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ORIGINAL PAPER

An electrochemiluminescence biosensor based on boron nitride quantum dots as novel coreactant for quantitative determination of concanavalin A

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Abstract

An electrochemiluminescence (ECL) analytical platform is constructed based on boron nitride quantum dots (BNQDs) as a novel coreactant of luminol for quantitative assay of concanavalin A (Con A). Different from previous research that mainly focuses on its superior optical properties, BNQDs are used for the first time as a coreactant for boosting ECL intensity of luminol, which has a 10-fold enhancement compared with individual poly(luminol/aniline) nanorods loaded on reduced graphene oxide (PLA-rGO) using GCE. On the basis that BNQDs contain an abundance of active amino, a possible mechanism of amino oxidation facilitating ECL emission is proposed. Firstly, luminol as light spices are oxidized to luminol$^{\bullet-}$ and BNQDs generate an abundance of BNQDs-NH$^{+\bullet}$ via electrochemical oxidization, producing reductive intermediates BNQDs-N$^{\bullet}$ in alkaline conditions. Finally, BNQDs-N$^{\bullet}$ react with luminol$^{\bullet-}$ to obtain the excited species AP^{2-*}, returning to ground state and emitting light. Due to the hindrance effect of Con A, the ECL intensity decreases gradually as various concentrations of Con A are modifying the electrode surface. Therefore, a sensitive ECL biosensor for detecting Con A is constructed exhibiting a wide linear range of 1.0 pg·mL^{-1} to 1.0 μg·mL^{-1} and a low detection limit of 0.15 pg·mL^{-1}.

Keywords Boron nitride quantum dots · Concanavalin a · Novel coreactant · Amino oxidation mechanism · Hindrance effect

Introduction

Concanavalin A (Con A) is a legume protein with some extraordinary biological properties [1]. Under neutral conditions, one site of Con A is for specific binding to R-mannose or D-glucose based on the affinity between lectin and carbohydrate ligand [2]. To our surprise, the special attractive force between Con A and carbohydrate ligands has a high affinity comparable to that observed for antigen-antibody interactions [3]. On account of the high affinity between carbohydrate and lectin, Con A is selected as a protein model to deeply study cell surface recognition and cell communication [4, 5]. Moreover, Con A can help the mature T cells to activate and proliferate [6]. Therefore, the interaction between Con A and carbohydrate plays a role key in clinical diagnostics, such as the determination of leukemia cell and cancer cell [7, 8]. By now, much efforts have been made to search an approach to detect Con A, containing fluorescence spectroscopy [9], surface plasmon resonance [10], photoelectrochemical [11], and electrochemical methods [12]. Although these analytical methods are efficient for the determination of Con A, some evident drawbacks including low sensitivity, complex test step, and expensive equipment still exist. Therefore, to search a sensitive, simple, and low-cost method for monitoring Con A concentrations has important clinical significance.

Electrochemiluminescence has attracted much attention owing to its remarkable advantages including high sensitivity, wide linear range, and good stability [13]. Luminol is the most popular luminescent in the ECL analysis method. As we all know, H_2O_2 is one of the most classical coreactant for luminol. However, H_2O_2 is unstable and easily

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decomposes at room temperature, leading to poor selectivity and low ECL intensity of the constructed sensor [14, 15]. Therefore, a number of novel coreactants and coreaction accelerators are developed for enhancing the ECL emission of luminol. For instance, Hanif et al. have successfully utilized tripropylamine (TPA) as a coreactant of luminol due to the oxidization of alkyl amine [16]. Besides, amino-modified perylenetetracarboxylic dianhydride (PTC-NH_2) was used as a novel coreaction accelerator for boosting the ECL intensity of luminol-H_2O_2 system [17]. Wang et al. also reported that poly-L-lysine (PLL) can be applied as a coreactant for enhancing the ECL signal of luminol owing to the primary amino and secondary amino groups on PLL [18]. Moreover, other studies also show that compounds containing amino functional groups can efficiently promote the ECL emission of luminol [19–22]. Boron nitride quantum dots (BNQDs), zero-dimensional nanomaterials, have aroused widespread research interest due to unique surface properties such as the quantum confinement, edge effects, and defect centers [23, 24]. For example, Xing et al. have reported that BNQDs containing an abundant of hydroxyl and amino functional groups can be used as efficient coreactants for ECL enhancement of $Ru(bpy)_3^{2+}$ [25]. In addition, BNQDs have many other fascinating merits such as low toxicity [23], good stability, good biocompatibility [25], and cheap raw materials [26]. To the best of knowledge, most studies on BNQDs mainly focus on the superior optical properties and explore various synthesis methods. Related reports using BNQDs as a coreactant of luminol are rarely involved.

