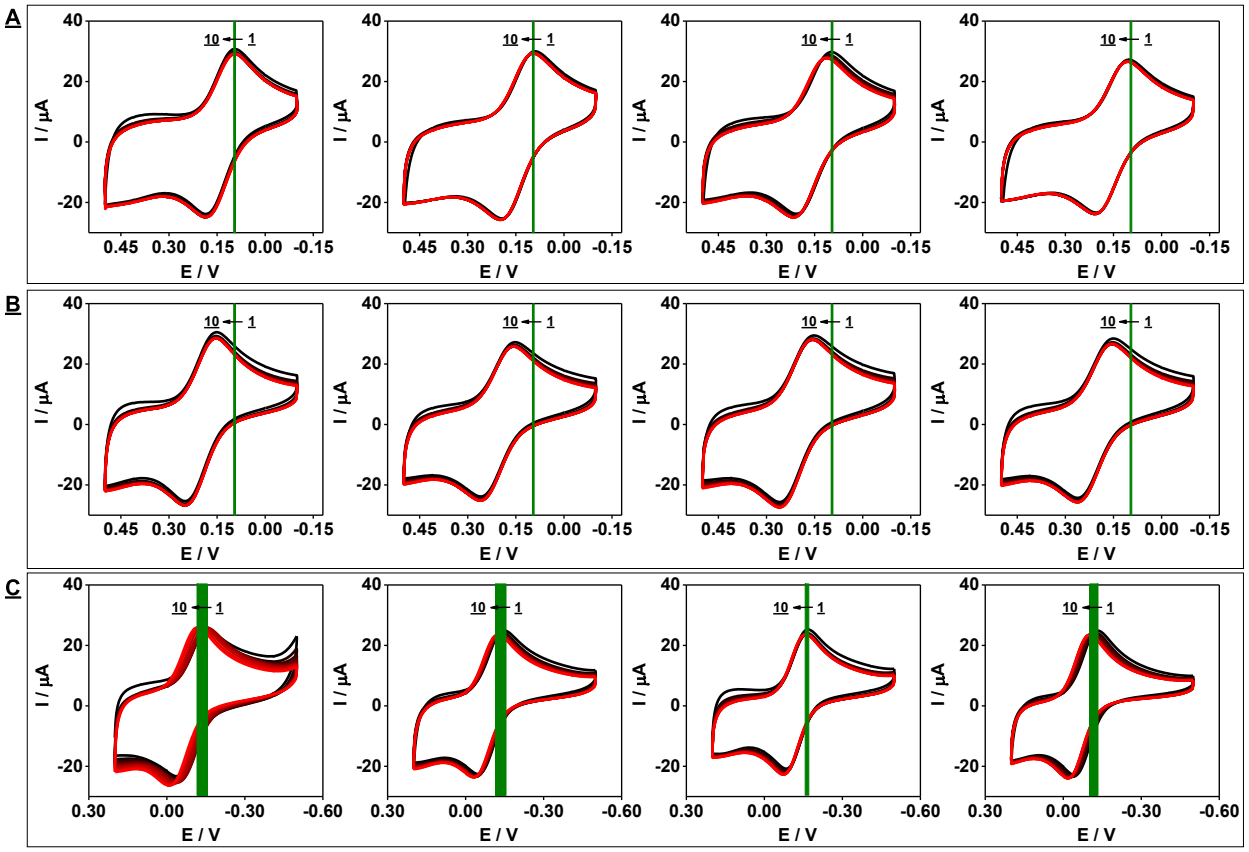

Figure S10. Cyclic voltammograms of 1 mM $K_3[Fe(CN)_6]$ solution in 0.1 M KCl produced using four different PEDs containing AuNP working and counter electrodes but with (**A**) AgNP pseudo-reference electrodes, (**B**) Standard Ag/AgCl reference electrodes, or (**C**) AuNP pseudo-reference electrodes. Ten scans were performed in total with a scan rate of 100 mV/s. The green bar represents shift in E_{pc} over the ten CV scans.

