Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary information

A Novel Photoelectrochemical Aptasensor Based on the Modulation of the Dye Sensitized TiO_2 Photoelectrode

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Experimental Section

Reagents and instruments Degussa P25 TiO_2 powder was used in the experiment to prepare the semiconductor film. N719 dye was acquired from Shanghai Materwin New Materials Co., Ltd.. Thrombin, (3-aminopropyl)triethoxysilane (APTES), glutaraldehyde (GA), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Co. LLC. All other chemicals were obtained from Sinopharm Chemical Reagent Corporation Ltd. and were used as received without further purification. Phosphate buffered saline (PBS, 0.1 M) was prepared by mixing the 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4 solutions to pH 7.4, and was diluted to obtain 10 mM PBS. All solutions were prepared by using Milli-Q pure water.

The 29-mer thrombin aptamer (TBA) and the complementary sequences (C1) were synthesized and purified by Invitrogen Biotechnology. The sequences were modified at the terminals by the NH_2 group, as following:

TBA, (5' to 3') NH_2-AGTCCGTGGTAGGGCAGGTTGGGGTGACT;

C1, (5' to 3') AGTCACCCCAAC-NH_2.

The photoelectrochemical signals were detected by CHI660D electrochemical workstation (CH Instruments, USA). UV-vis absorption spectra were performed using a Lambda 25 spectrometer (PerkinElmer, USA) with Milli-Q pure water as blank reference solution. UV-vis diffuse reflectance spectroscopy (DRS) measurements were recorded by a V-660 spectrometer (JASCO, Japan).

Preparation of the biosensor The TiO_2 thin film was prepared by a simple doctor blade method. First, FTO glass was cleaned by ultrasonic with detergent, water, iso-propanol and ethanol respectively. After that, 3 g ethyl cellulose (EC) was mixed with 20 g terpineol and the mixture was stirred for several minutes to disperse the EC powder. Thereafter, the mixture was kept at 80 °C for 2 h until a transparent solution was formed. 3.8 g above viscous solution and 0.5 g TiO_2 powder was added into a mortar and grinded for 30 min to form a uniform paste. Then, the TiO_2 paste was cast onto the FTO glass by doctor blade with 3M tape as spacer. The result TiO_2 film was dried at 50 °C and was annealed at 450 °C for 2 h to remove the organics. The film was carefully scratched by lens cleaning paper and sealed with silastic to control the exposed area as 0.25 cm^2.

The prepared TiO_2 film was wetted by several drops of water and illuminated by a Xe lamp for 30 min. Next, the film was modified with amino group by immersing in 1% (v:v) APTES/ethanol solution for 1 h and rinsing with ethanol and water, respectively. Then the electrode was transferred into 2.5% (v:v) GA aqueous solution for 2 h and completely washed with water to remove the unstable adsorption GA. 20 μL 10 mM PBS containing 50 μM amino modified TBA was dropped onto the surface of the GA modified TiO_2 film and kept at 4 °C for 12 h. The result electrode was rinsed carefully with PBS and stocked in the 10 mM PBS solution at 4 °C.

Photoelectrochemical experiment The photoelectrochemical detections were carried out with a three electrode system including the sample electrode as working electrode, a platinum foil as auxiliary electrode and a Ag/AgCl electrode as reference electrode. The electrolyte was 0.1 M PBS (pH=7.4) containing 10 mM ascorbic acid (AA) as electron donor. The light resource was a 500 W Xe lamp (Shanghai Lanpu, China) with a UV cut-off filter (λ>420 nm). Before the photoelectrochemical testing, the samples were carefully washed with 0.1 M PBS (pH=7.4) and immersed in the electrolyte for 10 min to fully remove the absorption molecules. After the background photocurrent was stabilized, the photocurrent response was subsequently recorded as the excitation light was turned on and off.

