Supporting Informations

Design of One-to-one Recognition Triple Au Nanoparticles DNA probe and Its Application in the Electrochemical DNA Biosensor

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Reagents

6-Mercapto-1-hexanol (MCH) was purchased from Acros (Geel, Belgium). Hexaammineruthenium(III) ($[Ru(NH_3)_6]^{3+}$) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were from Sigma (St. Louis, USA), $HAuCl_4 \cdot 4H_2O$ was obtained from Guoyao Chemical Company (Guoyao, China).

All solutions were prepared with triply distilled water. The buffers involved in this work are as follows. DNA immobilization buffer: 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP (pH 7.4); DNA hybridization buffer: 10 mM PBS, 1.0 mM EDTA, and 1.0 M NaCl (pH 6.8); Buffer for electrochemistry: 10 mM Tris-HCl buffer (pH 7.4); 10 mM acetate buffer (pH 5.6); 100 mM phosphate buffer (pH 7.4) containing 0.3 M NaCl.

All synthetic oligonucleotides were purchased from SBS Genetech Co. Ltd. (Beijing, China). Their sequences were presented in Table S1.

Table S1. DNA Sequences Used in This Work

Name	Sequence	Description
Capture DNA (S1)	5'-SH-$(CH_2)_6$-GCA GTA ACG CTA TGT GAC-3'	Thiolated probe immobilized on gold electrode
Target DNA (S2)	5'-GGG CTC AAG ACT ACA AAT CGC GTC ACA TAG CGT TAC TGC-3'	Complementary to S1 and S3
DNA (S3)	5'-ATT TGT AGT CTT GAG CCC CAG TGT ATC GCA ATG ACG CGT GCT TTG GAC CTG TCA-3'	Fashion S3 and S4 into a bridge DNA; complementary to S2 and S6-1
DNA (S4)	5'-ACT GTC CAG GTT TCG TGC GTC ACA TAG CGT TAC TGC CGT GCT TTG GAC CTG TCA-3'	Fashion S3 and S4 into a bridge DNA; complementary to S6-1 and S6-2
Reporter probe DNA (S5)	5'-TGG AAA ATC TCT AGC AGT CGT-$(CH_2)_6$-SH-3'	Thiolated loaded on AuNP and noncomplementary to S3 and S4
Conjunctive DNA (S6-1)	5'-SH-$(CH_2)_6$-TGA CAG GTC CAA AGC ACG-3'	Thiolated loaded on AuNP and complementary to S3 and S4
Conjunctive DNA (S6-2)	5'-GCA CGA AAC CTG GAC AGT-$(CH_2)_6$-SH-3'	Thiolated loaded on AuNP and complementary to S4
Two-base mismatched DNA (S7)	5'-GGG CTC AAG ACT TCA AAT CGC GTC ACA TAG CAT TAC TGC-3'	Underline was the mismatched base
Noncomplementary DNA (S8)	5'-CAT AGT GGA CGC GAG CCG ATG ACA GAG GCT ATC GCA GTA-3'	
Conjunctive (S9)	5'-ATT TGT AGT CTT GAG CCC-SH-3'	Thiolated loaded on AuNP and complementary to S2 in the comparison experimental

Apparatus

The electrochemical measurements for cyclic voltammetry (CV), chronocoulometry (CC) and electrochemical impedance spectroscopy (EIS) were carried out on a CHI 660C electrochemical working station (Texas, USA) using a three-electrode system consisted of a platinum wire as an auxiliary electrode, an Ag/AgCl electrode as reference electrode, and a 4-mm-diameter Au disk electrode as

working electrode. UV-visible spectra were carried out on a Cary 50 UV-Vis-NIR spectrophotometer (Varian, USA). Transmission electron microscopy (SEM) image was taken with a JEOL JSM-6700F instrument (Tokoy, Japan).