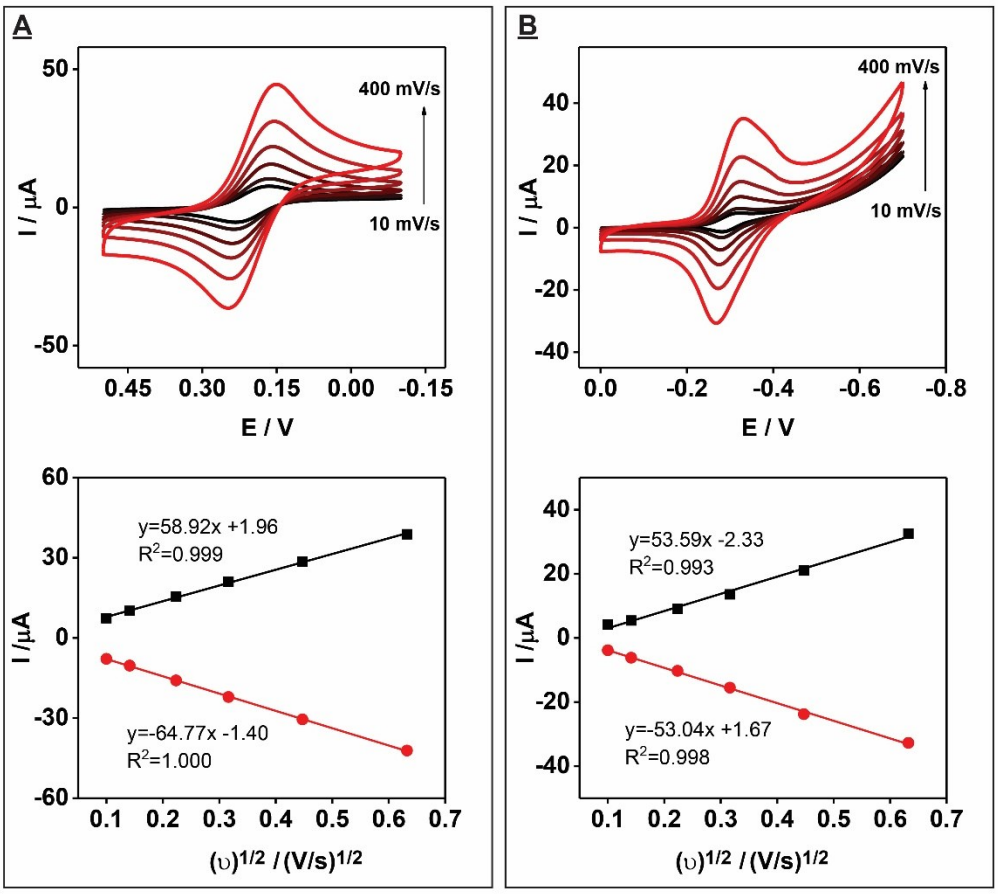

Figure S11. Electrochemical performance of the commercial gold slide with electroactive small molecules. Cyclic voltammetry scans at different scan rates (top) (increasing scan rate is indicated by the black to red color gradient, scan rates: 10, 20, 50, 100, 200 and 400 mV/s.) and plots of the relationship between the square root of the scan rate and the observed current (bottom) for (**A**) 1 mM $K_3[Fe(CN)_6]$ or (**B**) 1 mM methylene blue in 0.1 M KCl. Black and red lines indicate oxidation and reduction peak currents, respectively.

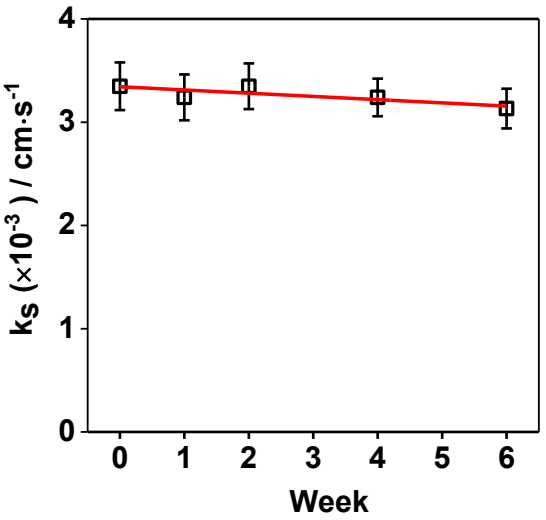

Figure S12. Change in the electron transfer rate (k_s) of PEDs containing AuNP working and counter electrodes and AgNP pseudo-reference electrodes after six weeks of storage at room temperature. Error bars represent the standard deviation of the measurements from two devices.

