Supporting Information for

Polymer Dots Synergized with NiO Hole Transporting Layer and Poly(amido amine) Dendrimer: Toward Sensitive Photocathodic Detection of Tyrosinase Level in Serum

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Contents

Materials and methods

Materials: Tyrosinase (TYR) from mushroom, photosensitizer tetraphenylporphyrin (TPP), conjugated polymer poly[(9,9′-dioctylfluorenyl-2,7-diyl)-co-(1,4-benzo-{2,1′,3}-thiadiazole)] (PFBT), poly(diallyldimethylammonium chloride) (PDDA, M_W = 200000−350000, 20% w/w in water), Poly(amido amine) (PAMAM G4 generation), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS) and $Ni(NO_3)_2 \cdot 6H_2O$ were purchased from the Sigma Aldrich (Shanghai, China). Poly(styrene-co-maleic anhydride) (PSMA) was obtained from Tianjin Heowns Biochem LLC (China). $NH_3 \cdot H_2O$ and hydrofluoric acid (HF) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydroquinone and tetrahydrofuran (THF) were purchased from Aladdin Biochemical Technology Co. Ltd. (Shanghai, China). 1,4-benzoquinone were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). The 96-well plates (MaxiSorp) were obtained from Thermo Fisher Scientific Inc. Human blood samples were collected from Yizheng People's Hospital Affiliated with the Medical College of Yangzhou University. All experiments were performed under the Guidelines "Helsinki Declaration" and approved by the ethics committee at Yangzhou University. Informed consent was obtained from human participants in this study. All chemicals were analytical reagents and used without further purification. All solutions were prepared in ultrapure water, using a Millipore Milli-Q water purification system (Billerica, MA).

Preparation of TPP-doped PFBT Pdots: The TPP-doped PFBT Pdots was prepared using the reprecipitation method described previously.[1-4] The conjugated polymer PFBT (1 mg mL^{-1}), PSMA (1 mg mL^{-1}), and photosensitizer TPP (1 mg mL^{-1}) were dissolved and mixed in THF with a PFBT concentration of 100 μg mL^{-1}, a PSMA concentration of 20 μg mL^{-1}, and a TPP concentration of 5 μg mL^{-1}. Then, the resulting solution was sonicated to form a homogeneous mixture. A total of 10 mL of deionized water was sonicated in a bath sonicator, while 2 mL of the mixture solution was injected into deionized water quickly. After that, THF was removed by nitrogen stripping, and the residual Pdots solution was filtered through a 0.22 μm Millipore filter. Finally, the Pdots solution was concentrated by rotary evaporation at 55 °C.

Fabrication of PAMAM/Pdots/NiO/FTO: FTO was cut into 0.7 cm × 3.3 cm pieces and then subjected to 5 min oxygen plasma treatment to enhance the hydrophilia. Next, the slices were dipped into the precursor solution containing 14 mL of 0.03 M $Ni(NO_3)_2 \cdot 6H_2O$, 14 mL of 0.09 M HF and 300 μL of $NH_3 \cdot H_2O$ at 60 °C for 2 h in an oven. Then, the as-treated slices were calcinated under 450 °C for 1 h to obtain the NiO/FTO. The Pdots/NiO/FTO was fabricated by first immersing the cleaned NiO/FTO into a solution of 2% PDDA containing 0.5 M NaCl for 10 min, and carefully washed with ultrapure water. Then, the electrode was immersed in the Pdots solution for 10 min and carefully washed with ultrapure water. This process was repeated three times to obtain the Pdots/NiO/FTO heterojunction. The carboxylic acids on the surface of the Pdots/NiO/FTO were then covalently bonded with the dendrimer Poly(amido amine) (PAMAM) through EDC/NHS activation approach. Specifically, the Pdots/NiO HTL was incubated with the solution containing 20 mg mL^{-1} EDC and 10 mg mL^{-1} NHS for 1 h at room temperature and carefully washed with 10 mM phosphate buffered saline (PBS, pH 7.4). The Pdots/NiO HTL was then placed in the 1 wt% PAMAM solution for 30 min and cleaned with 10 mM PBS to remove the non-immobilized PAMAM. Finally, the PAMAM/Pdots/NiO/FTO was used for further study.

PEC strategy for probing TYR-based bioassays: The PAMAM/Pdots/NiO/FTO was exploited toward sensitive photocathodic bioanalysis of TYR level with a standard addition method. Specifically, we prepared a TYR incubation solution containing 20 μL of 1.0 mM hydroquinone, 20 μL of different concentrations of TYR (0, 1, 5, 10, 50, 100, 1000, 5000, 10000 U L^{-1}) and 160 μL of 10 mM PBS. TYR reaction mixture was set in 96 wells plate for 2 h at 37 ℃ to allow BQ generation. Then, 20 μL of the reaction mixture was dropped on the PAMAM/Pdots/NiO/FTO for 10 min. Finally, the PAMAM/Pdots/NiO/FTO was carefully washed with 10 mM PBS. Finally, the PAMAM/Pdots/NiO/FTO was tested in 10 mM PBS at 0 V.

