Quantum-Dot-Functionalized Poly(styrene-*co*-acrylic acid) Microbeads: Step-Wise Self-Assembly, Characterization, and Applications for Sub-femtomolar Electrochemical Detection of DNA Hybridization

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A novel nanoparticle label capable of amplifying the electrochemical signal of DNA hybridization is fabricated by functionalizing poly(styrene-*co*-acrylic acid) microbeads with CdTe quantum dots. CdTe-tagged polybeads are prepared by a layer-by-layer self-assembly of the CdTe quantum dots (diameter = 3.07 nm) and polyelectrolyte on the polybeads (diameter = 323 nm). The self-assembly procedure is characterized using scanning and transmission electron microscopy, and X-ray photoelectron, infrared and photoluminescence spectroscopy. The mean quantum-dot coverage is $(9.54 \pm 1.2) \times 10^3$ per polybead. The enormous coverage and the unique properties of the quantum dots make the polybeads an effective candidate as a functionalized amplification platform for labelling of DNA or protein. Herein, as an example, the CdTe-tagged polybeads are attached to DNA probes specific to breast cancer by streptavidin–biotin binding to construct a DNA biosensor. The detection of the DNA hybridization process is achieved by the square-wave voltammetry of Cd^{2+} after the dissolution of the CdTe tags with HNO_3. The efficient carrier-bead amplification platform, coupled with the highly sensitive stripping voltammetric measurement, gives rise to a detection limit of 0.52 fmol L^{-1} and a dynamic range spanning 5 orders of magnitude. This proposed nanoparticle label is promising, exhibits an efficient amplification performance, and opens new opportunities for ultrasensitive detection of other biorecognition events.

1. Introduction

The rapid detection of nucleic-acid sequences has been extensively applied in different fields. Various approaches have been developed for the highly sensitive detection of DNA by amplifying the sample using the polymerase-chain-reaction (PCR) technique,[1–3] accelerating the reaction to produce a detectable signal with enzymatic cycles,[4,5] and amplifying the detectable signal using nanoparticles.[6,7] The unique physical and chemical properties of inorganic nanoparticles (NPs), as well as their unusual biomolecular binding capability, have attracted considerable attention in biological applications.[8–13] In particular, some NP-based optical methods, such as colorimetry[12] and surface-enhanced Raman spectroscopy (SERS) detection,[13] can offer superior performance over conventional strategies. However, these measurements still suffer from several drawbacks, such as complex handling procedures, easy contamination and high cost, etc. Electrochemical methods have attracted considerable attention in nucleic-acid detection due to their high sensitivity, simplicity and low cost, as well as their rapid compatibility with microfabrication technology.[14,15] Various electrochemical DNA biosensors based on labelling technologies with enzymes,[16] electroactive substances,[17,18] and nanoparticles[19–28] have been developed to amplify the hybridization signal. Among these methods, NP-based electrochemical-signal amplification platforms with low cost and simple operation have been proposed. These techniques usually involve the chemical solubilization of the semiconductor NP labels and subsequent detection of released ions by highly sensitive electrochemical techniques. However, the optimized detection limit of these assays is only at a pmol L^{-1} target concentration,[23,24] which is much higher than those of some methods coupled with the PCR technique (zmol)[2] and enzymatic cycles (0.01 pmol L^{-1}).[5] Thus new approaches are urgently needed to meet the demands of high sensitivity for the detection of

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nucleic-acid sequences with limited amounts or very-low concentrations of genomic DNA segments.

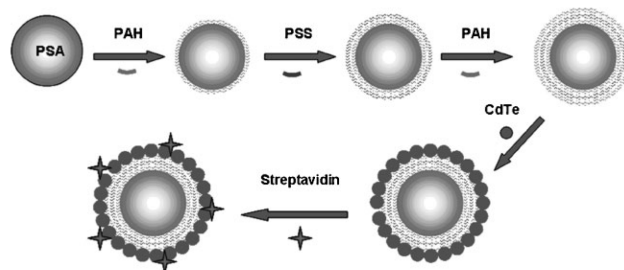
A major approach to improve the sensitivity of electrochemical detection is to amplify the transduction of the recognition events by increasing the quantity of NPs in each binding event. This can be conveniently achieved by loading or encapsulating numerous NP tags on or inside polybeads.[29–33] For example, CdS-NP-loaded carbon nanotubes[29] and Au-NP-tagged polystyrene spheres[31] have been employed as labels for the detection of DNA, Au-NP-loaded carbon nanospheres have been prepared for an amplified electrochemical immunoassay,[32] and silver-NP-encapsulated polyelectrolyte shells have been used for femtomolar electrochemical detection of DNA hybridization.[33] In particular, a significant aspect in this field is the application of the layer-by-layer (LBL) assembly technique to construct the NP-tagged polybeads.[34,35] With this technique, a Au-NP-loaded latex has been prepared to amplify the signal for sub-femtomolar electrochemical detection of DNA hybridization.[36] This work has developed a novel electrochemical-signal amplification platform by applying quantum dots (QDs) to tag layer-by-layer polyelectrolyte microbeads for the first time. The QD-functionalized polybeads provided an effective candidate for labelling and ultrasensitive detection of DNA or protein targets.

The polybead templates, prepared simply by the emulsifier-free copolymerization of styrene with acrylic acid to form poly(styrene-*co*-acrylic acid) (PSA) cores and the layer-by-layer deposition of a three-layer polyelectrolyte (PE_3) film, consisting of a poly(sodium 4-styrenesulfonate) (PSS) layer sandwiched between two layers of poly(allyaminehydrochloride) (PAH) on the cores, showed smooth and uniform positively charged surfaces, which facilitated the chemisorption of negatively charged mercaptoacetic acid-capped CdTe QDs on the templates. These CdTe-tagged polybeads possess advantages, such as outstanding biocompatibility, good dispersion and solubility, as well as size-tailored properties. Using the polybeads as a label, a promising method for the sub-femtomolar electrochemical detection of DNA hybridization by coupling highly sensitive stripping voltammetric measurements was thus developed. Notably, the suggested technique could be easily adopted to functionalize other template cores with different types of semiconductor nanocrystals, to obtain desired optical and electrochemical labels for bioanalysis and diagnostic applications.

2. Results and Discussion

2.1. Characterization of CdTe-tagged Polybeads

The UV-vis absorption spectrum of the as-prepared CdTe QDs shows a well-resolved maximum absorption of the first electronic transition at 534 nm (Fig. S1, Supporting Information), indicating a sufficiently narrow size distribution of the QDs. The result was also verified by the photoluminescence (PL) spectrum with a half-wave width less than 44 nm. The mean size of the QD particles and the concentration of the QD solution were estimated from the adsorption peak by Peng's empirical equation[37] to be 3.07 nm and 1.4×10^{-5} mol L^{-1}, respectively.