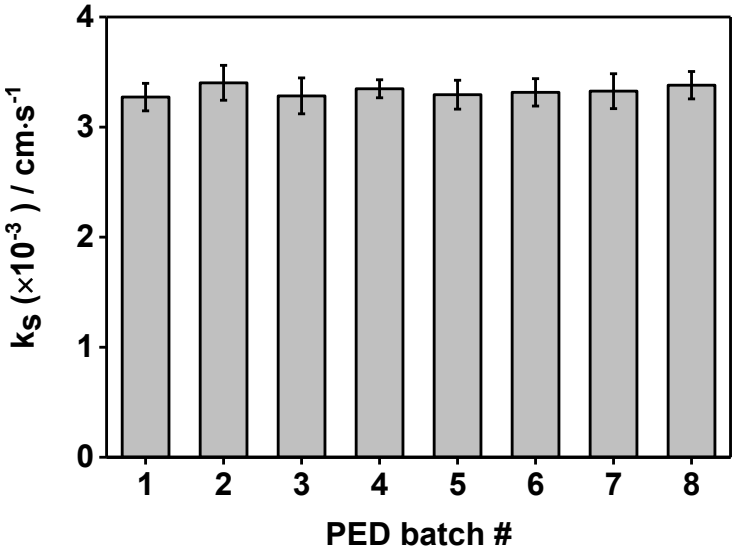

Figure S13. Measurements of electron transfer rate (k_s) of the gold working electrodes from 32 PEDs fabricated in 8 batches. Error bars are for electrodes from four individual devices fabricated from the same filter paper. k_s values were obtained from cyclic voltammetry measurements of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl at the following scan rates: 10, 20, 50, 100, 200 and 400 mV/s.

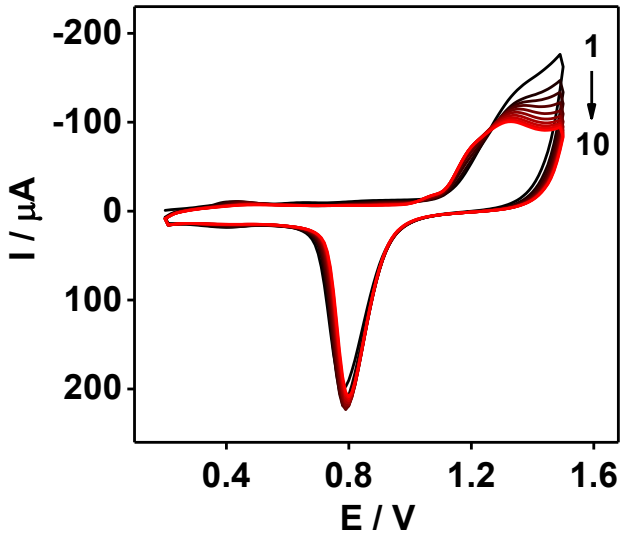

Figure S14. Electrochemical cleaning of a AuNP-working electrode of the PED in sulfuric acid. Cyclic voltammograms in 0.05 M H_2SO_4 in the potential range of 0.2 to 1.5 V with a scan rate of 100 mV/s using a PED. A standard Ag/AgCl reference electrode was used for the cleaning step. The black to red gradient represents scans 1 through 10, respectively.

