High-porosity hybrid bilayer enabled a portable LED plasmonic biosensing

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Experimental section

Preparation of HP-TWN/TWA/AuNP-PM hybrid layer

It was prepared through firstly growing AuNP-SP film onto the surface of glass substrates by sputtering deposition followed by the microwave plasmon heat-treatment and then growing the TiO_2 nanowires (TNW) onto it by the sputtering deposition process followed with alkaline thermal treatment. The base process for the AuNP-PM film fabrication was a sputter deposition of the gold thin film ~3 nm followed by rapid thermal annealing under microwave condition to produce the isolated AuNPs on the surface. The base process was then repeated for 6 times to produce the final AuNP-PM film owing to the optimal absorption efficiency in the wavelength range of 350-750 nm (Figure S7). Then the fabrication of the TNWs with cavities onto AuNP-PM film was performed by firstly coating a 50-nm-thick Ti film onto the surface of AuNP-PM film through a magnetic sputter instrument (TVC-M8C8TV, Transvac co. Ltd) and subsequently thermal-alkaline treatment in a 2.5 M NaOH (ACS reagent, ≥ 97.0%, Sigma-Aldrich) aqueous solution at 80 °C for 1hr to form the porous TiO_2 nanowires according to our previous reported work,[1] in which 2.5 M NaOH solution for pH control and 1hr for reaction time control of thermal-alkaline treatment were selected as the optimal experimental conditions for the growth of TiO_2 porous nanowires owing to its most satisfying sample porosity and surface area (Figure S7). Following, after washing by ethanol several times, drying at 50 °C overnight, and eventually annealing at 500 °C for 1 hour, the prepared plasmoelectric photoanodes with HP-TWN/TWA/AuNP-PM configuration on glass surface are accomplished.

Characterizations

The optical absorption properties were studied through a Shimadzu UV3600i UV-vis-NIR spectrometer. The absorption value is calculated by the equation $A = 1 - R - T$, where R and T are the normalized reflection and transmission, respectively. Scanning electron microscopy (SEM) was carried out with a Zeiss Ultra-Plus field emission scanning electron microscope (FESEM) with an accelerating voltage of 3 kV. The morphology, structure, and element mapping of the products were further characterized by a JEM 2010 high-resolution transmission electron microscope (HRTEM), operating at accelerating voltages of 200 kV. The sample for TEM investigation was prepared by a FIB milling. Antibody-antigen bilayer immune complex formation was monitored by Fourier Transform Infrared spectroscopy (FTIR) analysis performed with a Thermo Nicolet 6700 FTIR spectrophotometer with a resolution of 4 cm^{-1} at 25 °C.

Construction of the HP-TWN/TWA/AuNP-PM based photoelectric immunosensor

To verify the photoelectric performances of our plasmoelectric film, a prototype device constructed by a three-electrodes test-strip designed with three electrodes composed of the as-

fabricated hybrid bilayer as the working electrode, conducting carbon as the counter electrode, and Ag/AgCl as the reference electrode was accomplished via a screen printing method. The configuration was shown in Figure S2.

For assembly of the immunosensor, the anti-AFP antibody (MyBioSource, MBS530361) was firstly diluted to 1000 ppb in 1X phosphate buffered saline (PBS) and stored at–20°C. Next, the working electrode site of the prepared HP-TWN/TWA/AuNP-PM based test strip was immersed by a 20-μL drop of 1000 ppb anti-AFP for 10mins for the formation of non-covalent conjugations between the anti-AFP antibody and chip surface. Then, the electrode was rinsed with PBS solution and Milli-Q water, followed by drying at ambient temperature under a nitrogen flow.

Analysis of AFP

Firstly, the working site of the immunosensor test strip conjugated with anti-AFP was immersed in AFP (MyBioSource, MBS537909) dispersed in 100 times diluted Human Serum at different concentrations at room temperature; that is, a 20-μL AFP antigen with the concentrations 0.01, 0.10, 1.00, 10.0, 100, and 400 ppb in human serum was dropped onto immunosensors and then incubated for 30mins at ambient temperature. After washing with PBS and drying in a N_2 stream, the electrode was used for photocurrent analysis. Detection for the quantitative analysis of AFP protein was directly examined by monitoring the photocurrent change at an applied potential of 0.4 V in 1X PBS solution through the proposed three-electrodes test-strip integrating into a portable ready-to-go potentiostat (Sensit Smart from PalmSens BV and Analog Devices Inc.) under visible LED-light illumination (Fig. S4). For each sample, three independent tests were undertaken.