Preparation of AuNPs

AuNPs were prepared according to the method reported previously with a slight modification.[1] $HAuCl_4$ and trisodium citrate solutions were filtered through a 0.22 μm microporous membrane filter prior to use, and then 1.0 mL of 1% trisodium citrate was added to 100 mL of boiling 0.01% $HAuCl_4$ solution and stirred for 10 min. at the boiling point. The final AuNPs prepared by this method have an average diameter of approximately 22 ± 2 nm as measured by TEM (Figure 1 (a)). The prepared AuNPs were stored in brown glass bottles at 4 °C.

Preparation of bridge DNA

Bridge DNA was prepared as follows: To 1 mL of 1.0×10^{-7} M S3 solution, 1 mL of 1.0×10^{-7} M S4 solution was added. And the mixture was stirred for 2 h at 37 °C. The prepared bridge DNA was kept in PBS buffer at 4°C until use.[2,3]

Modification of AuNPs with conjunctive DNA (S6-1or S6-2) and reporter probe DNA (S5)

The process of modification of AuNPs with conjunctive DNA and reporter probe DNA was performed as follows.[4,5] The mixture of 2.0×10^{-8} M conjunctive DNA (S6-1 or S6-2) and 1.0×10^{-7} M of reporter probe DNA (S5) was activated with acetate buffer (pH 5.6) and 2 μL of 10 mM TCEP for 1 h, and then added to 3 mL of freshly prepared gold nanoparticles and shaken gently overnight. Over the course of

24 h, the DNA-AuNPs conjugates were aged in salts (0.1 M NaCl, 10 mM acetate buffer) for 24 h. Excess reagents were removed by centrifuging at 10,000 rpm for 30 min.. The red precipitate was washed, and centrifuged repeatly for three times. The resulting nanoparticles were dispersed into 2 mL of 100 mM phosphate buffer (pH 7.4) containing 0.3 M NaCl and stored at 4 °C.

Preparation of one-to-one recognition Tri-AuNPs DNA probe

The one-to-one recognition Tri-AuNPs DNA probe were prepared as following. Briefly, 2 mL S6-1 and S5 labeled AuNPs and 1 mL S6-2 and S5 labeled AuNPs were mixed and formed 3 mL DNA labeled AuNPs mixed solution. Then they were added to 2 mL of 2.0 $\times 10^{-9}$ M bridge DNA solution. After shaking gently for 24 h, the solution was allowed to stand for another 48 h, followed by centrifugation for at least 30 min. at 10, 000 rpm to remove excess reagents. Following removal of the supernatant, the red oily precipitate was washed with 5 mL of 0.1 M pH 7.4 Tris-HCl buffer containing 0.1 M NaCl, recentrifuged, and redispersed in 5 mL of 0.1 M pH 7.4 phosphate buffer containing 0.3 M NaCl. The solution of prepared one-to-one recognition Tri-AuNPs DNA probe was stored at 4 °C.

Preparation of DNA sensor based on one-to-one recognition Tri-AuNPs DNA probe

The process for the fabrication and CC detection of the DNA biosensor was schematically shown in Scheme 1 (see the main text). A gold electrode was polished carefully with alumina slurries (1, 0.3, 0.05 μm) and washed ultrasonically with deionized and doubly distilled water. Then it was electrochemically cleaned in 0.5 M

H_2SO_4 solution by cyclic potential scanning between 0.3 and 1.5 V until a standard CV was obtained. Subsequently, the gold electrode was rinsed with deionized and doubly distilled water and absolute ethanol in turn.

Subsequently the electrode was immersed in 1 mL of 10 mM Tris-HCl buffer containing 0.025 μM capture DNA and incubated for 5 h. In order to avoid consequent nonspecific adsorption in the following hybridization steps, the modified electrode was immersed in the sodium phosphate solution containing 0.2 mM MCH for 1 h to block the uncovered gold surface. The sandwich-type format assay used consists of two steps. First, the modified electrode was immersed into 10 mM PBS buffer (pH 6.8) containing target DNA with the different concentration at 37 °C. One hour later, the modified electrode was immersed into 10 mM PBS buffer containing one-to-one recognition Tri-AuNPs DNA probe for 12 h. Rinsing the electrode surface with 100 mM phosphate buffer (pH 7.4) after each step of fabrication process is very important to remove nonspecifically adsorbed sequences.

