Electronic Supplementary Information

Efficient quenching of electrochemiluminescence from K-doped graphene-CdS:Eu NCs by G-quadruplexes/hemin and target recycling-assisted amplification for ultrasensitive DNA biosensing

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Experimental section

Materials: Labeled DNA oligonucleotides were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China). The sequences of these oligonucleotides employed are given as following:

Hairpin DNA: 5'-NH_2-$(CH_2)_6$-AAAGGGTTGGGCGG***GATGGGTT***GGAT CAGTG▼CTTATTCGA***AACCCATC***-3'

(The italic bold letters are the sequence of the stem arms; the arrow indicates the nicking position).

Target DNA：5'-TCGAATAAGCACTGATCC-3'

(The underlined letters are the recognition sequence of NEase).

One-base mismatch DNA: 5'-TCGAATAAGCACTG*C*TCC-3'

(The mismatched position is highlightened in italic).

Noncomplementary DNA: 5'-ATCGCCTTAACTGACATT-3'

NEBuffer 2 solution and NEase (Nt.AlwI) were obtained from New England Biolabs, Inc. H_2O_2 with analytical grade was purchased from Shanghai Chemical Reagent Company (Shanghai,China). Sodium sulfide ($Na_2S·9H_2O$) was purchased from Nanjing Chemical Co. Ltd. Cadmium nitrate tetrahydrate (Cd $(NO_3)_2·4H_2O$) and europium(III) oxide (Eu_2O_3) was supplied by Sinopharm Chemical Reagent Co. Ltd. Reduction of graphene oxide (GR) was obtained from JCNano Co. Ltd. (Nanjing), Potassium (K) were purchased from Alfa Aesar. Poly(diallyldimethylammonium

chloride) (PDDA), bovine serum albumin (BSA), hemin, 3-mercaptopropionic acid (MPA), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

Apparatus: The electrochemical and ECL emission measurements were conducted on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Remax Electronic Instrument Limited Co., Xi'an, China) at room temperature. Both electrochemical and ECL properties were investigated by cyclic potential scan at a glassy carbon electrode (GCE, 3 mm diameter). A three-electrode configuration was used where a Pt wire served as the counter electrode, saturated calomel electrode (SCE) as reference electrode and GCE as working electrode (WE), respectively. The ECL emission measurements were carried out in 0.1 M pH 8.5 PBS using 5.0 mM H_2O_2 as a coreactant. The emission window was placed in front of the photomultiplier tube (PMT) biased at －600 V.

Synthesis of CdS:Eu NCs.

The CdS:Eu NCs were prepared using MPA as stabilizing agent according to our previous work with some modification.[1] Briefly, 112.5 μL of 0.08 M $Eu(NO_3)_3$ solution was added to 30 mL aqueous solution containing $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) and MPA (80 μL) under stirring. After adjusting the pH to 10, a freshly prepared solution of $Na_2S \cdot 9H_2O$ (0.7205 g) in 30 mL ultra-pure water was injected and orange–yellow precipitates were obtained instantly. The reaction was held at 70 °C for 3 h with continuous refluxing. The final reaction precipitates were centrifuged and washed thoroughly with absolute ethanol three times, followed by washing with ultra-pure water to get rid of any Eu^{3+} and other ions remaining outside the clusters. Then the as-resulting precipitate was ultrasonically dispersed into water for centrifugation to collect the upper yellow solution of CdS:Eu NCs. The final solution could be stored in a refrigerator at 4 °C.

Synthesis of the K-doped graphene and K-doped graphene-CdS:Eu NCs composites.

K-doped graphene was prepared according to our previous work.[2] Briefly, phenanthrene/K solution complex was made by reacting K (200 wt% to graphene) with 0.2 M of phenanthrene (98%) in 20 mL of 1, 2-DME (99.5%) solution. Then, the GR (20 mg) were added to the

phenanthrene/K solution complex for the K-doping into graphene by π-stacking interaction between the graphene and phenanthrene. Finally, the reaction was conducted for 48 h with stirring using a magnetic bar at 500 rpm at room temperature. The resulting product was thoroughly washed several times with ethanol, and then dried at 60 °C under air atmosphere.