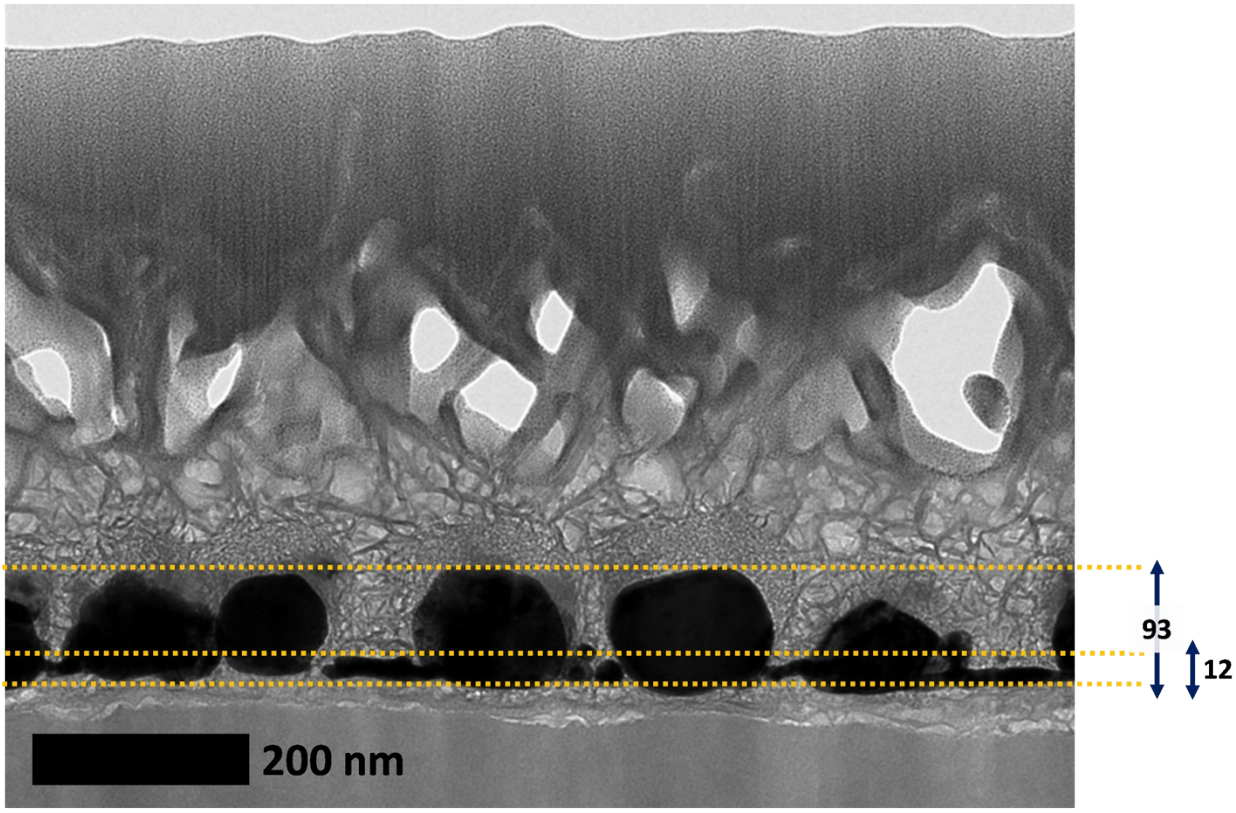

Figure S1. TEM images of cross-section of HP-TWN/TWA/AuNP-PM film, showing the bottom-layer is composed of Au (12 nm-height)/(93 nm-height)-based substrate.

