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Electrochemiluminescent biosensor with DNA link for selective detection of human IgG based on steric hindrance

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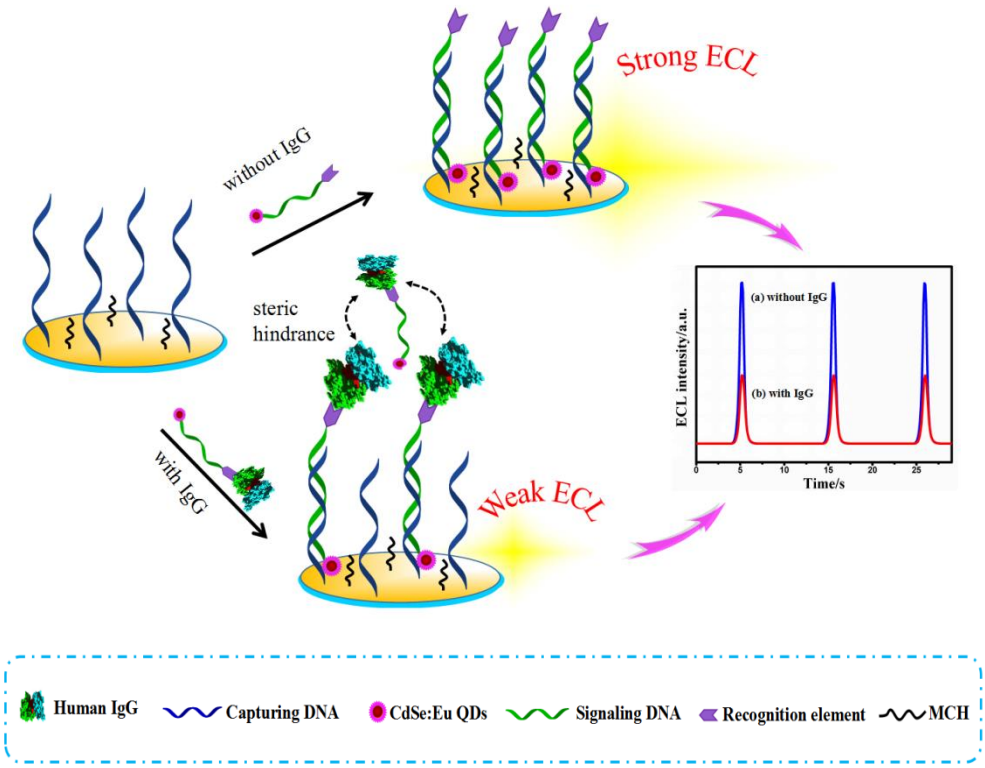
ABSTRACT

A highly selective DNA-based electrochemiluminescence (ECL) based biosensor is described for the detection of human IgG. It is exploiting the effect of steric hindrance that affects the strength of the ECL signal in the presence of IgG. Digoxin-linked signalling DNA was specifically bound to IgG, and this causes steric hindrance which limits the ability of DNA to hybridize with capturing DNA attached to a gold electrode. Europium (II) doped CdSe quantum

dots were covalently linked to the DNA in order to generate the ECL signal. Using this steric hindrance hybridization method, the ECL signal of the biosensor were proportional to the concentration of IgG with a wide linear range and a 14 pM detection limit. Conceivably, the method can be expanded to the detection of a wide range of proteins for which homologous recognition elements are available.

Graphical abstract



In the manuscript, a highly sensitive DNA-based electrochemiluminescence (ECL) biosensor was developed for the detection of human IgG (IgG), which takes advantage of steric hindrance

effects to influence the ECL signal in the presence of target protein. The prepared ECL biosensor presented higher sensitivity and could be applied in other proteins with homologous recognition elements.

Keywords: Electrochemiluminescence; Biosensor; Steric hindrance effects; Human IgG; Europium (II) doped CdSe quantum dots; Digoxin-linked signalling DNA

1. Introduction

Immunoglobulin G (IgG) is an important immunoglobulin as it can neutralize toxins and viruses, activate complement aggregation and other functions, and is an important biomarker in the clinical diagnosis of certain diseases such as chronic infection, chronic liver disease, lymphoma, and some autoimmune diseases such as systemic lupus erythematosus, multiple myeloma and various immunodeficiency diseases, and in leukemia [1]. Therefore, it is necessary to provide effective and satisfactory analytical strategies for the detection of human IgG in pathological analysis and clinical diagnosis.