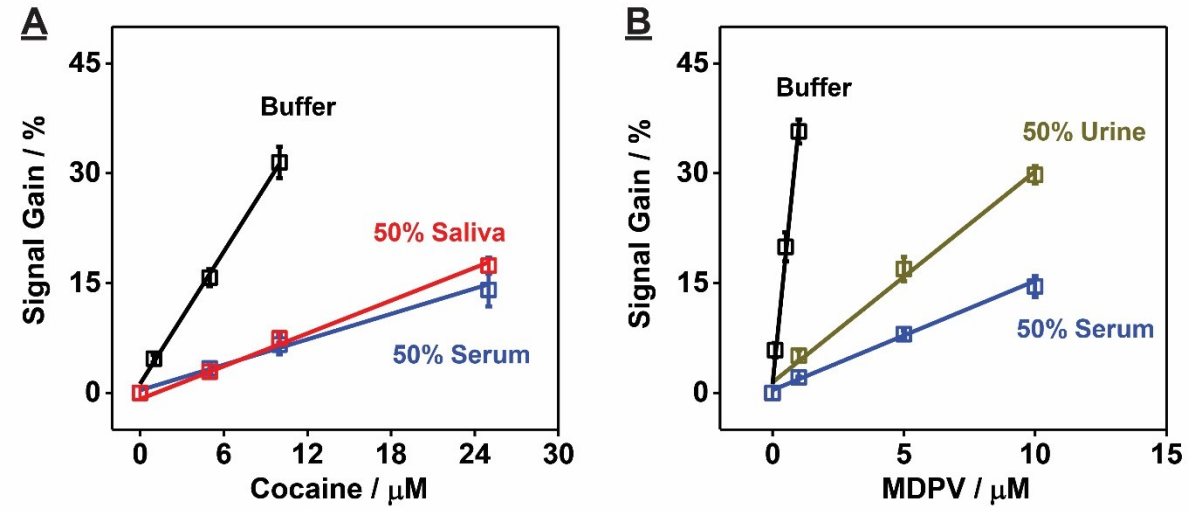

Figure S15. Performance of aptamer-based PEDs for the specific detection of small-molecule targets in various sample matrices. Linear range of detection for (**A**) cocaine and (**B**) MDPV in buffer (black lines), 50% calf serum (blue lines), 50% saliva (red line), or 50% urine (gold line). Error bars represent standard deviation of three experiments using three different devices.

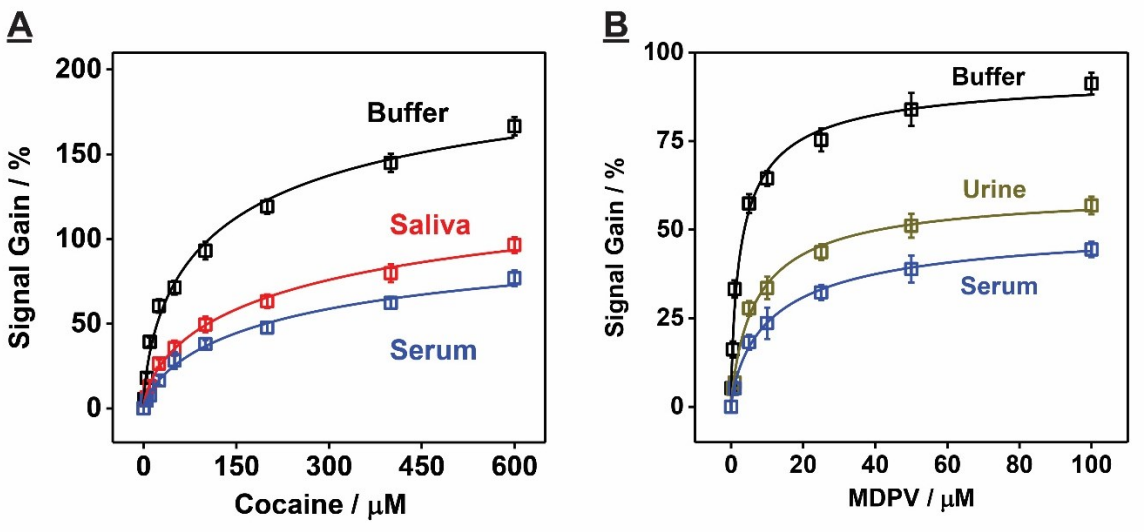

Figure S16. Sensing performance of aptamer-based gold disk electrodes for the specific detection of (**A**) cocaine and (**B**) MDPV in buffer (black lines), 50% calf serum (blue lines), 50% saliva (red line) or 50% urine (gold line). Error bars represent standard deviation of three experiments using three different electrodes.

