Supporting Information

Target-switchable DNA hydrogels coupled with $Bi_2Sn_2O_7/Bi_2S_3$ heterojunction based on in situ anion exchange for "signal-on" photoelectrochemical detection of DNA

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Experimental section

Reagents and Materials. Exonuclease (Exo III) and 10×Exonuclease III buffer were purchased from New England Biolabs Ltd. (Beijing, China). L(+)-Ascorbic acid (AA) was purchased from Sinopharm Chemical Reagent Co. $SnCl_4 \cdot 5H_2O$ and $Bi_2(NO_3)_3 \cdot 5H_2O$ were purchased from Aladdin Co., Ltd. (Shanghai, China). Sodium dodecyl sulfate (SDS), Tris(hydroxymethyl)aminoethane (Tris), Ethylene Diamine Tetraacetic Acid (EDTA), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), acrylamide, ammonium persulfate (APS), N,N,N',N'-Tetramethylethylenediamine (TEMED), sodium sulphide (Na_2S), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were obtained from Shanghai Sangon Biotechnology Co. (Shanghai, China). All reagents were of analytical grade and used as received. Ultrapure water from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used throughout the experiment. All the DNA sequences were synthesized and purified by Sangon Biotech (Shanghai) Co., Ltd. The DNA sequences used in this work are listed in Table S1.

Table S1. Sequences of the DNA

Name	From (5'-3')
P1	Acrydite-TTAATCGAAATCGTGATAGG
P2	Acrydite-GGTAAGAGTGCTTCACACGG
LK	CGGCCTGAAGCCTCCGTGTGAAGCACTCTTACCCCTATCACGATTTCGATTAA
PD	TTAATCGAAATCGTGATAGGGGTAAGAGTGCTTCACACGGAGGCTTCAGGCCGGTG
CP	NH_2-TTTTTTCGGCCTGAAGCCTCCGTGTGAAGCACTCTTACCCCTATCACGATTTCGATTAAGTCTTCCAGTGTGATG
Target	TCATCACACTGGAAGACGC
T1	TCTTCACACTGGAAGACGC
T2	TCTTCACACTGGAAGATGC

R ATCGATCGATCGATCGATC

Apparatus. Transmission electron micrograph (TEM) image was acquired using a JEM-2100 instrument (JEOL, Japan). Scanning electron microscopy (SEM) image was acquired using a S-4800 instrument (Hitachi, Japan) with the voltage of 20 kV. All of the photoelectrochemical (PEC) signals were recorded on a PEAC 200A PEC analyzer (Aida Hengyi Technology Development Co., Ltd., Tianjin, China). The reference electrode was an Ag/AgCl electrode, the working electrode was Indium tin oxide (ITO) glass (0.4 cm×4 cm), and a Pt wire was the counter electrode. UV-vis absorption spectra were measured with Lambda 35 UV Visible Spectrometer (Japan). Polyacrylamide gel electrophoresis (PAGE) analysis was performed on the Beijing JUNYI electrophoresis analyzer and imaged on the Biorad ChemDoc XRS (USA). X-ray photoelectron spectroscopy (XPS) was obtained on Multifunctional imaging electron spectrometer (Thermo ESCALAB 250Xi). JSM-6700F field emission scanning electron microscopy (FESEM, Japan). X-ray diffraction (XRD) analysis was performed with Cu Kα radiation on a D/Max 2500V/PC Rigaku diffractometer. Fourier transform infrared (FT-IR) spectra were obtained on a BRUKER OPUS 80V FT-IR spectrometer (BRUKER, Karlsruhe, Germany).