Due to its characteristics including time-saving operation steps, excellent sensitivity and selectivity, immunoassay has been widely used in clinical diagnosis [2] biomedicine [3], pharmacology [4] and environmental science [5]. In recent years, with the study in depth on the immunosensors, various detection techniques have been developed, including optics [6],

electrochemistry [7], radio-immunoassays (RIAs) [8], and piezoelectric detection analysis [9]. These techniques, although they have been shown to be extremely powerful, still have some limitations. For example, fluorescence immunoassay methods have problems such as interference due to promiscuous light and light scattering, which limit their application in the field of immunity [10]. Although the RIA has been revolutionized in the bioanalytical chemistry field due to its extremely high sensitivity, good specificity and extensive application [11], the potential radioactive hazards and the technical disposal of radioactive waste limit its application. Current methods for the quantitative detection of proteins, such as antibodies and antigens, still mainly rely on the enzyme-linked immunosorbent assay (ELISA), and although ELISA has been widely used in biological measurement, the experimental procedure is time-consuming, arduous and complicated due to frequent washing during the immunoassay process [12]. Hence, there is an urgent need to develop an accurate and simple analytical method and minimize the analysis time for the detection of proteins.

Electrochemiluminescence (ECL) is a chemiluminescence phenomenon caused by an electrochemical reaction [13, 34, 37], and compared with other analytical methods, it is a simple process, has an ultra-low background signal, good temporal control, and high sensitivity [14, 15]. ECL biosensors, as an experimental detection strategy, have been widely used in immunoassays [16, 35, 36], microRNA analysis [17], and medical diagnosis [18] due to their sensitive diagnostic system, high specificity, miniaturization for portability and low cost. ECL biosensors are currently used to detect macromolecules such as proteins mainly involved in the

antibody−antigen reaction and immunocomplexes generating steric hindrance to delay the transfer of co-reactant, which constitutes a simple signal-off type assay protocol [19]. Pang et al. previously synthesized GO/MWCNTs-COOH/Au@CeO_2 nanocomposites modified on the surface of an electrode as antibody carriers and an ECL signal probe, and achieved highly sensitive detection of carcinoembryonic antigen [20]. Liu's group first reported a potential-resolved ECL biosensor for the detection of multiplex proteins with high sensitivity and specificity [16]. Subsequently, the use of DNA-labeled recognition events for the analysis of multiple antibodies bound to antigens was demonstrated [21]. From these features of the detection mechanism in sensing areas, it is envisaged that a simple signal-off type detection mechanism caused by distinctive steric hindrance can also be applied in DNA-based ECL biosensors. To date, various DNA-based ECL biosensors have been developed, most of which were used to detect small-molecules or ions [22]. However, DNA-based ECL biosensors due to the coupling of steric hindrance for highly sensitive detection of proteins has never been reported.

Quantum dots (QDs) which are a popular luminescent material, have unique advantages, such as high emission efficiency, exquisite conductivity, good water solubility and biocompatibility in biosensors [23-25]. In addition, to further enhance the luminous efficiency of QDs, metal ion doped QDs have attracted attention in recent years, especially in the field of ECL bioassay. In our previous work [18], we successfully synthesized Europium(II) doped CdSe quantum dots (CdSe:Eu QDs) which were used in an embryonic antigen assay and showed fascinating ECL

intensity and stability when modified on the electrode surface and served as an ECL signal generator.

In the present study, a simple and highly selective DNA-based ECL biosensor was fabricated that exploits steric hindrance effects for the first time to detect human IgG. This steric hindrance mechanism was demonstrated using ECL by employing CdSe:Eu QDs modified signaling DNA and a gold electrode that contained complementary capturing DNA. CdSe:Eu QDs were used as the ECL signal reporter by combining with signaling DNA. When the target analyte IgG bound to the signaling DNA, a decrease in the number of signaling DNA strands or signaling DNA-QDs/IgG conjugate (S1/IgG) hybridized with capturing DNA which are immobilized to the gold surface due to steric hindrance, thus generating lower ECL signals. Using this steric hindrance hybridization mechanism in an ECL format, a remarkable ECL response was obtained for quantitative detection of IgG. The details of the preparation, characterization, optimization of conditions and the response performances of the biosensor are as followings.

2. Experimental

2.1. Apparatus

The ECL measurement was carried out on a MPI-E detection system (Xi'an Rimax, China) contain the three-electrode system: a gold working electrode (GE,Φ=4 mm), a Ag/AgCl reference electrode, and a platinum counter electrode. The photomultiplier tube (PMT) was biased at −800 V in the process of detection. The electrochemical impedance spectroscopy (EIS) measurements and the cyclic voltammetry (CV) analysis were recorded on an Autolab

electrochemical analyzer (EcoChemie, The Netherlands) and a CHI660D electrochemical workstation (Shanghai Chenhua Instrument, China) in 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl, respectively. The potential was set -0.18 V, within the frequency range of 0.1–100 kHz in the process of EIS measurement and the scan rate was at 100 mV/s and scan range was from -0.2 V to 0.6 V in the process of CV analysis. For characterization of the materials, a Transmission electron microscope (TEM) (JEM- 2100, JEOL Co., Japan), UV-vis absorption photospectrometer (UV-3600, Shimadzu Co.) and Photoluminescence (PL) spectrophotometer (RF-540, Shimadzu, Tokyo, Japan) was used.

