

## Supplementary Material

### **Digital Electrogenerated Chemiluminescence Biosensor for the Determination of Multiple Proteins Based on Boolean Logic Gate**

Honglan Qi\*, Xiaoying Qiu, Chen Wang, Qiang Gao, Chengxiao Zhang

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of  
Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an, 710062, P.R.  
China

## EXPERIMENTAL SECTION

### Reagents and apparatus

A peptide (8 mer, CHSSLKQK, MW=930.1) was purchased from Sinoasis Pharmaceuticals, Inc. (China). Prostate-specific antigen (PSA) from human semen was obtained from Fitzgerald Industries International, Inc. (USA). Bleomycin sulfate were purchased from Melone Pharmaceutical Co., Ltd. (China). The BLM samples were prepared by mixing BLM with Fe(II) ion in 1:1 molar ratio. Bis(2,2'-bipyridine)-4,4'-dicarboxybipyridine-ruthenium di(N-succinimidyl ester) bis(hexafluorophosphate) (Ru(bpy)<sub>2</sub>(dcbpy)NHS) and 6-Mercaptohexanol (MCH) was obtained from Sigma-Aldrich (USA). Oligonucleotide probe (5'-SH-(CH<sub>2</sub>)<sub>6</sub>-CGCTTTAAAAAAGCG-(CH<sub>2</sub>)<sub>6</sub>-NH<sub>2</sub>-3') was synthesized and purified by HPLC by Sangon Biotech Co., Ltd (China). Millipore Milli-Q water (18.2 MΩ·cm) was used to prepare solution throughout.

ECL measurements were performed with a MPI-A ECL detector (Xi'an Remax Electronics, China). The experimental set-up for ECL measurement was same as the previous paper (Li, Y.; Qi, H.; Peng, Y.; Yang, J.; Zhang, C., *Electrochem. Commun.*, **2007**, *9*, 2571-2575). A commercial cylindroid glass cell was used as an ECL cell, which contained a conventional three-electrode system consisting of either a gold electrode (2.0 mm diameter) or a ECL biosensor as the working electrode, a platinum plate as the counter electrode, and an Ag/AgCl (saturated KCl) as the reference electrode, respectively. ECL emissions were detected with a photomultiplier tube (PMT) that was biased at -900 V unless otherwise stated.

### Preparation of ECL probes

The ECL DNA probe, Ru(bpy)<sub>2</sub>(dcbpy)NHS labeled DNA, were synthesized according to literatures.(Li, Y.; Qi, H.; Peng, Y.; Yang, J.; Zhang, C., *Electrochem. Commun.*, **2007**, 9, 2571-2575) The concentration of Ru(bpy)<sub>2</sub>(dcbpy)NHS labeled DNA (Ru-ss-DNA) solution was estimated to be  $3.64 \times 10^{-5}$  M according to the value of UV absorption of Ru(bpy)<sub>2</sub>(dcbpy)NHS at 457 nm.

The ECL peptide probe, ruthenium bis (2, 2'-bipyridine) (2, 2'-bipyridine-4,4'-dicarboxylic acid)-N-hydroxysuccinimide ester -peptide (Ru-peptide), were synthesized according to literatures with some modifications. (Miao, W.J.; Bard, A.J., *Anal. Chem.*, **2003**, 75, 5825-5834; Shimdzu, T.; Iyoda, T.; Izaki, K., *J. Phys. Chem.* **1985**, 89, 642-645). Two milligrams of peptide (0.00215 mmol) was dissolved in 10 mL of absolute ethanol. The peptide was labeled by adding a 10-fold molar excess of Ru(bpy)<sub>2</sub>(dcbpy)NHS to the stirred peptide solution, followed by an overnight incubation at 4 °C. The labeled peptide was purified by gel filtration chromatography on Sephadex G-15 using 50 mM phosphate buffer solution (PBS) containing 0.15 M NaCl (pH 7.2). The concentration of Ru-peptide solution was calculated to be  $1.77 \times 10^{-5}$  M according to the value of UV absorption of Ru(bpy)<sub>2</sub>(dcbpy)NHS at 457 nm.

