Electronic supplementary information

Ferroelectric perovskite-enhanced photoelectrochemical immunoassay with photoexcited charge-transfer of built-in electric field

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Chemical and Reagents. Carcinoembryonic antigen (CEA), cancer antigen 125 (CA 125), alpha-fetoprotein (AFP), and bovine serum albumin (BSA) were achieved from Sigma-Aldrich (Shanghai, China). PSA standards and PSA ELISA kits were purchased from Wuhan Cusabio Biotech. Inc (https://www.cusabio.com/). Monoclonal anti-PSA antibody (mAb_1) and polyclonal anti-PSA antibody (pAb_2) were supplied by Abcam Inc. (Cambridge, MA) and Immuno Reagents Inc. (Raleigh, NC), respectively. Glucose oxidase (GOx) from *Aspergillus niger* were purchased from Dingguo Biotechnol. Inc. (Beijing, China) (Type X-S, lyophilized powder, 100,000 – 250,000 units g^{-1} solid). Bismuth nitride [$Bi(NO_3)_3 \cdot 5H_2O$], iron nitrate [$Fe(NO_3)_3 \cdot 9H_2O$], lanthanum nitrate [$La(NO_3)_3 \cdot 6H_2O$], 2-methoxyethanol, ethanolamine, graphite powder, sodium nitrate, sodium tungstate dihydrate [$Na_2WO_4 \cdot 2H_2O$] were purchased from Aladdin (Shanghai, China). Acetic acid, acetic anhydride, sulfuric acid, and hydrochloric acid were purchased from Sinopharm Chem. Re. Co., Ltd. (Shanghai, China). All high-binding polystyrene 96-well microplates were acquired from Greiner Bio-One (Frickenhausen,705071, Germany). Gold colloids (AuNP) were prepared as described in our previous work.[1,2] All reagents used were of A.R. grade. Ultrapure water obtained from a Millipore water purification system (18.2 MΩ cm^{-1}, Milli-Q, Millipore) was used throughout the experiment.

Preparation of mAb_1-Coated Microplate. Monoclonal antibodies to PSA were prepared and bonded in microtiter plates 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 PBA (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 PSA assay.

Preparation of Glucose Oxidase and Detection Antibody-Conjugated AuNP (GOx-AuNP-pAb2). GOx-AuNP-pAb_2 conjugates were prepared according to our previous reports.[3,4] 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 GO*x* (0.5 mg mL^{-1}) and 50 μL of pAb_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, GO*x*-AuNP-pAb_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.

Immunoreaction Protocol. A high-binding polystyrene 96-well microtiter plate were coated overnight at 4 °C with 50 μL per well of mAb1 capture antibody at a concentration of 10 μg mL^{-1} in 0.05 M sodium carbonate buffer (pH 9.6). The microplates were covered with adhesive plastics plate sealing film to prevent evaporation. On the following day, the plates were washed three time with pH 7.4 PBS, and then incubated with 300 μL per well of blocking buffer for 60 min at 37 °C with shaking. The plates were then washed as before. Following that, 50 μL of PSA standards/samples with various concentrations were added into the microtiter plates, and incubated for 40 min at 37 °C under shaking. After washing, 50 μL of the above-prepared GOx-AuNP-pAb_2 suspension (C[AuNP] ≈ 25 ng mL^{-1}) was added into the well and incubated for 40 min at 37 °C with gentle shaking to form the sandwiched immunocomplex. The plates were washed again. 110 μL of pH 6.0 PBS (50 mM) containing 4 mM glucose was added to each well, and reacted for 12 min at 37 °C. During this process, glucose was oxidized to D-glucono-β-lactone and hydrogen peroxide. After that, 50 μL of the reaction solution including H_2O_2 was injected into the PEC detection cell from the microplate by using micro syringe to implement the photoelectrochemical measurement. All measurements were made at least in duplicate.

Enzyme-Linked Immunosorbent Assay (ELISA) for PSA. 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-PSA 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.

Optimization of Experimental Conditions. To obtain the best analytical performance, it was necessary to optimize the incubation time and pH value in the immunoreaction. The thickness of

ferroelectric thin films with different thickness also had an impact on the test results. We also optimized the number of vacuum spin-coating process. The optimal conditions were determined based on the photocurrent changes in the presence of the target PSA (2.0 ng mL^{-1}). It could be seen from Fig. S3A that the intensity of the photocurrent increased with the increasing PSA incubation time before 30 min. To save the detection time, 30 min was chosen as the incubation time. According to Fig. S3B, we chose pH 6.0 as the optimal pH for incubation because the photocurrent density first increased and then decreased with increasing pH, and the maximal current density was at pH 6.0. The thickness of the BLFO also affected the photocurrent response, and its thickness was controlled by the number of spinning coats. The parameters of spin coating were set to 800 rpm for 10 s pre-coating treatment and 2000 rpm for 30 s spin coating treatment. The relationship between the number of spinning coats and the photocurrent density is showed in Fig. S3C. The number of spinning coats was finally chosen to be 2 for the PEC system.