Inspired by these perspectives, we construct a novel ECL biosensor based on BNQDs as a coreactant for the ultrasensitive assay of Con A. To our surprise, about 10-fold enhancement ECL intensity is obtained compared with the individual PLA-rGO using GCE, which endows BNQDs as effective coreactant to enhance ECL intensity of luminol. Simultaneously, the possible luminescence mechanism has been discussed and the role of BNQDs in the ECL system is further confirmed using ECL and CV characterization. In this strategy, PLA-rGO acted as ECL species and material carrier for immobilizing D-glucose through schiff-based reaction (Scheme 1a) and BNQDs applied as a coreactant for ECL enhancement of luminol. After immobilization of Glu/PLA-rGO, the electrode modified with bovine serum albumin (BSA) to eliminate the non-specific binding effect. Finally, as a various concentration of Con A is modified on electrode through a specific carbohydrate-Con A interaction, ECL intensity of luminol correspondingly decrease, which can be used for quantitative determination Con A (Scheme 1b). Moreover, this work will offer a new insight into the knowledge of BNQDs, which was beneficial for the further application of BNQDs.

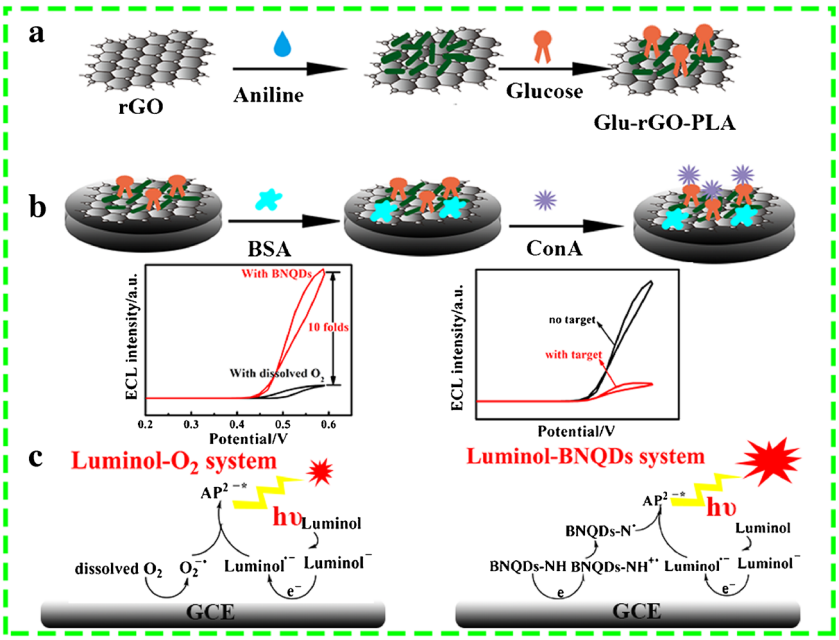
Experimental section

Reagents and chemicals

Luminol and glucose were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China; http://www.macklin.cn). Ammonium persulphate ($(NH_4)_2S_2O_8$) boric acid (H_3BO_3), ammonia ($NH_3{\cdot}H_2O$), aniline, and hydrazine hydrate ($N_2H_4{\cdot}H_2O$) were bought from Aladdin (Shanghai, China; http://www.aladdin-e.com). Graphene oxide was obtained from J&K Scientific Ltd. (Beijing, China; https://www.jk-scientific.com). Concanavalin A (Con A) from *Canavalia ensiformis* (Jack bean), bovine serum albumin (BSA, 98%), and phytohemagglutinin (PHA) and cytochrome c (Cyt c) were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China; http://www.shyuanye.com). Poly(diallyldimethylammonium chloride) (PDDA, 20 wt%) was bought from Sigma-Aldrich (St. Louis, MO, USA; https://www.sigmaaldrich.com). All reagents were used without further purification. Ultrapure water was employed all over the experiments.

Instrument

Electrochemical impedance spectroscopy (EIS) was performed on a CHI 760E electrochemical workstation (Chenhua, Shanghai, China; http://www.chinstr.com). XPS was carried out on an ESCALAB 250Xi electron spectrometer. Elements content of Glu/PLA-rGO composite were confirmed on vario El cube elemental analyzer (Germany, http://www.elementar.com). The Fourier-transform infrared spectroscopy (FT-IR) was recorded on a Nicolet IS10 FT-IR spectrophotometer using KBr pellets (USA, https://www.thermofisher.com). The thickness of BNQDs was characterized using Bruker atomic force microscope (AFM) (USA, https://www.bruker.com) by the tapping mode on the mica substrate. The size and morphology of BNQDs were obtained by the FEI talos F200X transmission electron microscope (TEM) (USA, https://www.fei.com) at an operating voltage of 200 kV. ECL emission was recorded on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Remax Electronic Instrument Limited Co., Xi'an, China; http://www.chinaremex.com), and the voltage of the photomultipliter tube (PMT) was set at − 700 V. All experiments were performed using conventional three-electrode system composed of a modified glassy carbon electrode (GCE, 4-mm diameter), a platinum wire counter electrode, and an Ag/AgCl reference electrode. The ECL signals were obtained in 0.1 M phosphate-buffered solution (pH = 8.6), and scanning potential was in the range of 0–0.6 V with a scanning rate of 0.2 $V{\cdot}s^{-1}$.