### **Fabrication of ECL biosensor**

The Ru-peptide (or Ru-ss-DNA) was immobilized on a cleaned gold electrode surface by self-assembling technique. (Zhao, N.; He, Y. Q.; Mao, X.; Sun, Y. H.; Zhang, X. B.; Li, C.Z.; Lin, Y. H.; Liu, G. D., *Electrochem. Commun.*, **2010**, 12, 471-474) Prior to the experiment, the gold electrode (2.0 mm diameter) was pretreated according to previously published protocols. (Li, Y.; Qi, H.; Peng, Y.; Yang, J.; Zhang, C., *Electrochem. Commun.*, **2007**, 9, 2571-2575) The self-assembly of Ru-peptide or Ru-ss-

DNA onto the gold electrode surface was performed at 4 °C by dipping the electrode into 10 µM Ru-peptide solution (or 3 µM Ru-ss-DNA solution). The Ru-peptide (or Ru-ss-DNA) modified gold electrode was thoroughly rinsed with ethanol to remove the unbinding the ECL probe on the gold electrode. The Ru-peptide (or Ru-ss-DNA) modified gold electrode was then immersed in 1 mM mercaptohexanol solution for 30 min. The resulting electrode was washed with water and used as the ECL-PB biosensor (or the ECL DNA biosensor).

For the multiple assay of PSA and bleomycin, Ru-peptide and Ru-ss-DNA were both immobilized onto the gold electrode at same time by dipping gold electrode into a mixture solution containing 10 µM Ru-peptide solution and 3 µM Ru(bpy)<sub>2</sub>(dcbpy)NHS labeled DNA solution. Then the ECL-PB and DNA biosensor was co-immobilized with 1 mM MCH and washed with water to remove the nonspecific adsorption. The surface densities of thiol compounds were evaluated by an electrochemical reductive desorption of the monolayers, according to the reported procedure (El-Deab, M, S., Ohsaka, T., *Electrochimica Acta* **2004**, 49, 2189–2194).

### **ECL measurement**

The ECL-PB biosensor fabricated was immersed in 100 µL 10 mM PBS (pH 7.4) containing different concentrations of PSA for 30 min at room temperature. The ECL DNA biosensor was immersed in 100 µL 10 mM PBS (pH 7.4) containing different concentrations of BLM samples for 30 min at room temperature. The ECL-PB and DNA biosensor fabricated was immersed in 100 µL 10 mM PBS (pH 7.4) containing different concentrations of PSA and BLM for 30 min at room temperature. After the reaction, the formed electrode was rinsed thoroughly with 10 mM PBS (pH 7.4) to remove the

nonspecific adsorption. The ECL measurement was performed at a constant potential of +0.85 V in 1.0 mL of 0.10 M PBS (pH 7.4) containing 50 mM tripropylamine (TPA) and 0.10 M NaCl. The concentration of PSA or BLM was quantified by a decreased ECL intensity ( $\Delta I = I_0 - I_s$ ), where  $I_s$  was the ECL intensity of ECL biosensor reacted with target protein and  $I_0$  was the blank ECL intensity of ECL biosensor. All experiments were carried out at room temperature.

