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Supporting Information

Introducing chemiluminescence resonance energy transfer into immunoassay in a microfluidic format for an improved assay sensitivity

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Chemicals and Materials

Estradiol (E2)、E2-BSA conjugate（Ag）, E2 antibody (Ab), N-(4-aminobutyl)-N-ethylisoluminol (ABEI) , fluorescein isothiocyanate (FITC) and N,N'-disuccinimidyl-carbonate (DSC) were purchased from Sigma-Aldrich Co.. Hydrogen peroxide was obtainedfromTaopu Chemicals (Shanghai, China). Horseradish peroxidase (HRP) was purchased from Dongfeng Biochemicals (Shanghai, China). Sodium dodecyl sulfate (SDS) and Tween 20 were provided by Shanghai Reagents (Shanghai, China). Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work.

Labeling of Ag with ABEI

Labeling of Ag with ABEI was based on a previous publication with minor modifications [1]. In brief, a 25 μL of 5 mM ABEI solution was added to an equal volume of 5 mM DSC solution and allowed to react at room temperature for 2 h. This ABEI–DSC solution was then added to an aliquot amount of Ag solution, and makes this

mixture solution with a 10-fold molar excess of ABEI–DSC. After vortex mixing, the mixture solution was allowed to stand at room temperature for 2 h. The resultant mixture was purified by dialysis in 20 mM PBS solution (pH=7.2) using dialyzing membrane with intercepting molecular weight 7000. The ABEI labeled Ag solution was diluted with running buffer prior to loading if necessary.

Labeling of anti-E2 with FITC

Labeling of anti-E2 with FITC was based on a previous publication with minor modifications [2]. Ab solution (4.1 g/L) was gradually added to a FTIC solution (4.1 g/L) in 0.5 M carbonate buffer (pH 9. 5) to obtained a 10-fold molar excess of FITC mixture solution. The reaction mixture was stirred at 20°C for 4 h. Excess, unconjugated FITC was removed by dialyzed against 20 mM PBS solution (pH=7.2). The FITC labeled Ab solution was stored at 4°C.

Immunological reaction

Immunological reaction was performed as follows: 20 μL of standard estradiol or sample solution were mixed with 20 μL of 0.5 μM ABEI labeled estradiol antigen and 20 μL of 0.25 μM FITC labeled anti-estradiol antibody in a 0.5-mL microcentrifuge tube. This solution was diluted to 100 μL, and the mixture solution then was incubated at 37°C for 40 min. The resulting solution was analyzed by MCE-CL.

Human serum samples

Human blood samples from a health volunteer was collected and centrifuged at 2000

rpm for 15 min to obtain serum. 250 μL of human serum sample was diluted with 500 μL of acetonitrile and shaken vigorously for 5 min to deposit proteins. After centrifuging at 12000 rpm for 20 min, the supernatant was transferred into another 1.5 ml vial and dried with a N2 stream. The residue was dissolved in 250 μL of 20 mM phosphate buffer solution (pH 7.4). The solution was vortexed and kept at 4 °C.

Analysis detection

CL spectra were measured with a LS-55 luminescence spectrometer (Perkin-Elmer, USA); UV-visible spectra were measured with a TU-1901 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd. China). The microfluidic immunoaasay was performed using a laboratory built system described previously [17]. Briefly, the glass/PDMS microchip assembly was mounted on the X-Y translational stage of an inverted microscope (Olympus CKX41) that also served as a platform of CL detection. Use of the X-Y translational stage allowed viewing any point of the microchannel. CL signal was collected by means of a microscope objective. After passing a dichroic mirror and a lens, CL photons were detected by a photomultiplier (PMT, Hamamatsu R105). The PMT was mounted in an integrated detection module including HV power supply, voltage divider, and amplifier. The CL emission was collected by a PMT and was recorded and processed with a computer using a Chromatography Data System (Zhejiang University Star Information Technology, Hangzhou, China). A multi-terminal high voltage power supply, variable in the range of 0–6000 V (Shandong Normal University, Jinan, China), was used for sample injection and MCE separation. The inverted microscope was placed in a black box.

Microchip fabrication

A home made glass/PDMS microchip was used in the whole work. The procedure of microchip fabrication was as described previously [1]. The channel between reservoir S and SW was used for sampling, the channel between B and BW was used for the separation and the channel between R and BW was used for the oxidizer introduction. The width of microchannels is 65 μm (except oxidizer introduction channel is 250 μm). The depth of all microchannels is 25 μm, and the double “T” size is 60 μm.

