Electronic Supplementary Material (ESI) for Analyst.

Electronic Supporting Information

New enzyme immunoassay of alpha-fetoprotein in a separate setup coupling aluminium/Prussian blue-based self-powered electrochromic display with digital multimeter readout

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■ EXPERIMENTAL SECTION

Material and Reagent. Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), potassium chloride (KCl), potassium ferricyanide ($K_3[Fe(CN)]_6$), hydrogen peroxide (30%), hydrogen chloride (HCl), glucose and bovine serum albumin (BSA) were purchased from Sinopharm Chem. Re. Inc. (Shanghai, China). The Al foil was obtained from local market. Alpha-fetoprotein (AFP) standards, monoclonal anti-AFP antibody (mAb_1) and polyclonal anti-AFP antibody (pAb_2) were achieved from Biospacific (CA, USA). Glucose oxidase (GOx, Type X-S, lyophilized powder, 100,000-250,000 units/g solid) was purchased from Sigma-Aldrich (Shanghai, China). Human AFP enzyme-linked immunosorbent assay (ELISA) kit was purchased from Biocell Biotechnol. Inc. (Zhengzhou, China). Clinical serum specimens were made available by Fujian Province Hospital, China. Ultrapure water from a Milli-Q purification system was used in all runs (18.2 MΩ/cm, Millipore). 0.1 M Phosphate-buffer saline (PBS) solution with different pH were prepared by NaH_2PO_4 and Na_2HPO_4 and mixed with 0.1 M KCl as the supporting electrolyte.

Apparatus. All electrochemical measurements were performed on CHI850D Electrochemical Workstation (Shanghai Chenhua Inc., China). The modified FTO electrode, saturated calomel electrode (SCE), and Pt wire electrode were used as the working electrode, reference electrode, and counter electrode, respectively. UV-vis absorption spectra of the Prussian blue film electrodes were recorded by the Infinite M200 Pro NanoQuant, (Tecan, Switzerland). The current of analysis detection was using a digital multimeter ($VC9801A^+$, VICTOR).

Preparation of Prussian Blue-Modified FTO Electrode (PB/FTO). Prior to modification, fluorine-doped tin oxide (FTO) glass electrode (1.0 × 5.0 cm^2) was treated with ultrasonic oscillation in acetone, ethanol and distilled water for 20 min, respectively. After drying, the conductive surface of FTO electrode was covered with adhesive tape and leave a circular area with a diameter of 6 mm. PB film was prepared on FTO electrode by electrochemical deposition referring to the literature.[S1] The electrolyte for PB film synthesis was a mixed aqueous solution containing HCl (0.1M), $K_3[Fe(CN)]_6$ (5.0 mM), $FeCl_3 \cdot 6H_2O$ (5.0 mM) and KCl (0.1 M). The electrochemical deposition was carried out by a three-electrode system

under a constant potential at 0.4 V for 360 s. The PB film-modified FTO was washed with distilled water for the subsequent use.

Preparation of GOx and pAb_2-Functionalized AuNP (GOx-AuNP-pAb_2). Glucose oxidase and polyclonal anti-AFP antibody-functionalized gold nanoparticle (GOx-AuNP-pAb_2) was prepared similar to our precious work.[S2] Initially, pH of the prepared gold colloid (AuNP, ~16 nm in diameter) was adjusted to about 8.5 by Na_2CO_3. Thereafter, 200 μL of GOx (0.5 mg/mL) and 50 μL of pAb_2 (0.5 mg/mL) were injected into the gold colloids (5 mL) and shaken for 2 h at room temperature. Following, 100 μL of polyethylene glycol (1.0 wt %) was added and further incubated for 12 h at 4 ℃. Finally, the mixture was centrifuged for 15 min at 14,000 rmp at 4 °C and the obtained precipitate, GOx-AuNP-pAb_2, was dispersed in 2 mL of pH 7.4 PBS containing 1.0 wt % BSA and 0.1 % sodium azide, and stored at 4 °C for further use.