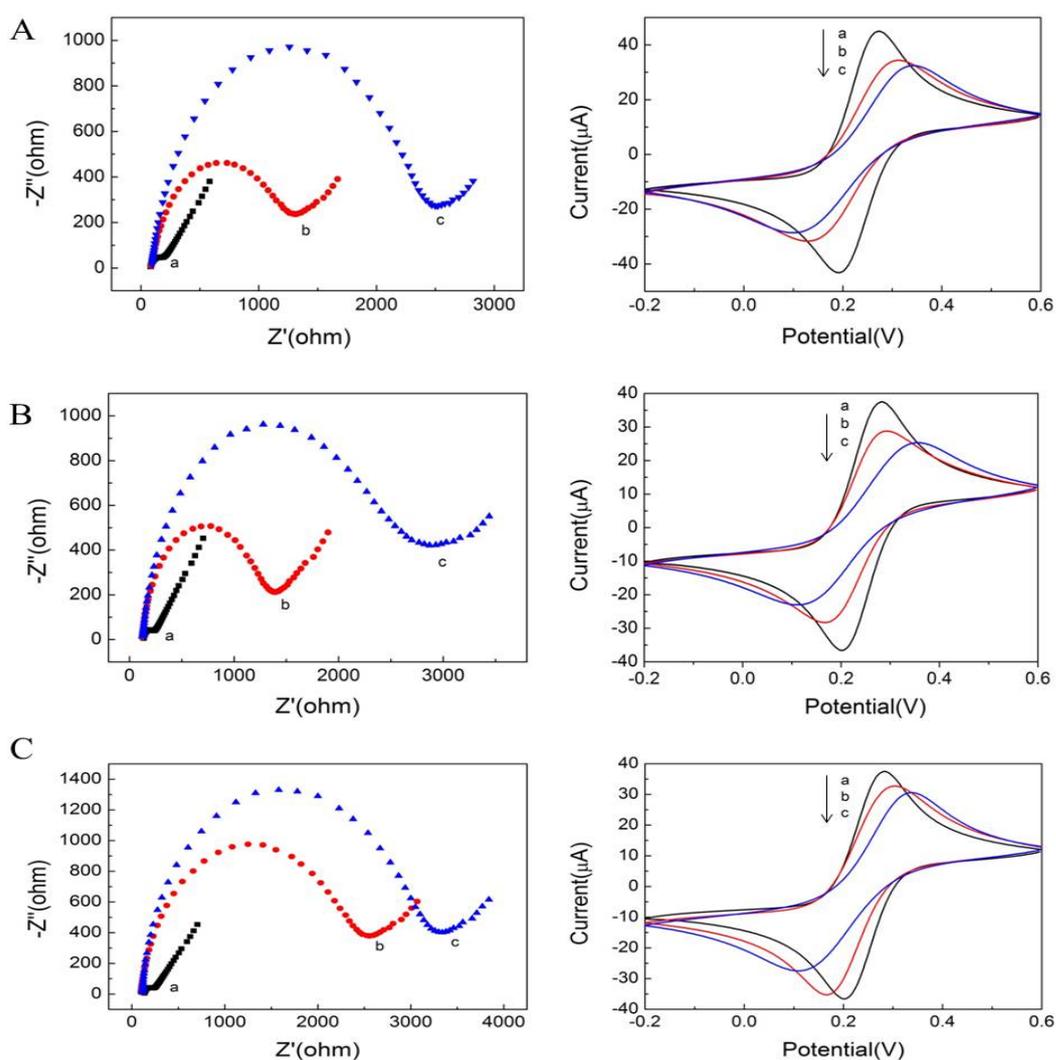


Fig. S-1 Nyquist plots (left) and cyclic voltammetry (right) obtained in 0.10 mol/L PBS containing 5 mmol/L  $\text{K}_3\text{Fe}(\text{CN})_6$ -5 mmol/L  $\text{K}_4\text{Fe}(\text{CN})_6$ -0.1 mol/L KCl (pH 7.4)

(A)(a) bare gold electrode, (b) Ru-ss-DNA modified electrode, (c) Ru-ss-DNA/MCH modified electrode.

(B) (a) bare gold electrode, (b) Ru-peptide modified electrode, (c) Ru-peptide /MCH modified electrode.

(C) bare gold electrode, (b) ss-DNA and Ru-peptide modified electrode, (c) ss-DNA and Ru-peptide /MCH modified electrode.

Scan rate in CV, 50 mV/s. The biased potential was 0.29 V vs Ag/AgCl; the frequency was from 100 kHz to 500 mHz and the amplitude was 5.0 mV for EIS.