Scheme 1. Schematic diagram of the assembly process for the preparation of streptavidin/CdTe-tagged polybeads.

The entire assembly process for the preparation of the CdTe-tagged microbeads is illustrated in Scheme 1. Typical scanning electron microscopy (SEM) images show that the obtained PSA cores, with an average diameter of 323 nm, were uniform (Fig. 1A and D). After the oppositely charged PAH polyelectrolytes, with $-NH_3^+$ groups, and PSS, with $-SO_3^-$ groups, were sequentially deposited on the cores to form PAH/PSS/PAH (PE_3) layers by the electrostatic interaction between the charged components, the formed PE_3/PSA had a diameter of 331 nm (Fig. 1B and 1E), and the outer PAH layer had a smooth and uniform surface with a positive charge, which facilitated the adsorption of the mercaptopropionic acid capped-QDs on the polybeads by electrostatic interactions to form CdTe-tagged polybeads.

High-resolution transmission electron microscopy (TEM) images display numerous, individual, dark "QD islands" on the carrier polybeads (Fig. 1C and F), which indicate the QDs were distributed on the polybead surface. The X-ray photoelectron spectroscopy (XPS) and PL spectra of the CdTe-tagged polybeads further confirmed the assembly of CdTe QDs on the polybead surface (Fig. 2). Compared with the spectrum of the PE_3/PSA microbeads (Fig. 2A, curve a), the spectrum of CdTe-tagged polybeads (Fig. 2A, curve b) shows two new and strong peaks at 405.45 and 573.1 eV, which correspond to Cd3d5 and Te3d5, respectively. Meanwhile, the PL spectrum of the CdTe-tagged polybeads presents a peak characteristic of the CdTe QDs (Fig. 2B,

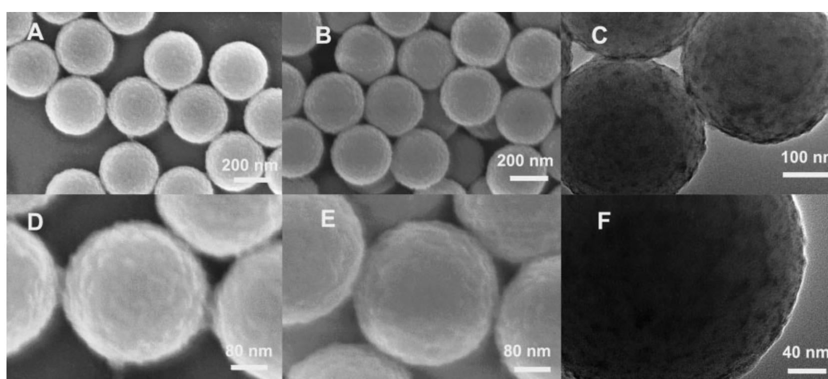


Figure 1. A) SEM image of PSA cores. B) SEM image of PE_3/PSA. C) TEM image of CdTe-tagged PE_3/PSA. D–F) Respective higher-magnification images of parts A–C.

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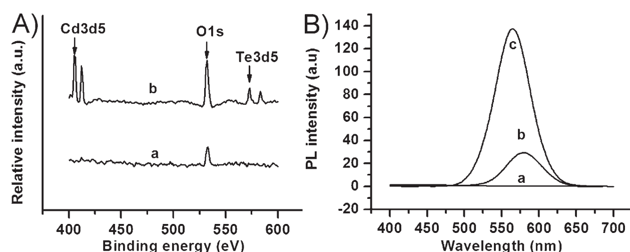

Figure 2. A) XPS spectra of PE_3/PSA (curve a) and CdTe-tagged polybeads (curve b). B) PL spectra of PE_3/PSA (curve a), CdTe-tagged polybeads (curve b) and CdTe QDs (curve c) suspensions in PBS (0.1 mol L^{-1}, pH 7.4) at $\lambda_{ex} = 350$ nm.

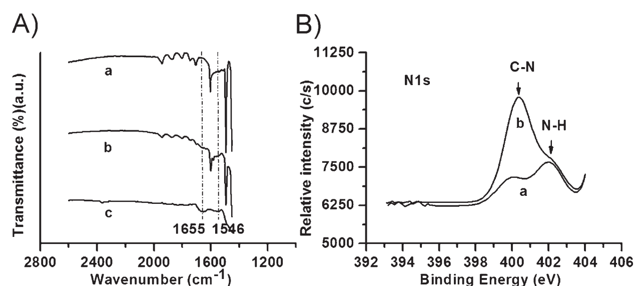

Figure 4. A) FTIR spectra of the CdTe-tagged polybeads (curve a), and the streptavidin/CdTe-tagged polybeads (curve b); curve c represents the difference between the FTIR spectra. B) XPS spectra of the CdTe-tagged polybeads (curve a) and the streptavidin/CdTe-tagged polybeads (curve b).

curve b). The red shift of the PL peak for the CdTe-tagged polybeads may be attributed to interparticle plasmon coupling caused by nanoparticle clusters.

Based on a density of 1.05 g cm^{-3} for the polystyrene core and 1.01 g cm^{-3} for the polyelectrolyte shell,[38] the mass of one polybead was estimated to be 2.0×10^{-14} g. In conjunction with the obtained mass of a 1 mL aliquot of particles after evaporation to dryness, the concentration of the polybeads was determined to be 1.25×10^{12} particles mL^{-1}. The total amount of CdTe QDs coated on the polybead was determined by measuring the QD-concentration change of the pure QD solution before and after the adsorption step, using UV-vis spectrophotometry. The nanoparticle coating was optimized by varying the concentration of the QDs (50 μL) for a fixed amount (50 μL, 2.5 wt%) of the polybeads used for adsorption. As shown in Figure 3, a maximum QD loading of as much as $(9.54 \pm 1.2) \times 10^3$ on each polybead could be achieved. The enormous coverage of the loading QDs provides a promising carrier-amplified label for the detection of DNA or protein in bioanalysis and clinical diagnosis.