The K-doped graphene-CdS:Eu NCs composites were obtained *via* the electrostatic interaction. Detailedly, 5% PDDA (50 μL) solution was added to 0.1 mg mL^{-1} K-doped graphene dispersion (1 mL) with sonication for 40 min. Then the final reaction substance was centrifugated and washed thoroughly with ultrapure water three times. Subsequently, the solution of PDDA modified K-doped graphene was mixed with the MPA modified CdS:Eu NCs solution with the ratio of 1/3 (v/v) under sonication for 40 min. Finally, K-doped graphene-CdS:Eu NCs composites were stored in PBS buffer at 4 ºC before use.

Preparation of the DNA biosensor.

Prior to surface modification, the GCE was polished with successively finer grades of SiC papers and then with 0.3 μm alumina powder to obtain a mirror-like surface. Then GCE was thoroughly rinsed with water and then sonicated in ultrapure water. The composite film was achieved by dropping 10 μL of K-doped graphene-CdS:Eu NCs composite solution onto the pretreated surface of GCE and evaporated in air at room temperature. At last, the K-doped graphene-CdS:Eu NCs composite modified GCE was stored in 0.1M NaCl + 0.1M PBS buffer (pH 7.4) for further use. To fabricate the biosensor, the K-doped graphene-CdS:Eu NCs composite modified GCE was immersed in 1.0 mL of 0.1 M imidazol-HCl buffer (pH 7.0) containing 20 mg EDC and 10 mg NHS for 1 h at room temperature to activate the terminal carboxylic acid groups. Then the electrode was rinsed with 0.1 M PBS buffer (pH 7.4) to wash off the excess EDC and NHS. Finally, the resulting composite film modified GCE was soaked in the stable colloidal solution of hairpin DNA (100 μL) for 2 h at room temperature. Finally, 2 wt% BSA solution was used at 4 ºC for 1 h for the purpose of blocking the active binding sites of the film. The electrode surface was rinsed with 0.1 M PBS buffer (pH 7.4) after each step to remove nonspecifically adsorbed species.

DNA hybridization and the nicking reaction at GCE electrode.

The prepared biosensor was reacted with 100 μL of target DNA with different concentrations. Upon incubation at 37 °C for 40 min, the sensor was incubated in a solution of NEase reaction buffer (0.5 U/μL) in 100 μL NEBuffer 2 at 37 °C. After a specified period of time, the electrodes

were rinsed with wash buffer and incubated in hemin solution of certain concentration to form G-quadruplex/hemin DNAzyme at 25 °C. ECL detection was accomplished in 0.1 M PBS (pH 8.5) containing 0.1 M KCl and 5.0 mM H_2O_2, with the PMT voltage of −600 V. ECL signals related to the target DNA concentrations could be measured. The data of three independent measurements are presented with an error margin of one standard deviation.

The mechanism of the proposed ECL DNA biosensor.

In our design, glassy carbon electrode (GCE) was modified by drop-coating of 10 μL K-doped graphene-CdS:Eu NCs composites and used as ECL emitter. During the cathodic potential scan, the CdS:Eu NCs were reduced to $CdS:Eu^{-\bullet}$, and the coreactant H_2O_2 could react with $CdS:Eu^{-\bullet}$ to obtain an excited state (CdS:Eu*). This state emitted light in the aqueous solution to produce an ECL signal (Fig. S5). The ECL mechanism was listed as Eqs. (1) - (3).[1] Upon addition of hemin to form the G-quadruplex/hemin DNAzyme, the ECL intensity decreased, which could be attributed to the electrochemical reduction of hemin. The reduced hemin then chemically reduced the H_2O_2 and resulted to the efficient consumption of the coreactant. The process could be described by the following mechanism (Eqs. (4) and (5)).[3]

$$CdS:Eu + e^- \longrightarrow (CdS:Eu)^{-\bullet} \quad (1)$$

$$2(CdS:Eu)^{-\bullet} + H_2O_2 \longrightarrow 2(CdS:Eu)^* + 2OH^- \quad (2)$$

$$(CdS:Eu)^* \longrightarrow CdS:Eu + h\nu \quad (3)$$

$$Fe(III)DNAzyme + e^- \longrightarrow Fe(II)DNAzyme \quad (4)$$

$$2Fe(II)DNAzyme + H_2O_2 \longrightarrow 2Fe(III)DNAzyme + 2OH^- \quad (5)$$

The cyclic voltammetry (CV) responses of graphene and K-doped graphene modified glassy carbon electrode (GCE).

