Electronic Supplementary Information

Fast Catalytic and Electrocatalytic Oxidation of Sodium Borohydride on Palladium Nanoparticles and Its Application to Ultrasensitive DNA Detection

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Chemicals. The silane copolymer, poly(TMSMA-*r*-PEGMA-*r*-NAS), was synthesized by radical polymerization as reported elsewhere.[S1] All buffer reagents and inorganic chemicals were supplied by Sigma, Aldrich, or Fluka, unless otherwise stated. Palladium(acetylacetonate)$_2$ (strem, 99%), trioctylphosphine (Aldrich, Tech., 90%), oleylamine (Aldrich, tech., 70%), benzyl ether (Aldrich, 99%, Reagent Plus) for Pd NP synthesis were used without further purification. Au-NP colloidal solution (10 nm, 0.01% $HAuCl_4$) was purchased from Sigma. The actual size of Au NP was 8.1 $\pm$ 0.8 nm. All aqueous solutions were prepared in doubly distilled water.

The phosphate buffer solution (0.1 M, pH 8) consisted of Na_2HPO_4 and NaH_2PO_4. The borate buffer solution (0.1 M, pH 11.0) was composed of H_3BO_3 and NaOH. The Tris buffer solution (pH 9.0) was composed of 50 mM tris(hydroxymethyl)aminomethane (Tris) and ca. 7 mM HCl. The washing buffer was 0.1 M phosphate buffer (pH 7.4) containing 0.1% sodium dodecyl sulfate (SDS). The hybridization buffer (pH 7.4) was composed of 20 mM Tris, 17.5 mM ethylenediaminetetraacetic acid disodium salt, 150 mM NaCl and 0.05% Tween, and the dehybridization buffer (pH 7.4) consisted of 30 mM Tris, 10 mM HCl, 0.3 M NaCl, and 0.1% SDS.

All DNAs were obtained from Genotech (Daejeon, Korea). The DNA sensor was designed for the detection of single nucleotide polymorphism for the encoding residue 1038 of exon 11 of the *BRCA1* gene.[S2,S3] DNAs had the following sequences: amine-terminated capture probe, NH_2-$(CH_2)_9$-5′-AAA GAA GCC AGC TCA A-3′; complementary target DNA, 5′-CTT CAT TAA TAT TGC TTG AGC TGG CTT CTT T-3′; single-base-mismatched DNA, 5′-CTT CAT TAA TAT TGC TTG AGC TGG CTc CTT T-3′; noncomplementary DNA, 5′-CTT CAT TAA TAT TGC TTG AGC GAC TTA AGAT-3′; thiolated detection probe, 5′-GCA ATA TTA ATG AAG-A_{20}-3′-$(CH_2)_3$-SH; rhodamine-labled detection probe, TAMRA-5′-GCA ATA

TTA ATG AAG-A_{20}-$(CH_2)_3$-3′-SH. The concentrations of DNAs (target, detection probe, and capture probe) refer to those of strands.

Electrochemical Measurements. The electrochemical experiments were carried out using a CHI405a (CH instruments, Inc.). The electrochemical cell consisted of a modified ITO working electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode. The area of the working electrode was 0.28 cm^2.

Synthesis of Water-Soluble SDS-stabilized PdNPs. A mixture of $Pd(acac)_2$ (150 mg), trioctylphosphine (2 mL), benzyl ether (8 mL), and oleylamine (0.68g) prepared in a 100-mL schlenk tube was placed under vacuum for 2 h. The mixture under 1 atm N_2 was heated to 260 °C and was kept at that temperature for 30 min with a magnetic stirring to give a black solution. During the reaction, the reaction mixture was constantly purged with a gentle stream of N_2. The precipitates, formed by adding methanol, were collected by centrifugation. The size of the synthesized Pd NPs was 3.2 ± 0.4 nm (Fig. S1a). The precipitates were dispersed in 10 mL of hexane saturated with SDS. Distilled water (10 mL) was added to the solution, and the mixture solution was stirred vigorously for 30 min. Pd NPs were slowly transferred from organic phase to aqueous phase, as SDS formed a hydrophilic micelle on the surface of Pd NPs. Finally, the aqueous solution of SDS-stabilized Pd NPs was obtained by discarding the organic phase.

