# Supporting Informations

# Disposable paper-based bipolar electrode for sensitive electrochemiluminescence detection of cancer biomarker

Qiu-Mei Feng, Jian-Bin Pan, Huai-Rong Zhang, Jing-Juan Xu* and Hong-Yuan Chen

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering,

Nanjing University, Nanjing 210093, China

## Chemicals and Materials

Human prostate specific antigen (PSA) standard solution and prostate specific antibody ($Ab_1$ and $Ab_2$) were purchased from Shenggong Bioengineering Ltd Company (Shanghai, China). Glutaraldehyde (25% aqueous solution), bovine serum albumin (BSA), (3-aminopropyl) triethoxysilane (APTES), Tri(2,2′-bipyridyl) dichlororuthenium (Ⅱ) hexahydrate, and chitosan (CS) were obtained from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). Glucose oxidase (GOD) and silica nanoparticles (99.5%, 50±5 nm) were from Aladdin (USA). Carboxylic multi-walled carbon nanotubes (MWCNTs, OD:＞50 nm, length: 0.5–2 μm, purity≧95％) dispersion liquid was obtained from Nanjing Xianfeng Nanomaterials Technology Co. Ltd (Nanjing, China). $H_2O_2$ (analytical reagent grade) was from Shanghai Lingfeng Chemical Reagent Co. Ltd (Shanghai, China). D-(+)-glucose and tripropylamine (TPrA) were bought from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Whatman grade 1 chromatographic paper (200.0mm×200.0mm, pure cellulose paper) was purchased from GE Healthcare Worldwide (Shanghai, China) and used with further adjustment of paper size. The ECL solution, which was comprised of $Ru(bpy)_3^{2+}$ and TPrA, were prepared in 0.1M phosphate buffer solution (PBS, pH7.4). The Millipore (model milli-Q) purified water was used in all the experiments. All the other chemicals were of analytical reagent grade without any further purification.

## Apparatus

The ECL measurements were monitored with a MPI- E electrochemiluminescence analyzer (Xi′an Remax Electronic Science & Technology Co. Ltd., Xi′an, China, 350-650 nm). UV-vis absorption spectra were collected with a UV–3600 UV-vis spectrophotometer (Shimadzu, Japan). Scanning electron micrographs (SEM) were performed with a Hitachi S-3000N scanning electron microscope (Japan).

## Fabrication of paper-based BPEs device

The paper-based BPEs immunodevice consists of two hydrophilic cells (10 mm long and 8 mm wide, sensing cell and reporting cell) patterned using hydrophobic wax boundaries. These two cells were connected by a carbon ink BPE (12 mm long and 2 mm wide) as electronic conductors. Carbon ink (the driving anode) and silver/silver chloride ink (the driving cathode) was screen printed on the end of cells. At last, the obtained paper-based BPEs device was flipped over and the

analytic solution was dropped in sensing cell and $Ru(bpy)_3^{2+}$/TPrA solution was dropped in the reporting cell.

## Preparation of $SiO_2$-GOD-$Ab_2$ nanocomposites

First, 20 mg $SiO_2$ nanospheres with an average diameter of about 50 nm were dispersed in 2 mL of ethanol and treated with 0.4 mL of APTES to produce amino-group on the surface of silica nanospheres. After stirring for 6 h, the suspension was centrifuged and washed with ethanol for four times. Second, the amino-functionalized silica nanopheres were incubated with 500 μL of 2.5% glutaraldehyde for 2 h. After centrifugation and washing three times with PBS, the resulting nanoparticles were redispersed in a solution of 450 μL containing 1 mg/mL GOD and 50 μL of 20 μg/mL anti-PSA. The solution was gently stirred to react for 10 h at room temperature. The unbound GOD and anti-PSA were removed by successively washing the $SiO_2$ nanoparticles with PBS. At last, the obtained $SiO_2$-GOD-$Ab_2$ nanocomposites were treated with blocking buffer for 24 h to block any nonspecific binding site on the surface. After centrifugation and washing three times, $SiO_2$-GOD-$Ab_2$ nanoparticles were redispersed in 1 mL of PBS, pH 7.4 containing 0.1% BSA, and stored at 4 ℃ for later experiments.