To investigate the electron transfer mechanism of the composite nanomaterials in the photoelectric process more deeply, we supplemented the specific energy diagram of the composite electrode with UV-vis diffuse reflection (Agilent Cary 7000) and Ultraviolet Photoelectron Spectroscopy (UPS, Reslove120MCD5) related calculations. The relevant characterization results showed that the band gaps of BLFO and WO were 2.67 eV and 2.84 eV, respectively, as shown in the Figure S4A. In other words, the suitable band gaps of BLFO and WO_3 were one of the prerequisites for the absorption of visible light. As shown in Figure S4B and C, the UPS measurement results showed that the spectral lines begin to rise sharply after 12.6 eV, indicating that there is a strong secondary inelastic scattering electron emission. Further, the secondary electron truncation at 17.82 eV and 18.06 eV were observed for WO_3 and BLFO. The valence bands of WO_3 and BLFO were determined to be 3.38 eV and 3.14 eV, respectively. A specific energy diagram of the electron transfer can be obtained by combining the UV-visible diffuse reflectance results, as shown in Fig. S5. In brief, the electron transfer of the nanocomposite in the photoelectronic process followed the traditional type II heterojunction transfer mechanism. Due to the spontaneous polarization of BLFO, the valence band and conduction band of BLFO would be bent in a certain range, which was conducive to the rapid transfer of electrons and holes, thus reducing the recombination rate of electrons and holes. Hydrogen peroxide was used as a sacrificial agent for reduction reaction, and electrons recombine with holes in the valence band of BLFO to generate cathodic photocurrent, which

was the same as the experimental results.

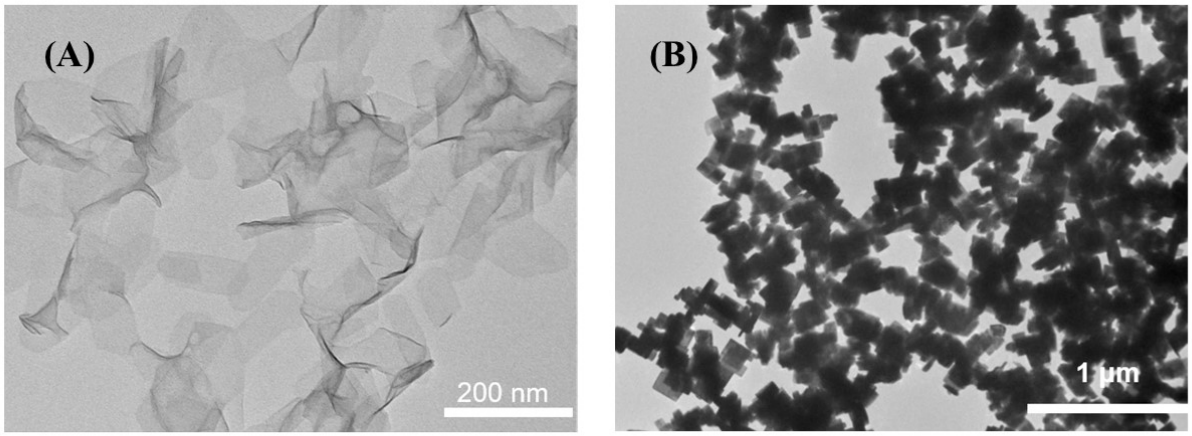

Fig. S1 SEM images of (A) rGO and (B) WO_3 nanoplates.

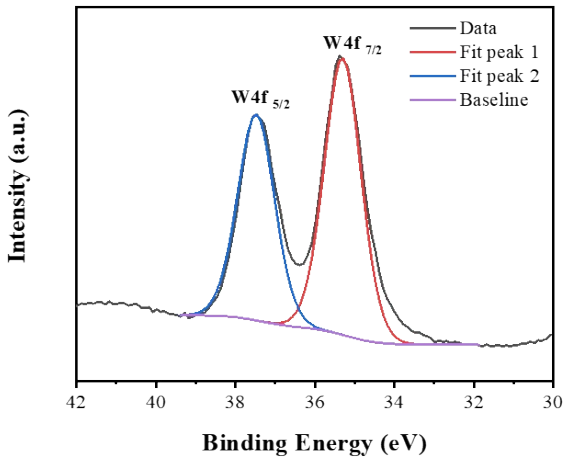

Fig. S2 High-resolution XPS spectra of W 4f.