Conjugation between Pd NPs and Detection Probes. To date, there is no report on the preparation of DNA-conjugated Pd NPs. The SDS-stabilized Pd NPs were conjugated with thiolated detection probes containing an A_{20} spacer by following our previous protocol with little modification.[S2,S3] To reduce disulfide bonds of detection probes, 120 μL of 100 μM DNA solution was mixed with 120 μL of 500 μM tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and the mixture was kept for 30 min at room temperature. The mixture was then mixed with 3 mL of a SDS-stabilized Pd NP solution (ca. 10 nM). The mixture was kept in refrigerator for 72 h with occasional shaking. This occasional shaking is important to keep all Pd NPs in dispersed state, because some of Pd NPs precipitate in a few hours. When all Pd NPs were conjugated with detection probes, no sedimentation of Pd NPs took place. By checking the disappearance of precipitated Pd NPs, we could know the completion of the conjugation. After 72-h incubation, DNA-conjugated Pd NPs were brought to 10 mM phosphate buffer (pH 7.4) containing 0.1 M NaCl. After 72-h aging, detection probe-conjugated Pd NPs were separated by centrifuging at 7,000 g for 15 min at room temperature by using Amicon ultra-4 (50,000 MW, Millipore Corporation, USA). The

conjugates were dissolved in 4 mL of PBS and again separated by centrifuging at 7,000 g for 15 min at room temperature by using Amicon ultra-4. Finally, detection probe-conjugated Pd NPs were dispersed in 9 mL of 10 mM phosphate buffer (pH 7.4) containing 0.25 M NaCl. The TEM image of detection probe-conjugated Pd NPs shows that detection probe-conjugated Pd NPs are well formed without aggregation of Pd NPs (Fig. S1b).

We also tried to use dithiothreitol (DTT) instead of TCEP to cleave disulfide bonds. Even a very low amount of DTT interfered with the conjugation between detection probes and Pd NPs. It was required to remove all DTT from the mixture of DTT and thiolated detection probe, but it was not easy to achieve the complete removal. It seems that thiolated detection probes on Pd NPs are readily replaced by DTTs. In our previous study, the concentration ratio of DNA to TCEP for the conjugation was 1:5000.[S2,S3] In this study, we initially tried to use the same ratio of DNA to TCEP. However, we could not obtain a reproducible conjugation. It seems that TCEP adsorbs on Pd NPs and that the bond strength between thiols and Pd NPs is weaker than that between thiols and Au NPs. When a low ratio of DNA to TCEP (i.e., 1:5) was used, we could obtain a reproducible conjugation. Whenever the conjugation between Pd NPs and DNAs was not good, either of two following problems occurred: a high nonspecific binding of Pd NPs on sensing electrodes, which caused high background currents, and no hybridization between target and detection probe, which caused very low signal currents.

Conjugation between Au NPs and Detection Probes. The citrate-stabilized Au NPs were conjugated with thiolated detection probes containing an A_{20} spacer by following our previous protocol.[S2,S3]

Surface Density of DNA on Detection Probe-Conjugated Pd NPs. To measure a surface density of DNA on detection probe-conjugated Pd NPs, we conjugated Pd NPs with rhodamine-labled detection probes. The conjugation procedure is the same as the mentioned procedure. Fluorescence of the rhodamine-labled conjugates was measured using a spectrofluorometer (FP-750, Jasco). The surface concentration of DNA was calculated by comparing the fluorescence intensity with the calibration curve that was obtained with standard solutions of rhodamine-labled detection probes. The excitation wavelength was 560 nm, and the emission wavelength was 580 nm. The calculated surface density of DNA on DNA-conjugated Pd NPs was ca. 50 pmol/cm^2. This surface density was higher than that of 15-nm AuNPs (14 ~19 pmol/cm^2) and lower than that of 2-nm AuNPs (64 pmol/cm^2).[S4]

Nonspecific Adsorption of Pd NPs and Au NPs on Dendrimer-Modified ITO Electrodes. The preparation of the ITO electrodes modified with amine-terminated G4 poly(amidoamine) dendrimer was previously described.[S3,S5] Detection probe-conjugated Pd NPs and Au NPs were dropped onto and spread over the dendrimer-modified ITO electrodes for 2 h, and then the electrodes were washed with a washing buffer.