2.2. Characterization of Streptavidin/CdTe-Tagged Polybeads

Biotin is a small molecule that can bind to the binding sites of tetrameric proteins, avidin (or streptavidin), with a very-high affinity. The formation of avidin (or streptavidin)-biotin complexes offers a useful means in a wide variety of applications.[22,39–41] This specific binding is often used to develop DNA biosensors. Streptavidin/CdTe-tagged polybead conjugates could be simply prepared by incubating a mixture of streptavidin and CdTe-tagged

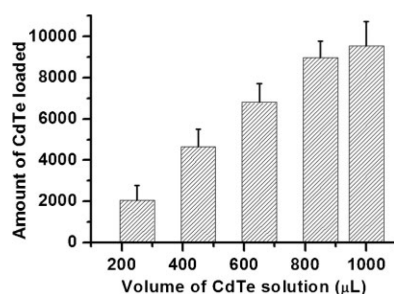

Figure 3. Effect of CdTe QD concentration on the loading amount of the CdTe QDs on each polybead.

polybead solutions at room temperature. In comparison with the FTIR spectrum of the CdTe-tagged polybeads (Fig. 4A, curve a), the FTIR spectrum of the resulting streptavidin/CdTe-tagged polybeads displays obvious absorption peaks corresponding to the amide bands I (1655 cm^{-1}) and II (1546 cm^{-1}) of streptavidin, respectively (Fig. 4A, curves b and c), which indicates that the streptavidin molecules were successfully adsorbed on the polybeads. Meanwhile, the N 1s XPS spectrum of the streptavidin/CdTe-tagged polybeads shows a strong C–N peak at 400.4 eV and a weak N–H peak at 402.0 eV (Fig. 4B, curve b), which are much stronger or weaker, respectively, than those of the CdTe-tagged polybeads (Fig. 4B, curve a), due to the different environments of the C–N and N–H bonds in the streptavidin molecules and the CdTe-tagged polybeads. These results further proved the presence of streptavidin molecules on the CdTe-tagged polybeads.

2.3. Preparation of Biosensing Surface for DNA Recognition

The process using the streptavidin/CdTe-tagged polybeads for the detection of DNA hybridization is depicted in Scheme 2. Briefly, after the surface of the substrate was grafted with glycidoxypropyltrimethoxysilane (GPTMS), an epoxy-group-rich compound, an amino probe (probe 1, for recognizing part of the sequence of the target DNA segment) was immobilized on the substrate through the coupling reaction between the epoxy group and the amino group. Then, the target DNA segment and the biotinized probe (probe 2, for recognizing the other part of the target DNA segment) were sequentially bound to the substrate by the interaction between complementary sequences for sandwiched hybridization. Next, streptavidin/CdTe-tagged polybeads were bound to probe 2 through biotin-streptavidin binding. The anchored CdTe QDs were finally dissolved in HNO_3 solution to release Cd^{2+} for stripping voltammetric measurements by square-wave voltammetry (SWV).

Contact-angle measurements were employed to monitor the hydrophilicity of the substrate during the immobilization and recognition procedures (Fig. S2, Supporting Information). The GPTMS-grafted, probe-1-modified, and streptavidin/CdTe-tagged polybead-conjugated substrates showed contact angles of 50°, 19°, and 30°, respectively. The lowest contact angle of the probe-1-modified substrate resulted from the presence of the many

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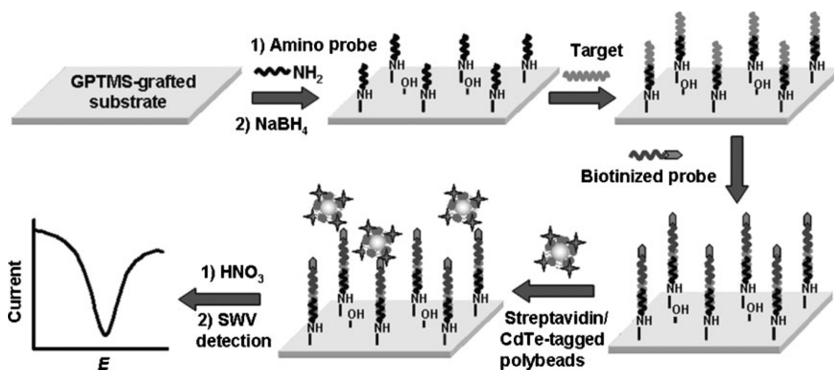

Scheme 2. Procedure for detection of DNA hybridization using streptavidin/CdTe-tagged polybeads.

phosphate groups along the chain. The binding of the streptavidin/CdTe-tagged polybeads to the probe-2-modified substrate decreased the hydrophilicity of the substrate, thus increasing the contact angle again.

2.4. Stripping Voltammetric Analysis of Target DNA

To eliminate the undesired covalent binding of the streptavidin/CdTe-tagged polybeads to the epoxy groups on the GPTMS-grafted substrate, an effective blockage of the epoxy-group binding sites is critical. Several blocking agents, such as bovine serum albumin (BSA), salmon sperm DNA and sodium borohydride reduction, were tested. The most-effective agent for eliminating the background was sodium borohydride solution. This solution, at 0.25 mg mL^{-1} in 3:1 phosphate buffered saline (PBS) (pH 7.0):ethanol could reduce the remaining active epoxy groups. After treating the probe-1-modified substrate with sodium borohydride solution, the background decreased from 31 to 13 nA, while the signal corresponding to 1.0 fmol L^{-1} target DNA decreased only from 51 to 38 nA (Fig. S3, Supporting Information). The ratio of the signal to the background increased from 1.6 to 2.9 upon the blocking step.

The hybridization efficiency was strongly influenced by the assay conditions. Therefore, the optimal concentrations of the two probes and the optimal incubation time of the target hybridization were investigated. At the substrates modified with 0.1, 0.2, 0.5 and 1.0 μmol L^{-1} probe 1, with the increasing concentration of probe 1, the response to 1.0 fmol L^{-1} target DNA at the secondary probe concentration of 1.0 μmol L^{-1} increased and tended to a stable signal at 0.5 μmol L^{-1} probe 1 (Fig. S4A, Supporting Information). Similarly, at the substrate modified with 1.0 μmol L^{-1} probe 1, on increasing the concentration of probe 2, the response to 1.0 fmol L^{-1} target DNA increased and tended to a stable signal at 0.5 μmol L^{-1} probe 2 (Fig. S4B, Supporting Information). In order to obtain a stable and sensitive signal, 1.0 μmol L^{-1} probe 1 and probe 2 were used in all subsequent work.

On increasing the incubation time from 20 to 80 min for both the target DNA and probe-2 hybridization, the response to 1.0 fmol L^{-1} target DNA increased and tended to a stable signal at an incubation time of 60 min (Fig. S5, Supporting Information). Therefore, a 60-min hybridization time was selected for the two hybridization steps.