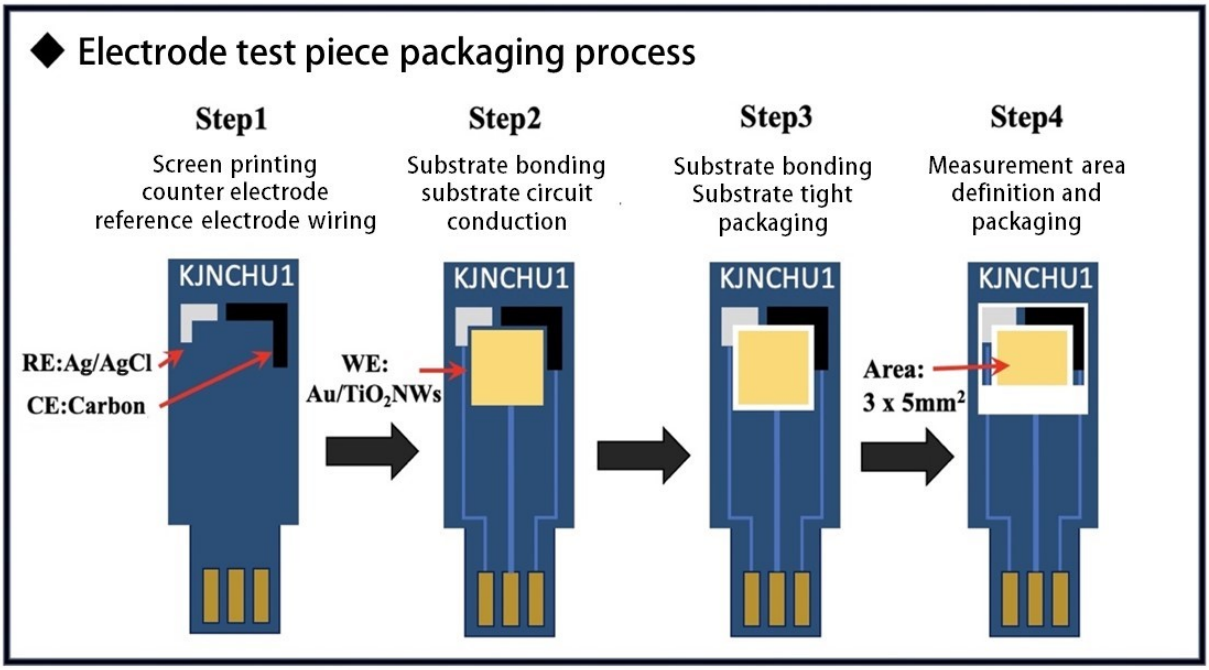

Figure S2. Schematic figure for the test strip preparation. A three-electrodes test strip was fabricated by screen printing methods, which composed of the as-fabricated hybrid bilayer as the working electrode, conducting carbon as the counter electrode, and Ag/AgCl as the reference electrode.

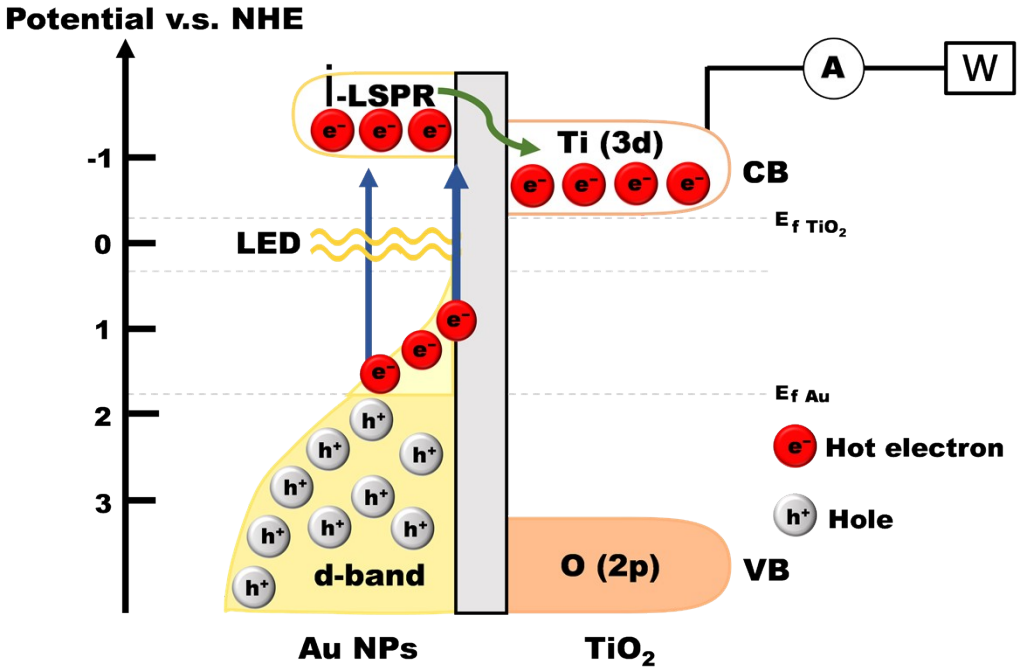

Figure S3. Mechanism of the photocurrent generation of the HP-TWN/TWA/AuNP-PM film. The AuNP layer absorbs photons to generate hot electrons, which have energy higher than the Schottky

barrier at the AuNP/TiO_2 interface to overcome the potential barrier to be injected into the conduction band of the TiO_2 and then contribute to a detectable photocurrent.