Self-assembled monolayer of one-to-one recognition Tri-AuNPs DNA probe for SEM

First gold-filled sheet silicon (2 × 3 mm) was washed ultrasonically with deionized and doubly distilled water. Subsequently the gold-filled sheet silicon was immersed in 1 mL 10 mM Tris-HCl buffer containing 0.025 μM capture DNA and incubated for 5 h. Then gold-filled sheet silicon was immersed into 0.1 nM target DNA 10 mM PBS buffer (pH 6.8) at 37 °C. One hour later, the gold-filled sheet silicon was immersed into one-to-one recognition Tri-AuNPs DNA probe 10 mM PBS

buffer (pH 7.4) containing for 12 h. Finally, the gold-filled sheet silicon surface was rinsed with 100 mM phosphate buffer (pH 7.4). The self-assembled monolayer of Tri-AuNPs was characterized by a transmission electron microscopy (TEM) for size and morphology.

Electrochemical measurements

All electrochemical measurements were carried out at room temperature in a single-compartment, three-electrode glass cell using an electrochemical analyzer. CV and CC was performed in 2 mL of 10 mM Tris-HCl solution (pH 7.4) containing 50 μM $[Ru(NH_3)_6]^{3+}$, with a scan rate of 500 mV/s for CV, and a pulse period of 250 ms for CC, respectively. EIS was carried out in a degassed Tris-HCl buffer (pH 7.4) containing 0.1 M KCl and 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$.

Calculation of the ratios of AuNPs, reporter probe DNA (S5), conjunctive DNA (S6-1) and (S6-2)

Preparation of the standard solutions of gold ion, reporter probe DNA (S5), and conjunctive DNA (S6-1), (S6-2).

The preparation of 100 μg/mL gold ion stock solution: A mixture of 0.1000 g pure gold, 20 mL aqua regia and 1 g potassium chloride was heat to dissolve. After cooled to room temperature, another 30 mL aqua regia was added and then the mixture was diluted to 1000 mL with water.[6]

A series of gold ion standard solution for calibration were obtained by diluting the corresponding stock solution with 2% aqua regia solution[7]. A 100 μL of the solution containing different concentration of gold ion was transferred into glass tubes,

and 100 μL of 1 μM luminol in 1 M NaOH solution was added to record the CL signal with a luminescence analyzer. The CL intensities calibration curve of gold ion standard solution was shown in Figure S1

The regression equation could be expressed as y = 7887.16 x + 448.47 (x was the concentration of gold ions, 10^{-6} g/mL; y was the CL intensity, n = 11, r = 0.9995).[8,9]

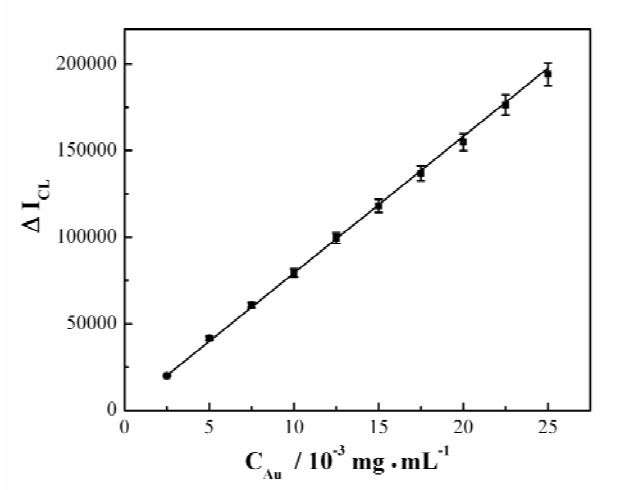

Figure S1. CL calibration curve of the standard Au ions solution

The mixture solution of reporter probe DNA (S5) and conjunctive DNA (S6-1) or (S6-2) was prepared in advance, a series of standard mixture DNA solution (S5/S6-1 or S5/S6-2) were prepared from the solution of 1.0 × 10^{-5} M mixture DNA solution (the proper proportion between S5 and S6-1 or S6-2 was 5:1) with water. The UV absorbance calibration curve of mixture DNA solution was shown in Figure S2, the regression equation could be expressed as y = 92958.84 x + 0.0017 (x was the concentration of mixture DNA solution, 10^{-7} M; y was the absorbance of UV, n = 10, r = 0.9986).

