Supporting Information

In Situ Modification of Semiconductor Surface by an Enzymatic Process: A General Strategy for Photoelectrochemical Bioanalysis

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EXPERIMENTAL SECTION

Materials and Apparatus.

Indium tin oxide (ITO) with type of N-STN-S1-10 was purchased from China Southern Glass Holding Co., Ltd. (Shenzhen, China), with coating thickness of 180±20nm and sheet resistance of 8.1±0.6 Ωcm^{-2}. $(NH4)_2TiF_6$ were obtained from Adamas Reagent Co., Ltd (Shanghai, China). H_3BO_3 was purchased from Nanjing Chemical Reagent Co., Ltd (China). Alkaline phosphate (ALP), ascorbic acid 2-phosphate (AAP), 2, 4-dichlorophenoxyacetic, acid (2, 4-DA) were purchased from Sigma-Aldrich (St. Louis, MO). Other chemicals were of analytical reagent grade and used as received. The dilution and usage of ALP should with 0.01 M Tris-HCl (pH 8.12) containg 5 mM $MgCl_2$ and 0.1 mM $ZnCl_2$.[1] The washing buffer solution was 0.01 M Tris-HCl (pH 8.12). All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

PEC measurements were performed with a homemade PEC system equipped with a 500W Xe lamp and a monochromator. Photocurrent was measured on a CHI 750a electrochemical workstation (China) with a three-electrode system: a modified ITO electrode with a geometrical area of 0.25 ± 0.01 cm^2 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 (vs saturated Ag/AgCl) and under the illumination of 410 nm visible light. To avoid the inbibitory effect of inorganic phosphate on ALP, 0.1 M Tris-HCl (pH 8.1) Tris-HCl buffer system, rather than the commonly used phosphate buffer solution (PBS), was used as the supporting electrolyte for photocurrent measurements. The solutions were deaerated by bubbling highly pure nitrogen for at least 15 min before experiment and a nitrogen atmosphere was kept over the test solution during the PEC detections. UV-vis absorption spectra were obtained on a Shimadzu UV-3600 UV/vis spectrophotometer (Shimadzu Corporation,

Japan). Scanning electron microscopic (SEM) images were recorded by a Hitachi S4800 scanning electron microscope (Hitachi Co., Japan).

Fabrication of the Liquid Phase Deposited TiO_2 Nanoparticles (NPs) Modified ITO Electrodes and the PEC Bioanalysis Development.

The ITO slices were cleaned by immersion in 2 M boiling KOH solution solved in 2-propanol for 20 min, followed by washing copiously with water and dried at 120 °C for 2 h.

Liquid phase deposition (LPD) of TiO_2 was performed according to previous report[2] with slight modification. $(NH_4)_2TiF_6$ (2.0096 g) and H_3BO_3 (1.8642 g) were separately dissolved in deionized water (100 mL). 0.3 M H_3BO_3 solution was added to 0.1 M $(NH_4)_2TiF_6$ solution with the volume ratio of 1:1. Then the freshly cleaned ITO substrates were immersed vertically into the mixed solution immediately. After keeping the solution at room temperature for 48 h, the as obtained TiO_2 film was rinsed with distilled water and followed by annealing at 500 °C for 60 min in air atmosphere to transform the amorphous TiO_2 into anatase TiO_2 and finally naturally cooled down to room temperature.

Subsequently, 20 μL of 2.5 mg/mL ALP was spread onto the ITO/TiO_2 electrode surface at 4 °C in a moisture atmosphere to avoid evaporation of solvent. After incubation for 5 h, the electrode was rinsed with the washing buffer to remove physically absorbed ALP. After rinsing, the ITO/TiO_2/ALP electrode was allowed for incubation in the 0.1 M Tris-HCl buffer (pH 8.12) containing variable concentrations of AAP at 37 ºC for 60 min. Thereafter, the electrodes were introduced for the respective absorbance or PEC measurements. For the detection of 2, 4-DA inhibitor, the incubation was performed in the 0.1 M Tris-HCl buffer (pH 8.12) containing 0.02 M AAP and variable concentrations of 2, 4-DA.

REFERENCES

(1) W. W. Zhao, Z. Y. Ma, D. Y. Yan, J. J. Xu and H. Y. Chen, *Anal. Chem.*, 2012, **84**, 10518.

(2) W. W. Zhao, C. Y. Tian, J. J. Xu and H. Y. Chen, *Chem. Commun.*, 2012, **48**, 895.