Scheme 1 **a** The illustration of the synthetic process of Glu/rGO-PLA composite. **b** The preparation of ECL sensor for Con A detection. **c** Possible luminescence mechanisms of luminol-O_2 system and luminol-BNQD system

Preparation of BNQDs

Functionalized BNQDs are obtained through a bottom-up hydrothermal route [27]. Briefly, 1.2 g of boric acid is dispersed into 30 mL of ultrapure water to acquire a uniform solution. Subsequently, 2.4 mL of concentrated ammonia water is added to the above solution quickly and the mixture reacted at high temperature (200 °C) for 12 h under nitrogen atmosphere in an autoclave. The prepared BNQDs are stored in 4 °C for use.

Synthesis of glucose functionalized poly(luminol/aniline) reduced graphene oxide nanocomposite (Glu/PLA-rGO)

In this strategy, reduced graphene oxide (rGO) and PLA-rGO nanocomposites are prepared according to previously reported methods [18]. Glu/PLA-rGO hybrids are obtained according to the literature with some modifications [28]. Generally, 160 mg glucose dissolve in 20 mL double distilled water then 40 mg prepared PLA-rGO composite is dispersed with the above solution under ultrasonic. Subsequently, the mixture was stirred vigorously in a 50 °C water bath for 6 h. Then, D-glucose is combined with PLA-rGO via schiff-based reaction. Finally, the precipitates are centrifuged and washed three times. Finally, composites are vacuum dried at 35 °C for 48 h.

Fabrication of ECL biosensor for detecting concanavalin A (ConA)

GCE is polished with alumina powder (0.3 and 0.05 μm), followed by successive sonication in distilled water, ethanol (v:v = 1:1), nitric acid (v:v = 1:1), and distilled water. After that, 4 μL of 2 mg·mL^{-1} Glu/PLA-rGO is dropped onto the dry GCE and dried in the air at room temperature. Subsequently, 4 μL of BSA (0.5 wt%) is dropped onto surface-modified GCE to block the nonspecific binding sites. Then, the surface of the modified electrode is incubated with different concentrations of Con A (4 μL) by a carbohydrate-Con A recognition effect for 40 min at 4 °C. The modified electrode is washed with phosphate-buffered solution to eliminate the nonspecific binding during the fabrication of the biosensor. The assembly process of the biosensor is presented in Scheme 1b, and the acquired ECL biosensor is stored at 4 °C before use.

Detection of ConA in the real human serum sample

Quantitative analysis of the ConA in real human serum sample was performed by standard addition method to eliminate matrix effect. Firstly, the human serum was diluted to 100 times. Then, 100 μL human serum above were spiked into 1.0 mL phosphate-buffered solution containing a certain amount standard ConA solution (from 0.1 to 10 ng·mL^{-1}). The modified electrode (BSA@Glu/PLA-rGO) was incubated with (4 μL) the human serum above containing different concentrations (from 0.1 to 10 ng·mL^{-1}) of Con A for 40 min in 4 °C. Finally, ECL signals were obtained in 0.1 M phosphate-buffered solution.

Results and discussion

Characterization of BNQDs

Functionalized BNQDs are prepared via one-pot in an autoclave. The size distribution of BNQDs is acquired via

HRTEM. It could be seen that BNQDs are uniformly distributed with an average size of 1.50 nm (Fig. 1b). Moreover, a good crystallization of BNQDs with clear lattice fringes of 0.21 nm is revealed through highly paralleled and ordered lattice fringes (Fig. 1a, inset), which corresponded to the (100) lattice space of the h-BN [23]. Furthermore, the surface topographies and thickness of the BNQDs are determined by using AFM. The height varies from 0.25 to 1.75 nm with an average thickness of 0.90 nm indicating a monolayered structure (Fig. 1c), which is consistent with results reported in previous literature [23].