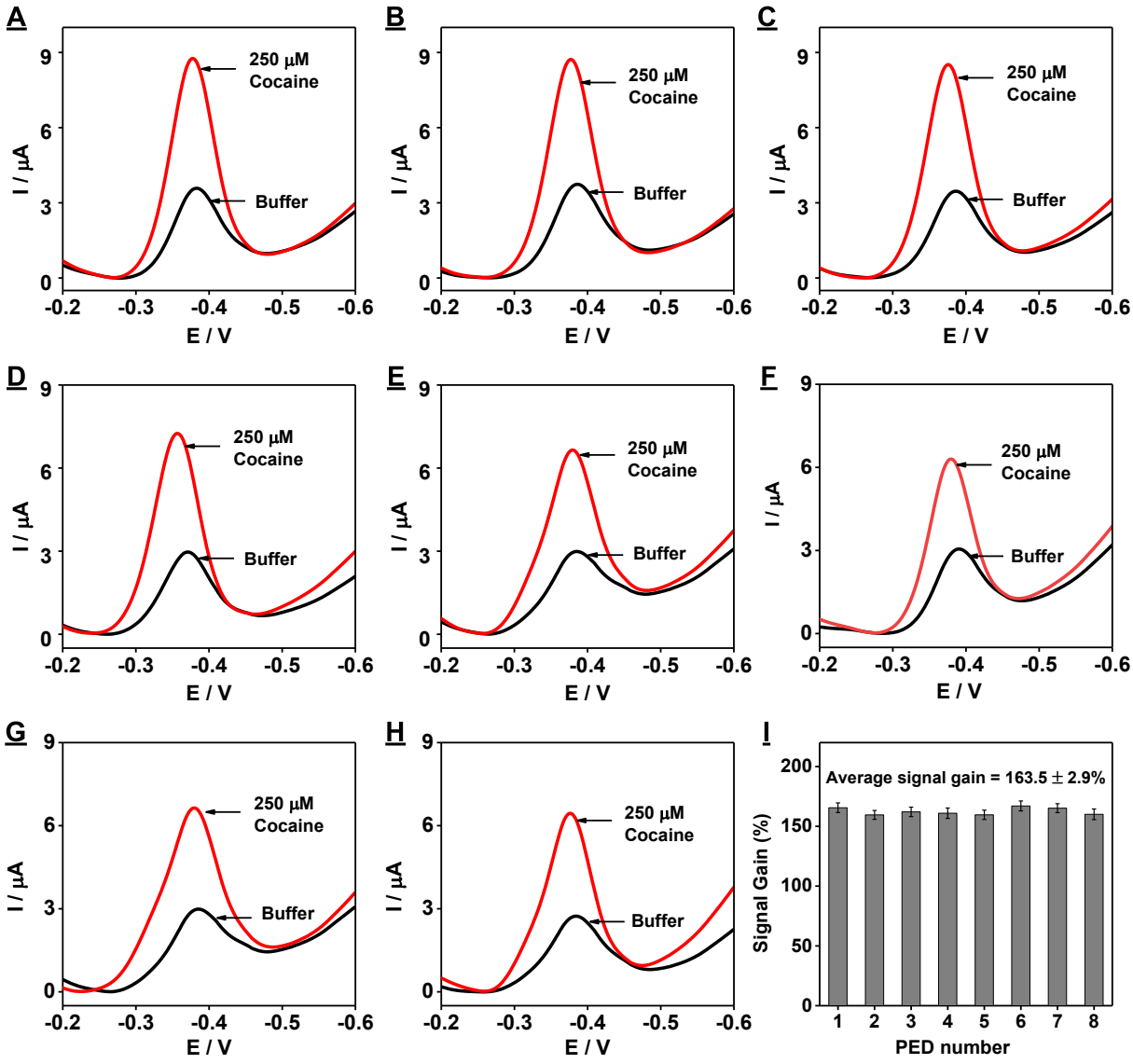

Figure S17. Reproducibility of the cocaine-binding aptamer-modified PEDs. (**A-H**) Square wave voltammetry plots (SWV) of eight different aptamer-modified PEDs recorded with and without 250 µM cocaine. (**I**) Signal gain obtained with each PED. Error bars represent standard deviation from six measurements for each PED.