Construction of Self-Powered Detection Device. Before assembling, an Al foil (1.0 × 5.0 cm^2) was washed with HCl and distilled water to remove the external Al_2O_3. After drying, the Al foil was attached to the conductive surface of the cleaned FTO electrode. Following, a home-made detection cell (made by polydimethylsiloxane, PDMS, Dow Corning Sylgard 184; the weight ratio of base to cross linker was 10 : 1) was placed between the Al electrode and PB electrode and the two electrodes were linked by a wire. Finally, the device was fixed by a clip. The photographs of the device were shown in Fig. 1 in the main text.

Immunoreaction Protocol. The visual self-powered sensor based on Al-PB/FTO for AFP detection is schematically represented in Scheme 1. Prior to measurement, a high-binding polystyrene 96-well microtiter plate was coated with 50 μL Ab_1 (10 μg/mL) per well overnight at 4 °C and covered with adhesive plastics plate sealing film to avoid evaporation. Following that, the microplate was washed with PBS solution three times and then blocked with the blocking buffer (300 μL PBS solution containing 1.0 wt % BSA per well, pH 7.4) for 1 h at 37 °C with gentle shaking. The microplate was washed as before. And then, 50 μL of AFP standards/samples with various concentrations were added into the microtiter plate, and incubated for 40 min at 37 °C under shaking. After washing, 50 μL of the above prepared GOx-AuNP-pAb_2 solution was added into the well and incubated for 35 min at 37 °C with gentle shaking to form the sandwiched immunocomplex. After the plate was washed again,

100 μL of pH 6.0 PBS containing 50 mM glucose was added into each well and reacted for 40 min at 37 °C. After that, 40 μL of the reaction solution including H_2O_2 was injected into the detection cell and reacted for 25 s. The current signal was detected by the DDM. All measurements were made at least in duplicate.

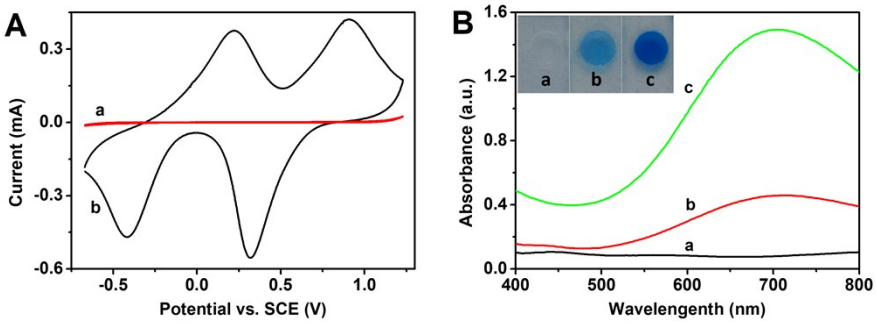

Fig. S1 (A) Cyclic voltammograms of (a) FTO electrode and (b) PB/FTO in 0.1 M PBS (pH 6.0); (B) UV–vis absorption spectra of (a) FTO electrode, (b, c) PB/FTO electrode at (b) 0 V and (c) 0.2 V (insets: the corresponding photographs of PB/FTO electrodes)

Characterization of Prussian blute. Firstly, the electrochemical behaviour of Prussian blue on fluorine-doped tin oxide (FTO) electrode was investigated in PBS (0.1 M, pH 6.0) (Fig. S1-A). Curve 'a' gives cyclic voltammogram of FTO electrode, and no redox peaks were achieved. When PB film was modified to the FTO electrode, however, two pairs of redox peaks were achieved (curve 'a'), which was similar to the previous report.[S3] These two-pair peaks were mainly ascribed to the redox reactions between Prussian white/Prussian blue (PW/PB) and Prussian blue/Berlin green (PB/BG), respectively. Such the redox peaks could provide the energy output for the self-powered sensing development.