ECL and spectral characterization of BNQDs as the novel coreactant

To explore the role of BNQDs in the ECL system, the ECL behavior of PLA-rGO (2 mg·mL^{-1}) is investigated in the presence of BNQDs. According to Fig. 2a, the ECL of individual PLA-rGO (2 mg·mL^{-1}) is weak in the absence of coreactant (about 1500 au). However, an obvious ECL enhancement (about 10 folds) can be observed in the presence of BNQDs (about 15,000 au), which indicates that BNQDs may significantly boost ECL of luminol. Simultaneously, to further confirm the source of the above ECL intensity, the ECL signal of individual BNQDs is collected. No ECL emission comes from the alone BNQDs indicating BNQDs may not act as ECL species under this condition. However, an obvious ECL emission is observed for individual PLA-rGO under the same condition, indicating ECL signal originates from luminol. The ECL spectra are recorded on ECL analytical system via using filters of different wavelengths to further verify the above conclusion. As shown in Fig. 2b, no ECL intensity of individual BNQDs is observed in the range of 330–500 nm, while pure PLA-rGO shows relatively weak ECL emission near-maximum emission wavelength of 420 nm. When BNQDs are added into detection solution, a significant ECL emission peak at 420 nm is observed, which is consistent with fluorescence spectrum of luminol. These spectra proved that luminol is acted as luminescent species in the luminol/BNQDs system, and the enhancement of ECL signals may be ascribed to the coreactant mechanism of BNQDs. CV curves also are recorded to further confirm the role of BNQDs. As shown in Fig. 2a, PLA-rGO gives a classical oxidation peak at 0.55 V in the absence of BNQDs. However, no oxidation peak of alone BNQDs is observed under the same potential range. Surprisingly, after the participation of BNQDs, the oxidation peak of PLA-rGO increases while the oxidation potential just shifts slightly, indicating BNQDs may catalyze the oxidation of luminol. Above results suggest that the ECL signals of PLA-rGO/BNQD system originate from luminol and enhanced by BNQDs.

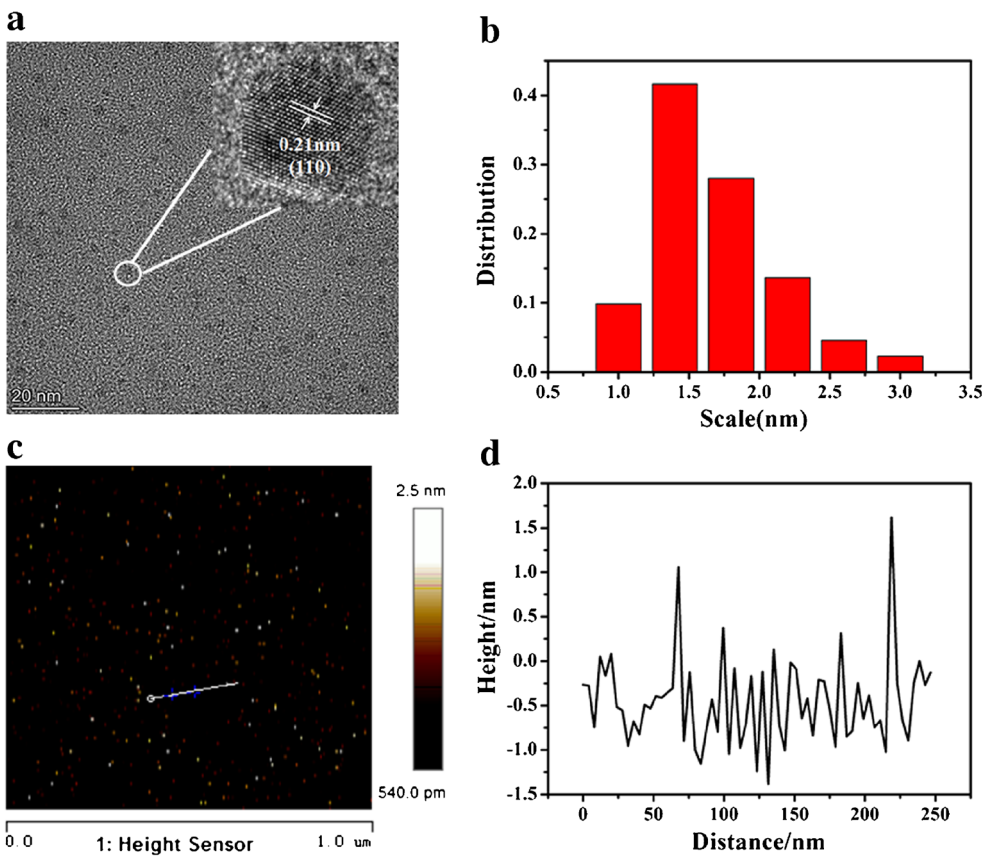


Fig. 1 **a** TEM images of BNQDs (inset, HRTEM image). **b** Size distribution of BNQDs (containing about 130 BNQDs). **c** AFM images of BNQDs. **d** The height profiles along the white line in part C