Preparation of DNA-Sensing Electrodes and Procedure for DNA Detection. A schematic representation of a DNA-sensing electrode is shown in Fig. 2. First, a monolayer of the silane copolymer was formed on ITO electrodes, and the monolayer was then modified with amine-terminated capture probes by following our previous protocol.[S5] Capture probe-modified electrodes were immersed into a 0.1 M borate buffer (pH 9) for 1 h to hydrolyze the unreacted *N*-acryloxysuccinimide group of the monolayer. Afterward, the electrodes were incubated at room temperature for 2 h in a hybridization buffer containing different concentrations of target DNA. After being rinsed with a washing buffer, the resulting assembly was immersed in a solution containing detection probe-conjugated Pd NPs for 2 h, followed by dipping the electrodes into a washing buffer for 5 min and then rinsing them with a washing buffer.

Investigation of the Possibility of DNA Detachment from DNA-Conjugated Pd NPs during Incubation in a $NaBH_4$ Solution. 100 μL of DNA-conjugated Pd NPs was mixed with 100 μL of a borate buffer solution containing 10 mM $NaBH_4$, and the mixture was kept at room temperature for 10 min. The mixture was then centrifuged at 7,000 g for 15 min at room temperature by using Amicon ultra-4. The residue was washed with 100 μL of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and again centrifuged at 7,000 g for 15 min at room temperature using the same centrifugal filter device. Two filtrates were mixed together. The mixed filtrate contains DNAs detached from Pd NPs if any. The filtrate stored for 9 h at room temperature to fully decompose $NaBH_4$. The detached DNAs in the filtrate were quantified by using a single-stranded DNA quantification kit (Oligreen, Invitrogen).[S4] The residue (containing DNA-conjugated Pd NPs) on Amicon ultra-4 was dissolved in 300 μL of Tris-EDTA buffer, and the DNAs in the residue were quantified by using the single-stranded DNA quantification kit. The fluorescence spectrum in the filtrate was similar to that of a solution containing no DNA-conjugated Pd NPs (Fig. S2), and the fluorescence intensity was much lower than that of the residue (Fig. S2). These results clearly show that the DNA detachment from DNA-conjugated Pd NPs does not occur or is negligible during 10-min incubation in a $NaBH_4$ solution. Considering that cyclic and linear sweep voltammetric experiments were performed within 1 min including incubation time, it seems that the enhancement of electrocatalytic activity of

DNA-conjugated Pd NPs in a $NaBH_4$ solution is not related to the DNA detachment from Pd NPs.[S3]

Incubation Time (in a $NaBH_4$ Solution) Required for the Enhancement of Electrocatalytic Activity of DNA-conjugated Pd NPs. Fig. S3 shows cyclic voltammograms obtained in a phosphate buffer solution (pH 8.0) containing 2 mM hydrazine at ITO/dendrimer electrodes on which detection probe-conjugated Pd NPs were electrostatically adsorbed. The electrocatalytic activity of DNA-conjugated Pd NPs was quite low before $NaBH_4$ treatment. The electrocatalytic activity was significantly enhanced after 30-s incubation, and the activity was similar to that after 30-min incubation. These results show that the electrocatalytic activity of DNA-conjugated Pd NPs is not initially high but that the electrocatalytic activity is enhanced in a short time in $NaBH_4$ solutions.

Surface Density of Capture Probe on DNA-sensing Electrodes. Chronocoulometry was performed in 10 mM Tris buffer (pH 7.4) in the presence and absence of 50 μM $[Ru(NH_3)_6]^{3+}$ after purging the solution thoroughly with argon gas with a pulse period of 1 sec and a pulse width of 0.6 V (from +0.15 V to −0.45 V), to calculate the surface density of capture probe DNA (Fig. S4).[S3] Following the published protocol,[S6,S7] the calculated surface density of capture probe DNA was $5.0 \pm 0.2 \times 10^{12}$ molecules/cm^2. This surface density is very efficient for hybridization of target DNA.[S8]

More Concentration Data. Fig. S5 shows the dependence of linear sweep voltammograms on the concentration of target DNA. More Pd NPs exist on the DNA-sensing electrodes at a higher concentration of target DNA. Accordingly, the electrocatalytic current increased with the concentration of target DNA. To test the ability of the electrode to discriminate single base mismatches, linear sweep voltammograms were obtained using single base-mismatched DNA and noncomplementary DNA (Fig. S6). To control thermal stringency, electrodes were washed twice with dehybridization buffer for 10 min at 40 °C prior to the hybridization of detection probe. The current for single base-mismatched DNA was significantly lower than that for complementary DNA. It shows that a small amount of single base-mismatched DNA hybridized with the capture probe. The current for the noncomplementary target was much lower than that for complementary DNA. These results clearly show that single base-mismatched DNA and noncomplementary DNA can be distinguished from complementary target DNA.