Under optimal conditions, as shown in Figure 5, the response of the DNA biosensor to the target DNA linearly increased with the increasing logarithm value of the target concentration in the range from 10^{-11} to 10^{-15} mol L^{-1}. The limit of detection (LOD) was 0.52 fmol L^{-1} at 4-times the standard deviation of the control (free of target DNA). This LOD of 0.52 fmol L^{-1} corresponds to the detection of 2.6 zmol of target in a5 μL of sample solution. When using streptavidin/CdTe QDs as a label without the presence of polybeads, a LOD of 1.0 pmol L^{-1} was obtained, while the proposed method showed a response 29.8 times that without the polybead carrier-amplified platform at the target concentration of 10 pmol L^{-1}. Thus the suggested nanoparticle signal amplification was very efficient for ultrasensitive electrochemical detection of DNA hybridization. Compared to other electrochemical methods[42–45] without the signal amplification, this proposed nanoparticle-based method showed a much-lower limit of detection, and was competitive with the highly sensitive detection of DNA by amplifying the sample with PCR technique.[46] In addition, this method was superior to some amplification techniques, such as liposome (0.75 amol),[28] magnetic particles (5.7 fmol),[47] bio-barcodes (2.5 fmol L^{-1})[48] and Au-NP-based amplification (10 zmol).[36]

The selectivity of the streptavidin/CdTe-tagged polybead-based amplification was studied using three kinds of DNA sequence including perfectly complementary targets, one-base mismatched strands and non-complementary strands, at concentrations of 100 fmol L^{-1}. The comparison of the three responses and background is shown in Figure 6. The perfectly complementary target shows a response 4.4 times that of the single-base mismatch sequence, indicating good selectivity. The response of the non-complementary strand is only 15% that of the perfectly complementary target, which mainly results from the background, 13.4% that of the perfectly complementary target. These results demonstrate that the electrochemical DNA biosensor is able to detect effectively a

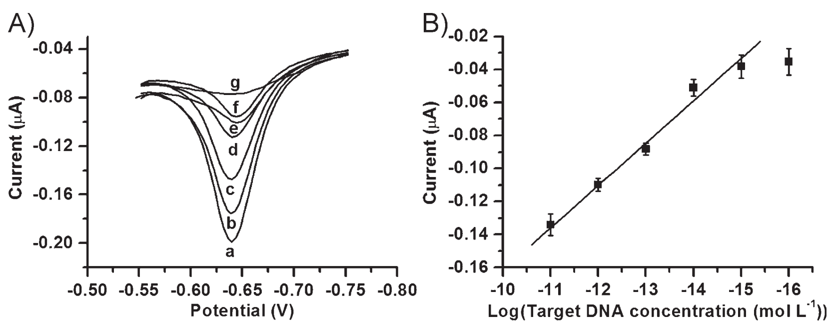

Figure 5. A) SWV curves at target DNA concentrations of 10, 1, and 0.1 pmol L^{-1}, and at 10, 1, 0.l, and 0 fmol L^{-1}, from (a) to (g) respectively. B) Linear relationship between the peak current and the logarithm of the target DNA concentration ($n = 4$).

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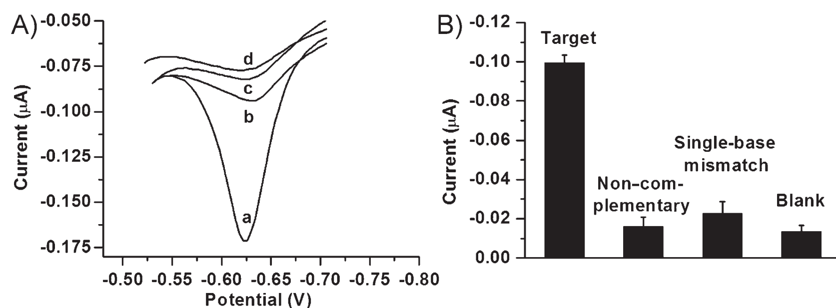

Figure 6. A) SWV curves for 1 pmol L^{-1} complementary (curve a), single-base mismatch (curve b), non-complementary sequences (curve c), and of a blank (curve d). B) Histograms for the curves in part A).

target with high specificity, and has great potential for single-nucleotide polymorphism analysis. The high selectivity possibly resulted from the "sandwich assay" with twice hybridization. Meanwhile, the proper position of the base mismatch site and the low concentrations of the two kinds of DNA molecules also contributed to the high selectivity. The presence of non-specific DNA and other surface-active components at levels similar to the target did not affect the sensitivity of the DNA detection in the real sample. However, as an assay method, a huge excess of interferents possibly decreased the detection sensitivity.

To demonstrate the extended practicality of the suggested system, a fluorescence-microscopy-imaging analysis was performed using the streptavidin/CdTe-tagged polybeads as a probe to monitor the hybridization events on a glass surface. As shown in Figure S6, a strong, blue fluorescence was observed at the DNA hybridization sites, indicating the potential of the probe for photoluminescence detection.

3. Conclusions

Highly uniform and size-controlled polybeads were obtained as a NP-amplified carrier by an emulsifier-free method and layer-by-layer assembly technology. With a simple assembly procedure, a novel streptavidin/CdTe-tagged polybead label was proposed for the construction of a carrier-amplified platform. The enormous coverage, coupled with the unique physical and chemical properties of QDs, enabled a promising and versatile amplified platform to be developed for applications in bioanalysis. An electrochemical DNA biosensor based on the streptavidin/CdTe-tagged-polybead label was constructed to evaluate their feasibility in bioanalytical application. By combining with a stripping voltammetric measurement, such a QD-carrier amplifier yielded an extremely high sensitivity, a wide linear range of 5 orders of magnitude, a sub-femtomolar detection limit and a high selectivity for detection of DNA hybridization. The proposed approach offers numerous flexible opportunities for obtaining beads with desired electrochemical, optical and biological characteristics by extending it to other template cores and to numerous types of semiconductor nanocrystals. The new detection route is superior to some other amplified techniques for application in detection of DNA, which demonstrates the application potential of the QD-tagged polybeads in bioassay.

4. Experimental

Materials and Reagents: Poly(sodium 4-styrene-sulfonate) (PSS) (molecular weight (MW) ≈ 70 000 g mol^{-1}), poly (allylamine) hydrochloride (PAH) (MW ≈ 56 000 g mol^{-1}), glycidoxypropyltrimethoxysilane (GPTMS) (98%), streptavidin and polyethylene glycol sorbitan monolaurate (TWEEN-20) were obtained from Sigma–Aldrich Inc. (USA). Styrene and acrylic acid were purified by distillation under vacuum and stored at −25 °C. Ammonium persulfate and hydrogen peroxide (30%) were purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China). Mercaptoacetic acid and cadmium chloride hemipentahydrate ($CdCl_2 \cdot 2.5H_2O$) were purchased from Alfa Aesar China Ltd. Analytical-grade sodium borohydride was purchased from Sinopharm Chemical Reagent Co. Ltd. (China). The tellurium rod was donated by Leshan Kaiyada Photoelectricity Co. Ltd. (China). All of the other reagents were of analytical grade. All of the aqueous solutions were prepared using ultrapure water (≥18 MΩ, Milli-Q, Millipore). Samples of phosphate buffered saline (PBS) (0.1 mol L^{-1}) were prepared by mixing stock solutions of NaH_2PO_4 and Na_2HPO_4 (0.2 mmol L^{-1}) and adjusting the pH with NaOH (0.1 mol L^{-1}) and H_3PO_4 (0.1 mol L^{-1}).