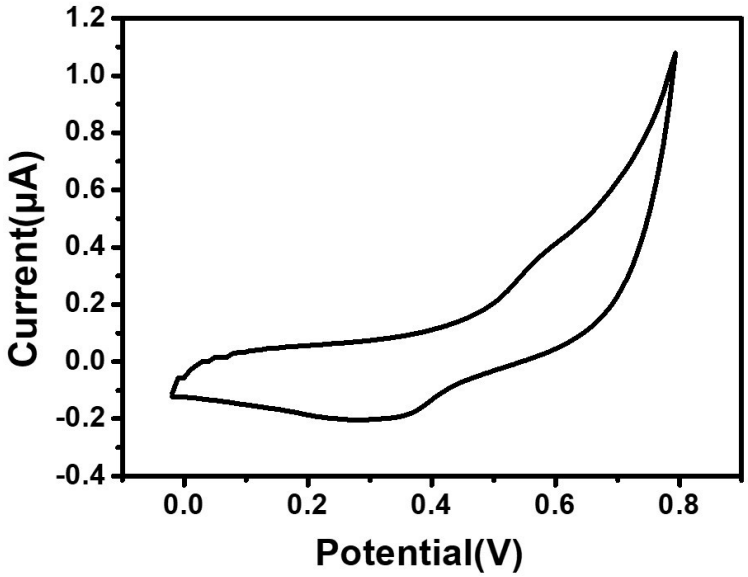

Figure S4. Cyclic voltammetry of HP-TWN/TWA/AuNP-PM film in 1X phosphate-buffered saline (PBS) solution. An anodic peak was observed at 0.5 V caused by the surface reaction and many small bubbles were observed on the surface of the electrode as the voltage raised to above 0.8 V due to the oxygen evolution, which would cause the film structure broken and the biomolecule inactivate. Thus, for maintaining the duration of our HP-TWN/TWA/AuNP-PM based biosensors, the best applied voltage of 0.4 V was chosen.

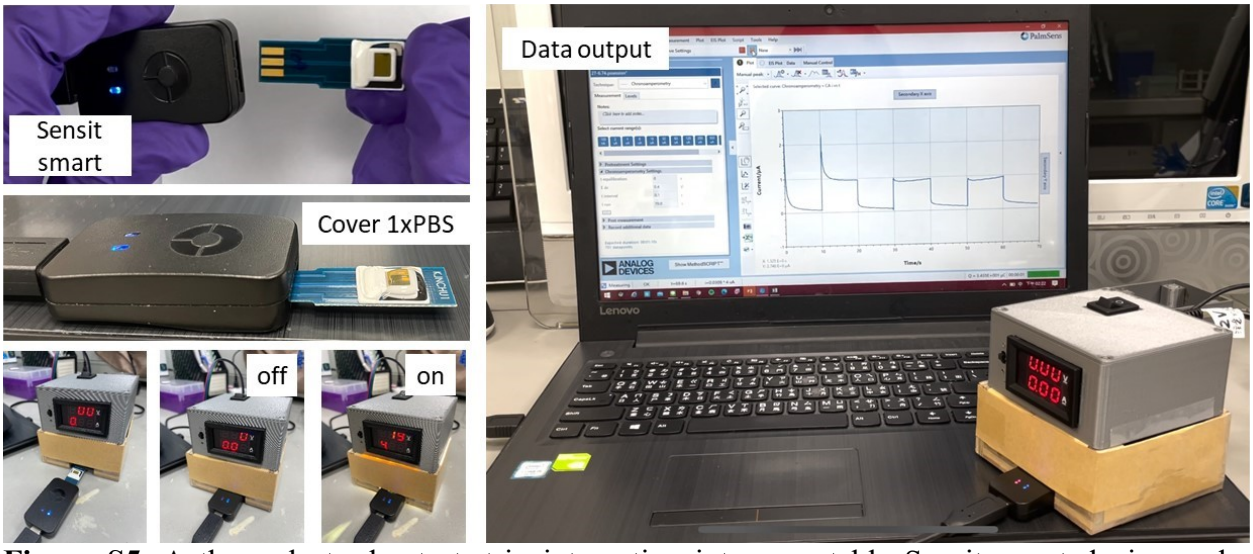

Figure S5. A three-electrodes test-strip integrating into a protable Sensit smart device under visible LED-light illumination.