2.2. Materials and Reagents

Cadmium chloride ($CdCl_2.2.5H_2O$, 99.0%) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Europium(III) nitrate hexahydrate ($Eu(NO_3)_3.6H_2O$, 99.9%) and Selenium (99.99%) were supplied by Aladdin reagent Co., Ltd.(Shanghai,China).6-mercapto-1-hexanol(MCH),L-Cysteine(L-Cys),N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide-hydrochloride(EDC),tri(2-carboxyethyl)phosphinehydrochloride(TCEP) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. All chemicals were analytical grade in this work. phosphate buffered saline (0.1M) were prepared with $Na_2HPO_4.12H_2O$ and $NaH_2PO_4.2H_2O$, containing 0.1M $K_2S_2O_8$, 0.1M NaCl and 0.1 M KCl served as ECL analysis electrolyte (pH 7.5). Double deionized water (18.2 MΩ·cm) obtained from a Millipore system was used to prepare all solutions. Human IgG was purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Oligonucleotide sequences were

custom-made by Takara Biotechnology Co., Ltd (Dalian,China). The oligonucleotide sequences are as follows:

Capturing DNA, 5'-AAGGAAAGAGAAGCAGTGT(CH_2)$_6$-SH-3'

Signaling DNA, 5'-NH2-ACACTGCTTCTCTTCCTT-Digoxin-3'

Reference DNA, 5'-NH2-ACACTGCTTCTCTTCCTT-3'

2.3.Preparation of CdSe:Eu QDs

The synthesis method was similar to that described in our previous work with some modification[26] are presented in the electronic supporting information(ESI).

2.4. Preparation of the signaling DNA-QDs conjugate (S1)

The carboxyl groups of the CdSe:Eu QD (80 μL) were activated by completely mixing them in EDC/NHS solution (C_{EDC}:C_{NHS}=2:1) for 0.5 h at 37 °C. Subsequently, 20 μL of the amino-functionalized signaling DNA (10 μM) was mixed with the activated CdSe:Eu QD solution, and next the mixed solution was incubated at 37 °C for 1 h with gentle stirring. The resultant solution was centrifuged with 30 kDa Millipore at 10000 rpm and 4 °C for 10 min. After removal of the supernatant, the S1 was re-suspended in Tris-HCl buffer (10 mM, 0.50 M NaCl, pH 7.4).

2.5. Fabrication of the ECL Biosensor

The GE (Φ=4 mm) was polished carefully on chamois leathers with 0.05 μm α-Al_2O_3 powders and cleaned ultrasonically with water, ethanol and water, respectively. After this treatment, 20 μL of freshly prepared piranha solution (30% H_2O_2/ 98% H_2SO_4 = 1:3, v/v) was dipped on the surface of gold electrode to incubate for 10 min, and then the electrode was

thoroughly rinsed with double distilled water. Finally, the GE was electrochemically cleaned in a 0.5 M H_2SO_4 solution with scan range from -0.3 and 1.6 V, then washed with double deionized water and dried with N_2. For capturing DNA assembling, the Au electrode was firstly incubated with 20 μL of 500 nM capturing DNA in Tris-HCl buffer (10 mM TCEP, 0.1 M NaCl, pH 7.4) at 4 °C overnight. Due to capturing DNA with a sulfydryl group at its 3'end, it can be immobilized closely on the surface of gold electrode through the formation of the Au−S covalent bond. Afterwards, the electrode was rinsed thoroughly with the Tris-HCl buffer (10 mM, pH7.4), and sealed with 10 μL of 3 mM MCH for 1 h to block blank sites and force the capturing DNA to hold an vertical surface orientation that benefits DNA hybridization. To remove nonspecifically adsorbed MCH, the modified electrode surface was rinsed with the Tris-HCl buffer (10 mM, pH7.4).

2.6. ECL Measurements

The above modified electrode was immersed into 250 μL of S1 conjugates solution including different concentrations of human IgG at 37 °C for 80 min (no pre-incubation was needed to label the target analytes with the signaling DNA). Thereafter, ECL detection was accomplished with the electrodes in 0.1 M PBS (pH 7.5) containing 0.1 M $K_2S_2O_8$ and the modified electrode was scanned from 0 to -1.6 V with the scan rate of 300 $mV \cdot s^{-1}$.

3. Results and discussion

3.1. Characterization of CdSe:Eu QDs and the signaling DNA-QDs

The morphology of the as-prepared CdSe:Eu QDs was characterized using transmission electron microscopy (Figure 1A), and showed a homogeneous spherical morphology and good monodispersity with particle sizes of approximately 2–3 nm. The average diameter of the CdSe:Eu QDs was further verified by dynamic light scattering (DLS) analysis (insets in Figure 1A). The UV−vis absorption and FL emission spectra of CdSe:Eu QDs without modification are shown in Figure 1B. The QDs showed a larger emission peak at 522 nm and a narrower absorption peak at 422 nm. As seen in Figure 1C, the FL emission spectrum of S1 had a similar Gaussian symmetry to that of pure QDs, indicating that the covalent conjugated S1 had good stability. However, the maximum emission wavelength of S1 conjugate showed slight red shifts from 522 to 531 nm compared to the QDs, which may be attributed to the size increase in QDs after DNA functionalization [29]. As shown in Figure 1D, the absorption peak at 260 nm was assigned to the characteristic absorption peak of the DNA strands, indicating that signaling DNA successfully bound to the surface of the QDs.

