Supporting Information for

Direct Electrochemiluminescence of Gold Nanoparticles Bifunctionalized by Luminol Analogues/Metal Complexes in Neutral and Alkaline Medium

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Experimental Sections

Materials and chemicals: A $HAuCl_4$ stock solution (0.2% $HAuCl_4$, w/w) was prepared by dissolving 1.0 g of $HAuCl_4 \cdot 4H_2O$ (Shanghai Reagent, China) in 412 mL of purified water and stored at 4 ºC. N-(aminobutyl)-N-(ethylisoluminol) (ABEI) and 2-aminoethanethiol and diethylenetriaminepentaceticacid (DTPA) bis-anhydride were purchased from TCI (Japan). Dimethylformamide (DMF), triethylamine, and other organic solvents were purchased from Sinopharm Chemical Reagent Col, Ltd. A 4.0 m mol/L stock solution of ABEI was prepared by dissolving ABEI in 0.1 mol/L NaOH solution and was kept at 4 ºC. A 0.01 mol/L stock solution of luminol was prepared by dissolving luminol (Sigma) in 0.1 mol/L NaOH solutions without further purification. Working solutions of luminol were prepared by diluting the stock solution. A 1.0 mmol/L stock solution of isoluminol was prepared by dissolving isoluminol (Sigma) in 0.1 mol/L NaOH solution without further purification and was kept at 4 ºC. Supporting electrolyte was 0.02 M phosphate buffer solution (PB). All other reagents were of analytical grade. Ultrapure water was prepared by a Milli-Q system (Millipore, France) and used throughout. All glassware used in the following procedures was cleaned in a bath of freshly prepared 3:1 (v/v) HNO_3-HCl, rinsed thoroughly in redistilled water, and dried prior to use.

Synthesis of ABEI-GNPs: ABEI-GNPs were prepared by the reduction of $HAuCl_4$ with ABEI in aqueous solution at room temperature as described previously.[1] A 7 mL portion of 6 mmol/L $HAuCl_4$ stock solution was mixed with 45 mL ultrapure water. While stirring vigorously, 5 mL of 4 mmol/L ABEI stock solutions were added rapidly, the solution was maintained at room temperature for 2 hours. Then another 6 mL portion of $HAuCl_4$ stock solution was added and the reaction was kept on for another 2 hours, during which a color change from yellow to black to purple was observed before a wine-red color was reached, then stored at 4 ºC.

Synthesis of luminol-GNPs: Luminol-GNPs were prepared via the reduction of $HAuCl_4$ by luminol according to the previous work.[2] In detail, a 100 mL portion of $HAuCl_4$ (0.01% w/w) solution was heated to boiling. While stirring vigorously, 1.60

mL of 0.01 mol/L luminol stock solution was added rapidly. The solution was maintained at the boiling point for 30 min, during which time a color change from yellow to black to purple was observed before a wine-red color was reached. The heating source was removed, and the colloid was kept at room temperature for another 20 min and then stored at 4 ºC.

Synthesis of isoluminol-GNPs: Isoluminol-GNPs were prepared according to the previous work with modification.[3] A 20 mL portion of 6 mmol/L $HAuCl_4$ stock solution was mixed with 100 mL ultrapure water. While stirring vigorously, 10 mL of 10 mmol/L isoluminol stock solution were added rapidly, the solution was maintained at room temperature for 2 hours. Then another 11 mL portion of $HAuCl_4$ stock solution was added and finally a wine-red color was reached and then stored at 4 ºC.

Synthesis of DTDTPA: The chelator DTDTPA was synthesized according to the previous work.[4] In a 50 mL round-bottom flask, 1 g diethylenetriaminepentacetic acid bis-anhydride was dissolved in 20 mL of DMF solution and then the mixture solution was heated to 70 ºC. In another flask, 0.55 g 2-aminoethanethiol was dissolved in 15 mL of DMF solution containing 0.87 mL of triethylamine and then added to the round-bottom flask. This mixture solution was stirred magnetically at 70 ºC overnight. Then, the resulting solution was cooled to room temperature and put in an ice bath. A white powder ($NEt_3 \cdot HCl$) precipitated and was filtered. The filtrate was concentrated at low pressure. After adding this solution to a chloroform solution, a white precipitate was formed. After filtration of solution, washing with 25 mL of chloroform and drying under vacuum, DTDTPA was obtained as a white powder.