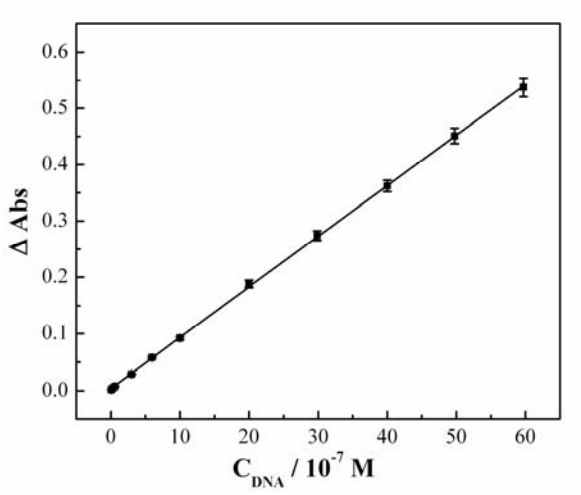

Figure S2. UV absorbance calibration curve of mixture DNA solution.

Determination of the ratios of AuNPs, mixture DNA (S5/S6-1 or S5/S6-2)

Briefly, 3 mL of the prepared gold colloid solution was added to 2 mL of different concentrations (1.2×10^{-8} M, 1.2×10^{-7} M and 1.2×10^{-6} M) of mixture DNA solutions. Meanwhile, the same amount of mixture DNA solution and 3 mL of water were mixed together, which was treated in the same way as the AuNPs solution in order to determine the total amount of mixture DNA solution through measuring the absorbance at 254 nm. After shaking gently for 24 h, the solution was allowed to stand for another 24 h, followed by centrifugation for 30 min. at 10000 rpm to remove excess reagents. Following removal of the supernatant, the red oily precipitate was washed with 0.1 M pH 7.0 phosphate buffer containing 0.1 M NaCl, recentrifuged, and redispersed in 2 mL of 100 mM pH 7.4 phosphate buffer containing 0.3 M NaCl. The absorbance of the supernatant was measured at 254 nm to obtain the amount of the mixture DNA which were not bounded with the AuNPs. The number of mixture DNA immobilized on the AuNPs can be quantitatively calculated from the

absorbance difference at 254 nm between the mixture DNA solution before and after immobilization, which was shown in Table S2.

Table S2. Quantitive calculation of the mixture DNA modified on AuNPs

Concentration of the mixture DNA solution for preparation of AuNPs modified with mixture DNA solution	UV absorbance of mixture DNA solution before immobilization*	UV absorbance of the supernatant after immobilization*	Moles of mixture DNA before immobilized on AuNPs (mole)	Moles of the supernatant mixture DNA after immobilized (mole)	Moles of mixture DNA immobilized on AuNPs (mole)
1.2×10^{-8}	0.0029	0.0001	2.58×10^{-11}	--**	2.58×10^{-11}
1.2×10^{-7}	0.0130	0.0022	2.43×10^{-10}	2.60×10^{-11}	2.17×10^{-10}
1.2×10^{-6}	0.1135	0.0424	2.41×10^{-9}	2.19×10^{-9}	2.20×10^{-10}

***** The average of three times measurements

****** Not detected

1 mL of the solution of prepared AuNPs functionalized mixture DNA was diluted to 2.5 mL and mixed with 2.5 mL of 0.01 M HCl-0.1 M NaCl-0.25 mM Br_2 solution and incubated for 10 min. to make sure that the Au dissolution was complete. The mixture was placed in the oven at 60 °C for 20 min. to remove any remaining bromine. A 100 μL of the solution was transferred into a glass tube, and 100 μL of 1 μM luminol in 1 M NaOH solution was added to record the CL signal with the luminescence analyzer. The calculation results were shown in Table S3.