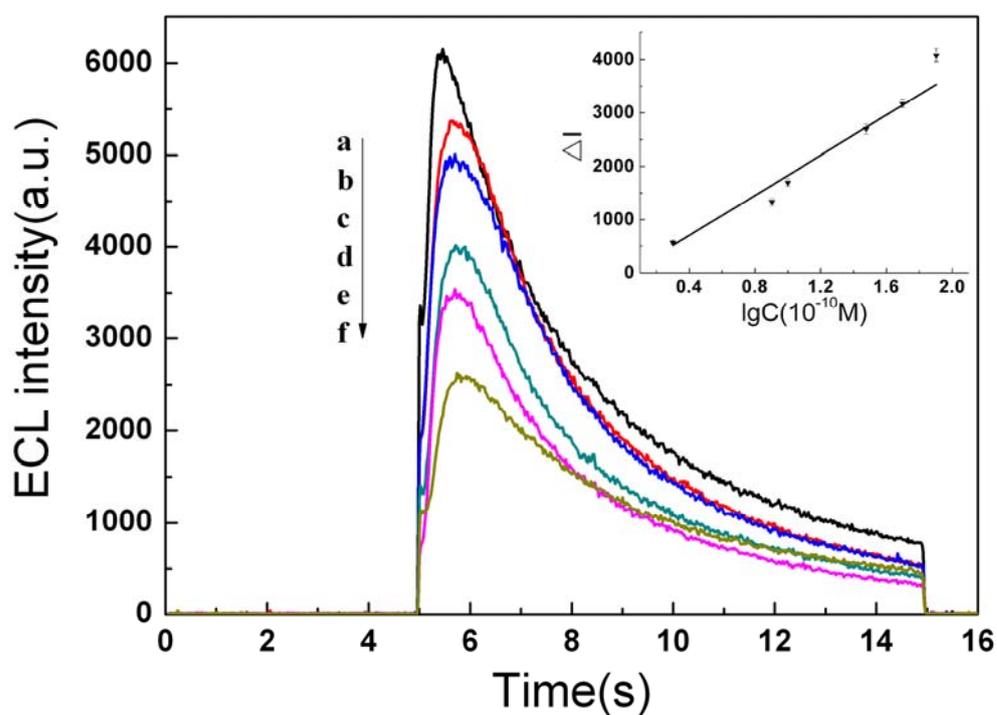


Fig. S-2 ECL responses of the DNA biosensor interacting with different concentrations of Fe(II)·BLMs. (a)  $2.0 \times 10^{-10} \text{ M}$ ; (b)  $8.0 \times 10^{-10} \text{ M}$ ; (c)  $1.0 \times 10^{-9} \text{ M}$ ; (d)  $3.0 \times 10^{-9} \text{ M}$ ; (e)  $5.0 \times 10^{-9} \text{ M}$ ; (f)  $8.0 \times 10^{-9} \text{ M}$ .

