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-Supporting Information-

A new visible-light-driven photoelectrochemical biosensor for probing the DNA-protein interaction

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Materials and Apparatus

DNA Oligonucleotides were acquired from Sangon Biotechnology Company, Ltd. (Shanghai, China) and contained the following sequences:

5'-NH_2-AGGACTTTTATAGTGGAGGCCGCG-SH-3' (DNA S1).

5'- CGCGGCCTCCACTATAAAAGTCCT-3' (DNA S2).

The ITO slices (type N-STN-S1-10, China Southern Glass Holding Co., Ltd) were used as the working electrode. 6-Mercapto-1-hexanol (MCH), tris (hydroxymethyl) aminomethane (Tris), tris (2-carboxyethyl) phosphinehydrochloride (TCEP), Poly(diallyldimethylammonium chloride) (PDDA; 20%, w/w in water, MW=200000-350000), hexaammineruthenium(III) chloride (RuHex), ascorbic acid(AA), TATA binding protein (Constituents: 20% Glycerol, 20 mM Tris HCl, 100 mM Potassium chloride, 1 mM DTT, 0.2 mM EDTA, pH 8.0), thrombin, alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were obtained from Sigma Aldrich. $CdCl_2 \cdot 2.5H_2O$ was obtained from Shanghai Jinshan Tingxin Chemical Plant. $Na_2S \cdot 9H_2O$ was obtained from Shanghai Lingfeng Chemical Reagent Co., LTD(Shanghai, China). Phosphate buffer solution (PBS, pH 7.4) is prepared from $Na_2HPO_4 \cdot 12H_2O$ and NaH_2PO_4. A stock solution of the DNA probe was prepared at a concentration of 100 μM in 10 mM Tris-HCl buffer solution (pH7.4) and was stored frozen. A solution of 10 mM Tris-HCl (pH 7.4) was used to dilute the stock solution when needed. All the other chemicals were of analytical grade. All aqueous solutions were prepared with ultrapure water (Milli-Q, Millipore).

Photoelectrochemical (PEC) measurements were performed with a homemade PEC system. A 500 W Xe lamp equipped with monochromator was used as irradiation source to produce the monochromatic illuminating light on the front of the electrode. Photocurrent was measured on a

CHI 750a electrochemical workstation (Shanghai Chenhua Apparatus Co., China) with a three-electrode system: a modified ITO electrode with geometrical circular area (radius-0.5 cm) as the working electrode, a Pt wire as the counter electrode and a saturated Ag/AgCl electrode as the reference electrode. All the photocurrent measurements were performed at a constant potential of 0 V (versus Ag/AgCl). A 0.1 M PBS containing 0.1 M AA was used as the blank solution for photocurrent measurements, which was degassed by highly pure nitrogen for 10 min before PEC experiments and then kept over a N_2 atmosphere for the entire experimental process. Transmission electron microscopic images (TEM) were performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage. UV-vis absorption spectra were acquired with a Shimadzu UV-3600 UV/vis spectrophotometer.

Fabrication of TGA-stabilized CdS QDs modified ITO electrode.

The utilized CdS QDs were synthesized according to the previous report with slight modification.[1,2] Briefly, 250 μL of TGA was added to 50 mL of 1.0×10^{-2} M $CdCl_2$ aqueous solution, N_2 was bubbled throughout the solution to remove O_2 for 30 min at 110 ℃. During this period, 1.0 M NaOH was added to adjust the pH of the above solution to the desired value of 11. Then, 5.5 mL of 0.1 M Na_2S aqueous solution was injected into this solution to obtain TGA-capped water-soluble CdS QDs and the reaction mixture was refluxed under N_2 atmosphere for 4 h. This procedure produced CdS QDs with a Cd to S (Cd/S) ratio of 1:1.1. Finally, the desired TGA-stabilized CdS QDs were obtained and then diluted with the same volume of water and stored in a refrigerator at 4℃ for further use.

ITO slices were cleaned by immersion in 2M boiling KOH solution solved in 2-propanol for 20 min, followed by washing copiously with water and dried at 120 ℃ for 2 h. The PDDA/CdS multilayer film was grown by alternately dipping of the cleaned ITO slices into a solution of 2% PDDA containing 0.5 M NaCl and the as-obtained CdS QDs solution for 10 min, respectively. The ξ-potential of the cationic polyelectrolyte PDDA and TGA-capped CdS QDs utilized in this work are determined as + 6.27 mV and -16.6 mV, respectively. This process was repeated 4 times to obtain desired photocurrent intensity. The films were carefully washed with doubly distilled water after each dipping step.

Immobilization of capture DNA and TBP detection

Immobilization of capture DNA to the CdS QDs modified electrodes was accomplished via the commonly used EDC coupling reactions between COOH groups on the surface of CdS QDs and the NH_2 groups of capture DNA. The CdS QDs modified electrodes were immersed in a solution containing 10 mM EDC and 20 mM NHS for 60 min at room temperature. After rinsing, 25 μL of 1μM DNA S1 was dropped onto the surface of the electrode and incubated at 4℃ overnight, then immersed in MCH solution (1mM in 10mM PBS) at the same pH for about 60 minutes to remove the nonspecifically attached DNA and simultaneously to eliminate the steric hindrance among attached DNA molecules. After removal of MCH by thoroughly rinsed with 10mM PBS solution, 25 μL of 1μM DNA S2 was dropped onto the surface of the electrode and incubated at 37℃ for 90 minutes to form the DNA duplex. Then the electrode was rinsed with 10 mM PBS buffer (pH 7.4) to wash off the excess DNA S2.