References

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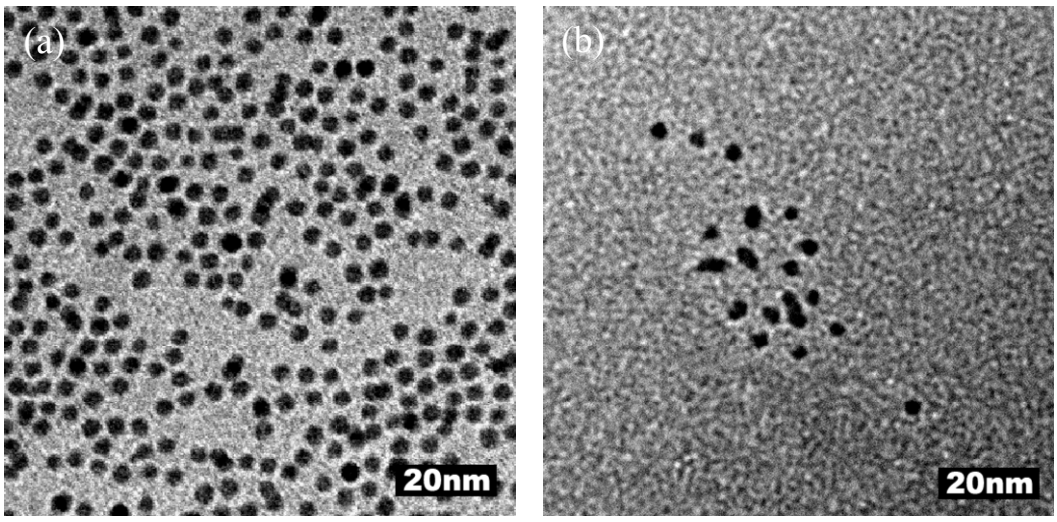

Fig. S1 TEM images of (a) Pd NPs and (b) detection-probe conjugated Pd NPs.

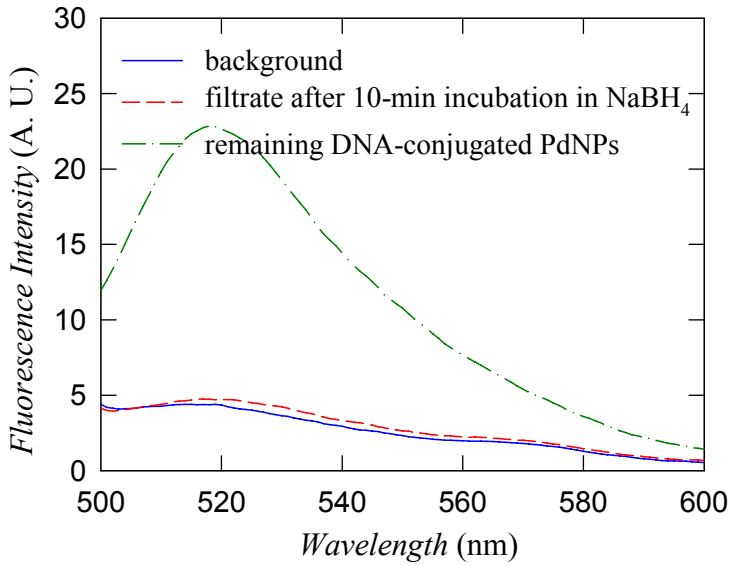

Fig. S2 Fluorescence spectra of oligreen-containing solutions. The background corresponds to the spectrum obtained in a solution containing no DNA-conjugated Pd NPs, the filtrate after 10-min incubation in $NaBH_4$ corresponds to the spectrum obtained with the filtrate after $NaBH_4$ treatment of DNA-conjugated Pd NPs, and the remaining DNA-conjugated Au NPs corresponds to the spectrum obtained from residual DNA-conjugated Pd NPs after filtration.