Light Absorption of TiO_2 and N719

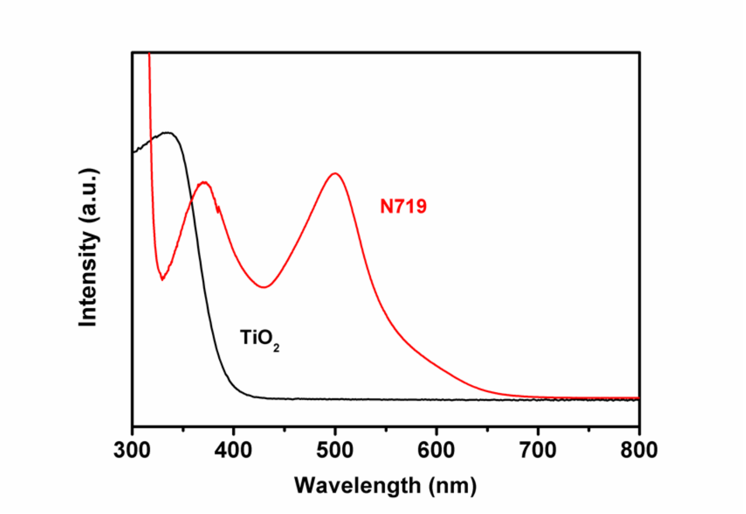

Fig. S1 The normalized diffuse-reflection spectra of TiO_2 film and UV-vis absorption spectra of N719 aqueous solution.

Optimizations of the Concentration of AA and pH Value

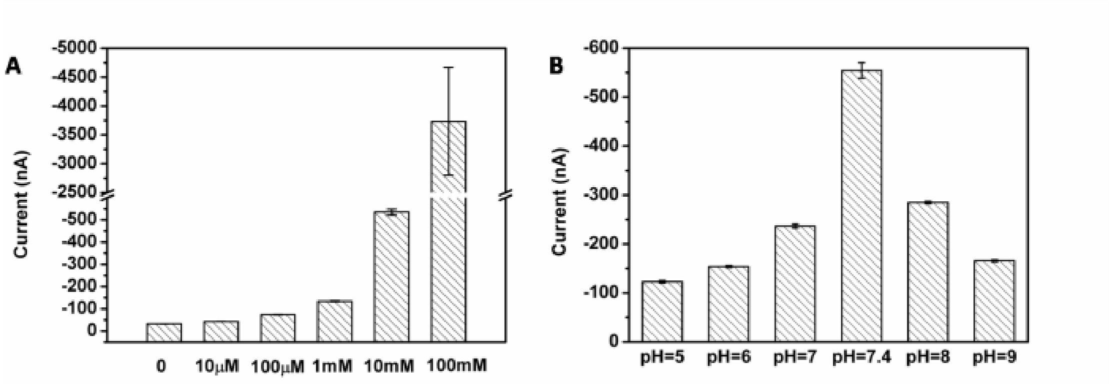

Fig. S2 Photocurrent responses of the TiO_2/TBA/C1-N719 electrode in 0.1 M PBS (pH=7.4) containing different concentrations of AA (A) and in 0.1 M PBS containing 10 mM AA with different pH values (B).

Selectivity of the PEC Aptasensor

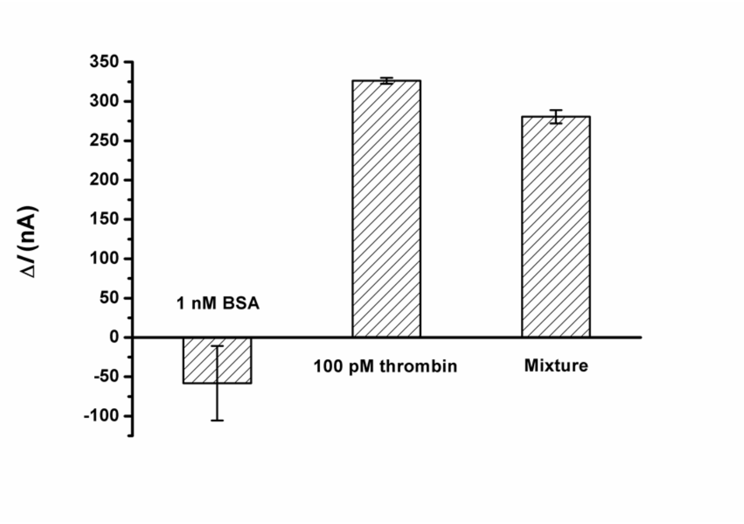

Fig. S3 Selectivity of the PEC aptasensor against BSA.