Table S3. Calculation of moles of AuNPs in a given preparation

Concentration of the mixture DNA for preparation of AuNPs modified with mixture DNA	Concentration of gold ion dissolved from AuNPs modified with mixture DNA (g/mL) *	Amount of AuNPs modified with mixture DNA	Moles of AuNPs modified with mixture DNA (mole)
1.2×10^{-8}	8.70×10^{-6}	8.04×10^{11}	1.34×10^{-12}
1.2×10^{-7}	8.71×10^{-6}	8.06×10^{11}	1.34×10^{-12}
1.2×10^{-6}	8.71×10^{-6}	8.06×10^{11}	1.34×10^{-12}

*****The average of three times measurements

Calculation of the moles of AuNPs when the concentration of the signal DNA probe was 1.2×10^{-7} M[7,10]

AuNP diameter = 22 ± 2 nm

AuNP Volume = $4/3\pi r^3 = 5.57 \times 10^{-18}$ cm^3

Mass AuNP = $\rho_{Au} \times V_{AuNP} = 19.3 \times 5.57 \times 10^{-18} = 1.08 \times 10^{-16}$ (g / AuNP)

The concentration of gold ions dissolved from the AuNPs modified with mixture DNA was as follow:

$C_{Gold\ ion} = 8.71 \times 10^{-6}$ g/mL

The moles of AuNPs aggregation with mixture DNA was as follow:

$8.71 \times 10^{-6} \times 5 \times 2 / 1.08 \times 10^{-16} = 8.06 \times 10^{11}$ AuNPs

$8.06 \times 10^{11} / 6.02 \times 10^{23} = 1.34 \times 10^{-12}$ moles AuNPs

When the concentration of the mixture DNA for preparation of AuNPs modified with mixture DNA was 1.2×10^{-7} M, the ratios of AuNPs, mixture DNA, were approximately 1/162

DNA assay based on DNA-AuNPs signal amplification technology and modified AuNPs bio bar codes technology

DNA-AuNPs signal amplification technology

Modification of AuNPs with conjunctive DNA S9. The process of modification of AuNPs with conjunctive DNA S9 was performed as follows. The 1.2×10^{-7} M of conjunctive DNA S9 were activated with acetate buffer (pH 5.6) and 2 μL of 10 mM TCEP for 1 h, and then added to 3 mL of freshly prepared gold nanoparticles and shaken gently overnight. Over the course of 24 h, the AuNP which modified with one kind of DNA conjugates were aged in salts (0.1 M NaCl, 10 mM acetate buffer) for 24 h. Excess reagents were removed by centrifuging at 10,000 rpm for 30 min.. The red precipitate was washed, and centrifuged repeatly for three times. The resulting nanoparticles were dispersed into 2 mL of 100 mM phosphate buffer (pH 7.4) containing 0.3 M NaCl and stored at 4 °C.[5]

Modified AuNPs bio bar codes technology

Modification of AuNPs with conjunctive DNA (S9) and reporter probe DNA (S5). The process of modification of AuNPs with conjunctive DNA (S9) and reporter probe DNA (S5) was performed as follows. The mixture of 2.0×10^{-8} M conjunctive DNA (S9) and reporter probe DNA (S5) was activated with acetate buffer (pH 5.6) and 2 μL of 10 mM TCEP for 1 h, and then added to 3 mL of freshly prepared gold nanoparticles and shaken gently overnight. Over the course of 24 h, the AuNP bio bar codes which modified with two kinds of DNA conjugates were aged in salts (0.1 M NaCl, 10 mM acetate buffer) for 24 h. Excess reagents were removed by centrifuging

at 10,000 rpm for 30 min.. The red precipitate was washed, and centrifuged repeatly for three times. The resulting nanoparticles were dispersed into 2 mL of 100 mM phosphate buffer (pH 7.4) containing 0.3 M NaCl and stored at 4 °C.