Synthesis of BF-GNPs: As described previously, DTDTPA/metal ions chelate were prepared by mixing 6 mmol/L DTDTPA aqueous solution with 6 mmol/L metal ions aqueous solution with the same volume.[4] 6 mL of DTDTPA/metal ion chelate aqueous solution was added to 20 mL of ABEI-GNPs, luminol-GNPs or isoluminol-GNPs colloid and stirred overnight. Then the resulting solution is centrifuged at a speed of 12500 rpm for 15 min. After the supernatant was removed, the soft precipitant was dispersed in pH 12.0 NaOH aqueous solution, and various BF-GNPs colloids were obtained.

Characterization of BF-GNPs: To characterize as-prepared BF-GNPs, Co^{2+} was chosen as a model of metal ions. BF-GNPs were subsequently characterized by transmission electron microscopy （TEM，JEOL Ltd, JEOL-2010, Japan), UV-visible spectroscopy (Agilent 8453 UV-visible spectrophotometer, USA), and X-ray photoelectron spectroscopy (XPS, an ESCALABMK II electron spectrograph (VG Scientific, UK) with Al Ka radiation as the X-ray source). The histograms were obtained by measuring the diameters of nanoparticles ($n>100$) in TEM photomicrographs. The precipitates were redispersed in the ultrapure water for the UV-visible spectrum detection and in the alcohol solution for the TEM detection. The precipitates were dried under vacuum at room temperature for XPS detection. Co^{2+}/DTDTPA/ABEI-GNPs were characterized as described previously.[4]

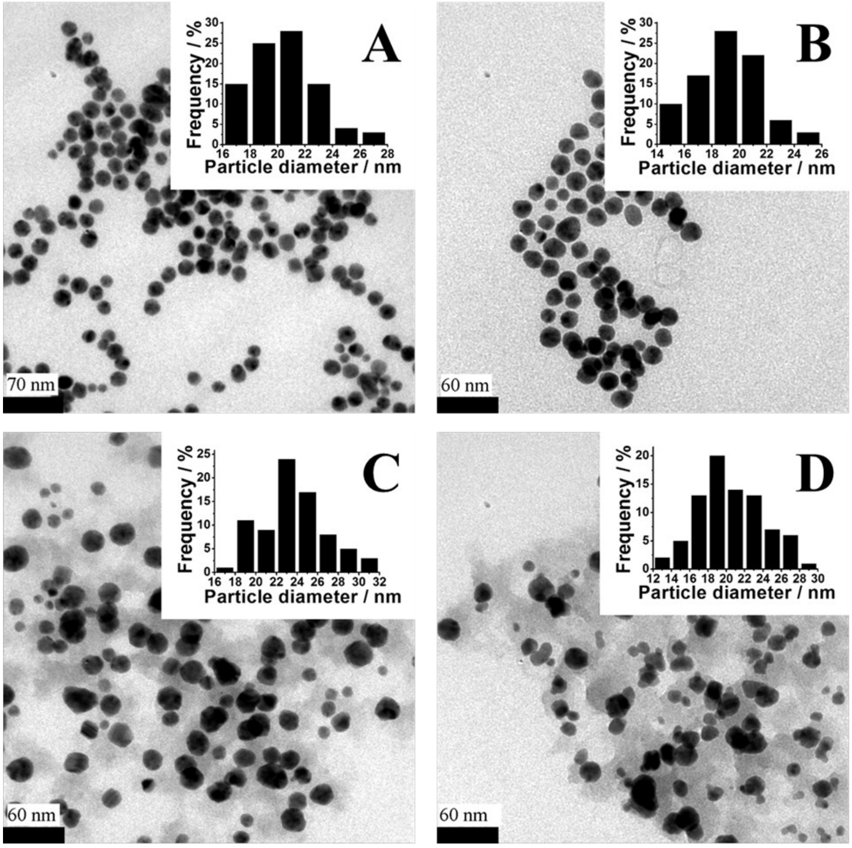

Fig. S1. TEM images of (A) luminol-GNPs, (B) Co^{2+}/DTDTPA/luminol-GNPs, (C) isoluminol-GNPs, (D) Co^{2+}/DTDTPA/isoluminol-GNPs.

Fig. S1 showed the TEM images of luminol-GNPs, isoluminol-GNPs, Co^{2+}/DTDTPA/luminol-GNPs and Co^{2+}/DTDTPA/isoluminol-GNPs. As shown in Fig. S1A and B, Co^{2+}/DTDTPA/luminol-GNPs were monodispersed sphericity with the diameter of 19 nm, which is similar to the diameter of luminol-GNPs. Similarly, it could be seen that isoluminol-GNPs and Co^{2+}/DTDTPA/isoluminol-GNPs (Fig. S1C and D) have the similar diameters. Moreover, the Co^{2+}/DTDTPA/luminol-GNPs and Co^{2+}/DTDTPA/isoluminol-GNPs had a better dispersibility than corresponding luminol-GNPs and isoluminol-GNPs, as the Co^{2+}/DTDTPA complexes are negatively charged, increasing the electrostatic repulsion of GNPs. It demonstrated that Co^{2+}/DTDTPA/luminol-GNPs and Co^{2+}/DTDTPA/isoluminol-GNPs have been synthesized successfully.