Preparation of DNA Hydrogel

Acrydite-modified DNA strand A (2 μL, 100 μM) and B (2 μL 100 μM) were added to 94 μL of stock solution, respectively. The stock buffer contained 10 mM Tris (hydroxymethyl) aminoethane (Tris), 1 mM Ethylene Diamine Tetraacetic Acid (EDTA, pH = 8.0), 140 mM sodium chloride (NaCl), 5 mM potassium chloride (KCl), 1 mM calcium chloride ($CaCl_2$), 1 mM magnesium chloride ($MgCl_2$) and 3% acrylamide. Then the solution was deoxygenated with N_2 for 15 min. Later, 1.5% (v/v) freshly prepared initiator ammonium persulfate (0.1 g/mL APS) and accelerator N, N, N', N'-Tetramethylethylenediamine (3% v/v TEMED) were quickly added to the solution for an additional 5 min to induce the polymerization, obtaining polyacrylamide-DNA polymer P-A and P-B. To prepare DNA hydrogel, P-A (100 μL, 2 μM), P-B (100 μL, 2 μM), linker DNA (2 μL, 100 μM), and sodium sulphide (Na_2S, final concentration: 50 mM) were mixed and incubated at 60 °C for 5 min, and then it gradually came down to room temperature to generate the hydrogel.

Assembly of capture strands CP on SiO_2 microsphere

1 μL of carboxyl-modified SiO_2 microspheres (2.5%, w/v) was activated with 24.5 μL of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC 5 mg/mL) and 24.5 μL of N-Hydroxysuccinimide (NHS 5 mg/mL) by incubating at 37 °C for 1.5 h in the centrifuge tube. The solvent used in this process is MES Buffer (pH=6.0, 1 wt% SDS). After centrifugation (6000 rpm, 5 min), the activated microspheres were redispersed in the MES Buffer (pH=6.0, 1 wt % SDS). Then the amino-modified capture strands CP (final concentration 5 μM) was added to the above solution and incubated at 37 °C overnight. The resulting solution was centrifuged to remove the excess sequence (6000 rpm, 5 min), and the resulting precipitation was redispersed to secondary water (50 μL) and stored at 4 °C for future use.

Exo III-assisted target cycling amplification reaction

10 μL of 1 μM PD DNA was added to 10 μL of SiO_2-CP solution and incubated at 37 °C for 2 h. Different concentrations of target DNA and Exo III (10U) were added to the above solution and incubated at 37 °C for two hours to obtain abundant product chains (PD). Then, the mixture was incubated at 70 °C for 30 min to inactivate Exo III. After centrifugation (6000 rpm, 5 min), the obtained supernate was stored at 4 °C before use.

The process of polyacrylamide gel electrophoresis

At first, we prepared 50×TAE buffer (tris 24.2 g, EDTA 3.72 g, glacial acetic acid 5.71 mL, adding water to 100 mL). Next, we take 10 mL centrifugal tube and add water (4256 μL), 40% Polyacrylamide gel (3500 μL), 50×TAE buffer (160 μL), APS (80 μL), TEMED (4 μL). The freshly prepared gel was added to the gel box and the comb was inserted into the gel. Then the gel polymerized at room temperature for 2 hours. Afterwards, the gel box was transferred to the electrophoresis tank and we added 1×TAE buffer to 2/3 of the electrophoresis tank. The comb in the gel was pulled out and the sample was added to the gel aperture. The electrophoresis was carried in 1× tris-acetic acid EDTA (TAE) (pH 8.0) at 180 V constant voltage for 3 min and 135 V for 1.5 h. After EB staining, the gel was scanned using a gel imaging analyzer (Saizhi Venture Technology Co., Ltd. Beijing, China).

Results and discussions

Investigation of the Optical Band Gap (Eg), Valence Band, and Conduction Band, for the semiconductor.

The band gap energies (Eg) of semiconductor can be obtained by the following Tauc plot formula: $(\alpha h\nu)^n=c(h\nu-Eg)$, α is absorption coefficient; h is Planck's constant; ν is frequency; c is constant; Eg is band gap energy. As for the direct bandgap semiconductor, n=1/2; For the indirect bandgap semiconductor, n=2. In addition, the valence band (VB) and conduction band (CB) of semiconductor can be calculated by the following formula: (1) $E_{VB}=X-E_e+0.5Eg$; (2) $E_{CB}=Eg-E_{VB}$. ($E_{VB:}$ valence band potential; $E_{CB:}$ conduction band potential; X: absolute electronegativity of semiconductor; Ee: potential energy of free electron in standard hydrogen electrode; Eg: band gap energy).