Preparation of DNA sensor based on DNA-AuNPs signal amplification technology and single modified AuNPs bio bar codes technology

The process for the fabrication and CC detection of the DNA biosensor based on DNA-AuNPs signal amplification technology and single modified AuNPs bio bar codes technology was same as one-to-one recognition Tri-AuNPs DNA probe schematically shown in Scheme 1 (see the main text). The results were shown in Figure S3 and Figure S4.

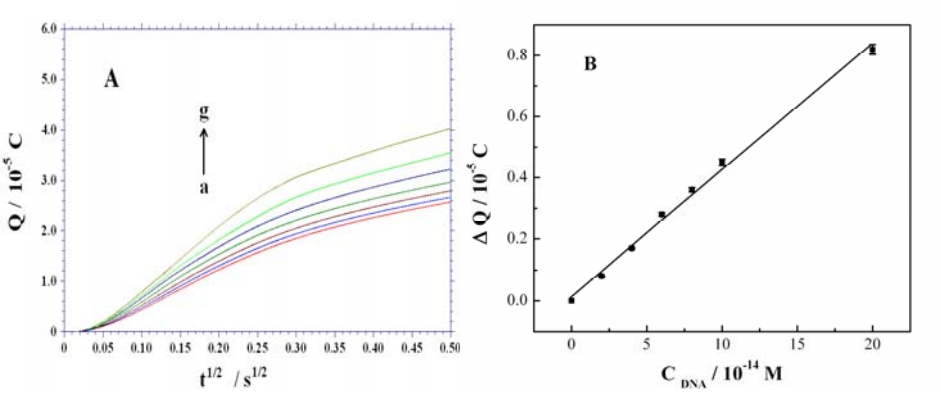

Figure S3. Chronocoulometry transients of $[Ru(NH_3)_6]^{3+}$ for different hybrids with CC DNA assay based on the DNA-AuNPs signal amplification technology. The concentrations of target DNA: (a) 0, (b) 2.0×10^{-14} M, (c) 4.0×10^{-14} M, (d) 6.0×10^{-14} M, (e) 8.0×10^{-14} M, (f) 1.0×10^{-13} M, (g) 2.0×10^{-13} M (A). The linear relationship between the CC intensity and the different concentrations of target DNA (B). The electrolyte was 10.0 mM Tris buffer (pH 7.4) containing 50 μM $[Ru(NH_3)_6]^{3+}$. Pulse period, 250 ms.

DNA-AuNPs signal amplification technology under our optimal experimentation condition can give the linear range 2.0×10^{-14} to 2.0×10^{-13} M with the detection limit of 5.0×10^{-15} M of target DNA and the regression equation y = 0.0414 x + 0.0132 (n = 7, r = 0.9977).