## Preparation of paper-based BPE immunodevice

Carboxylic MWCNTs dispersion liquid was dispersed in 0.1% CS solution (in 0.1 M HAc) to obtain a black homogeneous CS-MWCNTs suspension with sonication, and then 2.0 μL of this suspension was cast onto cathode working zones on the back of carbon ink printed BPEs. After drying at room temperature, the modified bipolar electrodes were incubated with 1 μL of 2.5% glutaraldehyde (in 0.05M PBS, pH7.4) for 2 h, and washed with PBS. Then, 1 μL of 20 μg/mL $Ab_1$ solution was dropped onto the corresponding cathode working zones of BPE, and reacted at room temperature for 30 min. Subsequently, the physically adsorbed antibodies were washed with PBS, and then we blocked possible remaining active sites against nonspecific adsorption by adding 1 μL of 1% BSA for 30 min. After washing with PBS, the resulting paper-based bipolar immunodevice was stored at 4℃ in a dry environment prior to use.

## Analysis procedure

A sandwich-type immunoassay was performed for simultaneous determination of PSA on the paper-based BPE device. The prepared paper-based bipolar electrochemical immunodevice was first incubated with 1 μL of the sample solution containing different concentrations of PSA for 30 min at room temperature, followed by washing with PBS. Then, it was further incubated with 1 μL of the prepared $SiO_2$-GOD-$Ab_2$ solution for 40 min at room temperature, followed by washing with PBS. Finally, 30 μL of pH7.4 PBS containing 10 mM glucose was dropped onto the sensing cell and 30 μL of the ECL solution containing 1.0 mM $Ru(bpy)_3^{2+}$ and 50 mM TPrA were added to the reporting cell.

The ECL measurements were performed using MPI-E electrochemical and electrochemiluminescence analyzer. The ECL voltage curves were obtained by applying a linearly increasing voltage from the initial voltage of -0.2 V to the different final voltage (from 3.0 to 3.5V) on the two ends of driving electrodes with the scan rate of 0.2 V/s. The voltage of photomultiplier tube (PMT) was set at 400 V during detection.

## Characterization of the pure cellulose paper

Due to its attractive features including ease of use, portability, low consumption of reagent and so on, pure cellulose paper has been introduced as a promising substrate material in the analytical devices.[1] As shown in Fig. S1A, the substrate paper has supramolecular cellulose structure and three-dimensional network of microfibers. More clear structure could be seen in the enlarged image (Fig. S1B). These obvious incompact porous structures can increase the surface areas of electrode and facilitate the diffusion of reagent. Owing to the three dimensional porous structure of paper, the melted wax can penetrate into the paper to decrease the hydrophilicity of paper remarkably (Fig. S1D). The unprinted area maintains good flexibility, hydrophilicity and porous structure (Fig. S1C).

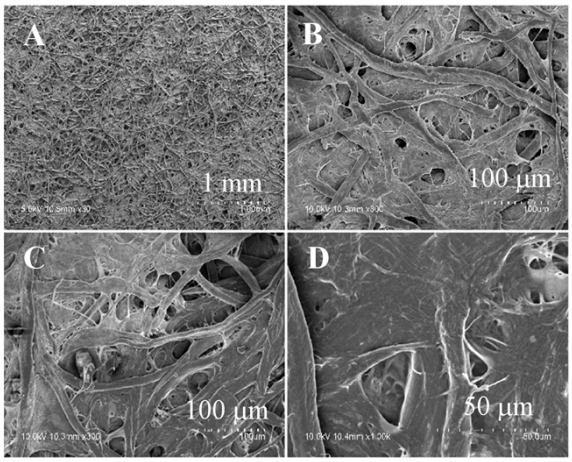


**Fig. S1.** SEM images of (A) pure cellulose paper; Enlarged images of (B) pure cellulose paper; (C) the boundary of the wax-pattern: pure cellulose (left) and wax-printed paper (right); (D) wax-penetrated pure cellulose paper.