The oligonucleotides were purchased from Jinsite Biotechnology Co. (Nanjing China) and purified using high-performance liquid chromatography. Their sequences were: probe 1: 5′-$NH_2(A)_{10}$-ATGGGGTCTGTC-3′; probe 2: 5′-GTTCAGCTTG-biotin-3′; target: (specific to HPV-16, E7 region) 5′-CAAGCAGAACCGGACAGACCCCAT-3′; single-base mismatch: 5′-CAAGCAGAACCGTACAGACCCCAT-3′; non-complementary: 5′-GTGATCTCCGGACTTGACAATATC-3′.

Apparatus: The morphology of the polybeads was examined using a Hitachi S-4800 scanning electron microscope and a JEM 2100 high-resolution transmission electron microscope. X-ray photoelectron spectroscopy (XPS) measurements were performed using an ESCALAB 250 spectrometer (Thermo-VG Scientific, USA) with an ultrahigh vacuum generator. The UV-vis absorption spectra were recorded using a Lambda 35 UV-vis spectrometer (Perkin–Elmer instruments, USA). The photoluminescence (PL) spectra were recorded at room temperature using a F900 fluorescence spectrometer (Edinburgh Instruments Ltd., UK). The IR spectra were recorded using a Nicolet 400 Fourier transform infrared (FTIR) spectrometer (Madison, WI). The static-water contact angles were measured at 20 °C using a contact-angle meter (Rame–Hart-100) employing drops of pure deionized water. The fluorescence microscopic image was taken using a Nikon TE2000-U inverted optical microscope. Square-wave voltammetric (SWV) measurements were preformed using a CHI 430A electrochemical workstation (CH Instruments Inc., USA) at room temperature using a conventional three-electrode system with a modified glassy carbon electrode (GCE) as the working electrode, a platinum wire as the auxiliary, and a saturated calomel electrode as the reference.

Preparation of the PSA Microbeads: The PSA microbeads were prepared according to a method described in the literature [49] with some modifications. Briefly, 40 mL of ultrapure water was filled into a three-necked flask submerged in a water bath and purged with nitrogen for 1 h. 5.25 g of styrene and 0.18 g of acrylic acid were added under stirring at 350 rpm at 70 °C while purging with N_2. The emulsifier-free emulsion copolymerization of the styrene and acrylic acid was started by adding 10 mL of 0.01 g mL^{-1} ammonium persulfate solution into the mixture and refluxing for 8 h. The resulting PSA suspension was centrifuged three times with ultrapure water at 13 000 rpt for 20 min, and stored at 4 °C.

Preparation of Streptavidin/CdTe-Tagged Polybeads: The CdTe QDs were prepared according to our previous report [50] with a reflux time of 8 h at 80 °C. The self-assembled, three-layer film was formed by incubating sequentially a PSA-particle dispersion (0.2 mL, 2.5 wt%) with PAH, PSS and PAH solutions (1 mL, 1 mg mL^{-1} in 0.5 mol L^{-1} NaCl) for 30 min for each polyelectrolyte layer. Before incubation in the next solution, the resulting particles underwent centrifugation three times (13 400 rpt) with ultrapure

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water to remove excess polyelectrolyte. The outermost PAH layer gave the PE_3/PSA beads a smooth, uniform and positively charged surface, which facilitated the absorption of negatively charged CdTe capped with mercaptopropionic acid. The CdTe-tagged PE_3/PSA beads were prepared by mixing a 50 μL dispersion of the microspheres and a 1.0 mL dispersion of the CdTe QDs (1.4×10^{-5} mol L^{-1}). After absorption for 60 min, the unattached CdTe QDs were isolated by centrifugation repeated 3 times (6000 rpt) with ultrapure water. The obtained CdTe-tagged polybeads were dispersed in 50 μL of ultrapure water and stored at 4 °C.

The streptavidin/CdTe-tagged polybeads were prepared according to the method of Ellen R. Goldman et al. [51] with some modifications. 10 μL of streptavidin (1 mg mL^{-1}) and 50 μL of CdTe-tagged polybead (~4×10^{10} microspheres) were mixed in 200 μL of PBS (pH 7.4, 0.1 mol L^{-1}). After the mixture was incubated for 15 min at room temperature under stirring and kept at 4 °C for 5 min, it was centrifuged at 6 000 rpt for 10 min and washed with 0.1 mol L^{-1} PBS (pH 7.4) three times. The resulting soft sediment was resuspended in 50 μL of PBS (pH 7.4, 0.1 mol L^{-1}).

DNA Hybridization: A glass substrate (1 cm × 1 cm × 0.1 cm) was firstly immersed in piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 12 h, washed thoroughly with water and then dried under a stream of nitrogen. The obtained substrate was silylanized by dipping it in a toluene solution of 1% GPTMS overnight at room temperature [52]. Afterwards, the substrate was washed thoroughly with toluene and ethanol to remove the physically absorbed GPTMS and dried under a stream of nitrogen.

Immobilization of ssDNA on the silylanized substrate was performed by dropping 10 μL of probe 1 solution (pH 7.6), which was composed of probe 1 (1 μmol L^{-1}), Tris-HCl (10 mmol L^{-1}) and ethylenediaminetetraacetic acid (EDTA) (1 mmol L^{-1}), on the substrate, and allowing them to react at room temperature for 3 h. After the substrate was washed three times with rinsing buffer (RB) (Tris-HCl (50 mmol L^{-1}, pH 7.4) and twice with ultrapure water, the remaining active epoxy groups were reduced with sodium borohydride (1 mg in a mixture of 3 mL of PBS at a pH of 7.0 and 1 mL ethanol). It was then rinsed twice with 0.2% sodium dodecylbenzenesulfonate and dried at room temperature. The hybridization reaction was carried out by dropping 5 μL of the target solution prepared with Tris-HCl solution (pH 7.6, 10 mmol L^{-1}) containing NaCl (1 mol L^{-1}) and EDTA (1 mmol L^{-1}) on the as-prepared, probe-1-functionalized substrate and incubating them at 25 °C for 1 h. After washing with RB containing EDTA (1 mmol L^{-1}) and TWEEN-20 (0.05%)) three times and twice with ultrapure water, 5 μL of the biotinylated DNA probe (probe 2) solution (1 μmol L^{-1}) prepared with Tris-HCl (10 mmol L^{-1}, pH 7.6) containing NaCl (1 mol L^{-1}) and EDTA (1 mmol L^{-1}) was introduced and incubated at 25 °C for 1 h. Afterwards, the substrate was washed three times with RB and twice with ultrapure water, and 5 μL of the obtained streptavidin/CdTe-tagged polybead suspension was transferred onto the substrate, which was left for 30 min and then washed three times with RB and twice with ultrapure water to remove the unbound streptavidin/CdTe-tagged polybeads.