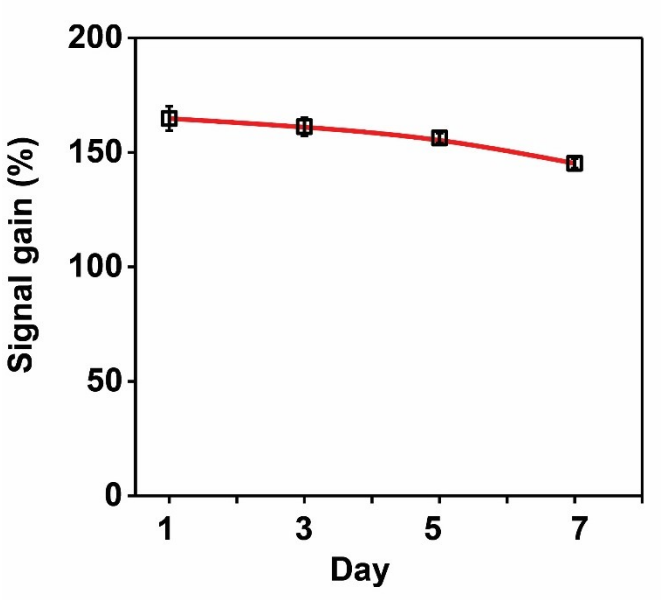

Figure S18. Variation of signal gain of a cocaine-detecting aptamer-based PEDs in the presence of 250 μM cocaine within one week of storage at room temperature. Error bars represent the standard deviation of signal gain of three different PED devices.

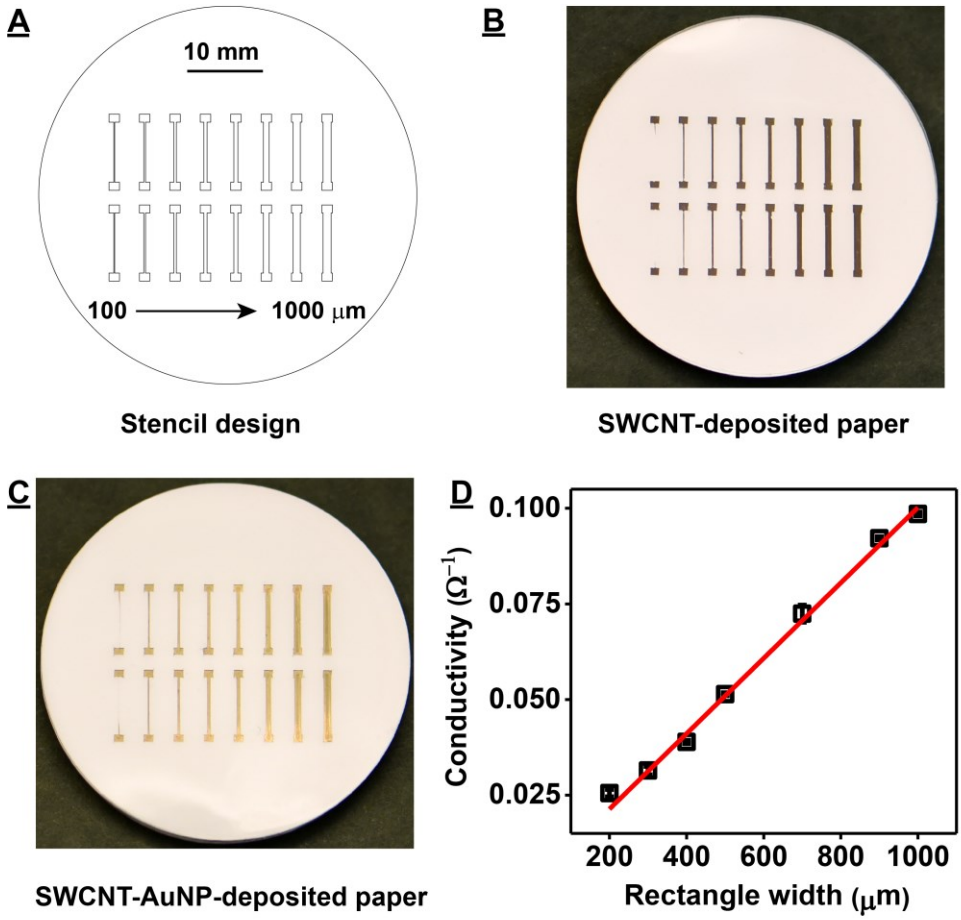

Figure S19. Architectural resolution of our PED fabrication method. (**A**) Stencil design containing two identical sets of eight rectangular shapes with lengths of 8 mm (excluding electrode contact sites at both ends) but different widths (100, 200, 300, 400, 500, 700, 900, and 1,000 μm). Photographs of (**B**) a SWCNT-coated paper and (**C**) an AuNP film formed on the SWCNT-coated paper utilizing our filtration method with the stencil shown in **A**. (**D**) The relationship between gold film width and film conductivity. Error bars are from measurements of two sets of rectangular patterns fabricated on the same filter paper.