References

[1] S. Zhao, Y. Huang, M. Shi, Y. M. Liu, *J. Chromatogr. A* **2009**, *1216,* 5515-5519.

[2 N. Monji, A. S. Hoffman, *Appl. Biochem. Biotech.* **1987**, *14*, 107-120.

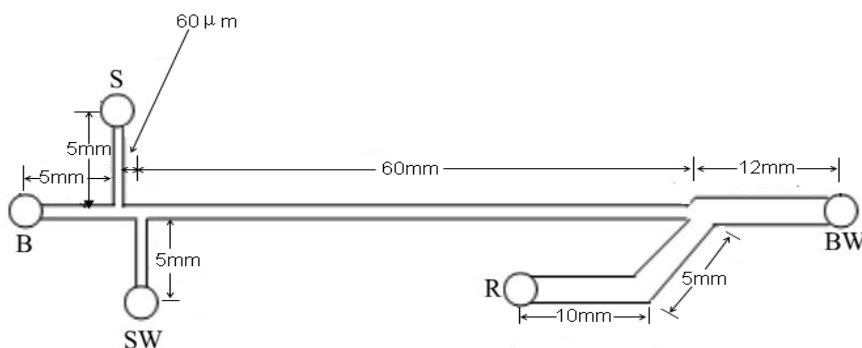

Figure S1. Schematic diagram of the layout of the glass/PDMS microchip. S: sample reservoir; B: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir; R: the oxidizer reagent reservoir.

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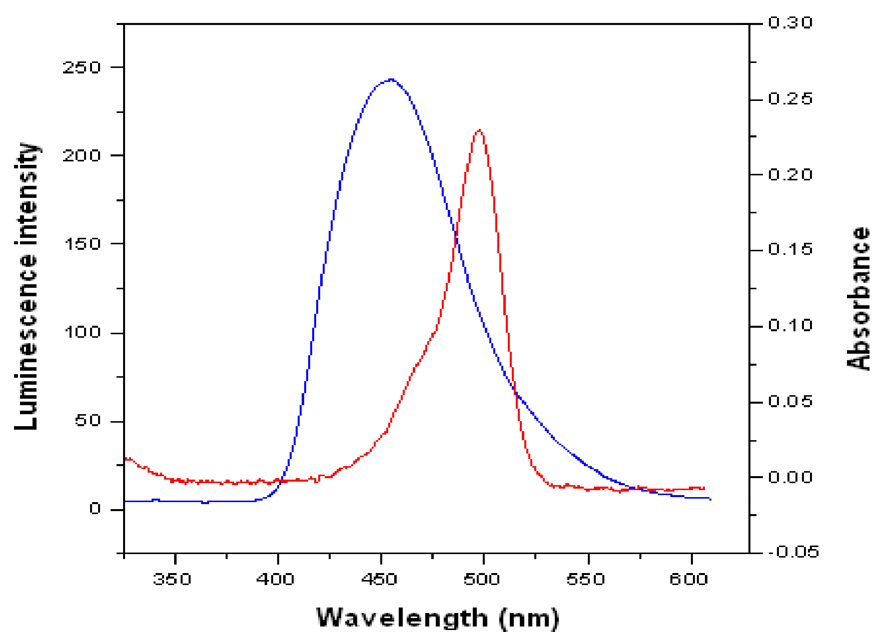

Figure S2. CL spectra of the ABEI-H_2O_2-HRP sytem (blue) and absorbance spectra of FITC (red). All spectra are normalized.

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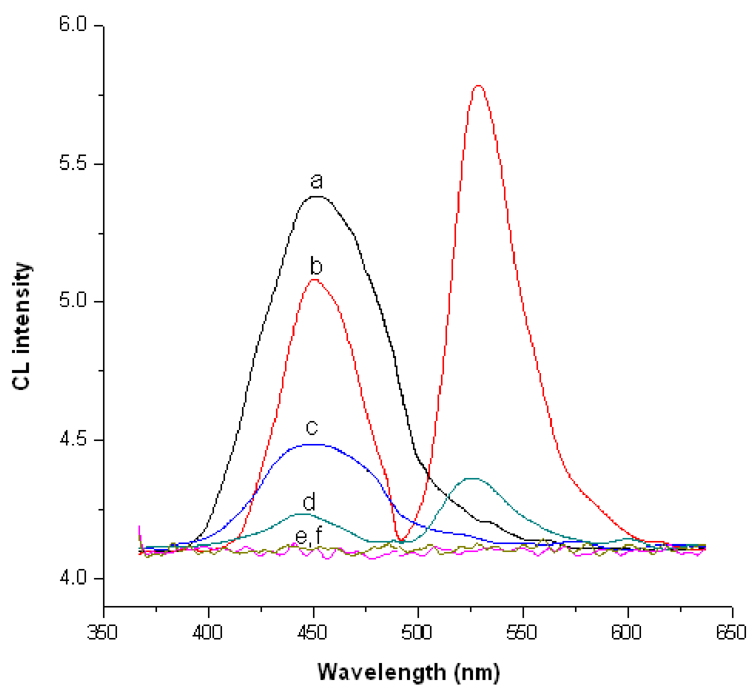

