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Spatial-resolved electrochemiluminescence ratiometry based on bipolar electrode for bioanalysis

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ABSTRACT

Herein, a spatial-resolved electrochemiluminescene (ECL) ratiometry based on a closed biopolar electrode (BPE) is reported for the highly sensitive detection of prostate specific antigen (PSA). Au@g-C_3N_4 NCs as one ECL emitter were firstly coated on the cathode of BPE, while the anode of the BPE served for calibration via another ECL substance, $Ru(bpy)_3^{2+}$. The electroneutrality across the BPE makes the reactions on each pole of BPE electrically coupled. Thus one electrochemical sensing reaction at one pole of BPE could be quantified at both ends. A composite, Pt-PAMAM-DNAzyme was assembled on the surface of cathode via DNA hybridization between probe DNA and PSA aptamer. It acted as an ECL quencher of g-C_3N_4 via resonance energy transfer (RET) and catalyzing the reduction of O_2, the co-reactant of g-C_3N_4. Meanwhile, it could promote the ECL of $Ru(bpy)_3^{2+}$ at anode, since the catalytic reduction of O_2 at the cathode increased the faradiac current flowing through the BPE. Based on this signal composite, an ECL "off-on" phenomenon was observed at the cathode, after Pt-PAMAM-DNAzyme was "peeled off" by PSA. Conversely, at the anode, an "on-off" ECL changing was obtained. Therefore, a sensitive ratiometry for PSA detection was achieved with a linear range from 0.10 to 200 ng/mL. Since the two ECL emitters were physically separated, the ratiometric system was relatively simple and neither optical filters nor spectrometer were required. The strategy combining the ECL ratiometry and BPE broadens the applications of BPE-ECL and shows good perspective in clinical application.

1. Introduction

Electrochemiluminescence (ECL) is a luminescence excitation process triggered electrochemically (Richter, 2004; Shan et al., 2009). Combining the advantages of zero optical background and easy reaction control by applying electrode potential, ECL has been proved to be a highly sensitive method in the areas of biological (Dai et al., 2015; Wu et al., 2015), environmental (McCall et al., 1999; McCall and Richter, 2000) and food analysis (Cheng and Stanker, 2013). Through monitoring the change in ECL intensity, species that directly take part in ECL reaction (Lei et al., 2015; Wang et al., 2013) or indirectly influence the reaction (Zhang et al., 2015b) could be quantified. However, other factors such as environmental conditions can interfere with the signal output, especially during trace analysis, which may cause false positive or negative errors.

Ratiometric detection is an ideal strategy to limit the interference factors via normalizing environmental variation by self-calibration, which has been widely developed in fluorescence (Wu et al., 2016). In the past 5 years, some works based on the methodology of ratiometric ECL have been reported in biological and environmental analysis (Wu et al., 2016; Zhang et al., 2014). The ratiometric ECL should include both dual-potential and dual-wavelength signal ratiometric assays. Since the first dual-potential ECL ratiometry for DNA sensing was reported by Xu and coworkers in 2013 (Zhang et al., 2013), a series of researches have been published for the detections of cancer cell, miRNA and Mg^{2+} using similar strategy (Cheng et al., 2014; Hao et al., 2014; Wang et al., 2016). Recently, applying graphite-like carbon nitride nanosheet (g-C_3N_4 NS), an effective, strong and stable ECL emitter, Xu et al. reported the first dual-wavelength ratiometric ECL approach for the highly sensitive detection of miRNA (Feng et al., 2016). Despite the advantage of the ratiometric ECL, it usually makes the detection system more complicated, since two ECL substances with special characters and typical co-reactants should

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exist in the same solution. How to choose the emitters and balance their ECL excitation potentials, intensities, wavelengths and co-reactants remains a challenge. In addition, optical filters or spectrometer have to be applied for dual-wavelength ratiometry, which decreased the ECL intensity to a great extent (Doeven et al., 2012). Therefore, pursuing a simple strategy with self-calibration function but easy to handle is valuable for further study.