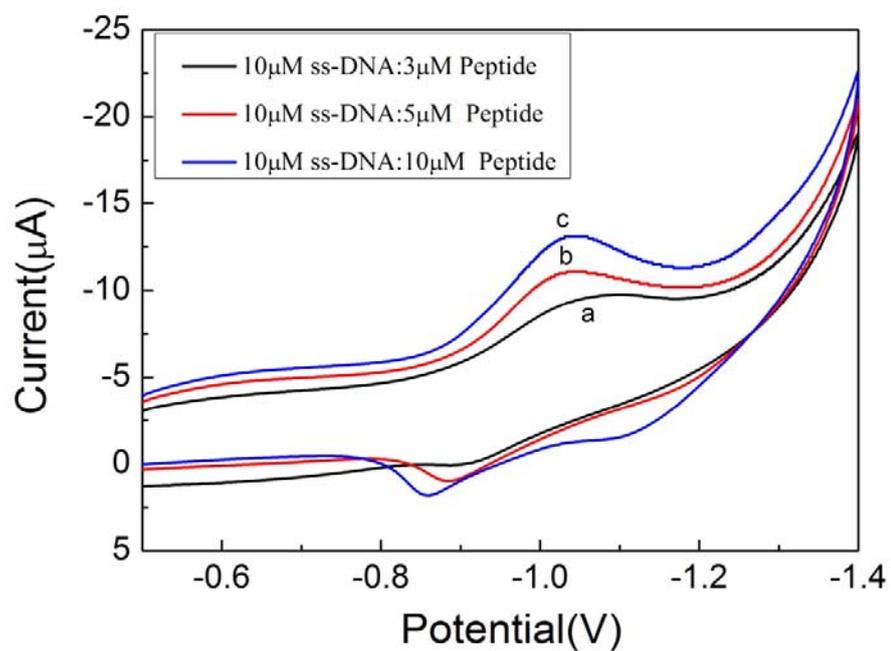


Fig. S-3. CVs for the reductive desorption of self-assembly of biosensor, in  $N_2$ -saturated 0.5 M KOH, formed on different gold electrodes, (a) electrode dipped in 10  $\mu$ M DNA: 3  $\mu$ M peptide, (b) electrode dipped in 10  $\mu$ M DNA: 5  $\mu$ M peptide, (c) electrode dipped in 10  $\mu$ M DNA: 10  $\mu$ M peptide for 30 min. Potential scan rate: 100 mV/s.

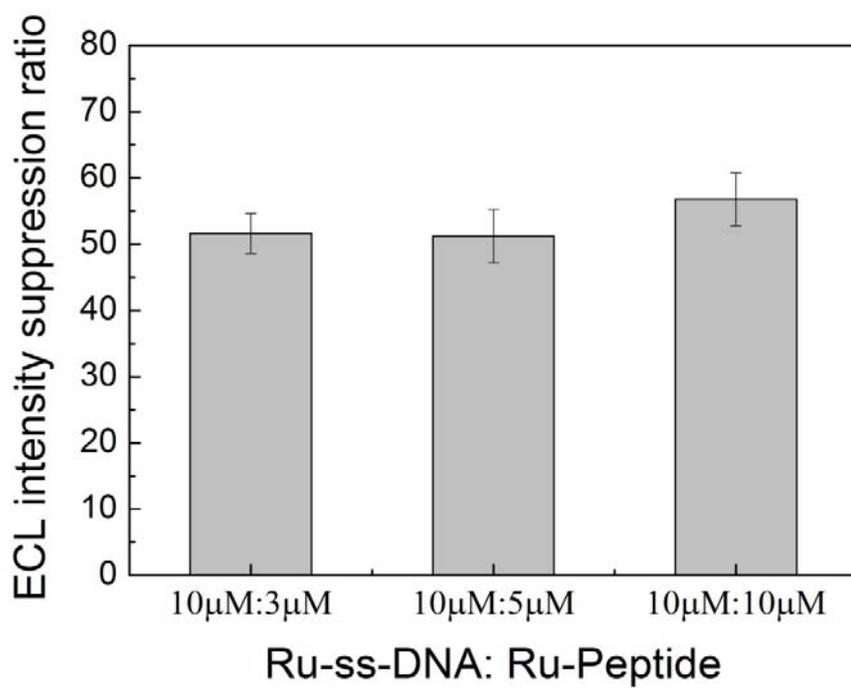


Fig. S-4 Effect of the concentration ratio of Ru-peptide and Ru-ss-DNA on the ECL intensity suppression ratio for  $1 \times 10^{-9}$  g/mL PSA