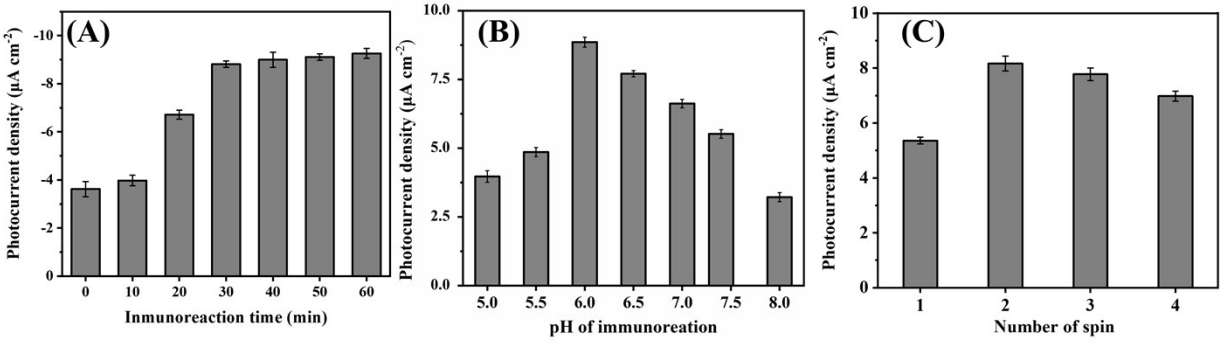

Fig. S3 The effect of (A) incubation time, (B) pH value for the mAb_1-PSA-pAb_2 immunoreaction, and (C) number of spin coating of ferroelectric BLFO on the photocurrent of PEC immunoassay (2.0 ng mL^{-1} PSA used in this case).

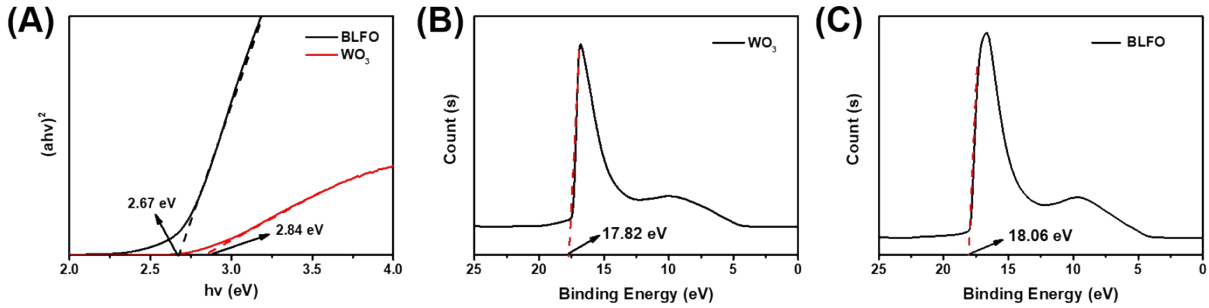

Fig. S4 (A) Tauc plot of BLFO film and WO_3 nanosheets. Ultraviolet photoelectron spectroscopy of (B) WO_3 and (C) BLFO.

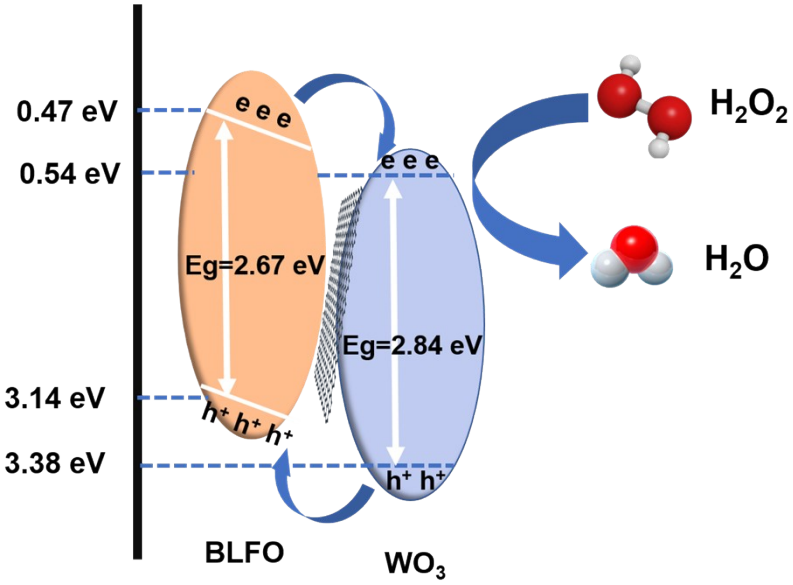

Fig. S5 Schematic diagram of energy level relationship of BLFO/rGO/WO_3 nanocomposites.

Table S1 Comparison of the assayed results by using PEC immunosensor and commercial PSA ELISA kit.

No.	Added PSA (ng ml^{-1})	Methods (mean ± SD, ng ml^{-1}, n = 3)		Recovery (%) by this work	Recovery (%) by PSA ELISA kit
		This work	PSA ELISA kit		
1	1	0.97 ± 0.07	1.02 ± 0.04	97.0	102.0
2	2	2.07 ± 0.06	2.02 ± 0.07	103.5	101.0
3	5	4.72 ± 0.13	4.91 ± 0.08	94.4	98.2
4	10	9.70 ± 0.17	10.29 ± 0.09	97.0	102.9
5	20	20.87 ± 0.13	19.71 ± 0.06	104.4	98.5

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