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A TiO_2-Au-polymer hybrid system for photoelectrochemical immunoassay of SirT1

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Experimental section

Chemicals and materials

N-hydroxysuccinimide (NHS), 1-ethyl-3-3(3-dimethylaminopropyl) carbodiimide (EDC), dopamine, hydroxylamine hydrochloride, *N*-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), pentaerythritol tetrakis(3-mercaptopropionate) (PTMP), tetrakis (hydroxymethyl) phosphonium chloride (THPC), SirT1, goat anti-rabbit IgG (Ab_2), goat anti-rabbit IgG-peroxidase (Ab_2-HRP), and bovine serum albumin (BSA) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Goat polyclonal anti-SirT1 ($Ab_1^{\#1}$), rabbit polyclonal anti-SirT1 ($Ab_1^{\#2}$) were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Hyper branched polymer was a gift from Dr. Liang Ding, Department of Chemistry, East China Normal University. TiO_2 (P-25, Φ = 30 nm) was purchased from Degussa Company. Chloroauric acid ($HAuCl_4$), trisodium citrate, and methanol were obtained from China National Medicines Corporation Ltd. (China). Disodium hydrogen phosphate, potassium dihydrogen phosphate, and hydrogen peroxide (30% w/v solution) were purchased from Shanghai Chemical Reagent Co. (China). Phosphate buffer solutions (PBS) with different pH were prepared by using Na_2HPO_4 and KH_2PO_4. Doubly distilled water was used throughout the experiments.

Apparatus and instruments

The scanning electron micrographs (SEM) were carried out on S-4800 (Hitachi Co., Ltd., Tokyo, Japan) equipped with an energy dispersive X-ray (EDX) analyzer. The TEM images were performed by using transmission electron microscopy (JEOL Model JEM 2100, Japan). The morphologies of the polymers were studied on a BioScope atomic force microscope (AFM) (NanoScope IIIa SPM System, Digital Instruments, Veeco Instruments Inc., USA). The element compositions of the products were measured by inductive coupled plasma-atomic emission

spectroscopy (ICP-AES) on an iCAP 6300 (Thermo Fisher Co., Waltham, MA, USA). 1H (500 MHz) NMR spectra were recorded using tetramethylsilane as an internal standard in dimethyl sulfoxide-d6 on a Bruker DPX spectrometer. Gel permeation chromatography (GPC) was performed at 45 °C at a flow rate of 1 mL/min against linear polystyrene standards (the molecular weights ranged from 10^3 to 10^6 Da) across a set of four 5 μm PL Gel columns (Polymer Laboratories) with porosities of 100, 500, and 1000 Å and Mixed C. Light absorption was measured using a UV-visible spectrophotometer (model Cary 50, Varian Corp., USA). A 300 W Xe lamp with a monochromator was used as the light source. Electrochemical experiments were achieved on a CHI 660D electrochemical system (CH Instruments, Austin, TX, USA), with a conventional three-electrode system comprising a platinum wire as auxiliary electrode, a saturated calomel reference (SCE) as reference electrode, and a bare or modified electrode as working electrode.

Results and discussion

Optimal conditions for photoelectrochemical detection

The applied potential is an important factor relevant to the photocurrent response. As seen in Figure S1A, with the increments of applied potential, the photocurrent was enhanced and leveled off at 0.6 V (curve a). This indicated that the photogenerated electrons were effectively driven to the counter electrode by the positive potential, which could be beneficial to charge separation. In addition, the introduction of GC-HBAP into the photoelectrochemical immunosensor leads to a prominent enhancement in photocurrent with the potential ranging from -0.1 V to 0.9 V (curve b). This phenomenon can be explained that the application of HBAP, which acts as an electron acceptor, provides a facile pathway for the transfer of electrons, resulting in the higher photocurrent. Therefore, 0.6 V was selected as the applied potential for the photoelectrochemical experiments in this study.

In the immunoassays, temperature and time for the antigen-antibody interaction greatly influence the sensitivity of the developed immunosensor. In Figure S1B, with an increasing incubation temperature from 10 to 50 °C, the immunosensor showed a maximum photocurrent response at 37 °C after incubated with 60 ng mL^{-1} of SirT1. Consequently, the temperature of 37 °C was selected for further studies.

At the optimized temperature, the photocurrent response increased with incubation time and reached a plateau at 60 min (Figure S1C). An incubation time longer than this did not clearly improve the response. Therefore, subsequent experiments employed 60 min as the optimum time for all the incubation steps of the assay.

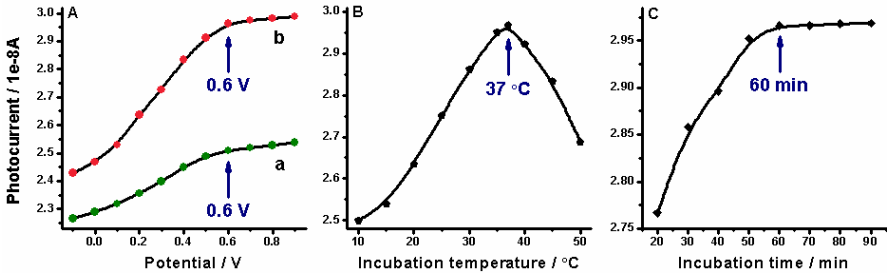

Figure S1. (A) Influence of the applied potential of {TiO_2-Au/Ab_2}/$Ab_1^{\#2}$/SirT1/$Ab_1^{\#1}$/PDOP/ITO (a) and {TiO_2-Au/Ab_2}/$Ab_1^{\#2}$/SirT1/$Ab_1^{\#1}$/GC-HBAP/PDOP/ITO (b) immunosensor on the photocurrent response; Effects of (B) incubation temperature, and (C) incubation time on the photocurrent response of the {TiO_2-Au/Ab_2}/$Ab_1^{\#2}$/SirT1/$Ab_1^{\#1}$/GC-HBAP/PDOP/ITO immunosensor toward 60 ng mL^{-1} SirT1 in 0.05 M Na_2SO_4.