As shown in Fig. S1, the CV peak currents of $Fe(CN)_6^{3-/4-}$ at the K-doped graphene modified GCE (curve c) greatly increased compared with those at the graphene modified GCE (curve b) and bare GCE (curve a) which indicated an increased surface area and a better electron acceleration behaviors of $[Fe(CN)_6]^{3-/4-}$ on the K-doped graphene modified electrode.

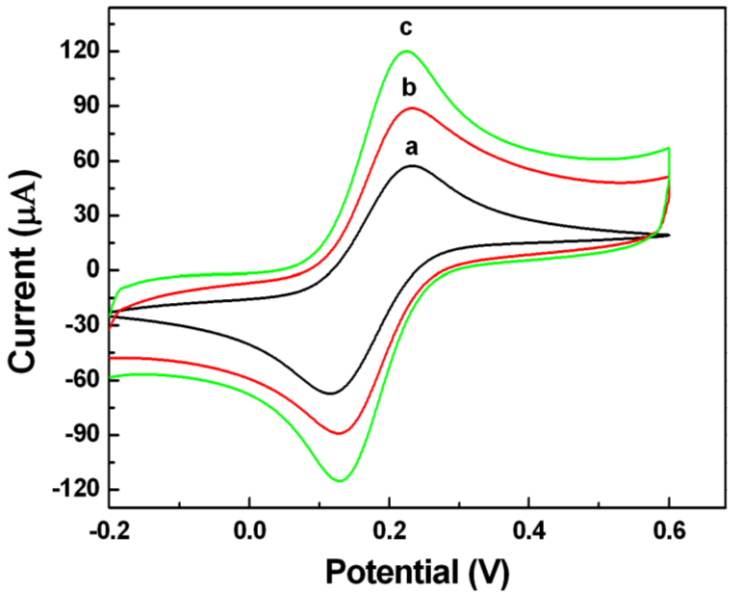

Fig. S1 The CV response of (a) bare, (b) graphene and (c) K-doped graphene modified GCEs in 0.1 M KCl solution containing 5.0 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$. Scan rate: 50 mV s^{-1}.

UV-vis absorption spectrum and Raman spectra of K-doped graphene and K-doped graphene-CdS:Eu NCs composites.

Fig. S2A showed the typical UV-vis absorption spectra of K-doped graphene, K-doped graphene-CdS:Eu NCs composites and pure CdS:Eu NCs solution. The spectrum of K-doped graphene showed an absorption band at about 266 nm (curve a), which corresponded to the chemically reduced graphene oxide (GO).[4] From the spectrum of pure CdS:Eu NCs (c), the size of the NCs particles is estimated to be 6.18 nm based on strong excitonic adsorption peak at ~470 nm and the empirical equations reported.[5,6] The absorption peak of K-doped graphene-CdS:Eu NCs composites was much weaker and slightly red shift comparison with that obtained at pure CdS:Eu NCs solution (curve b), which meant that the CdS:Eu NCs have been strongly combined with K-doped graphene sheets.

The Raman spectrum in Fig. S2B also confirmed the presence of K-doped graphene as substrate. Raman spectrum of the K-doped graphene displayed two prominent peaks at around 1580 cm^{-1} and 1350 cm^{-1}, corresponding to the G and D bands, respectively (curve a). In the case of K-doped graphene-CdS:Eu NCs composites, the G and D bands still occurred (curve b), indicating that the presence of K-doped graphene as substrate.