Optimization of the experimental conditions

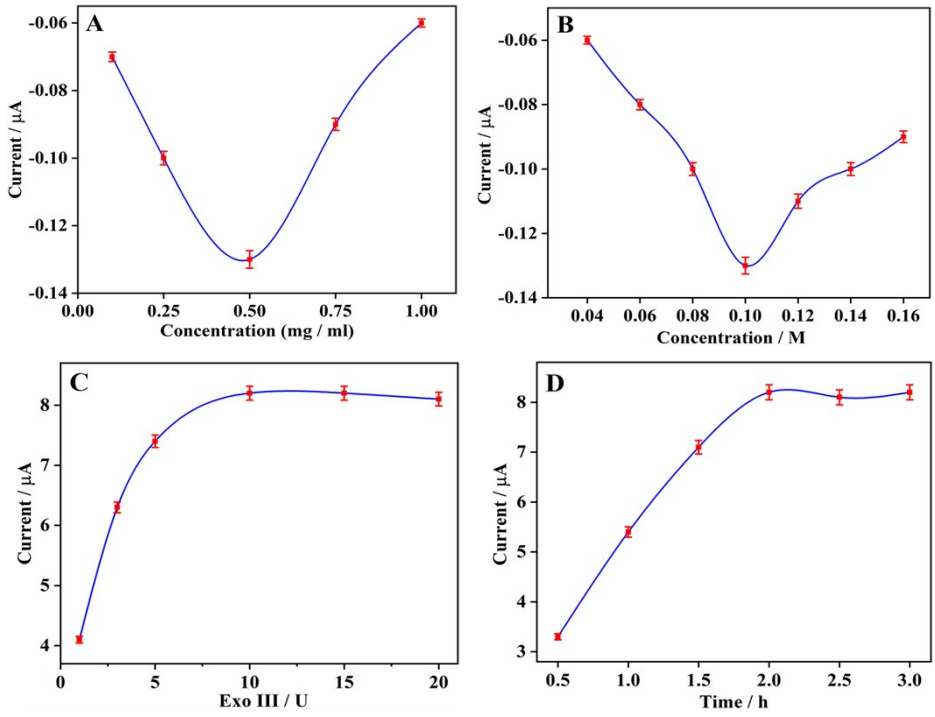

Figure S1. Optimization experiments: Effects of (A) The concentration of $Bi_2Sn_2O_7$; (B) The concentration of AA; (C) The dosage of Exo III; (D) The incubation time of Exo III on the PEC signal of the biosensing system.