PEC strategy for sensing TYR in human serum samples: TYR activity in human serum samples was used to assess the feasibility of the detection potential of this PEC sensor for real clinical analysis. After 10 min of ultracentrifugation at 12,000 rpm at room temperature, the blood supernatant was used as a serum sample. TYR activity was detected in the diluted

human serum (0.01%, the sample was diluted 10^4-fold) with 10 mM PBS. Then different concentrations of TYR containing 0.1 mM hydroquinone were injected into the diluted human serum samples. The detection of TYR in diluted human serum samples was similar to the standard addition method for detecting TYR in 10 mM PBS.

Characterization: Scanning electron microscopy (SEM) images were recorded by a Hitachi S4800 scanning electron microscope (Hitachi Co., Japan). The transmission electron microscope (TEM) images were characterized by the JEM-2000 instrument (JOEL Ltd., Japan). The X-ray photoelectron spectrum (XPS) was recorded on an ESCALAB 250 spectrometer (Thermo Scientific Co. USA) with an ultrahigh vacuum generator. UV-vis absorption spectra were gained from a Shimadzu UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co., Japan). The fluorescence emission spectra were obtained on a Shimadzu fluorescence S-3 spectrophotometer (RF-5301PC, Shimadzu Co., Japan). Photocurrents were performed with a CHI 760e electrochemical workstation (China) in a self-regulating three-electrode system: the PAMAM/Pdots/NiO/FTO with a fixed circular area (diameter 5 mm) served as the working electrode, a Pt wire and a saturated Ag/AgCl electrode were employed as the counter electrode and the reference electrode, respectively. A LED lamp with monochromatic emitting at 455 nm was used as an irradiation source.

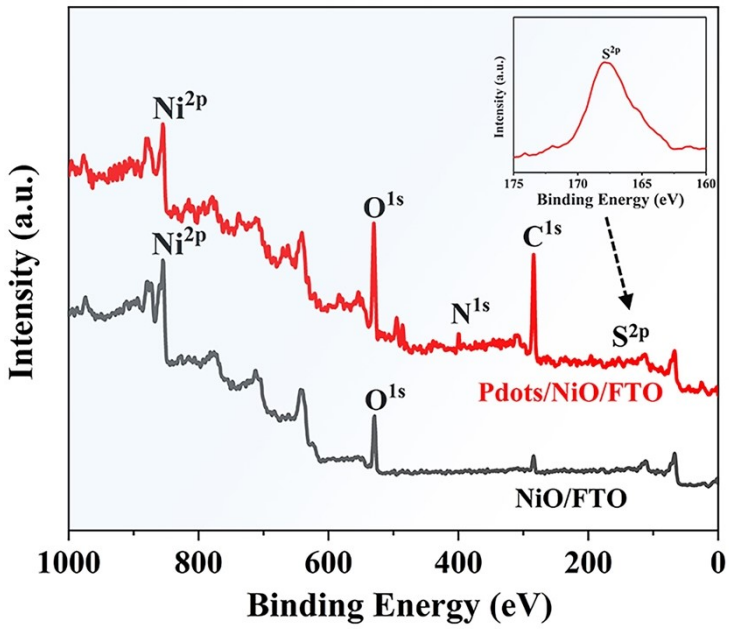

Fig. S1 Full-scan XPS spectrum of the as-fabricated NiO/FTO, Pdots/NiO/FTO, and the high-resolution XPS spectra of the S^{2p} region (inset).

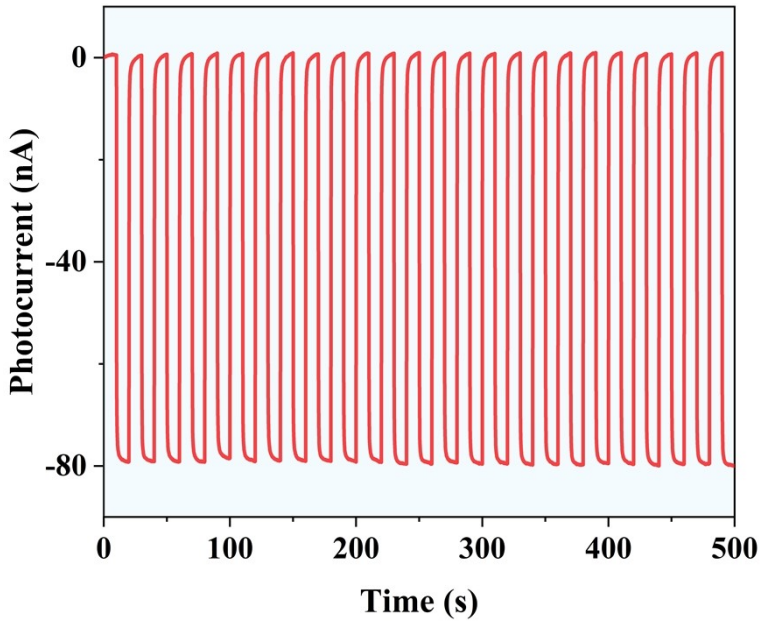

Fig. S2 The stability of the photocurrent response of the PAMAM/Pdots/NiO/FTO by repeated on/off illumination cycles.

References

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