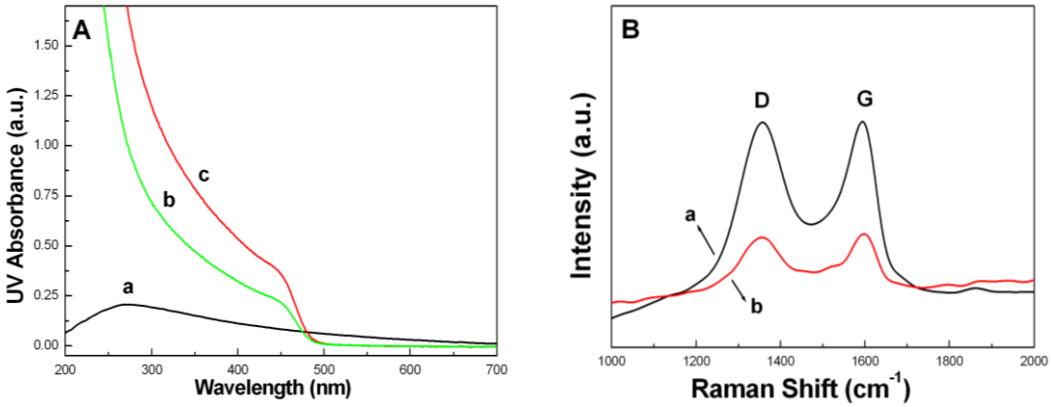

Fig. S2 (A) UV-vis absorption spectrum of K-doped graphene (a), K-doped graphene-CdS:Eu NCs composites (b) and free CdS:Eu NCs (c); (B) Raman spectra of K-doped graphene (a) and K-doped graphene-CdS:Eu NCs composites (b).

Electrochemical impedance spectroscopy (EIS) of different modified electrodes.

Electrochemical impedance spectroscopy (EIS) is an effective method for probing the interfacial properties of modified electrodes which employing $[Fe(CN)_6]^{3-/4-}$ as the redox probe in the supporting electrolyte solution. The impedance spectra include a semicircle portion and a linear portion. The semicircle diameter at higher frequencies corresponds to the electron-transfer resistance (Ret), and the linear part at lower frequencies corresponds to the diffusion process. Fig. S3 shows the EIS of the GCE at different stages. Obviously, it can be observed that the EIS of bare electrode is similar to a straight line (curve a), which is characteristic of a diffusion process. When CdS:Eu NCs are assembled on the electrode surface, the Ret increase obviously (curve b), which indicates that the CdS:Eu NCs decrease the electron-transfer efficiency. Nevertheless, after K-doped graphene-CdS:Eu NCs composite coating on the electrode surface, a smaller Ret is observed (curve c), which indicates that the high electrical conductivity of K-doped graphene could lower the impedance of the film and facilitate the electron transfer between the electrode and the electroactive probe. The addition of the hairpin DNA layer results in a larger electron-transfer resistance (Ret) (curve d), mainly due to the large stereospecific blockade of the stem-loop structure, and the electrostatic repulsion between negative charges of the DNA backbone and the $Fe(CN)_6^{3-/4-}$, then inhibits the interfacial charge transfer. On hybridization with the target sequence, then treatment with NEase and hemin, the Ret dramatically increases (curve e). It is expected that the formed G-quadruplex/hemin complexes make a negatively charged interface on the electrode

surface more orderly and dense, repelling the negatively charged redox species more strongly.

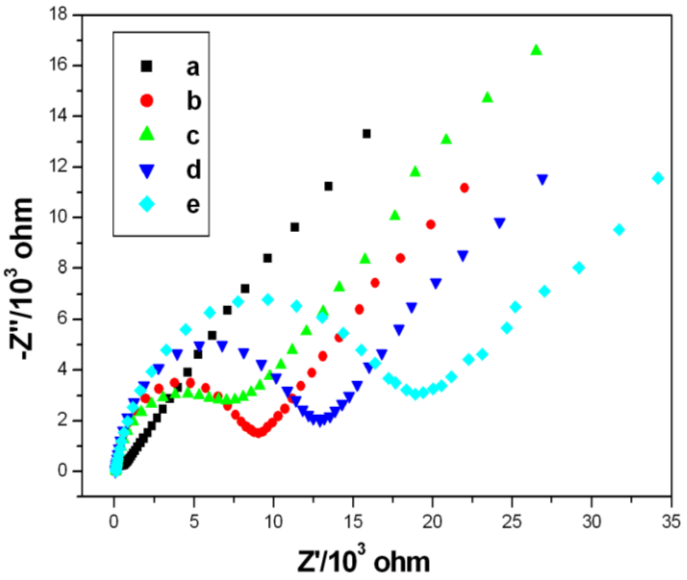

Fig. S3 EIS of the GCE at different stages. (a) bare GCE; (b) GCE/CdS:Eu NCs; (c) GCE/K-doped graphene-CdS:Eu NCs composites; (d) GCE/K-doped graphene-CdS:Eu NCs/hairpin DNA; (e) GCE/K-doped graphene-CdS:Eu NCs/ G-quadruplex/hemin. EIS are measured in 0.1 M KCl containing 5.0 mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$.