<<Herein Figure 1 >>

3.2. Electrochemical characterization of the modified electrode

Electrochemical impedance spectroscopy is an effective means of monitoring the assembly process of a modified electrode. As shown in Figure 2A, the bare gold electrode displayed a smaller electron-transfer resistance (R_{ct}) (curve a), indicating that the gold electrode had relatively good electrical conductivity. After capturing DNA was modified on the gold electrode

surface (curve b), the R_{ct} was much larger, which was due to electrostatic repulsion between the negative charges of the capturing DNA oligonucleotides backbone and the redox probe $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. By incubating the modified electrode with the MCH and S1 solution, successively, the resistance continually increased (curve c and curve d), which suggested that MCH and S1 were successfully immobilized on the electrode surface. The R_{ct} markedly increased following the introduction of S1/IgG (curve e), which may be attributed to an increase in steric hindrance due to IgG. These results indicated the successful fabrication of the proposed biosensor.

<<Herein Figure 2>>

Cyclic voltammetry (CV) was used to characterize the interface state of the fabricated electrodes at each step in $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ solution. As shown in Figure 2B, a couple of larger reversible redox peaks were observed at the bare gold electrode (curve a), indicating that the redox probe of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ had easy access to the gold electrode surface and achieved good electron transfer. The peak current diminished following self-assembly of capturing DNA (curve b), suggesting that the capturing DNA was successfully immobilized on the gold electrode surface. The CV response decreased further when the modified electrode was incubated in MCH (curve c) and S1 (curve d) solution, successively. The reason for this may be that MCH and S1 as insulation layers impeded electron transfer by the redox probe. Following

incubation of S1/IgG, the electrochemical signal reduced significantly (curve e), which indicated that the S1/IgG complex further increased steric hindrance to hinder the electron transfer of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. The CV results were in agreement with the results of electrochemical impedance spectroscopy measurement, which further confirmed successful fabrication of the biosensor.

3.3. Mechanism of the ECL Biosensor

In this study, initially, the CdSe:Eu QDs with signaling DNA bound to the gold electrode surface and were reversibly reduced to $(CdSe:Eu)^{\bullet-}$ (eq a), and the co-reactant $S_2O_8^{2-}$ was reduced to $SO_4^{\bullet-}$ (eq b). The radical anion of $SO_4^{\bullet-}$ then obtained an electron from $(CdSe:Eu)^{\bullet-}$ to generate the excited state CdSe:Eu* (eq c), indicating the important role played by the radical anion of $SO_4^{\bullet-}$ in efficient production of the CdSe:Eu* ECL emission source. In the presence of human IgG, the target analyte IgG specifically bound to its recognition element modified on the signaling DNA forming a steric hindrance effect. This effect significantly blocked signaling DNA-QDs complexes (S1) or S1/IgG access to the electrode interface to hybridize with capturing DNA and further impeded production of abundant CdSe:Eu*, thus resulting in a decrease in ECL emission (eq d). The ECL mechanism was determined as follows (eqs a−e) [27]:

$$CdSe:Eu + ne^- \rightarrow n(CdSe:Eu)^{\bullet-} \text{ (eq a)}$$

$$S_2O_8^{2-} + ne^- \rightarrow SO_4^{2-} + SO_4^{\bullet-} \text{ (eq b)}$$

$$(CdSe:Eu)^{\bullet-} + SO_4^{\bullet-} \rightarrow (CdSe:Eu)^* + SO_4^{2-} \text{ (eq c)}$$

$$(CdSe{:}Eu)^* \rightarrow CdSe{:}Eu + hv \quad \text{(eq d)}$$

Scheme 1 illustrates a schematic representation of the DNA-based biosensor construction and the mechanism of IgG analysis. Two single-stranded DNAs (signaling DNA and capturing DNA) were employed in the detection process. The 3′-thiol-modified capturing DNA was assembled on the gold electrode surface through an Au-S bond and the introduction of signaling DNA hybridized with capturing DNA to form dsDNA. The signaling DNA was labeled at 5′end with an amino which was incorporated with CdSe:Eu QDs by an amide bond self-assembly and used as the ECL signaling reporter, and at 3′end with the small hapten digoxigenin (Dig) which was recognized by IgG. When binding to the capturing DNA, S1 generated a large ECL signal by bringing CdSe:Eu QDs close to the electrode surface (Scheme 1, a peak). Conversely, the ECL signals markedly declined when the target analyte IgG was incubated with signaling DNA. The reason for the decrease in ECL signals when the detection process was carried out is as follows. The specific binding of IgG to the recognition element on signaling DNA blocked the approach of the major S1 conjugate or S1/IgG to the gold electrode surface to hybridize with capturing DNA due to the steric hindrance effect and thus generated lower ECL signals (Scheme 1, b peak). On the basis of the target analyte binding signaling DNA-induced steric hindrance effect, the ECL signal for the detection of IgG was significantly diminished.