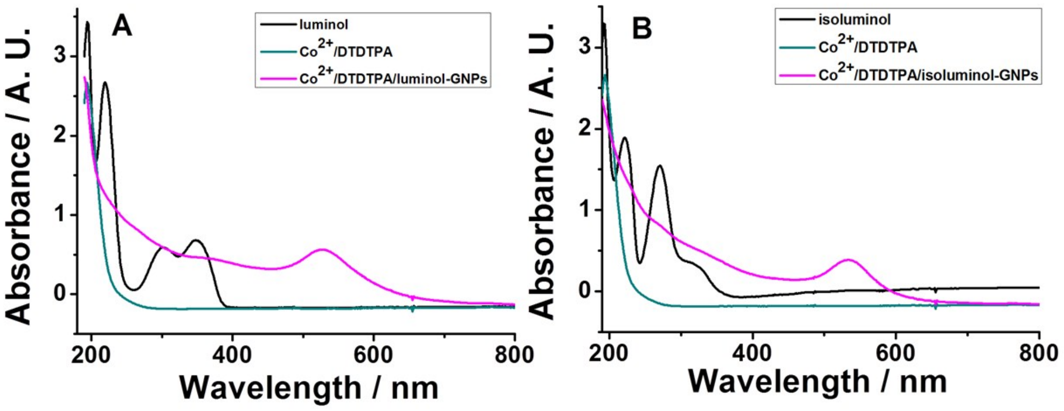

Fig. S2. UV-Visible absorption spectra of (A) pure luminol, Co^{2+}/DTDTPA, and redispersed Co^{2+}/DTDTPA/luminol-GNPs, (B) pure isoluminol, Co^{2+}/DTDTPA, and redispersed Co^{2+}/DTDTPA/isoluminol-GNPs.

Fig. S2A showed the UV-visible spectroscopy of luminol, Co^{2+}/DTDTPA and Co^{2+}/DTDTPA/luminol-GNPs. The two peaks around 300 nm and 350 nm were the characteristic absorption peaks of pure luminol. The maximal surface plasmon absorption wavelength of Co^{2+}/DTDTPA/luminol-GNPs appeared around 350 nm and 527nm. The broad peak around 350 nm demonstrated that luminol molecules existed on the surface of Co^{2+}/DTDTPA/luminol-GNPs. The peak around 527 nm was attributed to the spherical morphology of Co^{2+}/DTDTPA/luminol-GNPs. As shown in Fig. S2B, the characteristic absorption peaks of pure isoluminol appeared around 280 nm and 320 nm, respectively. The maximal surface plasmon absorption wavelength of Co^{2+}/DTDTPA/isoluminol-GNPs showed two broad peaks around 320 nm and 528 nm, respectively. The peak around 528 nm was attributed to the spherical morphology of Co^{2+}/DTDTPA/isoluminol-GNPs. The peak around 320 nm demonstrated that isoluminol molecules existed on the surface of Co^{2+}/DTDTPA/isoluminol-GNPs.