Optimization of experimental conditions.

The factors including the concentration of hairpin DNA, H_2O_2 and hemin, are examined in detail with quenching efficiency, defined as I_E = 1- I/I_0, where I and I_0 are the ECL intensity of the presence and absence of target DNA, respectively. As shown in Fig. S4A, the biosensor with the hairpin DNA concentration of 2.0 μM shows the highest quenching efficiency. This is probably due to the stronger steric hindrance effect of the hairpin DNA at higher immobilization concentration (>2.0 μM). On the contrary, low concentration of hairpin DNA can provide more space for unfolding of the hairpin probe. Based on Fig. S4A, an optimal hairpin DNA concentration of 2.0 μM is chosen for immobilization. For H_2O_2, quenching efficiency is increased with increasing H_2O_2 concentration, and the quenching efficiency reaches the highest at 5.0 mM H_2O_2, and then decreases sharply (Fig. S4B). For hemin, quenching efficiency is also increased with hemin concentration increasing, and is almost the highest from 1.0 μM (Fig. S4C). Hence, 5.0 mM H_2O_2 and 1.0 μM hemin are selected for the following experiments. Fig. S4D shows the time of nicking reaction with different concentration of target DNA. When the target concentration is 0 fM, we don't see apparent changes in ECL intensity (curve a), and the target concentration is 0.01 fM, there is also no any

apparent changes in ECL intensity (curve b). With the target concentration increasing to 0.6 pM, 6 pM and 10 pM, the ECL intensity obviously decreases with time (curve c, d and e), and the ECL intensity plateaus after 40 minutes in the presence of 6 pM and 10 pM of target DNA, therefore, we choose the 40 minutes as the best time of nicking reaction.