<<Herein Scheme 1 >>

To further study the steric hindrance hybridization mechanism of the ECL DNA-based biosensor, the capturing DNA was fully saturated by S1 on the electrode surface, and then incubated in IgG solution. Figure 3A shows that almost no reduction in ECL signal was observed when the analyte IgG bound the signaling DNA which hybridized with capturing DNA on the surface of the gold electrode compared to that without target analyte. These results demonstrate that the ECL steric hindrance hybridization mechanism is not linked to a binding-induced reduction in the electron transfer rate of the CdSe:Eu QDs and co-reagent $K_2S_2O_8$. Furthermore, these results also suggest that ECL intensity reduction is closely related to less S1 binding to the electrode surface when bound to target analyte IgG. The reason for this may be that the target analyte formed a thick protein membrane on the surface of the capturing DNA, and other S1/IgG or S1 were unable to penetrate this layer due to steric hindrance, thus diminishing the ECL signals [28].

In order to better clarify the ECL steric hindrance hybridization mechanism come about when the target analyte specifically binds the recognition element (Dig) on signaling DNA, a reference signaling DNA without Dig as a comparison DNA in the presence of IgG was used. This showed an ECL response similar to signaling DNA in the absence of IgG, when compared with the signaling DNA in the presence of IgG (Figure 3B). These results showed that the hybridization of this reference signaling DNA with capturing DNA on the electrode surface was not affected by the presence of the target analyte, and indicated that Dig played an important role in the ECL steric hindrance hybridization process.

<<Herein Figure 3 >>

3.4. Analytical performance

<<Herein Figure 4 >>

Under optimized experimental conditions, the designed ECL DNA-based biosensor was used to detect concentrations of IgG. As shown in Figure 4A, a significantly enhanced ECL signal was observed without target analyte, as S1 was free to interact with all the capturing DNA. In the presence of target analyte, S1 was captured by the target analyte through binding of the recognition element, which limited its ability to hybridize with capturing DNA on the gold electrode surface due to the steric hindrance effect. Hence, the ECL intensity decreased with increased IgG concentration. Figure 4B shows the linear response between ECL intensity and the logarithm of IgG concentration ranging from 6.7×10^{-11} to 6.7×10^{-6} M, and the linear regression equation was $I = -1328.2\ \lg C_{Ab} - 4685.9$ with a correlation coefficient (R) of 0.9965 and the detection limit was 1.4×10^{-11} M (S/N = 3). This detection limit and response range were superior to those reported in previous studies (Table 1), indicating that this strategy was an effective method for the detection of human IgG.

<<Herein Table 1 >>

3.5. Stability, reproducibility and specificity of the biosensor

Stability is an essential factor in the application of biosensors for biological sensing. As shown in Figure 4C, the ECL intensity of the biosensor for the detection of 10^{-5} M human IgG was determined by consecutive scanning for 25 cycles, and showed that the ECL signal of the proposed biosensor was quite stable with a relative standard deviation of 3.18%.

In addition, the reproducibility of the fabricated biosensor was tested by detecting 10^{-6} M human IgG with five electrodes prepared independently with a relative standard deviation of 4.85%, indicating that the proposed strategy had acceptable reproducibility. Specificity is one of the potential advantages of using hapten digoxigenin as a recognition element in a biosensor. Other possible interfering agents such as carcinoembryonic antigen (CEA), bovine serum albumin (BSA), and α-fetoprotein (AFP) were used to evaluate the selectivity of the biosensor. The biosensor was exposed to 10^{-6} M IgG solutions with interference (10^{-4} M) or without interference in the presence of S1, respectively. As shown in Figure 4D, the ECL signal changed obviously for the target IgG compared with the interfering agents, suggesting that the proposed ECL biosensor for human IgG showed good specificity.

4. Conclusions

In summary, we successfully developed a new electrochemiluminescence method for the detection of human IgG based on the steric hindrance effect. Due to the unique properties of the hapten digoxin as a recognition element modified on the signaling DNA, it can specifically recognize IgG and results in good selectivity. The ends of signaling DNA strands functionalized with the recognition element and amino, respectively, cannot capture target analyte IgG but hybridized with complementary DNA resulting in ECL signals by conjugating CdSe:Eu QDs on the surface of the electrode. This study shows the potential application of steric hindrance effects as the preferred ECL method for the detection of human IgG. This bioassay was also rapid and simple without tedious separation steps and showed high detection sensitivity. Importantly, the developed bioassay is feasible and will be used in the clinical identification of antibodies in various diseases on a larger scale.