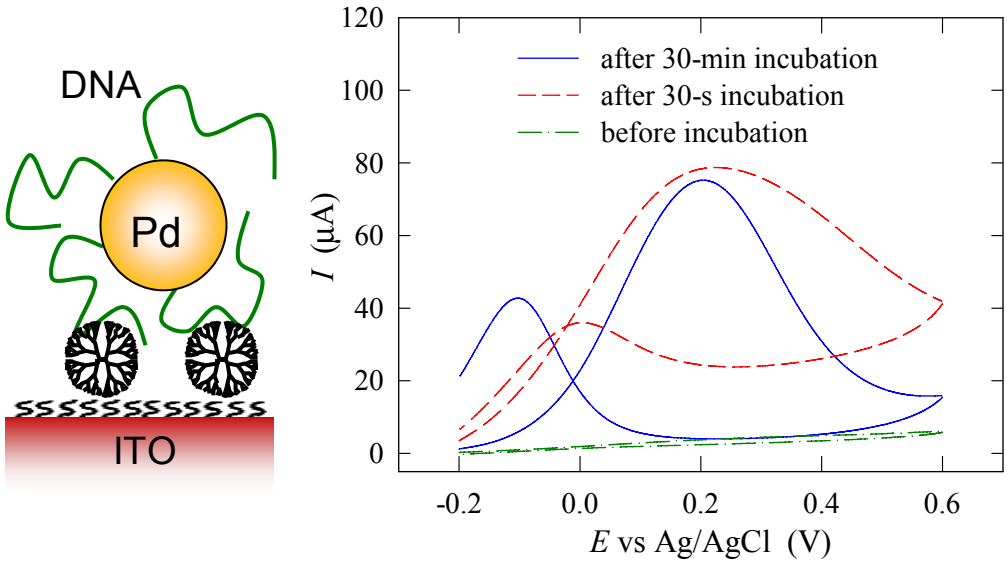

Fig. S3 Cyclic voltammograms obtained in a phosphate buffer solution (pH 8.0) containing 2 mM hydrazine (at a scan rate of 50 mV/s) at the ITO/dendrimer electrodes on which detection probe-conjugated Pd NPs were electrostatically adsorbed, before and after incubation in a Tris buffer containing 10 mM $NaBH_4$ (pH 9).

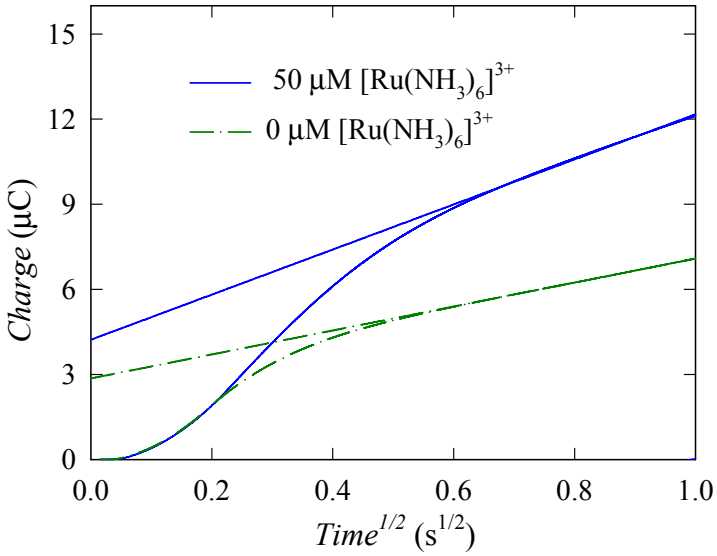

Fig. S4 Chronocoulometric data obtained at capture probe-modified DNA-sensing electrodes in a 10 mM Tris buffer solution (pH 7.4) in the presence and absence of 50 μM $[Ru(NH_3)_6]^{3+}$.