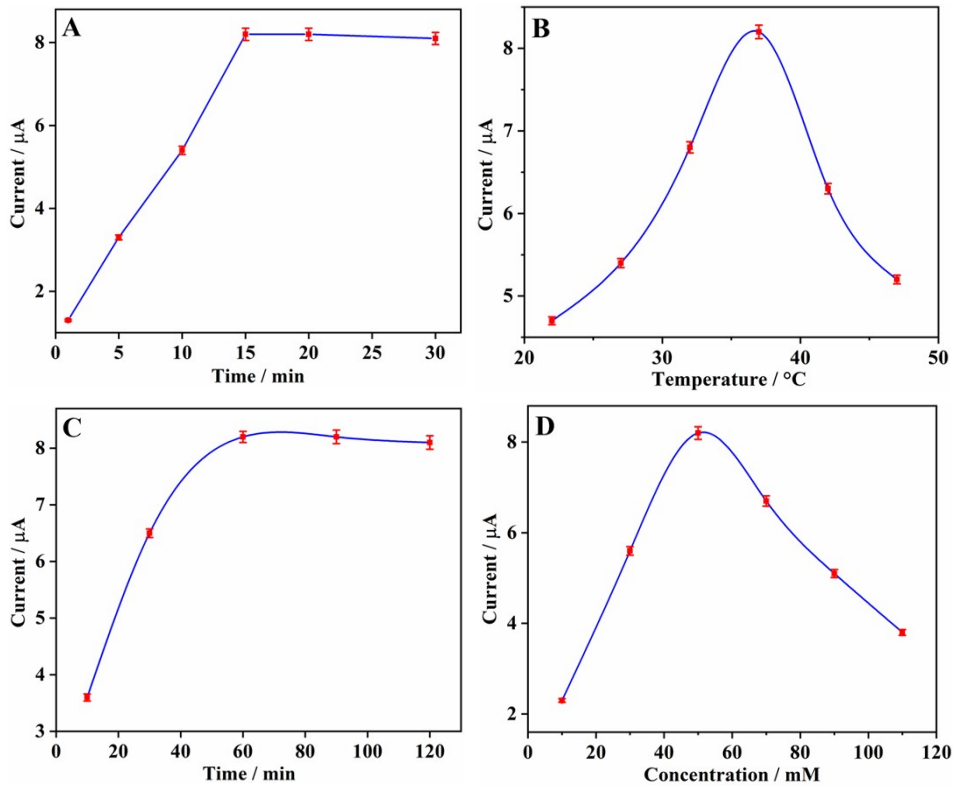

Figure S2. Optimization experiments: Effects of (A) The time of deoxygenation with N_2; The incubation temperature (B), and time (C) of opening the DNA hydrogel; (D) The concentration of Na_2S on the PEC signal of the biosensing system.