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Figure captions

Figure 1. (A) Transmission electron microscopy image of synthesized QDs and (inset) the dynamic light scattering (DLS) size distribution of the QDs; (B) UV–vis absorption spectrum and fluorescence spectrum of QDs (excited at 424nm); (C) Fluorescence spectra of QDs (curve a) and S1 (curve b); (C) UV–vis absorption spectra of DNA (curve a) and S1 (curve b).

Figure 2. Nyquist plots (A) and cyclic voltammogram (B) of the electrode at different stages in 0.1 M KCl and 5 mM $[Fe(CN)_6]^{4-/3-}$: (a) bare Au electrode, (b) Capturing DNA/Au electrode, (c) MCH/Capturing DNA/Au electrode, (d) S1/MCH/Capturing DNA/Au electrode, (e) IgG/S1/MCH/Capturing DNA/Au electrode. The concentration of IgG is 10^{-9} M. The frequency range: 0.1–1.0×10^5 Hz. The CV scan rate: 100 mV s^{-1}, scan range: -0.2 V to 0.6 V.

Scheme 1. Schematic diagram of the electrochemiluminescence DNA-based assay used to detect human IgG

Figure 3. (A) Investigation of the ECL steric hindrance hybridization mechanism in the absence and presence of 200 nM IgG in 10 mM Tris–HCl buffer (pH = 7.4) containing 0.50 M NaCl, respectively; (B) ECL response to different conditions: (a) reference signaling DNA in the presence of IgG, (b) and (c) signaling DNA in the absence and presence of IgG, respectively. The inset shows the ECL curves.

Figure 4. (A) ECL response of the designed biosensor in the presence of different concentrations of IgG: (a) 0 M, (b) 6.7×10^{-11} M, (c) 6.7×10^{-10} M, (d) 6.7×10^{-9} M, (e) 6.7×10^{-8} M, (f) 6.7×10^{-7} M, (g) 6.7×10^{-6} M; (B) Calibration curve of the biosensor for the determination of different IgG concentrations. (C) ECL stability of the biosensor for detection of 10^{-5} M IgG under continuous cyclic scans for 25 cycles in PBS (pH=7.4) containing 0.1 M KCl and 0.1 M $K_2S_2O_8$; (D) Selectivity of the proposed ECL biosensor for the detection of human IgG in the presence of different interferences: blank, CEA (10^{-4} M), BSA (10^{-4} M), AFP (10^{-4} M) and IgG (10^{-6} M), and a mixture (10^{-4} M CEA, 10^{-4} M BSA, 10^{-4} M AFP and 10^{-6} M IgG). Error bars

represent standard deviations of measurements (n= 5). The CV scan rate: 100 mV s^{-1}, scan range: -0.2 V to 0.6 V.