Stripping Voltammetric Detection: The streptavidin/CdTe-tagged polybead-conjugated substrate was placed in a polypropylene tube (diameter: 1.2 cm) containing 400 μL of HNO_3 (0.1 mol L^{-1}) and sonicated for 1 h to dissolve the CdTe quantum dots. The resulting solution was transferred to a HAc-NaAc buffer (1.6 mL, 0.2 mol L^{-1}, pH 5.2). The dissolved Cd^{2+} was then measured using square-wave stripping voltammetric detection with a mercury-film-modified glassy carbon electrode (diameter of 1 mm) as the working electrode, which was prepared by 4 cycles of alternate deposition at −1.0 V for 40 s and scanning from −0.9 to −0.2 V in HAc-NaAc buffer (0.2 mol L^{-1}, pH 5.2) containing 40 μg mL^{-1} Hg^{2+} under a N_2 atmosphere. The anodic stripping detection was performed by electrodepositing cadmium at −1.1 V for 6 min and then stripping from −0.9 to −0.2 V under a N_2 atmosphere using square-wave voltammetry with a potential step of 4 mV, a frequency of 25 Hz, and an amplitude of 25 mV.

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[1] Y. K. Ye, H. X. Ju, *Sensors* **2003**, *3*, 128.
[2] Y. K. Ye, J. H. Zhao, F. Yan, Y. L. Zhu, H. X. Ju, *Biosens. Bioelectron.* **2003**, *18*, 1501.
[3] H. X. Ju, Y. K. Ye, J. H. Zhao, Y. L. Zhu, *Anal. Biochem.* **2003**, *313*, 255.
[4] D. J. Caruana, A. Heller, *J. Am. Chem. Soc.* **1999**, *121*, 769.
[5] F. Patolsky, A. Lichtenstein, I. Willner, *Nature Biotechnol.* **2001**, *19*, 253.
[6] T. Deng, J. S. Li, J. H. Jiang, G. L. Shen, R. Q. Yu, *Adv. Funct. Mater.* **2006**, *16*, 2147.
[7] P. Hazarika, J. Irrgang, M. Spengler, C. M. Niemeyer, *Adv. Funct. Mater.* **2007**, *17*, 437.
[8] P. Alivisatos, *Nature Biotechnol.* **2004**, *22*, 47.
[9] N. L. Rosi, C. A. Mirkin, *Chem. Rev.* **2005**, *105*, 1547.
[10] R. Gill, M. Zayats, I. Willner, *Angew. Chem. Int. Ed.* **2008**, *47*, 7602.
[11] S. J. Guo, S. J. Dong, *Trends Anal. Chem.* **2009**, *28*, 96.
[12] R. Elghnian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078.
[13] Y. W. C. Cao, R. Jin, C. A. Mirkin, *Science* **2002**, *297*, 1536.
[14] E. Bakker, Y. Qin, *Anal. Chem.* **2006**, *78*, 3965.
[15] T. G. Drummond, M. G. Hill, J. K. Barton, *Nat. Biotechnol.* **2003**, *21*, 1992.
[16] Z. Gao, S. Rafea, L. H. Lim, *Adv. Mater.* **2007**, *19*, 602.
[17] F. Patolsky, E. Katz, I. Willner, *Angew. Chem. Int. Ed.* **2002**, *41*, 3398.
[18] Y. Xiao, A. A. Lubin, B. R. Baker, K. W. Plaxo, A. J. Heeger, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 16677.
[19] A. Numnuam, K. Y. Chumbimuni-Torres, Y. Xiang, R. Bash, P. Thavarungkul, P. Kanatharana, E. Pretsch, J. Wang, E. Bakker, *J. Am. Chem. Soc.* **2008**, *130*, 410.
[20] L. Ding, W. Cheng, X. J. Wang, S. J. Ding, H. X. Ju, *J. Am. Chem. Soc.* **2008**, *130*, 7224.
[21] R. J. Cui, H. C. Pan, J. J. Zhu, H. Y. Chen, *Anal. Chem.* **2007**, *79*, 8494.
[22] C. H. Liu, Z. P. Li, B. A. Du, X. R. Duan, Y. C. Wang, *Anal. Chem.* **2006**, *78*, 3738.
[23] J. Wang, D. Xu, A. N. Kawde, R. Polsky, *Anal. Chem.* **2001**, *73*, 5576.
[24] J. Wang, G. Liu, A. Merkci, *J. Am. Chem. Soc.* **2003**, *125*, 3214.
[25] G. Liu, J. Wang, J. Kim, M. R. Jan, *Anal. Chem.* **2004**, *76*, 7126.
[26] X. Yu, B. Munge, V. Patel, G. Jensen, A. Bhirde, J. D. Gong, S. N. Kim, J. Gillespie, J. S. Gutkind, F. Papadimitrakopoulos, J. F. Rusling, *J. Am. Chem. Soc.* **2006**, *128*, 11199.
[27] X. W. Zhao, Z. B. Liu, H. Yang, K. Nagai, Y. H. Zhao, Z. Z. Gu, *Chem. Mater.* **2006**, *18*, 2443.
[28] W. C. Liao, J. A. Annie Ho, *Anal. Chem.* **2009**, *81*, 2470.
[29] J. Wang, G. D. Liu, M. R. Jan, Q. Zhu, *Electrochem. Commun.* **2003**, *5*, 1000.
[30] A. S. Angelatos, B. Radt, F. Caruso, *J. Phys. Chem. B* **2005**, *109*, 3071.
[31] A. N. Kawde, J. Wang, *Electroanalysis* **2004**, *16*, 101.
[32] R. J. Cui, C. Liu, J. M. Shen, D. Gao, J. J. Zhu, H. Y. Chen, *Adv. Funct. Mater.* **2008**, *18*, 2197.
[33] P. Rijiravanich, M. Somasundrum, W. Surareungchai, *Anal. Chem.* **2008**, *80*, 3904.
[34] D. Trau, W. Yang, M. Seydack, F. Caruso, R. Renneberg, *Anal. Chem.* **2002**, *74*, 5480.
[35] W. C. Mak, K. Y. Cheung, D. Trau, A. Warsinke, F. Scheller, R. Renneberg, *Anal. Chem.* **2005**, *77*, 2835.
[36] S. Pinijsuwan, P. Rijiravanich, M. Somasundrum, W. Surareungchai, *Anal. Chem.* **2008**, *80*, 6779.
[37] W. W. Yu, L. Qu, W. Guo, X. G. Peng, *Chem. Mater.* **2003**, *15*, 2854.

