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# A novel electrochemiluminescence resonance energy transfer system for ultrasensitive detection of prostate-specific antigen

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ABSTRACT

A novel electrochemiluminescence resonance energy transfer (ECL-RET) system from $Eu^{3+}$-doped CdS nanocrystals (CdS:Eu NCs) to $Ru(bpy)_3^{2+}$-doped silica nanoparticle (RuSi) was designed for ultrasensitive detection of prostate-specific antigen (PSA). The platform consists of a CdS:Eu NCs film on glassy carbon electrode (GCE) as ECL emitter and a sandwich-type immunocomplex formed by the primary antibody, PSA and RuSi-tagged second antibody. This system showed a maximum of 4.83-fold enhancement of ECL intensity at 620 nm due to the efficient ECL-RET and high $Ru(bpy)_3^{2+}$ quantum yields. By the signal amplification of RuSi and the specific antibody–antigen interactions, this ECL-RET system could sensitively respond down to 1.0 fg $mL^{-1}$ PSA.



## 1. Introduction

Förster resonance energy transfer (RET) is a well-established molecule spectroscopy method which is caused by the nonradiative energy transfer between a suitably matched donor and acceptor pair [1,2]. RET has been proved to be a powerful technique for probing changes in the distance between donors and acceptors and sensitive monitoring molecular binding events [3–6]. Electrochemiluminescence resonance energy transfer (ECL-RET), which converts electrical energy into radiative energy as excitation source, is a newly developed technique for biological applications, due to its high sensitivity, rapid biological response, and excellent controllability [7–11], but it also meets difficulty to find a suitable pair of donor–acceptor due to the limited kinds of ECL donors and the electrochemical instability of the acceptor itself in electric field [12].

Nowadays, semiconductor nanocrystals (SNCs) as promising ECL emitters, have recently attracted much attention as ECL-RET donor due to the diversity and tunable emission wavelength [7,13,14]. Previous studies have demonstrated the effective ECL-RET between CdS NCs and Au nanoparticles (NPs), CdTe NCs, or $Fe_3O_4$ NPs to enhance or quench the donor's ECL emission [7–9]. More recently, $Ru(bpy)_3^{2+}$ has been applied as an excellent ECL acceptor of CdS NCs or nanorods [15,16] due to its good electrochemical stability, high quantum yields, long excited state lifetime and strong luminescence [17–19]. Many approaches have been aimed at improving the energy transfer ratio between donor and acceptor, thus introducing abundant signal amplification elements could be a simple way [20–23]. Silica nanospheres as a traditional carrier were employed to encapsulate large number of $Ru(bpy)_3^{2+}$ with the aim to amplify the signal [15,16].

In medicine and molecular diagnostics, it is in high demand to develop extremely sensitive and accurate clinical tools for biomarkers of interest for early diagnosis and monitoring of diseases [24–27]. Here, prostate-specific antigen (PSA), the main clinical protein biomarker for prostate cancer [28], was employed as model biomarker. Based on the classic sandwich-type immunocomplex and ECL-RET technique, an ideal means was developed. $Eu^{3+}$-doped CdS nanocrystals (CdS:Eu NCs), the donor used in this ECL-RET system, possess improved ECL intensity and efficiency with multi-emission. $Ru(bpy)_3^{2+}$-doped silica nanoparticles (RuSi), as a novel acceptor applied in the assay could greatly amplify the efficiency of ECL-RET.

## 2. Experimental

### 2.1. Materials and apparatus

Two mouse anti-human total prostate-specific antigen (PSA) monoclonal antibodies, clone P27A10 (primary capture antibody (Ab1)) and clone P27B1 (secondary detection antibody (Ab2)), total PSA, fetoprotein (AFP), and carcinoembryonic antigen (CEA) were obtained from Shuangliu Zhenglong Biochemical Lab (Chengdu, China). Clinical serum samples of PSA with different concentrations were provided by the Affiliated Hospital of Nanjing University. Bovine serum albumin (BSA), mouse immunoglobulin G (IgG), $Ru(bpy)_3^{2+}$, 3-mercaptopropionic acid (MPA), 1-methylimidazol, N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were of analytical