Table 1. Comparison of different biosensors for the determination of human IgG

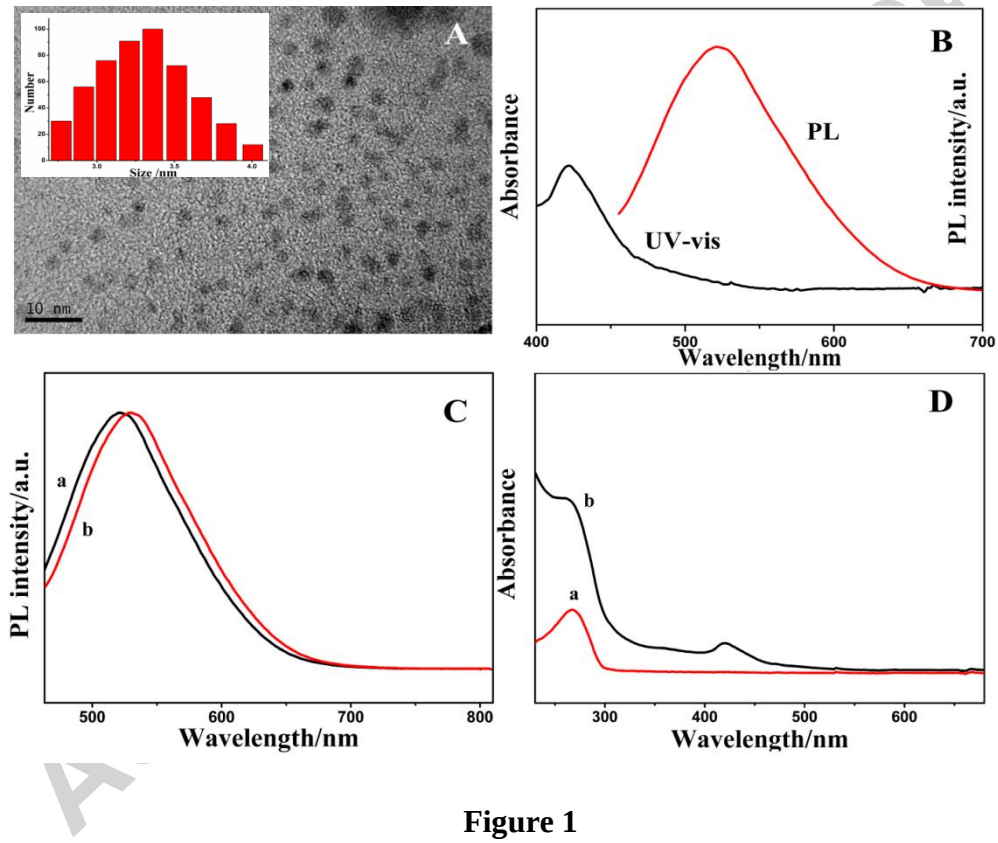


Figure 1

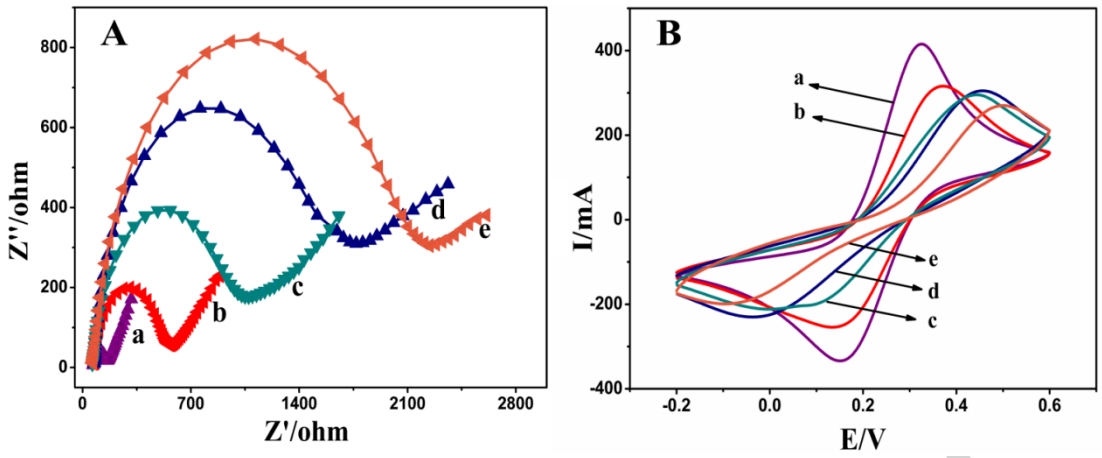
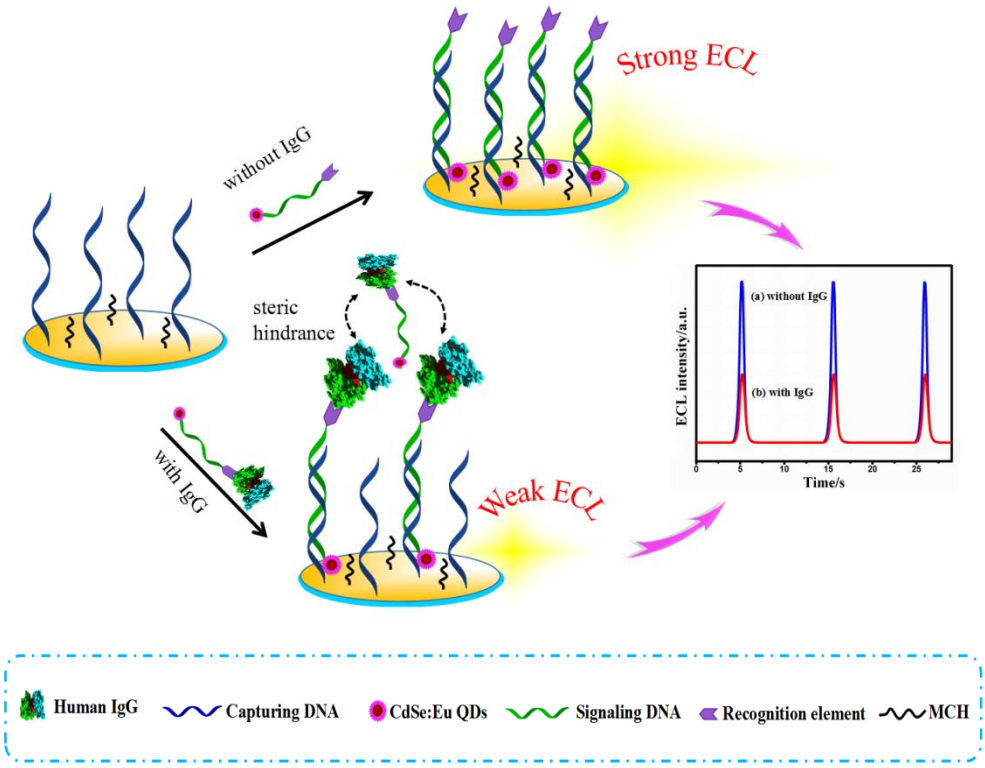