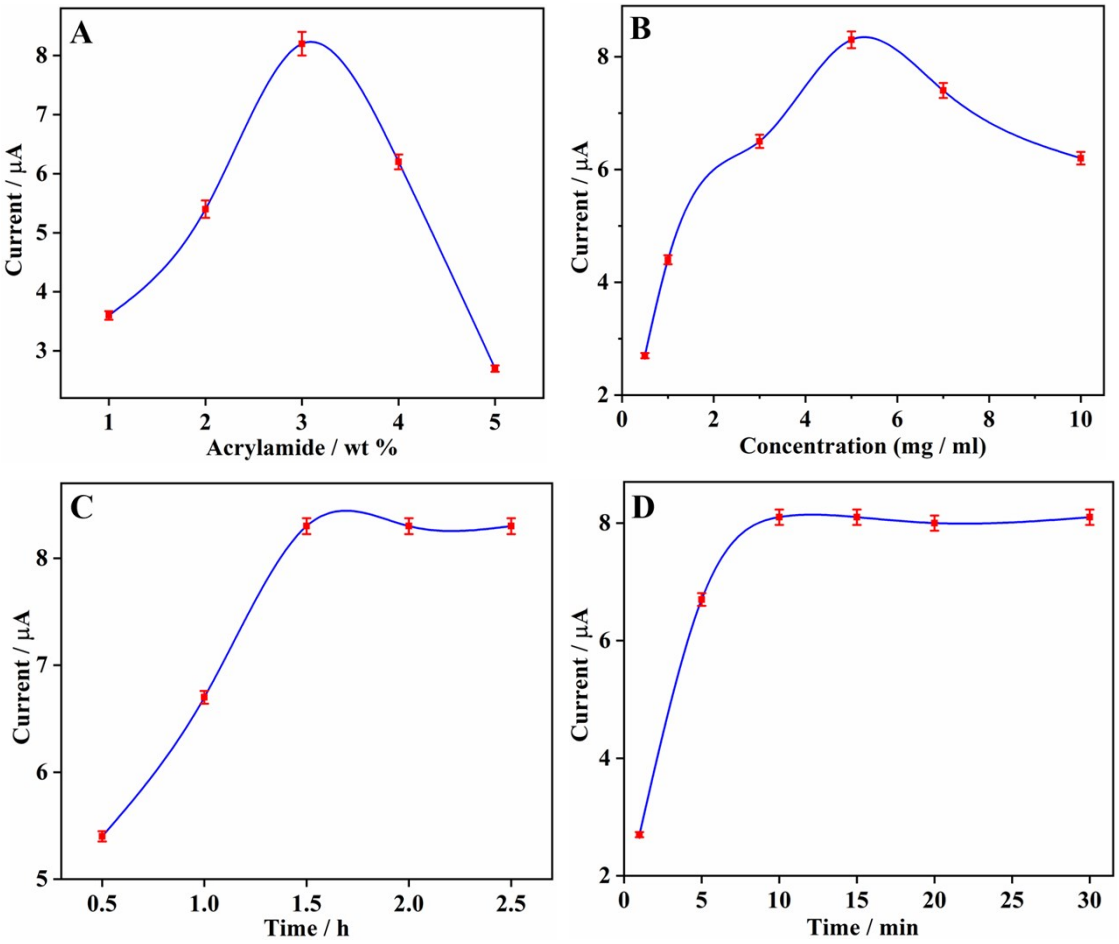

Figure S3. Optimization experiments: Effects of (A) The dosage of acrylamide; The concentration (B) and activation time (C) of EDC and NHS; (D) The time of ion-exchange reaction the PEC signal of the biosensing system.

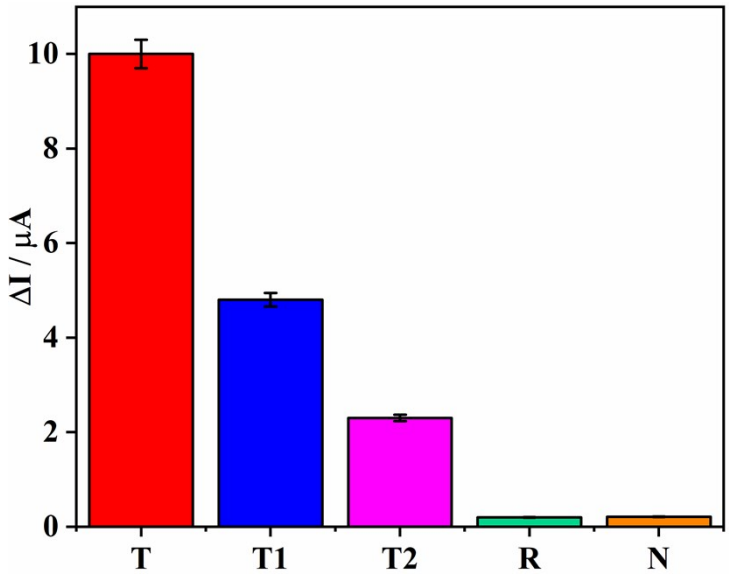

Figure S4. The selectivity of the PEC biosensor (T:Target; T1: single-base mismatched target; T2: two-base mismatched target; R: random sequence; N: non-complementary DNA).

Table S2. The comparison of the proposed biosensor with other various biosensors

Detection methond	detection range	detection limit	Reference
Fluorescence	100 fM-10 pM	81 fM	(S1)
PEC	10 pM-1 nM	35 fM	(S2)
ECL	100 fM-100 nM	33.6 fM	(S3)
ECL	50 fM-500 fM	36 fM	(S4)
PEC	100 fM-10 nM	20 fM	(Our work)

Table S3. Results of Target DNA Detection with PEC biosensor in Diluted Serum Samples

Sample	Add (pM)	Found (pM)	Recovery (%)	RSD (%, n=3)
1	0	6.24		2.87
2	10	15.82	95.8	3.64
3	20	25.70	97.3	3.28
4	50	57.99	103.5	3.82

References

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[S4] Wang, M. Q. He, F. H. Zhai, J. Wang, R. H. He, Y. L. Yu, Autonomous DNA nanomachine based on cascade amplification of strand displacement and DNA walker for detection of multiple DNAs. Biosens. Bioelectron. 105 (2018) 159-165.