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grade and used as received. 0.10 M phosphate buffer solution (PBS, $KH_2PO_4$-$K_2HPO_4$) containing 0.050 M $K_2S_2O_8$ (pH 8.3) was used for ECL detection, 0.10 M Tris–HCl buffer containing 0.10 M NaCl (pH 7.4) was used for storing modified GCE, and 0.10 M PBS containing 0.10 M NaCl (pH 7.4) was for preparation of antigen and antibody stock solutions. The clinical PSA serum samples were diluted 10,000 times with 0.10 M PBS containing 0.10 M NaCl (pH 7.4) for the PSA detection. Millipore ultrapure water (resistivity of 18.2 M Ω cm) was used throughout this study.

The ECL emission measurements were conducted on an MPI-E multifunctional electrochemical and chemiluminescent analytical system (Remax Electronic Instrument Limited Co., Xi'an, China, 350 nm–650 nm) by cyclic potential scan at room temperature, and the voltage of the PMT was set at −500 V in the process of detection. Potential scan was from 0 V to −1.35 V. Scan rate was 100 mV $s^{-1}$. Electrochemical impedance spectroscopy (EIS) was carried out with an Autolab potentiostat/galvanostat (Eco Chemie B.V., Netherlands) and detected in 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$. The experiments were carried out with a conventional three-electrode system. The working electrode was a 3 mm diameter glassy carbon electrode (GCE) modified with NCs composite film, while a Pt wire and saturated calomel electrode (SCE) served as the counter and reference electrodes, respectively. ECL spectra were obtained by a series of optical filters (from 400 nm to 660 nm, spaced 20 nm, Omega Optical Inc., USA). The UV–vis absorption spectra were obtained on a UV-3600 UV–vis–NIR photospectrometer (Shimadzu Co., Japan).

### 2.2. Synthesis and of $Ru(bpy)_3^{2+}$-doped silica nanoparticles (RuSi) and preparation of RuSi tagged-Ab2 (RuSi-Ab2) composites

The RuSi NPs were prepared according to our previous work [29] and the number of $Ru(bpy)_3^{2+}$ molecules in single NP was calculated to be about $2.78 \times 10^4$ [30,31]. For the preparation of RuSi-Ab2, 2 mL 50 μg $mL^{-1}$ Ab2 solution was added into 3 mL 2.5% glutaraldehyde modified RuSi monodisperse solution. The mixed solution was kept for 2 h at 37 °C. The ratio between Ab2 and RuSi NPs was calculated to be about 8:1 [30]. The excess amino-group and nonspecific binding sites of the centrifuged Ab2-RuSi were blocked in 5 mL 1 wt% BSA solution. Finally, the Ab2-RuSi were washed thoroughly at each step with 0.1 M PBS (pH 7.4) and stored in 2 mL PBS for further use.

### 2.3. Fabrication of the ECL sensor

CdS:Eu NCs and CdS:Eu NCs film on GCE were prepared according to our previous work [32]. The prepared CdS:Eu NCs film was firstly immersed in MPA solution to immobilize MPA on CdS:Eu NCs film by Cd–S bond. Then the terminal carboxylic acid groups of MPA were activated by EDC and NHS [13]. Afterward, the electrode was soaked in 100 μL 50 μg $mL^{-1}$ Ab1 solution for 12 h at 4 °C. After that the electrode was incubated in 2 wt% BSA for 1 h at 4 °C to block the excess active groups on the surface. The as-prepared electrode was washed thoroughly at each step with 0.1 M PBS (pH 7.4) and stored in the 0.1 M NaCl + 0.1 M Tris–HCl buffer (pH 7.4) for the following PSA detection.

### 2.4. ECL detection of PSA

The above Ab1/CdS:Eu NCs-GCE was incubated with different concentrations of PSA for 1 h and was further incubated with 100 μL solution of RuSi-Ab2 composites for 1 h at 37 °C. In order to remove nonspecifically adsorbed species, the electrode was thoroughly washed with PBS at each step. Then, a sandwich immunoassay was conducted for the detection of PSA.