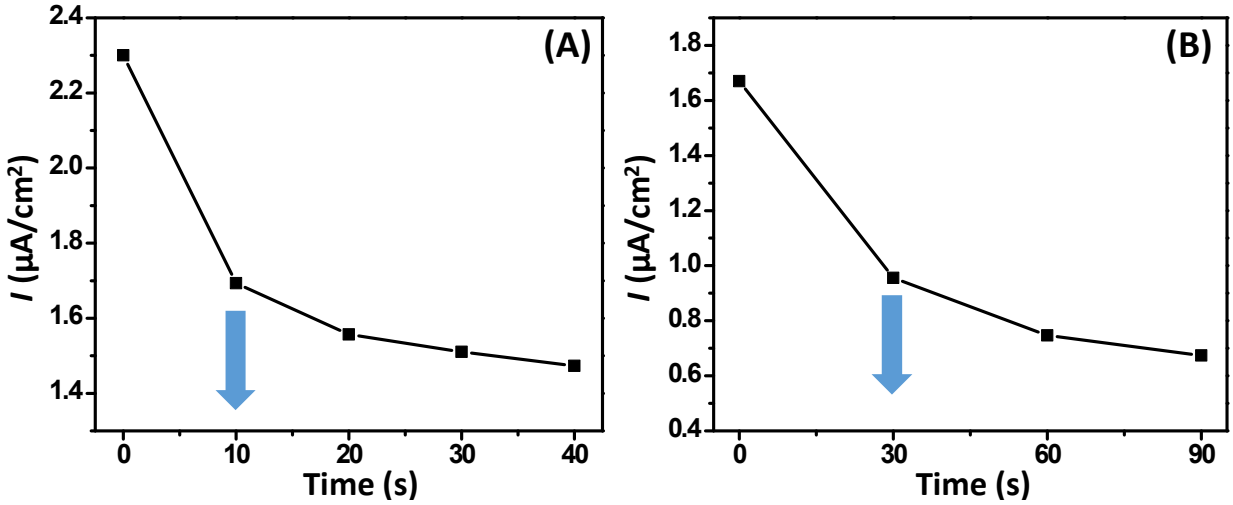

Figure S6. Effect of incubation time with (A) anti-AFP adsorption and (B) AFP antigen on the photocurrent response. Both figures indicated that the photocurrent signal response decreased with increasing reaction time, and then maintained a steady value, in which the optimal incubation time for anti-AFP and AFP antigen were 10mins and 30mins, respectively.

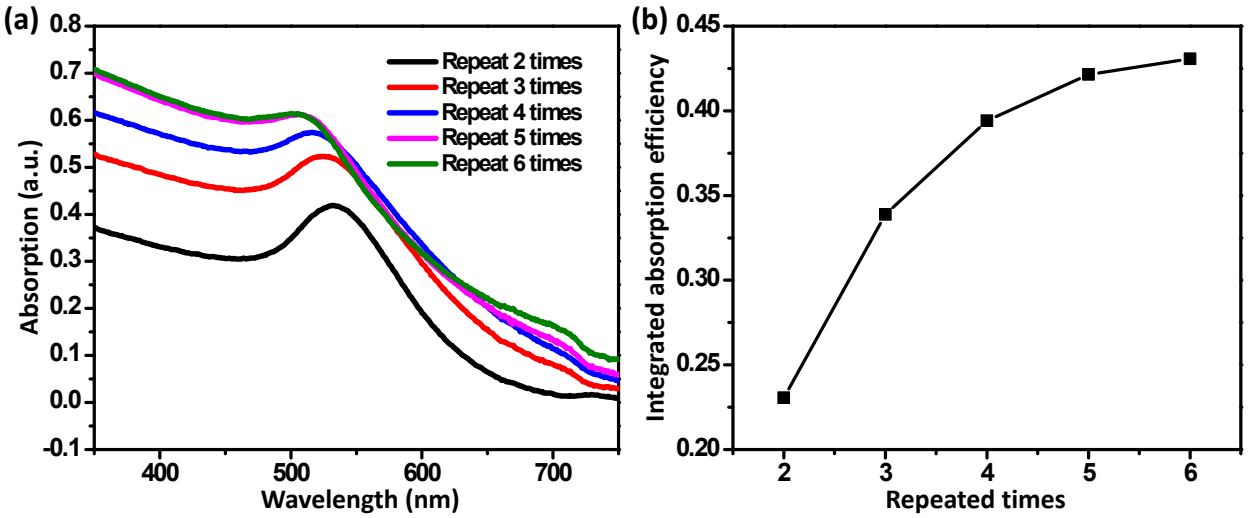

Figure S7. (a) Absorption spectra of the AuNP-PM film synthesized under different repeated times of base process. (b) Integrated absorption efficiency of light over the wavelength range from 350 to 750 nm in the active layer as a function of the repeated times of base process for the formation of AuNP-PM film.

References:

1. J.-Z. Chen, W.-Y. Ko, Y.-C. Yen, P.-H. Chen and K.-J. Lin, *ACS Nano*, 2012, **6**, 6633-6639.