Figure 2



Scheme 1

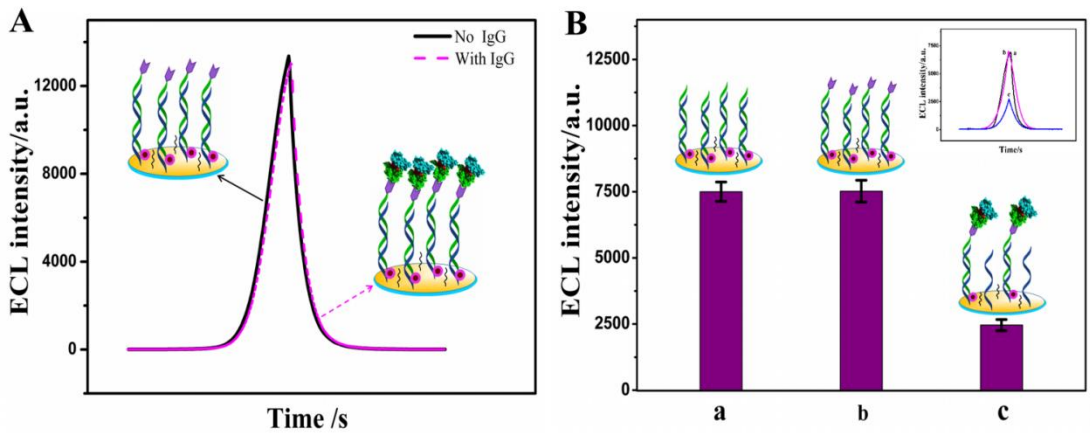


Figure 3

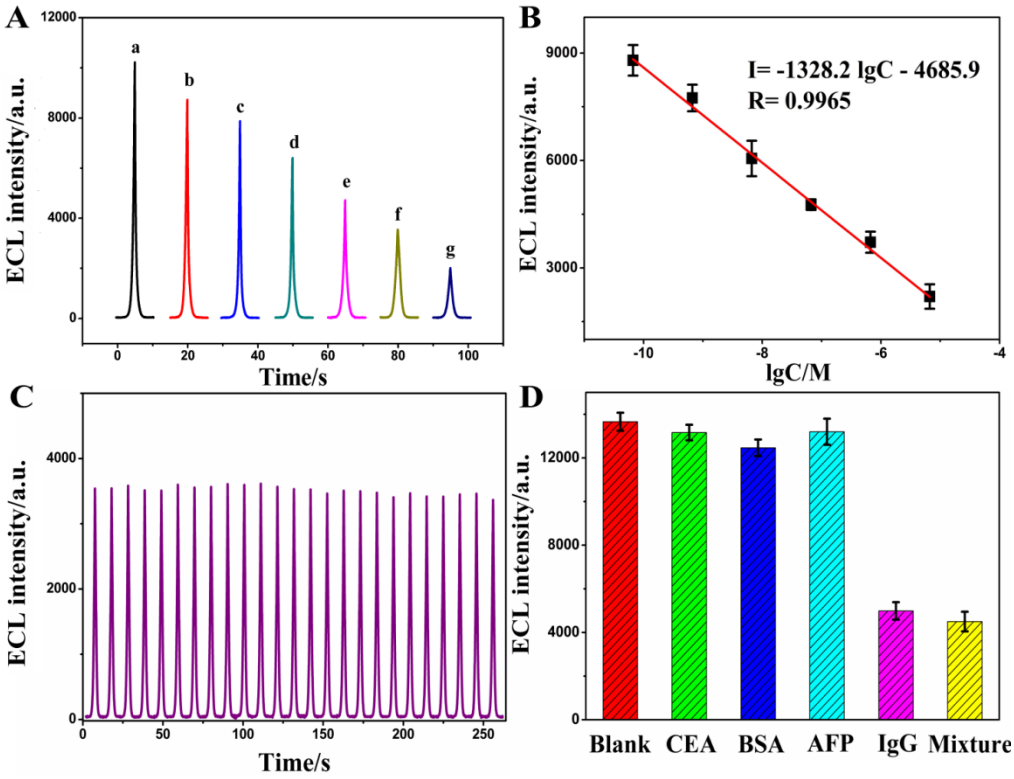


Figure 4

Method	linear range	detection limit	Reference
Electrochemical	5.0 μM—960 μM	0.5 μM	[30]
Electrochemical	0.06 pM—0.6 nM	0.02 pM	[31]
Fluorescent	0.06 pM—5.3 nM	0.04 pM	[22]
ECL	50 pM—31 nM	7.1 pM	[23]
ECL	67 pM—6.7 μM	14 pM	This work

Table 1

Highlight

1. A highly sensitive DNA-based electrochemiluminescence (ECL) biosensor was developed for the detection of human IgG (IgG).
2. The biosensor takes advantage of steric hindrance effects to influence the ECL signal in the presence of target protein.
3. This method can be expanded for the detection of a wide range of proteins with homologous recognition elements and could achieved good results in the analysis of real samples.