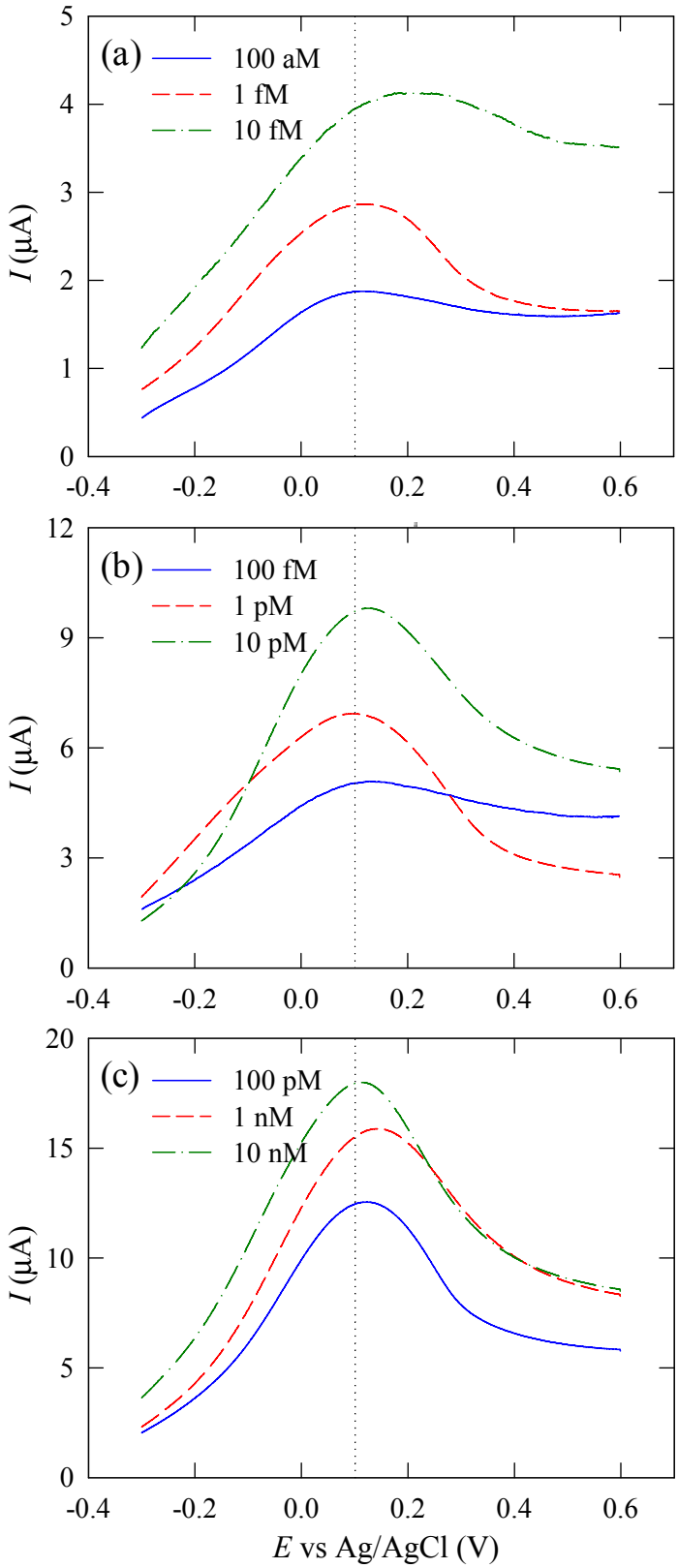

Fig. S5 Concentration dependence of linear sweep voltammograms obtained after 20-sec incubation in a 0.1 M borate buffer solution (pH 11) containing 5 mM $NaBH_4$ (at a scan rate of 50 mV/s).

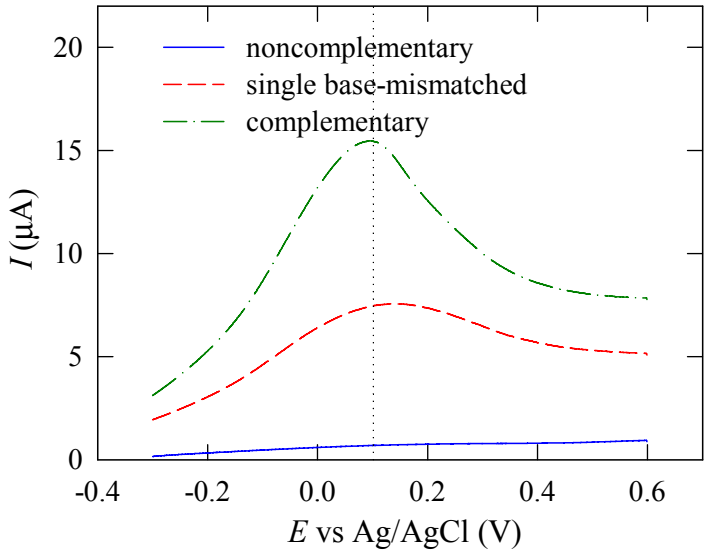

Fig. S6 Linear sweep voltammograms obtained after 20-sec incubation in a 0.1 M borate buffer solution (pH 11) containing 5 mM $NaBH_4$ (at a scan rate of 50 mV/s) after incubation with a solution containing 1 nM complementary, single base-mismatched, or noncomplementary target DNA.