## 3. Results and discussion

### 3.1. Mechanism of ECL-RET immunoassay for PSA

The principle of ECL-RET system was shown in Fig. 1. After CdS:Eu NCs (1.5%) was used as ECL emitter firstly coated on GCE surface, Ab1 was immobilized on CdS:Eu NCs film through EDC and NHS. In the presence of PSA, the Ab1/PSA/Ab2-RuSi complexes could be formed. The ECL-potential curves and EIS of the CdS:Eu NCs film at each step were illustrated in Fig. 2A. The resistance increased with the processes of immobilization. However, only when the immunoreaction between PSA and RuSi-tagged Ab2 occurred, the ECL signal was greatly enhanced (curve d). For no cathodic ECL emission of RuSi (curve f), the enhancement was attributed to the ECL-RET between CdS:Eu NCs and RuSi.

Obviously, the detection sensitivity of the immunoassay depended on the efficiency of ECL-RET between CdS:Eu NCs film and RuSi. As we know, the factors that affect the efficiency involve the spectral overlapping integrals of the donor's emission and the acceptor's absorption, the distance between the donor and acceptor, the extinction coefficient of the acceptor, and the lifetime of the donor and the acceptor. Here, the separation length between CdS:Eu NCs and RuSi NPs was controlled by the sandwich-type immunocomplex of Ab1/PSA/Ab2. Our previous work has demonstrated that the CdS:Eu NCs at 1.5% doping level showed 4-fold stronger and more stable cathodic ECL signals, improved excited state lifetime as well as multi-emission compared to pure CdS NCs [32], which made CdS:Eu NCs excellent donor. Likewise, $Ru(bpy)_3^{2+}$ has good extinction coefficient, high quantum yields, long excited state lifetime and strong luminescence, which made it a good acceptor and also enhancer. To further amplify the enhancement, RuSi encapsulating abundant $Ru(bpy)_3^{2+}$ molecules were applied as the acceptor in this system. Thus, the overlap of emission/absorption spectra came to be the point to influence the efficiency.

As shown in Fig. 2C, the broaden absorption from RuSi (red line) greatly overlapped the double peaks from 450 to 550 nm of recombination emission of host CdS surface states of CdS:Eu NCs, especially the peak at 480 nm (blue line), while $Ru(bpy)_3^{2+}$ just partly overlapped (black line). When RuSi-Ab2 were captured by PSA on the film, the ECL emission at 480 nm decreased (the inset of Fig. 2B, curve b′), while the ECL emission at 620 nm increased substantially (Fig. 2B, curve b), indicating the efficient energy transfer from the recombination emission to RuSi. The increment of ECL at 620 nm was larger than the decrement of ECL at 480 nm and no cathodic ECL was generated by RuSi alone (Fig. 2D, curve c), indicating that the transferred energy could excite a large number of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{2+*}$ in RuSi and exhibited signal amplification due to the high quantum yields.

To further investigate the effect of ECL-RET from CdS:Eu NC to RuSi, the ECL emission spectra of PSA/Ab1/CdS:Eu-GCE before and after the capturing of RuSi-Ab2 were tested and shown in Fig. 2D. Without the attached RuSi-Ab2, there existed double peaks from 450 to 550 nm of recombination emission, a high peak of the characteristic transitions of $Eu^{3+}$ ions around 620 nm and also a shoulder peak around 580 nm. After the capturing, the emission from 450 to 550 nm distinctly decreased, especially the peak at 480 nm reduced 67.2%, while the emission from 580 to 640 nm substantially increased, especially the peak at 620 nm enhanced to 4.83-fold. As a result, the total ECL intensity obtained 3.17-fold rise due to the quenching of recombination emission and the enhancement of red emission from $Ru(bpy)_3^{2+}$. For largest increment at 620 nm and the effect of background signals reducing through optical filters, the following ECL intensity of PSA detection was recorded at 620 nm.

### 3.2. ECL detection of PSA

Owing to ECL-RET effect, the more the RuSi-Ab2 attached, the larger the ECL intensity enhanced, as shown in Fig. 3A. When the PSA concentration exceeded to 1.0 pg $mL^{-1}$, the signals changed slightly. Thus, the