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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Ultrasensitive photoelectrochemical biosensing platform based target-triggered biocatalytic precipitation reaction on flower-like Bi_2O_2S super-structured photoanode

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Reagents. Monoclonal rabbit anti-human CEA capture antibody (mAb_1), monoclonal rabbit anti-human CEA secondary antibody (mAb_2) and humans CEA ELISA kit were acquired from Sangon Biotech. Prostate-specific antigen (PSA) and immunoglobulin G (IgG), and other interferences were purchased from Zhengzhou Biocell Biotech. Co., Ltd. (Zhengzhou, China). Bovine serum albumin (BSA), Horseradish peroxidase (HRP) from were purchased from Dingguo Biotechnol. Inc. (Beijing, China). Other raw materials for photoanode nanomaterial synthesis are purchased from Aladdin. The high-binding polystyrene 96-well microplates were gotten from Greiner Bio-One (Frickenhausen, Germany). Ultrapure water purified by a Millipore water purification system (18.2 MΩ cm, Milli-Q, Millipore) was used in all runs. The washing and blocking buffer solutions were prepared by adding BSA (1.0%, w/v) and Tween 20 (0.05%, v/v) to PBS solution, respectively.

Preparation of mAb_1-Coated Microplate. Monoclonal antibodies to CEA were prepared and bonded in microplates as follows. The purchased mAb_1 (50 μL per well, 10 μg mL^{-1}) was added into a high-binding 96-well microplate containing sodium carbonate buffer (0.05 M, pH 9.6), and then incubated 24 h at 4 °C. A plastic film is wrapped over the microplate to prevent evaporation of the liquid. The completed incubated microplates were removed and washed three times with PBS (pH 7.4) buffer containing 0.05% Tween 20 (v/v). This was followed by incubation for 1 h by adding closure buffer (300 uL of PBS solution per well (10 mM, pH 7.4) including 1.0 wt % BSA). The aforementioned prepared microtiter plates were prepared for the CEA assay.

Preparation of HRP and Detection Antibody-Conjugated AuNP (HRP-AuNP-mAb_2). HRP-AuNP-mAb_2 conjugates were prepared according to previous reports[1]. Firstly, gold colloids (5.0 mL, 5.0 ng mL^{-1}) were adjusted to pH 9.5 by using 0.1 M Na_2CO_3 aqueous solution. Then, 200 μL of HRP (0.5 mg mL^{-1}) and 50 μL of mAb_2 (0.5 mg mL^{-1}) were injected into colloidal gold nanoparticles and gently shaken for 60 min at room temperature on a shaker (MS, IKA GmbH, Staufen, Germany). 100 μL of polyethylene glycol (1.0 wt %) was added into the suspension and the mixture was further incubated for 12 h at 4 ℃. Finally, HRP-AuNP-mAb_2 conjugates were obtained by centrifugation at 4 ℃ (10 min, 13 000 g), and dispersed in 1.0 mL of 2 mM sodium carbonate solution ($C_{[Au\ NP]} \approx 25$ ng mL^{-1}) containing 1.0 wt % BSA and 0.1 wt % sodium azide, pH 7.4, and stored at 4 ℃ for further use.

Enzyme-Linked Immunosorbent Assay (ELISA) for CEA. A commercially available ELISA kit was utilized for method comparison. In the sandwich ELISA with standard polystyrene 96–well

plates, 50 μL serum sample suspension was incubated at 37 °C for 60 min, and the wells were rinsed 3 times (3 min each) with 0.1 M PBS (pH 7.4) containing 0.05% (v/v) Tween 20. Then we added 50 μL HRP-anti-CEA conjugate and incubation continued for 60 min. After washing, TMB reagent (50 μL) and H_2O_2 (50 μL) was added and incubated at 37 °C for 10 min. The enzymatic reaction was stopped by adding 50 μL of 2.0 M H_2SO_4 to each well. The results of ELISA were measured by a spectrophotometric ELISA reader at a wavelength of 450 nm.