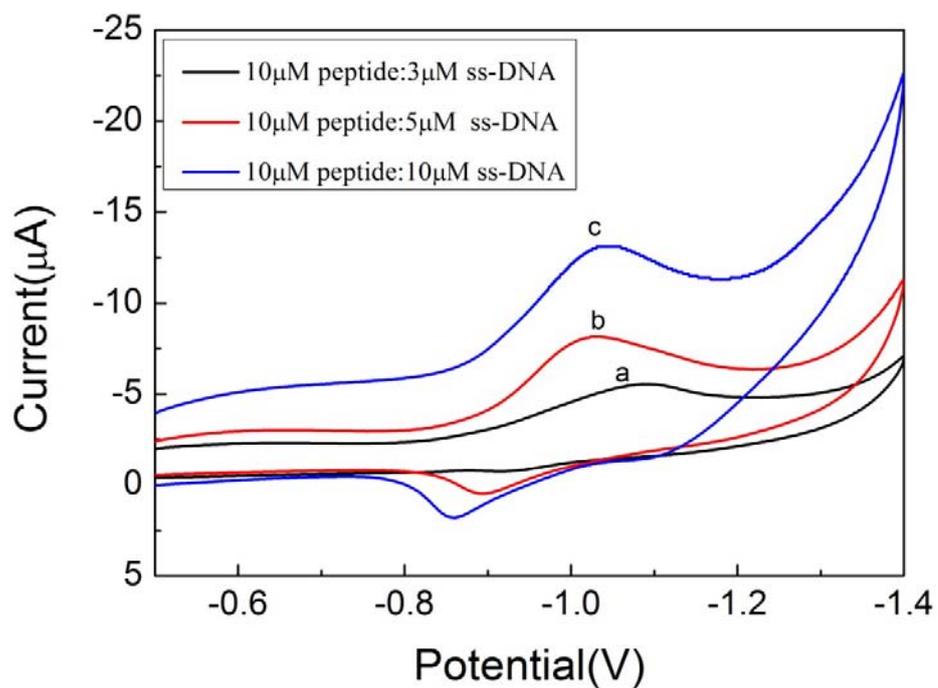


Fig. S-5. CVs for the reductive desorption of self-assembly of biosensor, in  $N_2$ -saturated 0.5 M KOH, formed on different gold electrodes, (a) electrode dipped in 10  $\mu$ M peptide: 3  $\mu$ M DNA, (b) electrode dipped in 10  $\mu$ M peptide: 5  $\mu$ M DNA, (c) electrode dipped in 10  $\mu$ M peptide: 10  $\mu$ M DNA for 30 min. Potential scan rate: 100 mV/s.

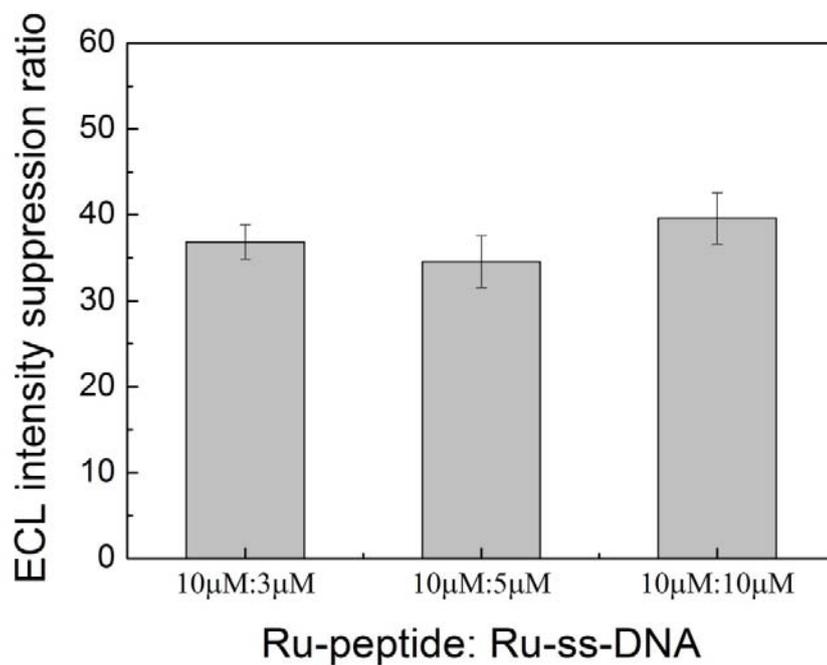


Fig. S-6 Effect of the concentration ratio of Ru-peptide and Ru-ss-DNA on the ECL intensity suppression ratio for  $1.0 \times 10^{-9}$  M Fe(II)•BLM