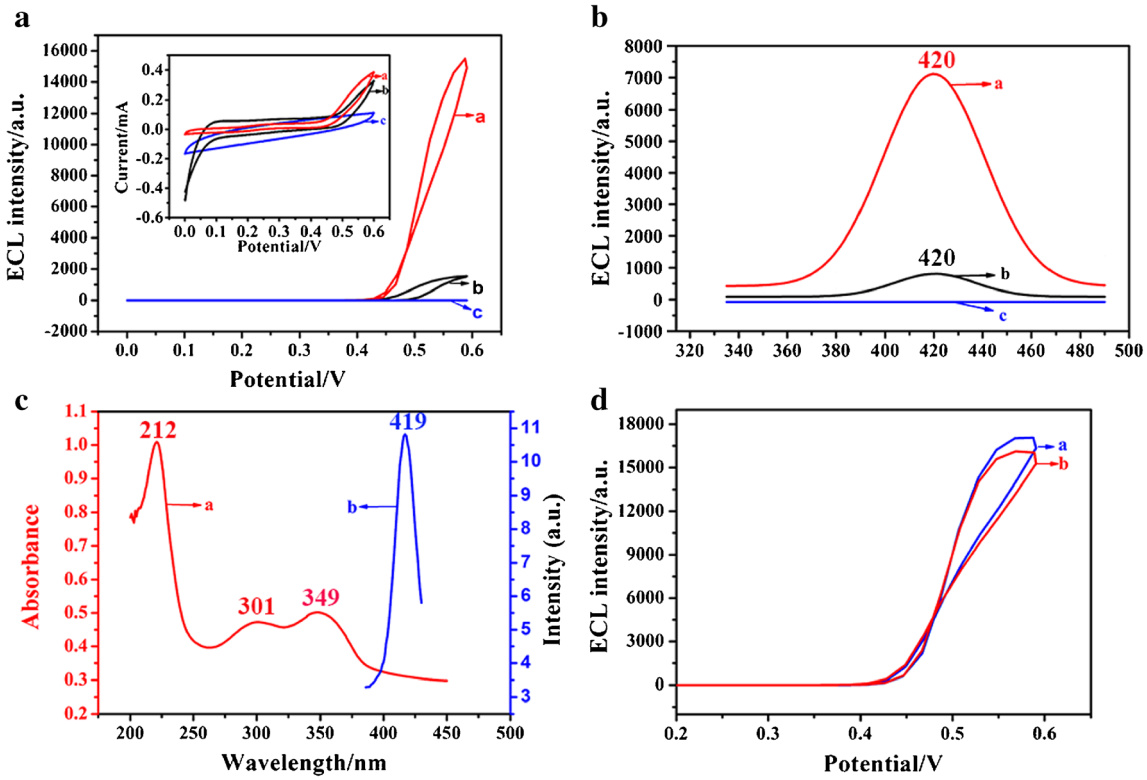


Fig. 2 **a** ECL behaviors of PLA-rGO with (a) and without (b) 0.69 mg mL^{-1} BNQDs and (c) bare GCE with 0.69 mg mL^{-1} BNQDs in 0.1 M PBS solution (pH 8.6) (inset, corresponding CV curves). **b** ECL spectra of 2 mg mL^{-1} PLA-rGO (a) with and (b) without 0.69 mg mL^{-1} BNQDs and (c) individual BNQDs. **c** The UV-VIS absorption spectrum of PLA-rGO (a) and the fluorescence emission spectrum of the BNQDs under the excitation of 366 nm (b). **d** ECL response of 2 mg mL^{-1} PLA-rGO in 0.1 M phosphate-buffered solution (pH 8.6) saturated with (a) Ar and (b) dissolved O_2. Scan rate: 200 mV s^{-1}