The obtained QDs-duplex DNA assembly was served as the work electrode in the following photoelectric chemical analysis and the initial photocurrent signal is measured in 0.1 M PBS solution containing 0.1 M AA. Next, 25 μL of analyte TBP with different concentrations were dropped onto the QDs-duplex DNA modified electrodes for an incubation of 60 min in moist environment at 37 ℃. Thereafter, the electrodes were rinsed with 10 mM PBS, pH 7.4, and then introduced for the respective PEC measurements.

Stability of the TBP biosensor

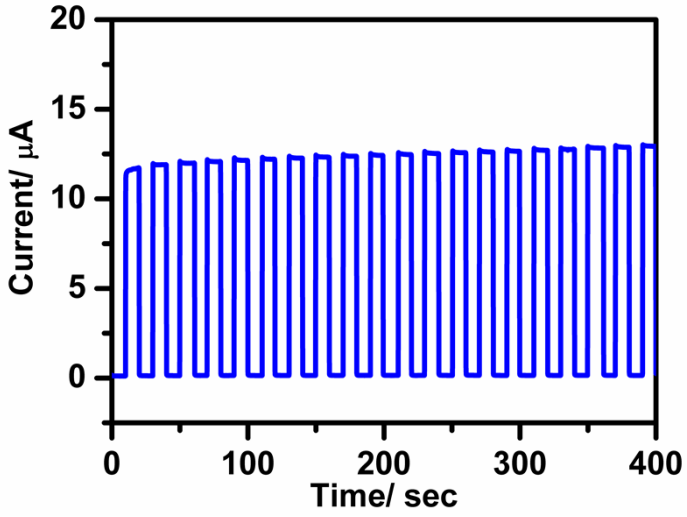

Fig. S1 Photocurrent stability of the proposed biosensor responding to 200 ng/mL TBP

DNA surface density determination

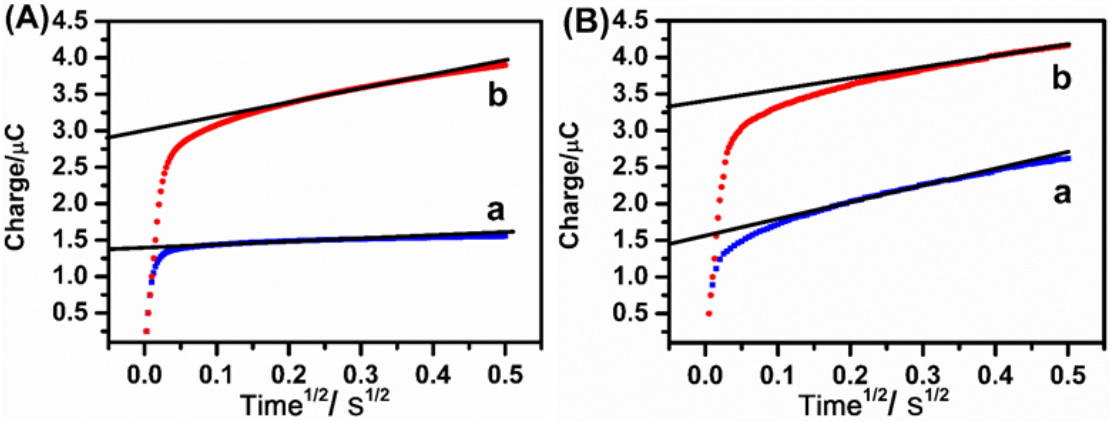

Fig. S2 (A) Chronocoulometric response curves for DNAS1 modified electrodes in the absence (a) and presence (b) of 50 μM RuHex. The lines represent the fit to the data used to determine the intercept at t=0 for the according curves. (B) Chronocoulometric response curves for duplex DNA modified electrodes in the absence (a) and presence (b) of 50 μM RuHex. The lines represent the fit to the data used to determine the intercept at t=0 for the according curves.

We calculated the surface densities of DNA probes according to the formulas:

$\Gamma_{DNA} = \Gamma_o$ (z/m)(NA); $Q(t=0) = Q_{dl} + nFA\Gamma_o$ (n is the number of electrons per molecule for reduction, F the Faraday constant (C/equiv), A the electrode area (cm^2), Q_{dl} the capacitive charge (C), Γ_o designates the surface excess and represents the amount of redox marker confined near the electrode surface, Γ_{DNA} is the probe surface density in molecules/cm^2, m is the number of bases in the probe DNA, z is the charge of the redox molecule, and N_A is Avogadro's number.), the surface densities of DNA S1 and duplex DNA probe for TBP in the biosensor are calculated to be 1.61×10^{12} and 1.26×10^{12} molecules/cm^2.

References.

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