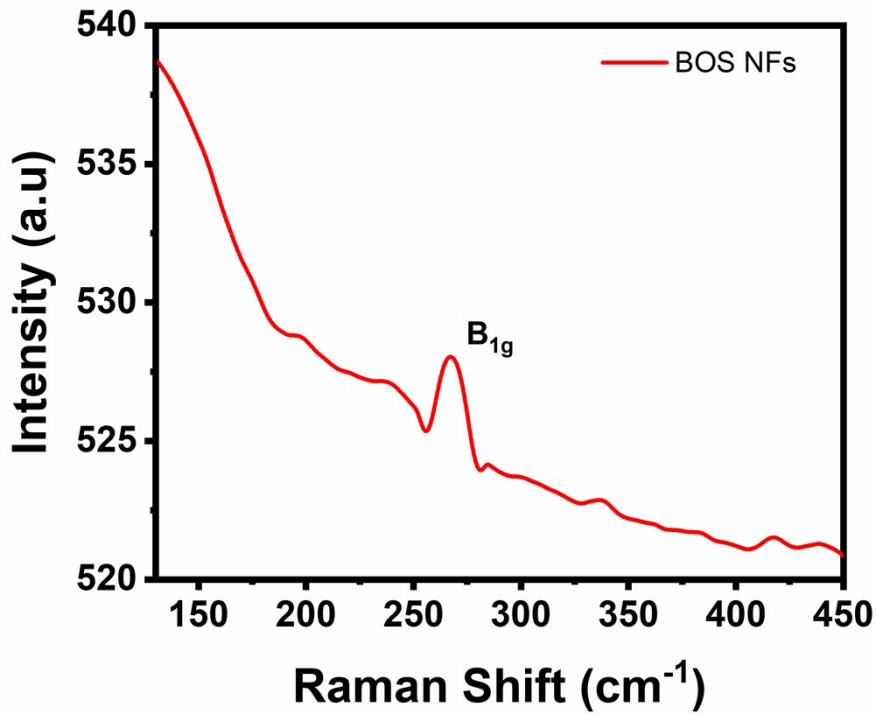

Fig. S1 Raman spectra of BOS NFs.