Recently, various amplified strategies are developed to enhance the ECL emission of luminol, such as resonance energy transfer (RET), coreactant, and coreaction accelerator. To confirm if there is resonance energy transfer between BNQDs and luminol, the fluorescence spectra of BNQDs is collected under the excitation of 366 nm (Fig. 2c). A significant fluorescence emission at 417 nm can be observed. Furthermore, the UV-VIS absorption spectrum of luminol is recorded from 200 to 500 nm. Interestingly, the absorption peaks at 212, 301, and 349 nm all cannot overlap well with fluorescence emission of BNQDs, indicating that BNQDs may not boost ECL intensity of luminol via resonance energy transfer. However, in a recent work, amino-modified perylenetetracarboxylic dianhydride (PTC-NH_2) can be as coreaction accelerator to facilitate the formation of $O_2^{\bullet-}$ [14]. As we all know, oxygen in air can be used as a coreactant of luminol. To prove whether BNQDs are coreactant or coreactant accelerator in the ECL system, the effect of dissolved oxygen is also discussed. As seen from Fig. 2d, the ECL intensity obtained in the solution saturated with dissolved oxygen is about 15,000 au. Interestingly, when the solution saturated with argon to substitute dissolved oxygen, the ECL intensity obtained is almost as high as above the ECL emission, suggesting BNQDs may not react with O_2 to facilitate the formation of $O_2^{\bullet-}$. In other words, BNQDs is not as a coreactant accelerator in luminol/BNQD system. Results above indicate that PLA is the ECL species and BNQD is the coreactant in this ECL system. Xing et al. report that BNQDs containing abundant amino functional groups via amine oxidization can be used for enhancing ECL of $Ru(bpy)_3^{2+}$ [25]. On the basis of this fact, a possible luminescence mechanism is proposed in Scheme 1c. Firstly, luminol is electrochemically oxidized to luminol$^{\bullet-}$ via losing single electron and two protons on surface of the electrode. Then, BNQDs with rich amino group generate an abundance of BNQDs-$NH^{+\bullet}$ via electrochemical oxidization, which can produce reductive intermediates BNQDs-$N^{\bullet}$ in alkaline conditions. Finally, BNQDs-$N^{\bullet}$ react with luminol$^{\bullet-}$ to obtain the excited species AP^{2-*}, which can return to ground state and emit light.

$$\text{Luminol–H} \rightarrow \text{Luminol}^{-} \quad (1)$$

$$\text{Luminol}^{-} – e \rightarrow \text{Luminol}^{\bullet} \quad (2)$$

$$\text{Luminol}^{\bullet} – H^{+} \rightarrow \text{Luminol}^{\bullet-} \quad (3)$$

$$\text{BNQDs–NH–e} \rightarrow \text{BNQDs–NH}^{+\bullet} \quad (4)$$

$$\text{BNQDs–NH}^{+\bullet} – H^{+} \rightarrow \text{BNQDs–NH}^{\bullet} \quad (5)$$

$$Luminol^{\bullet -} + BNQDs{-}NH^{\bullet} \rightarrow AP^{2-*} + prouducts \quad (6)$$

$$AP^{2-*} \rightarrow AP^{2-} + h\upsilon \quad (7)$$

Electrochemical and ECL behaviors during the assembly process of electrode

The electrochemical impedance spectroscopy (EIS) is a useful method to monitor the assembly process of a biosensor. According to Fig. 3a, it reveals the electronic transfer resistance (R_{et}) of each modified process in 10.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution containing 0.1 M KCl. Compared to bare GCE (curve a, R_{et} = 156 Ω), the PLA-rGO modified GCE (curve b, R_{et} = 123 Ω) exhibits smaller the semicircle domain due to the excellent conductivity of polyaniline (PANI) and rGO [18]. For Glu/PLA-rGO-modified GCE (curve c, R_{et} = 273 Ω), the semicircle domain increases obviously owing to the presence of nonconductive material (glucose). As BSA (curve d, R_{et} = 316 Ω) and Con A (curve e, R_{et} = 340 Ω) are successively modified on the above electrode, the semicircle domain all increase due to the hindrance effect of proteins. Above results indicate the successful assemble process of the ECL sensor.

In order to better understand the fabrication process of ECL biosensor, the electrochemiluminescence behavior is also investigated during the assembly process step by step (Fig. 3b). As expected, no ECL intensity is obtained from bare GCE, while the PLA-rGO-modified GCE exhibits an obvious ECL intensity under the same condition, indicating ECL emission originated from luminol. Furthermore, for Glu/PLA-rGO-modified electrode, the ECL signals decrease greatly compared with PLA-rGO-modified GCE owing to the poor conductivity of glucose. After BSA and Con A are modified to the electrode, the ECL intensity further decreases due to protein molecules obstructing electron transfer.