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[38] P. Rijiravanich, K. Aoki, J. Chen, W. Surareungchai, M. Somasundrum, *Electroanalysis* **2004**, *16*, 605.
[39] X. H. Fang, X. J. Liu, S. Schuster, W. H. Tan, *J. Am. Chem. Soc.* **1999**, *121*, 2921.
[40] S. L. Pan, L. Rothberg, *Langmuir* **2005**, *21*, 1022.
[41] Z. J. Yang, Z. Y. Xie, H. Liu, F. Yan, H. X. Ju, *Adv. Funct. Mater.* **2008**, *18*, 3991.
[42] Y. Jin, X. Yao, Q. Liu, J. Li, *Biosens. Bioelectron.* **2007**, *22*, 1126.
[43] L. Alfonta, A. K. Singh, I. Willner, *Anal. Chem.* **2001**, *73*, 91.
[44] L. Moreno-Hagelsieb, B. Foultier, G. Laurent, R. Pampin, J. Remacle, J.-P. Raskin, D. Flandre, *Biosens. Bioelectron.* **2007**, *22*, 219.
[45] E. Komarova, M. Aldissi, A. Bogomolova, *Biosens. Bioelectron.* **2005**, *21*, 182.
[46] A. Ramanaviciene, A. Ramanavicius, *Anal. Bioanalytical Chem.* **2004**, *379*, 287.
[47] Ó. A. Loaiza, S. Campuzano, M. Pedrero, M. I. Pividori, P. García, J. M. Pingarrón, *Anal. Chem.* **2008**, *80*, 8239.
[48] H. D. Hill, R. A. Vega, C. A. Mirkin, *Anal. Chem.* **2007**, *79*, 9218.
[49] V. R. Polpanich, A. Currao, G. Calzaferri, *Polym. Sci.* **2005**, *284*, 183.
[50] C. W. Ge, M. Xu, J. Liu, J. P. Lei, H. X. Ju, *Chem. Commun.* **2008**, 450.
[51] E. R. Goldman, E. D. Balighian, H. Mattoussi, M. K. Kuno, J. M. Mauro, P. T. Tran, G. P. Anderson, *J. Am. Chem. Soc.* **2002**, *124*, 6378.
[52] C. M. Ruan, L. J. Yang, Y. B. Li, *Anal. Chem.* **2002**, *74*, 4814.

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Quantum-Dot-Functionalized Poly(styrene-*co*-acrylic acid) Microbeads: Step-wise Self-Assembly, Characterization, and Applications for Sub-femtomolar Electrochemical Detection of DNA Hybridization

By *Haifeng Dong*, *Feng Yan*,* *Hanxu Ji*, *Danny K. Y. Wong*,* and *Huangxian Ju**

Supporting Information

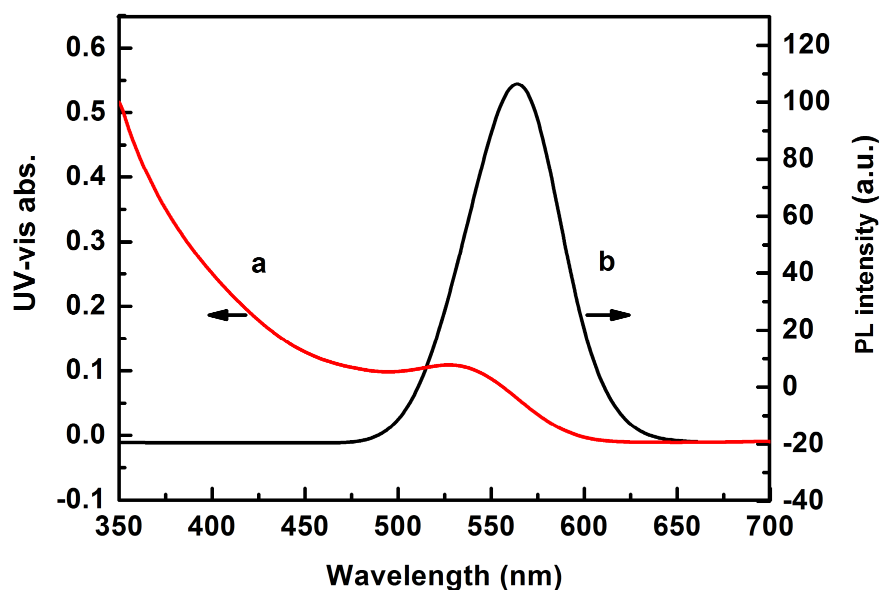

Figure. S1 UV–vis absorption (a) and PL (b) spectra (λ_{ex} = 350 nm) of MAP-capped CdTe QDs in PBS (0.1 mol L^{-1}, pH 7.4).

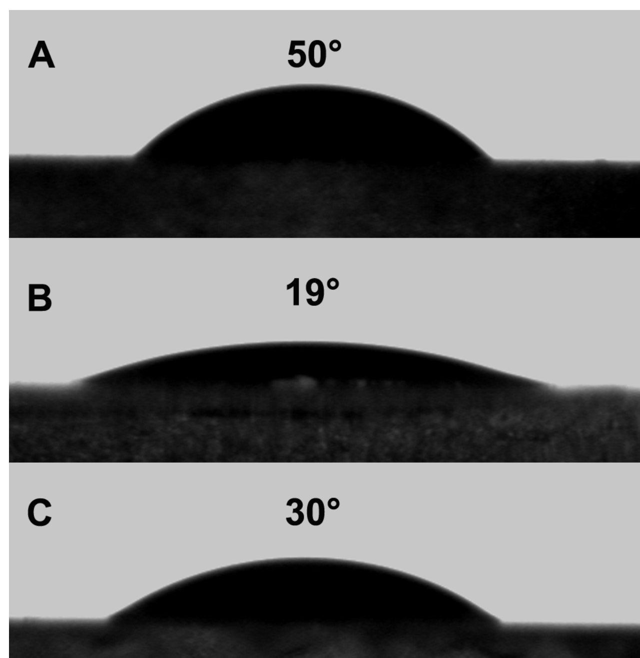

Figure S2. Contact angles of GPTMS-silylanized (A), amino probe-modified (B), and streptavidin/CdTe-tagged polybeads-conjugated (C) substrates.