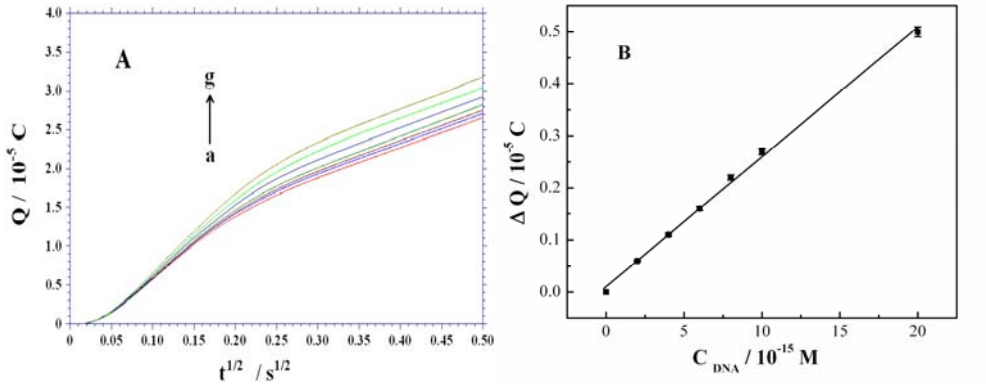

Figure S4. (A) Chronocoulometry transients of $[Ru(NH_3)_6]^{3+}$ for different hybrids. the concentrations of target DNA based on modified AuNPs bio bar codes technology: (a) 0, (b) 2.0×10^{-15} M, (c) 4.0×10^{-15} M, (d) 6.0×10^{-15} M, (e) 8.0×10^{-15} M, (f) 1.0×10^{-14} M, (g) 2.0×10^{-14} M. (B) The linear relationship between the CC intensity and the different concentrations of target DNA. The electrolyte was 10.0 mM Tris buffer (pH 7.4) containing 50 μM $[Ru(NH_3)_6]^{3+}$. Pulse period, 250 ms.

Modified AuNPs bio bar codes technology under our optimal experimentation condition showed the CC intensities of $[Ru(NH_3)_6]^{3+}$ increased with the increase of the concentration of the target DNA ranging from 2.0×10^{-15} to 2.0×10^{-14} M with the detection limit of 4.7×10^{-16} M. And the regression equation is y = 0.02492 x + 0.01054, n = 7, r = 0.99878.

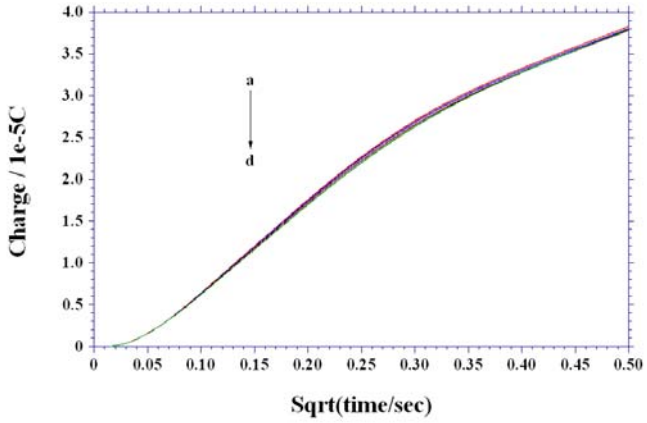

Figure S5. Chronocoulometry transients of $[Ru(NH_3)_6]^{3+}$ on DNA probe denaturation/regeneration working cycles. The original hybrid (a), first cycle (b), second cycle (c), third cycle (d). The concentration of the target DNA was 2.0×10^{-15} M.

Table S4. Comparison between the Proposed Assay and Other Reported Techniques for the Determination of DNA Hybridization with Electrochemical Detection

Label	Detection techniques [a]	Detection limit of ssDNA	Ref.
AuNPs (cross-linked)	electrochemical	100 fM	12
AuNPs	PSA	15 nM	13
AuNPs	chronocoulometric	10 fM	5
liposome	liposome-amplified electrochemical	50 fM	14
AuNPs with Ag amplification	Scanometric	50 fM	15
AuNPs with Ag amplification	electrochemical	500 fM	16
AuNPs with Ag amplification	PSA	32 pM	17
silver NPs	ASV	0.5 pM	18
ZnS, CdS, PbS NPs	stripping voltammetry	270 pM	19
AuNPs with Ag amplification	bio bar codes amplified scanometric	500 aM	20
AuNPs	bio bar codes amplified electrochemical	28 aM	4
multiwalled carbon nanotubes	ac impedance	10 nM	21
conducting polypyrrole	CV	0.16 fmol	22
poly(pyrrole-co-3-pyrrolylacrylic acid)	EIS	0.98 nM	23
magnetic nanoparticles	CC and EIS	1.7 nM	24
tin oxide NPs	photoelectrochemical	0.18 nM	25
AuNPs with Ag amplification	electrochemical	500 fM	18
one-to-one recognition Tri-AuNPs DNA probe	CC	53 aM	This method

[a] Abbreviations: ASV, anodic stripping voltammetry; CC, chronocoulometry; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; PSA, potentiometric stripping analysis

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