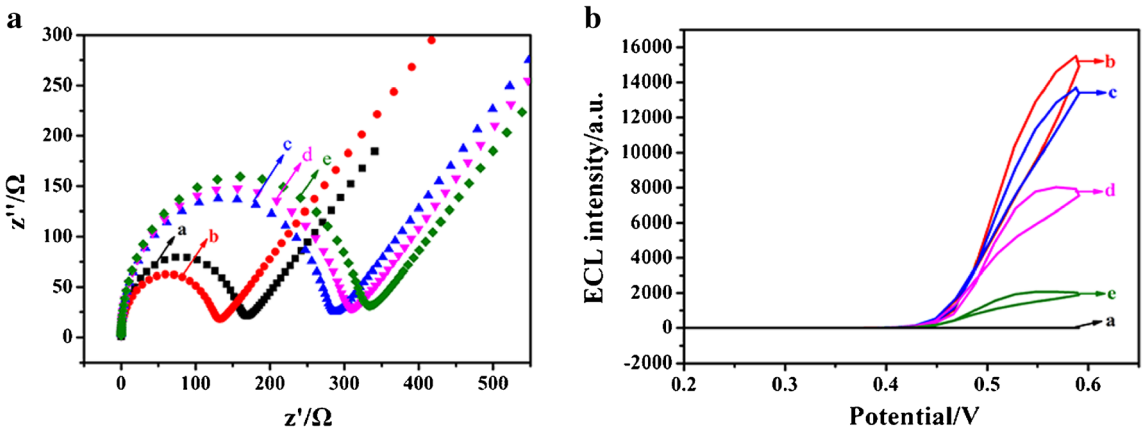


Fig. 3 **a** The EIS of assemble process in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and **b** ECL response in pH 8.6 PBS containing 0.69 mg mL^{-1} BNQDs at (a) bare GCE, (b) 2 mg mL^{-1} PLA-rGO, (c) 2 mg mL^{-1} Glu/PLA-rGO, (d) 2 mg mL^{-1} BSA/Glu/ PLA-rGO, and (e) 2 mg mL^{-1} Con A/BSA/Glu/PLA-rGO. Scan rate: 200 mV s^{-1} for ECL

Optimization of experimental parameters

Some important influencing parameters including the concentration of BNQDs, incubation time of biosensor, and pH of solution have been discussed in supplementary materials.

The performance of ECL biosensor

The concentration-dependent ECL performance for the detection of Con A is investigated in optimal condition. As exhibited in Fig. 4a, the ECL emission decreases gradually with an increasing concentration of Con A. Accordingly, a good linear relationship (in Fig. 4b) is obtained between the ECL emission and natural logarithm of Con A concentration ranging from 1.0 pg·mL^{-1} to 1.0 μg·mL^{-1} with a low detection limit (LOD) of 0.15 pg·mL^{-1} (S/N = 3, detailed calculation process presented in supplementary materials). The homologous linear equation is I = 3330–985lg c with the correlation coefficient (R^2) of 0.994. Compared with previous works (Table 1), the constructed ECL biosensor displays a wider liner range and lower detection limit, suggesting a high sensitivity of this strategy and a great potential for the quantitative determination of Con A.

Stability, reproducibility, and selectivity of the ECL intensity in concanavalin A (ConA) detection

Stability, reproducibility, and selectivity are key criteria of biosensor performance. As illustrated in Fig. 4c, the ECL emission was observed under 12 cycles of successive pulse potential. It is obvious that a relatively stable ECL emission has been achieved with the relative standard deviation (RSD) of 1.4%, indicating the proposed biosensor has a good stability in Con A determination. To evaluate the storage stability of biosensor, the fabricated sensors were stored at 4 °C for a few days and periodically checking its relative activity for 10 ng·