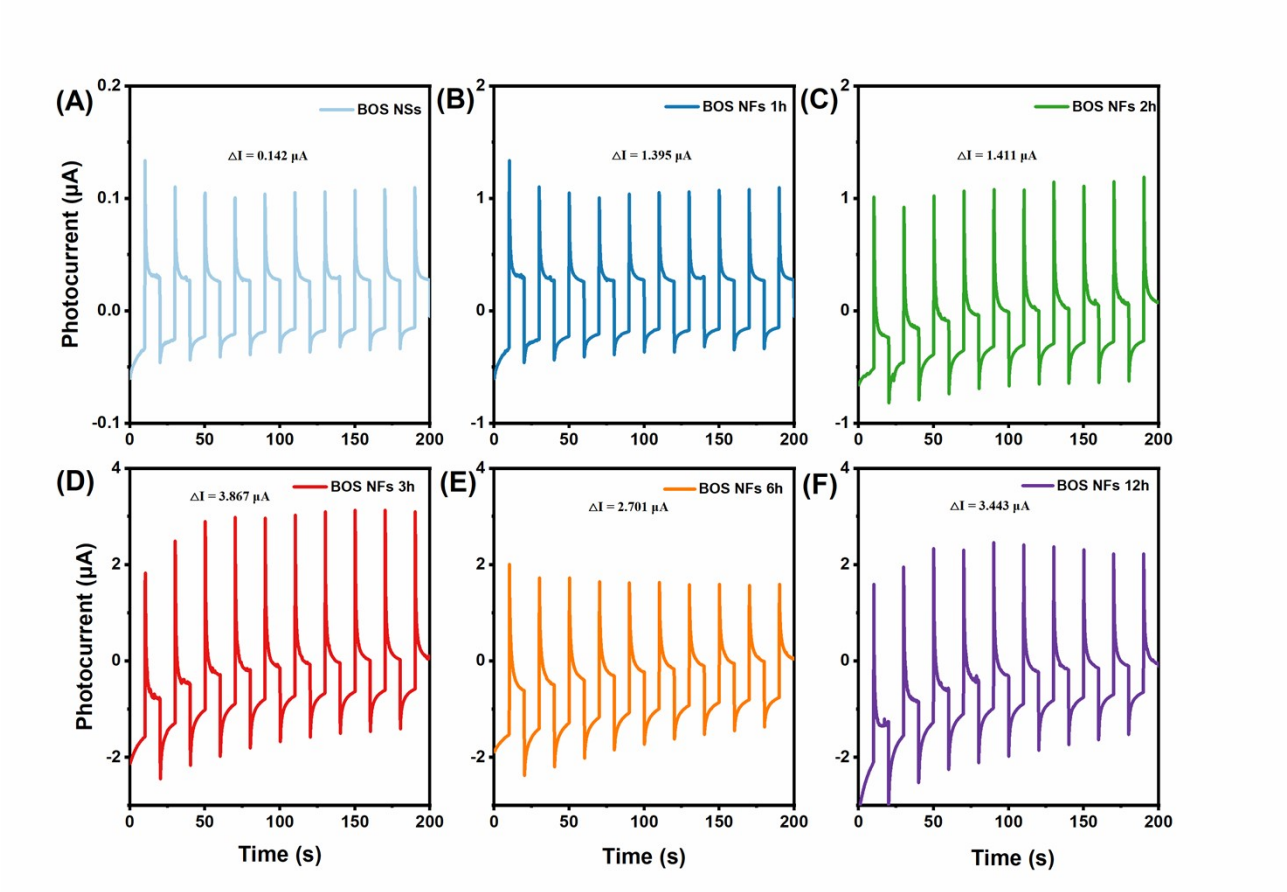

Fig. S2 Photocurrent response of BOS NSs and BOS NFs: (A) BOS NSs; (B) 1h BOS NFs; (C) 2h BOS NFs; (D) 3 h BOS NFs; (E) 6 h BOS NFs; (F) 6 h BOS NFs.

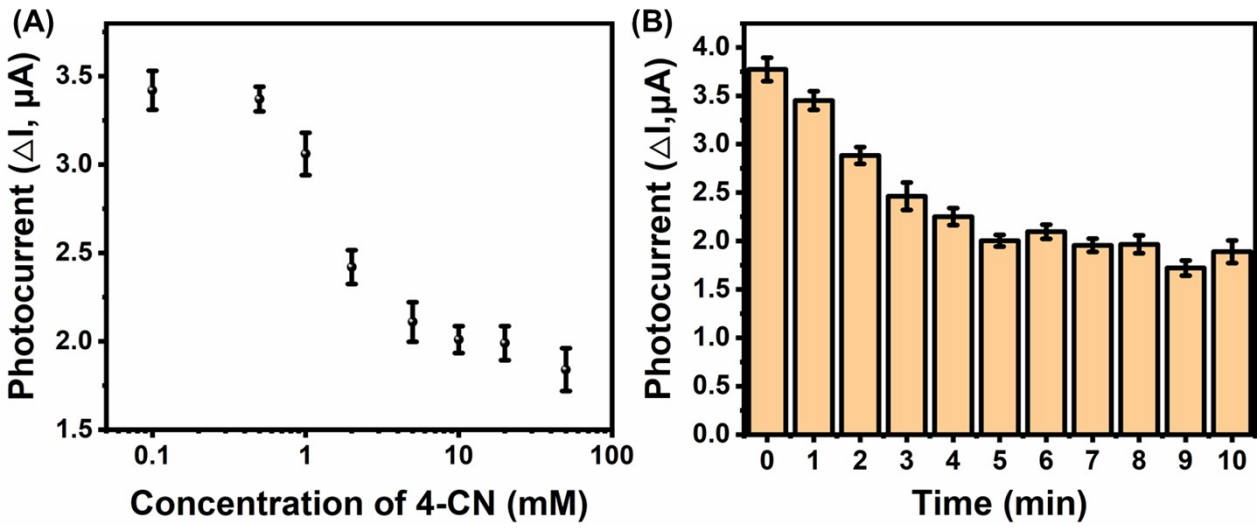

Fig. S3 Condition optimization results. (A) Photocurrent response curves of different substrate concentrations for biocatalytic precipitation; (B) Corresponding photocurrent histograms for different incubation times of biocatalytic precipitation.

Reference

1. X. Huang, Q. Lin, L. Lu, M. Li and D. Tang, *Anal. Chim. Acta*, 2022, **1228**, 340358.