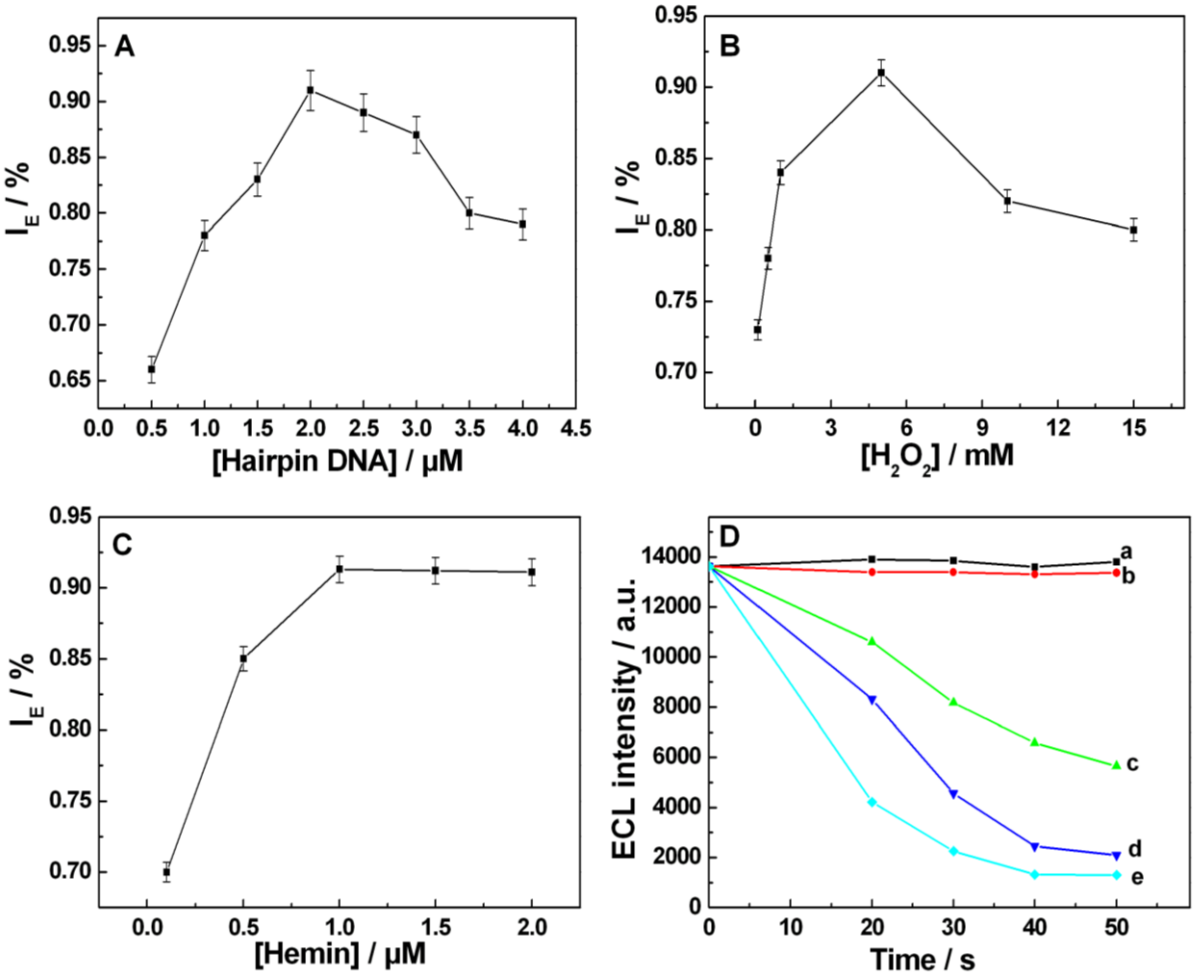

Fig. S4 Effects of concentrations on quenching efficiency: (A) varying concentrations of hairpin DNA with the concentrations of H_2O_2 and hemin fixed at 3.0 mM and 5.0 μM, respectively; (B) varying concentration of H_2O_2 with the concentrations of hairpin DNA and hemin fixed at 2.0 μM and 5.0 μM, respectively; (C) varying concentration of hemin with the concentrations of hairpin DNA and H_2O_2 fixed at 2.0 μM and 5.0 mM, respectively. (D) Effect of nicking time on ECL intensity. Experimental conditions: target DNA, (a) 0 fM, (b) 0.01 fM, (c)0.6 pM, (d) 6 pM, (e) 10 pM; NEase, 0.5 U/μL; hemin, 1.0 μM; ECL detection buffer: 0.1 M PBS (pH 8.5) containing 0.1 M KCl and 5.0 mM H_2O_2. Scan rate, 100 mV s^{-1}.

Characterization of the K-doped graphene-CdS:Eu NCs composites film on GCE.

The ECL signal-time curve under continuous potential scanning was shown in Fig. S5. The stable and high ECL signals suggested that the K-doped graphene-CdS:Eu NCs composites film is an excellent platform to construct ECL-based biosensors.

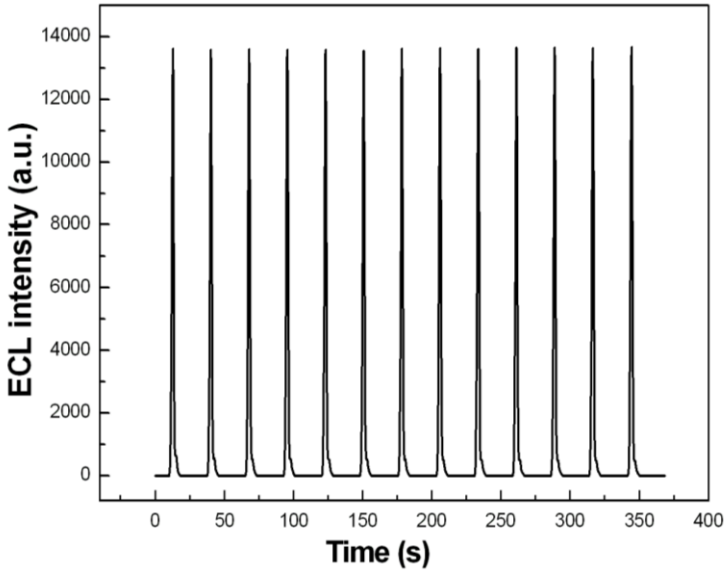

Fig. S5 ECL emission from K-doped graphene-CdS:Eu NCs composites film on GCE in 5.0 mM H_2O_2 + 0.1 M KCl + 0.1M PBS (pH 8.5) under continuous cyclic potential scan for 13 cycles. Scan rate, 100 mV s^{-1}.

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