During the cyclic scanning process, an obvious change in the visual color on the PB film was obvious by naked eyes from the colourless to blue to yellow-green with the increasing potential. To elucidate this point, two potentials including 0 and 0.2 V were selected as the examples in this case. As seen from the insets in Fig. S1-B, the different colors between the PB electrodes at various potentials could be easily distinguished by the naked eyes. Further, we also used UV-vis absorption spectroscopy to monitor the resulting phenomena. As shown in Fig. S1B, almost no absorption peak was observed within 400-800 nm for bare FTO electrode (curve 'a'). After PB film was deposited on the electrode, however, a broad

absorption peak at about 700 nm was appeared, which was in consistence with the literature.[S4] Compared with the absorbance of PB film at different potentials, an obvious increase in the absorbance was displayed when the potentials increased from 0 V to 0.2 V (curve 'b' and curve 'c'). Hence, the color could be triggered at the different potentials for the development of electrochromic display.

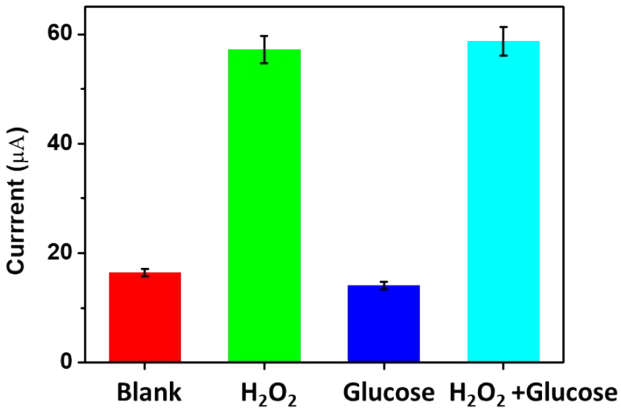

Fig. S2 Responses of aluminium/Prussian blue-based self-powered electrochromic display sensing platform toward 10 mM glucose and 0.1 mM H_2O_2.

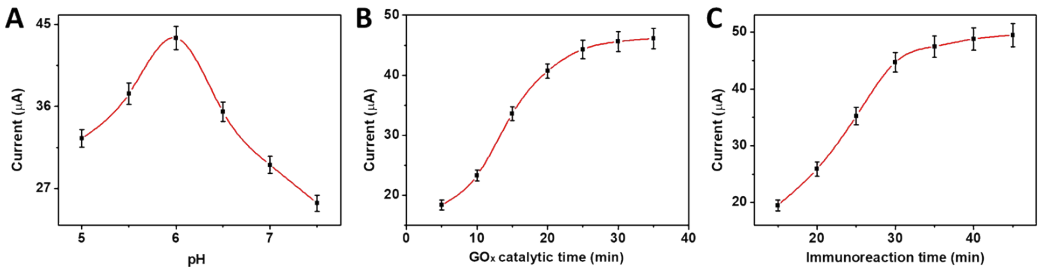

Fig. S3 Effects of (A) pH of PBS, (B) GOx catalytic time and (C) immunoreaction time on the response of the self-powered electrochromic display immunoassay (20 ng/mL AFP used in the case).

■ References

S1 X. Yu, J. Liang, T. Yang, M. Gong, D. Xi and H. Liu, *Biosens. Bioelectron.*, 2018, **99**, 163-169.

S2 B. Zhang, B. Liu, D. Tang, R. Niessner, G. Chen and D. Knopp, *Anal. Chem.*, 2012, **84**, 5392-5399.

S3 F. Ricci and G. Palleschi, *Biosens. Bioelectron.*, 2005, **21**, 389-407.

S4 X. Yu, J. Y. Liang, T. G. Yang, M. J. Gong, D. M. Xi and H. Y. Liu, *Biosens. Bioelectron.*, 2018, **99**, 163-169.