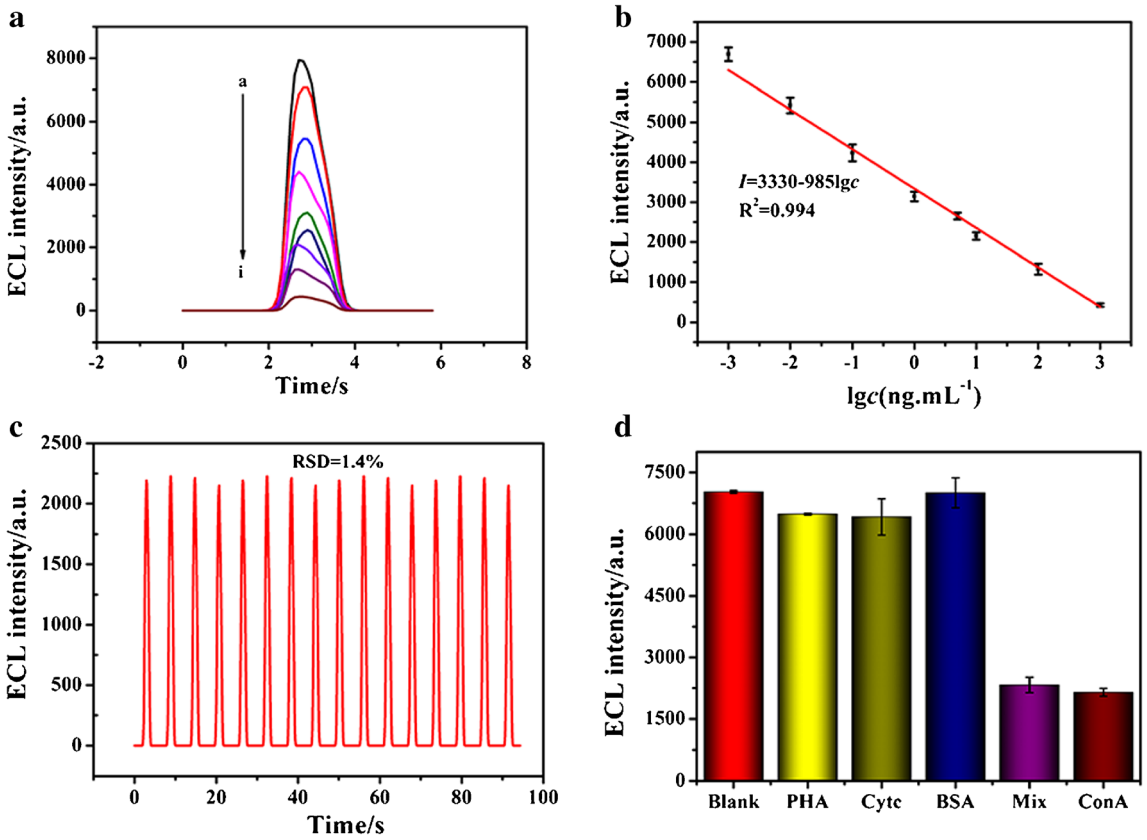


Fig. 4 **a** ECL-time curves of constructed sensor incubated with different Con A concentrations (ng·mL^{-1}): (a) 0, (b) 1.0×10^{-3}, (c) 1.0×10^{-2}, (d) 1.0×10^{-1}, (e) 1.0, (f) 5.0, (g) 10, (h) 1.0×10^{2}, and (i) 1.0×10^{3}. **b** Calibration curves of ECL biosensor. **c** The stability of the prepared ECL platform under continuous cyclic scans for nine cycles with 10 ng·mL^{-1} Con A. **d** The selectivity of proposed ECL sensor: blank, PHA (10 μg·mL^{-1}), CytC (10 μg·mL^{-1}), BSA (10 μg·mL^{-1}), mix (10 ng·mL^{-1} Con A and 10 μg·mL^{-1} interference), and Con A (10 ng·mL^{-1})

mL^{-1} Con A. As exhibited in Fig. S4A, the ECL intensity could retain 93% of its original emission, indicating that it had acceptable stability upon storage. The reproducibility of the sensor was estimated by determining ConA (10 ng·mL^{-1}) with five the same modified electrodes. As shown in Fig. S4B, five electrodes exhibited similar ECL responses and RSD was 4.7%, showing acceptable fabrication reproducibility of the biosensor. To further explore whether the detection of Con A is selective or not, some biomolecules including BSA (10 μg·mL^{-1}), cytochrome c (Cyt c, 10 μg·mL^{-1}), and phytohemagglutinin (PHA, 10 μg·mL^{-1}) are selected as interference substances. It can be seen in the Fig. 4d, and the mixture and 10 ng·mL^{-1} Con A can trigger obvious decline of ECL. Although the ECL intensities incubate with Cyt C and BSA has a slight influence compare with blank sample, concentrations of interference substances are much higher than target (1000-fold). Therefore, the biosensor is used for the assay of Con A with a relatively good selectivity.

Analysis of real samples

The recovery analysis is performed via standard addition method in the real human serum sample (provided by the Southwest University Hospital) to further monitor the practical application of the prepared sensor platform (Table S1). The recovery (100.70–105.00%) and its corresponding RSD

Table 1 Comparison of different methods for concanavalin A (ConA) determination

Method	Linear range (ng·mL^{-1})	LOD (ng·mL^{-1})	References
Surface plasmon resonance	1.0×10^{3}–2.0×10^{4}	3.9×10^{2}	[10]
Fluorescence	5.1×10^{2}–25.5×10^{3}	2.0×10	[9]
Photoelectrochemical	5.0×10^{-2}–5.0×10^{2}	1.7×10^{-2}	[11]
ECL	1.0×10^{-3}–10.0	2.0×10^{-4}	[29]
ECL	1.0×10^{-3}–1.0×10^{3}	1.5×10^{-4}	This work

(0.22–1.48%) are within a reasonable error range, indicating the satisfactory applicability of the proposed ECL sensor for Con A determination in human serum samples.

Conclusion

In conclusion, BNQDs are demonstrated as a novel coreactant of luminol for the first time. Surprisingly, the ECL enhancement is about 10-fold compared with individual PLA-rGO and the amino units on BNQDs are suggested to be active sites for the coreactants. Simultaneously, based on the Lectin-carbohydrate affinity, an ECL biosensor using glucose as a recognition element and BNQDs as a coreactant for detecting Con A is fabricated. Furthermore, BNQDs may be an attractive candidate of coreactant, owing to possessing merits of facile synthetic steps, good stability.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing of interests.

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