## Characterization of $SiO_2$-GOD-$Ab_2$

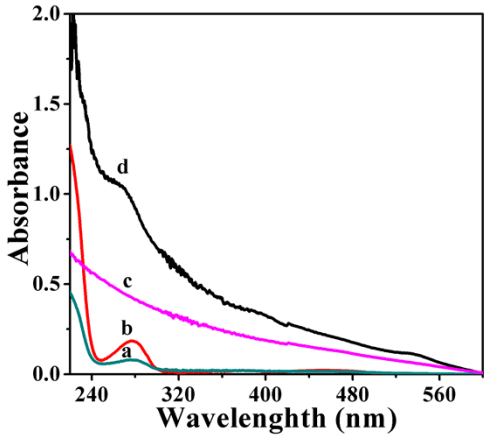


**Fig. S2** UV-vis absorbance spectra of (a) $Ab_2$; (b) GOD; (c) silica nanosphere and (d) $SiO_2$-GOD-$Ab_2$ nanocomposites.

Due to its good biocompatibility and large specific surface area, silica nanosphere was an excellent carrier for loading different signal enzymes and antibodies. In this paper, both of $Ab_2$ and GOD were loaded on the surface of silica nanosphere via GA which was used as a cross-linking reagent. In Fig. S2, we could find that the bare silica nanosphere do not appear absorption peak (curve c). However, $SiO_2$-GOD-$Ab_2$ nanoparticle exhibited a 280 nm

absorption peak (curve d), which corresponded to the typical protein absorption peak of pure antibody (curve a) and GOD (curve b). All these results confirmed that antibody and GOD were successfully assembled on the silica nanosphere.

## The influence of driving voltage on the detection

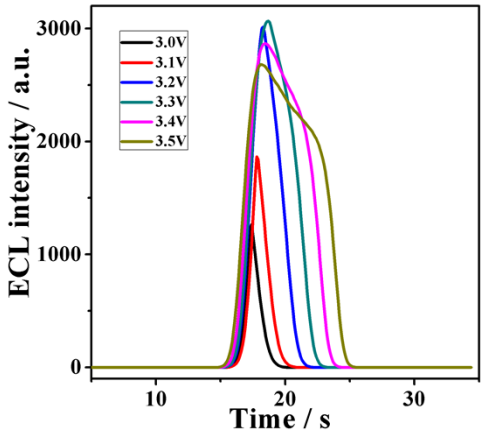


**Fig. S3** The ECL voltage curves obtained by applying a linearly increasing voltage from the initial voltage of -0.2 V to the different final voltage (from 3.0 to 3.5V) on the two ends of driving electrodes with the scan rate of 0.2 V/s.

## Stability of the immunodevice

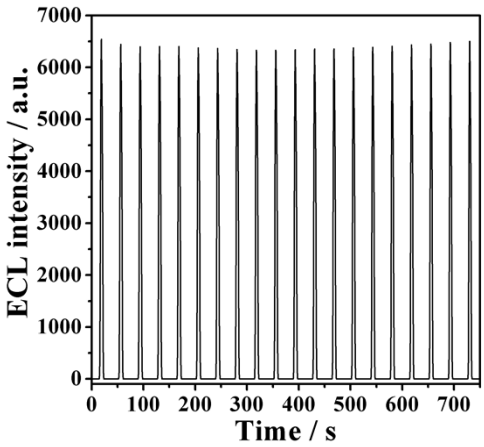


**Fig. S4** ECL emission stability of the proposed paper-based BPE biosensor to 100 ng/mL PSA.

## Reference

1 (a) D. L. Clegg, *Analytical Chemistry*, 1950, **22**, 48; (b) A. W. Martinez, S. T. Phillips, M. J. Butte and G. M. Whitesides, *Angewandte Chemie-International Edition*, 2007, **46**, 1318.