Aiming at this issue, we developed a closed bipolar electrode (BPE)-ECL device in the present work for ratiometric detection of prostate specific antigen (PSA), which belongs to the human kallikrein family and has been recognized as the premier tumor marker for the detection of early stage prostate cancer and to monitor the recurrence of the disease after treatment. BPE is an electrically conductive material that promotes electrochemical reactions at its extremities (Chow et al., 2008; Wu et al., 2012, 2011). The electroneutrality across the BPE makes the reactions on each pole of BPE electrically coupled (Fosdick et al., 2013; Shi et al., 2014; Wu et al., 2013; Zhang et al., 2016). Thus one electrochemical sensing reaction at one pole of BPE could be quantified at both ends. The closed BPE is an excellent choice for ratiometric assay since the solutions contacting the BPE anode and cathode are physically separated from one another. Therefore, two ECL emitter/co-reactant systems could be separated in space which limits mutual interference. The sensing principle is shown in Scheme 1. The cathode modified with Au@g-C_3N_4 as the ECL substance served as sensing pole. The anode of the BPE served for calibration via another ECL substance, $Ru(bpy)_3^{2+}$ adding in the reservoir. As a sensing probe, Pt-PAMAM-DNAzyme was prepared and assembled at the cathode through the combination of probe DNA and PSA aptamer. The composite acted as the quencher of cathode ECL emission, but the promoter of anode ECL ascribed to the electric equilibrium of the BPE. In the presence of target PSA, its recognition by aptamer led to the release of Pt-PAMAM-DNAzyme from the cathode and caused partial recover of the ECL emission. Accordingly, the anode ECL emission decreased because of the decreased faradiac current flowing through BPE. Calculating the ratio of ECL cathode to ECL anode resulted in the quantitative analysis of PSA. Compared with conventional dual-potential and dual-wavelength ECL ratiometries, the spatial-resolved ECL ratiometry based on closed BPE is more simple and sensitive since the two ECL reactions could be spatially separated and no optical filters were required for the data acquisition.

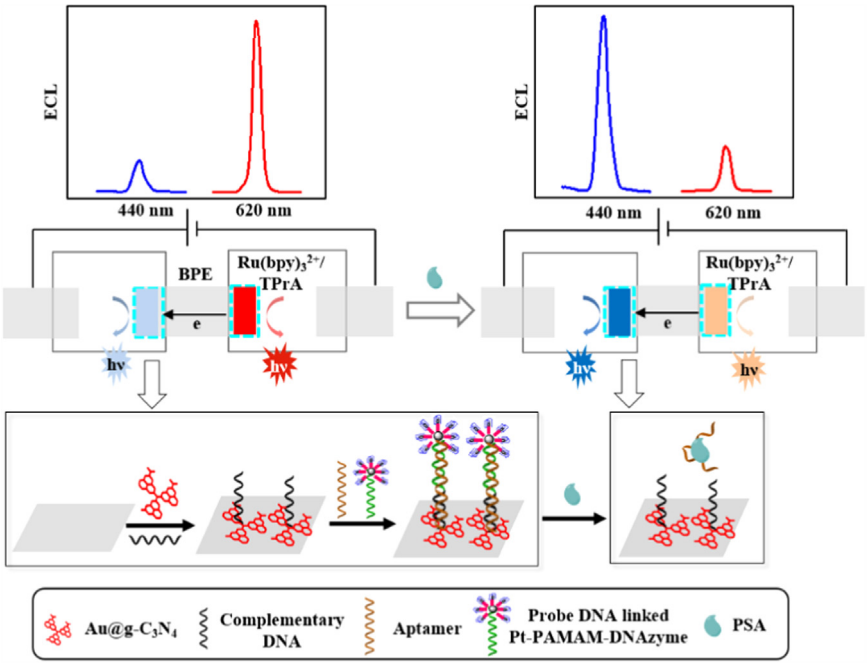
2. Experimental section

2.1. Materials and reagents

Indium-tinoxide (ITO)-coated (thickness: 100 nm; resistance: 10 Ω/square) aluminosilicate glass slides were obtained from China Southern Glass (Shenzhen, China). Sylgard 184 (including poly (dimethylsiloxane) (PDMS) monomer and curing agent) was from Dow Corning (Midland, MI). Melamine was obtained from Fuchen Chemical Reagent Co. (Tianjin, China). H_2SO_4 was supplied by Shanghai Chemical Reagent Co. Ltd., China. $HAuCl_4$, H_2PtCl_6, $NaBH_4$, trisodium citrate, PAMAM dendrimer (ethylenediamine core, generation 4.0, 10 wt% in methanol, 64 surface primary amino groups), bovine serum albumin (BSA, 96–99%), N-hydroxy succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), $Ru(bpy)_3^{2+}$, tripropylamine (TPrA) (3-mercaptopropyl)triethoxysilane (MPTES) and tri (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). PSA antigen was purchased from Key GEN Biotech. Hemin was purchased from Aladdin (Shanghai, China) and used without further purification. Complementary DNA (cDNA), probe DNA (pDNA), PSA aptamer and G-quadruplex DNA were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China), and their sequences were as follow:

c DNA: 5′-NH_2-$(CH_2)_6$-TTTTTGCTATTTGATG-3′
p DNA: 5′-GCGAGCTTTAATTTTTT-$(CH_2)_6$-SH-3′
PSA aptamer: 5′-ATTAAAGCTCGCCATCAAATAGC-3′
G-quadruple: 5′-TGGGTAGGGCGGGTTGGGTTTT-TT-$(CH_2)_6$-SH-3′

The phosphate buffered saline (PBS) (pH 7.4) contained NaCl (100 mM), $Na_2HPO_4 \cdot 12H_2O$ (10 mM), and NaH_2PO_4 (10 mM). All other reagents were of analytical reagent grade. The water used in



Scheme 1. Schematic illustration of the ECL-BPE modification process and ratiometric sensing of PSA.

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