Figure S3. CL spectra of ABEI and ABEI-E2-FITC-anti-E2 immunocomplex in the presence of H_2O_2 and HRP. (a) ABEI; (b) a mixture of ABEI-E2-FITC-anti-E2 immunocomplex and E2; (c) a mixture of ABEI and E2, 20 amino acids, GSH and catecholamines (each concentrations are 4.0×10^{-6}M); (d) a mixture of ABEI-E2-FITC-anti-E2 immunocomplex and E2, 20 amino acids, GSH and catecholamines (each concentrations are 4.0×10^{-6}M); (e) ABEI in the serum samples (diluted 4 times); (f) ABEI-E2-FITC-anti-E2 immunocomplex in the serum samples (diluted 4 times).

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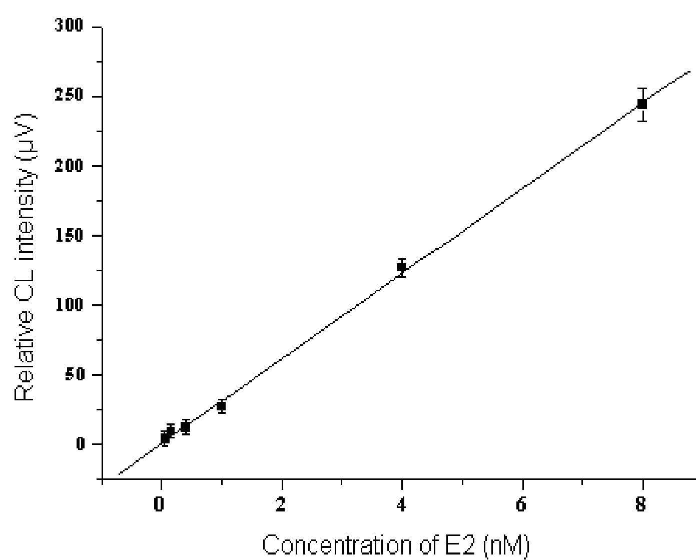

Figure S4. Calibration curve for E2 detection with different concentration. Error bars are standard deviation of three repetitive measurements.

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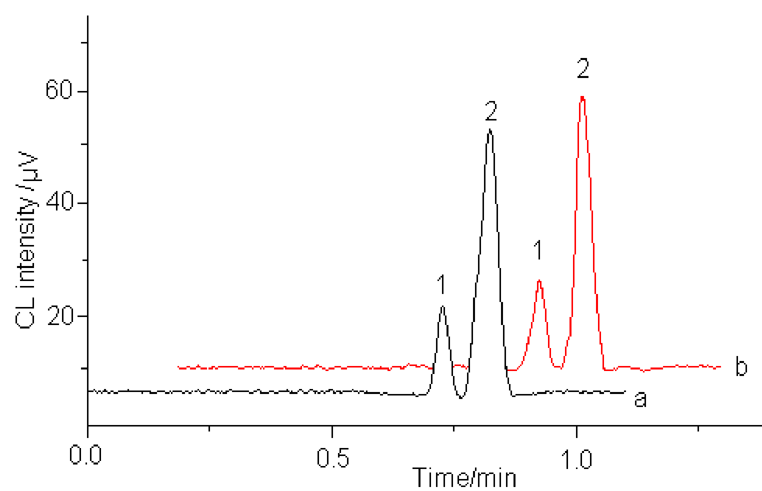

Figure S5. Electropherograms obtained from analyzing a women's serum sample (a) and the serum sample spiked with E1 and E3 at 5 nM (b). Electrophotetic buffer is 20 mM borate solution (pH 9.0) containing 10 μM HRP and 40 mM SDS; Oxidizer solution (pH 10.5) is 100 mM H_2O_2 in 35 mM $NaHCO_3$ buffer. Peak identification: (1) ABEI-E2-FITC-anti-E2 immunocomplex; (2) ABEI-E2.