Reproducibility and stability of the photoelectrochemical immunosensor

The repeatability and reproducibility of the photoelectrochemical immunosensor were assessed by detecting SirT1 at six levels for five repeated measurements, and the mean current responses are listed in Table S1. The results suggest that the reproducibility of the {TiO_2-Au/Ab_2}/$Ab_1^{\#2}$/SirT1/$Ab_1^{\#1}$/GC-HBAP/PDOP/ITO immunosensor was satisfactory.

The regeneration of the immunosensor was investigated by rinsing it with a stripping buffer, namely, pH 2.8 Gly-HCl solution, to dissociate the antigen–antibody complex. The renewed immunosensor could restore 96.2% of the initial photocurrent after six cycles, which is indicative of high reusability and stability.

The steady-state value of the photocurrent was quite stable with time. The irradiation process was repeated 25 times over more than 1000 s, providing reproducible photocurrent responses without noticeable decrease in their intensity (Figure S2). The long-term stability of the immunosensor was also examined. When the immunosensor was stored in pH 7.4 PBS at 4 °C after a storage period of two weeks, it retained 95.8% of its initial photocurrent response to 60 ng mL^{-1} SirT1, indicating its good long-term storage stability.

Table S1. Reproducibility of the photoelectrochemical immunosensor.

C_{SirT1} [ng mL^{-1}]	5	10	50	100	200	500
I [μA][a]	2.788	2.816	2.931	3.047	3.279	3.863
R.S.D [%]	2.5	2.3	2.2	1.9	1.8	1.5

[a] The mean value of five assays.

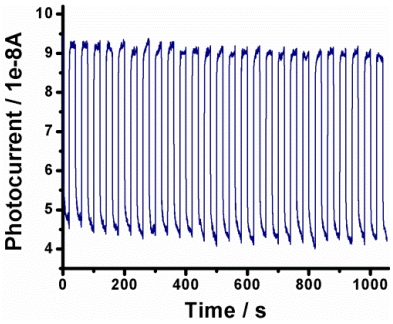

Figure S2. Time-based photocurrent response of the {TiO_2-Au/Ab_2}/$Ab_1^{\#2}$/SirT1/$Ab_1^{\#1}$/GC-HBAP/PDOP/ITO immunosensor in 0.05 M Na_2SO_4 as the excitation light turned on and off.

Analysis of etoposide-treated cells

Etoposide is an important chemotherapeutic agent that is used to treat a wide spectrum of human cancers. As etoposide could enhance SirT1 expression, we also investigated the dose-dependent relationship between SirT1 expression and etoposide concentration, and the time-dependent relationship between SirT1 levels and the treatment time. As displayed in Figure S3A, with the increments of time from 0 to 12 hours, the SirT1 concentration was increased. In Figure S3, SirT1 levels increased with doses of etoposide from 0 to 10 μM for 6 hours. The measurements obtained from the photoelectrochemical immunosensor were more clearly than that from western blot analysis, and also suggest that SirT1 levels are sensitized to the doses of etoposide and treatment time. In order to study SirT1 expression in different cell lines, we treated H1299, MCF-7, and HCT116 cells with 20 μM etoposide for 6 hours. Figure S3C revealed that SirT1 levels increased accordingly after etoposide treated. All these results were consistent with the result by western blot analysis in Figure S3D, S3E and S3F.

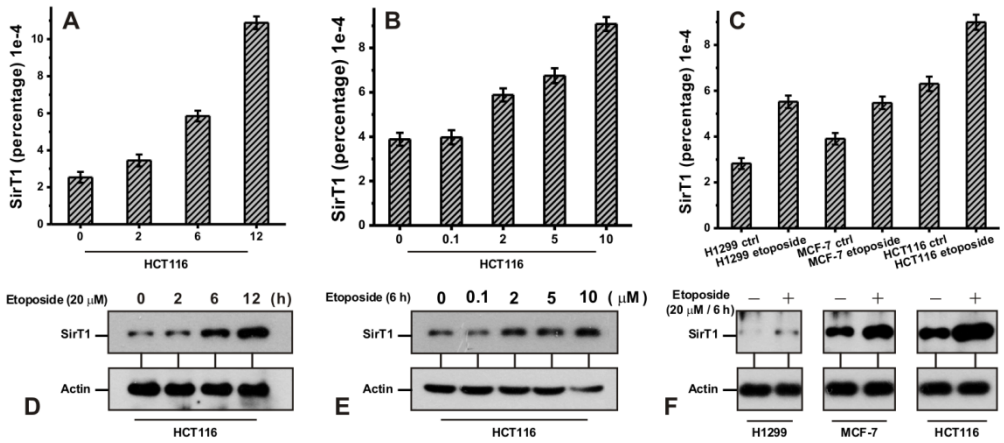

Figure S3. The percentage of SirT1 expression analyzed by photoelectrochemical immunosensors (A), (B), and (C); SirT1 expression analyzed by western blot (D), (E), and (F). HCT116 cells were treated with 20 μM etoposide for 0, 2, 6, 12 hours (A) and (D); HCT116 cells were treated with different doses of etoposide (0, 0.1, 2, 5, 10 μM) for 6 hours (B) and (E); H1299, MCF-7, and HCT116 cells were treated with 20 μM etoposide for 6 hours (C) and (F).