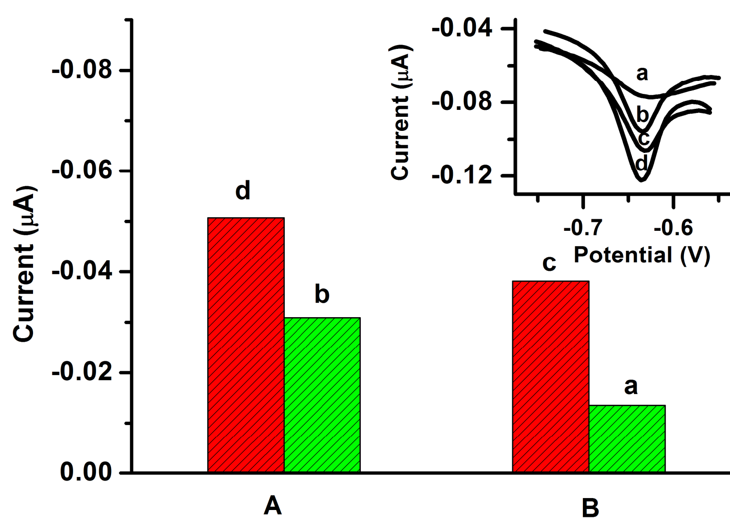

Figure S3. SWV response to unblocked (A) and blocked (B) hybridization of 0 (a) and (b), and 1 fmol L^{-1} (c) and (d) target DNA in Tris-HCl (10 mmol L^{-1}, pH 7.6) containing NaCl (1 mol L^{-1}) and EDTA (1 mmol L^{-1}). Inset shows the SWV curves.

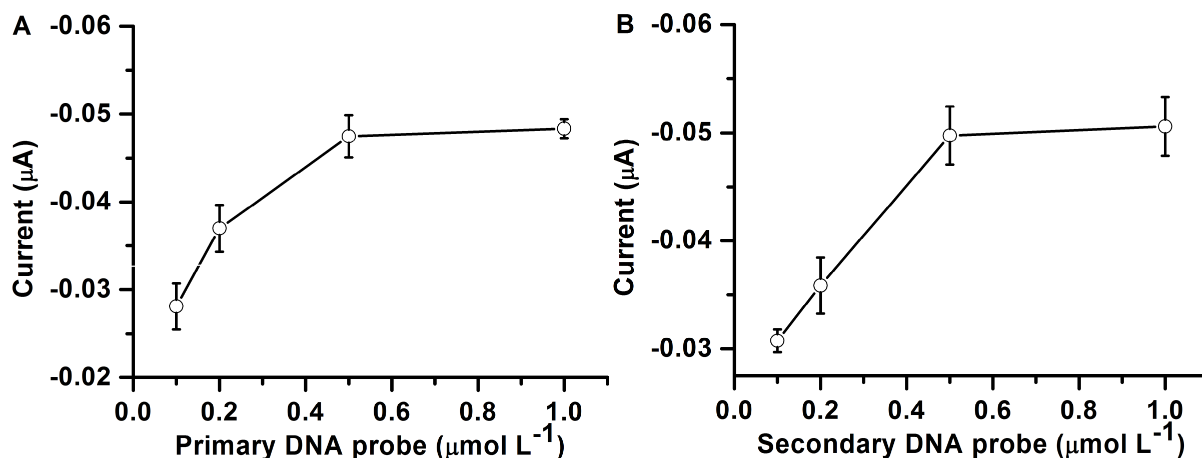

Figure S4. Influences of probe 1 concentration at 1 μmol L^{-1} probe 2 (A) and probe 2 concentration at 1 μmol L^{-1} probe 1 (B) on SWV response to 1 fmol L^{-1} target DNA in Tris-HCl (10 mmol L^{-1}, pH 7.6) containing NaCl (1 mol L^{-1}) and EDTA (1 mmol L^{-1}) with 60-min incubation (error bars: SD, n = 4).

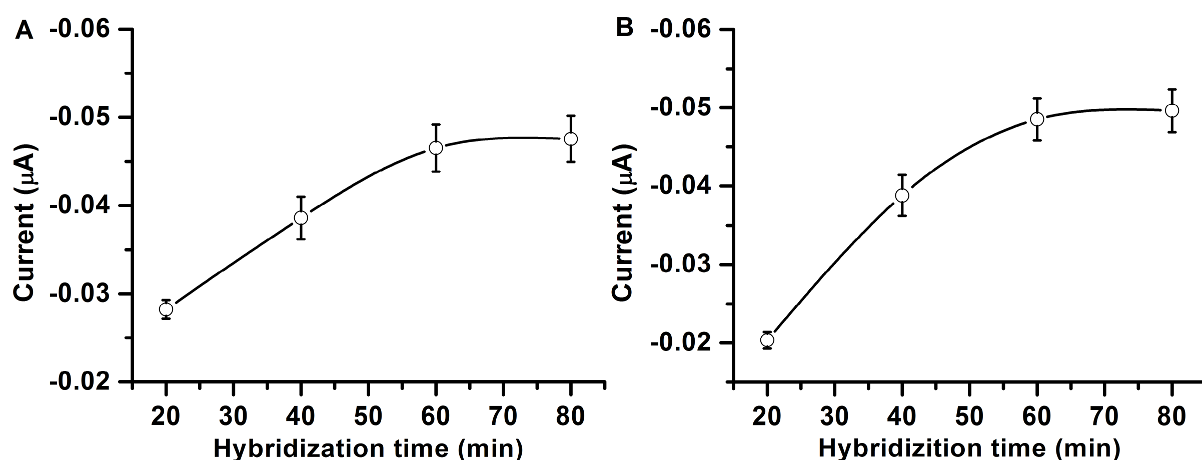

Figure S5. Effects of the primary (A) and secondary (B) DNA hybridization time on SWV response to 1 fmol L^{-1} target DNA in Tris-HCl (10 mmol L^{-1}, pH 7.6) containing NaCl (1 mol L^{-1}) and EDTA (1 mmol L^{-1}) at probes 1 and 2 concentration of 1 μmol L^{-1} (error bars: SD, n = 4).

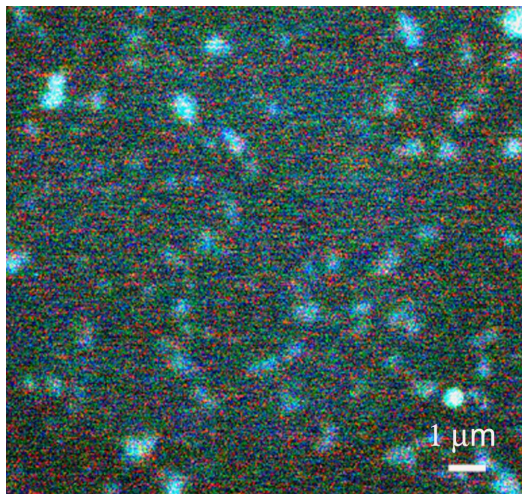

Figure S6. Fluorescence microscopic image of the resulting streptavidin/CdTe-tagged polybeads-conjugated substrate with the target concentration of 1 pmol L^{-1}. Other conditions are the same as Figure 5.