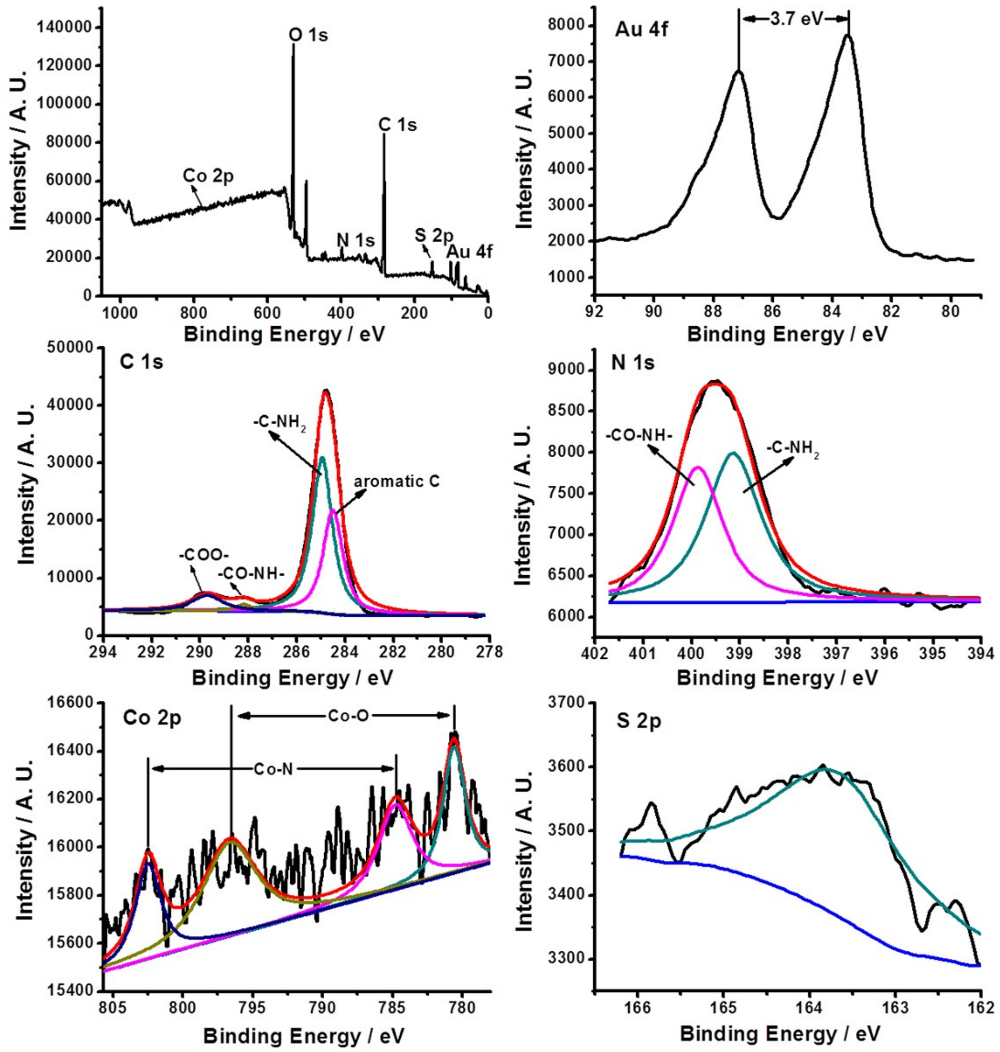

Fig. S3. X-ray photoelectron spectra of Co^{2+}/DTDTPA/luminol-GNPs

Fig. S3 shows the survey spectrum and the Au 4f, C 1s, N 1s, Co 2p, S 2p X-ray photoelectron spectra of Co^{2+}/DTDTPA/luminol-GNPs, after further treatments. All binding energies (BEs) were calibrated with respect to the C 1s BE at 284.6 eV. The spin-orbit splitting of doublet components for Au $4f_{7/2}$ and Au $4f_{5/2}$ were measured to be 3.7 eV, which is in good agreement with previous results. This finding is supporting evidence for the presence of Au_0 in as-prepared AuNPs. As can be seen from Fig. S3, the C 1s spectrum of Co^{2+}/DTDTPA/luminol-GNPs was curve-fitted into four components at 284.6, 285.4, 288.0 and 288.7 eV. The maximal peak centered at 284.6 eV was attributed to the aromatic carbon atoms in the luminol molecules. The component at 285.4 eV was associated with the carbon atom in the aromatic ring linked to the amine group ($C-NH_2$). The component at 288.0 eV was due to the carbon atom in the amide group (-CO-NH-). The component centered at 288.7 eV was attributed to the carbon atom in the car-boxylic group (-COO-). The N 1s spectrum of Co^{2+}/DTDTPA/luminol-GNPs was curved-fitted into two components at 399.0 and 399.8 eV. The component at 399.0 eV was attributed to the nitrogen atoms in the $-NH_2$. The component at 399.8 eV was attributed to the nitrogen atoms in –NH-CO- group in luminol molecule and DTDTPA molecule. The Co 2p spectrum of Co^{2+}/DTDTPA/luminol-GNPs was curved-fitted into four components. The components at 802.2 and 785.4 eV attributed to the cobalt atom in the Co-N group and 796.8 and 781.2 eV attributed to the cobalt atom in the Co-O group, respectively. The spin-orbit splitting of doublet components for Co $2p_{1/2}$ and Co $2p_{3/2}$ were measured to be 15 eV, which is in good agreement with literature results. The S 2p spectra of Co^{2+}/DTDTPA/luminol-GNPs at 163.6 eV indicated the existence of S element. These results strongly supported the notion that luminol and Co^{2+}/DTDTPA coexisted on the surface of the AuNPs and DTDTPA were grafted on the surface of GNPs through the Au-S bond.

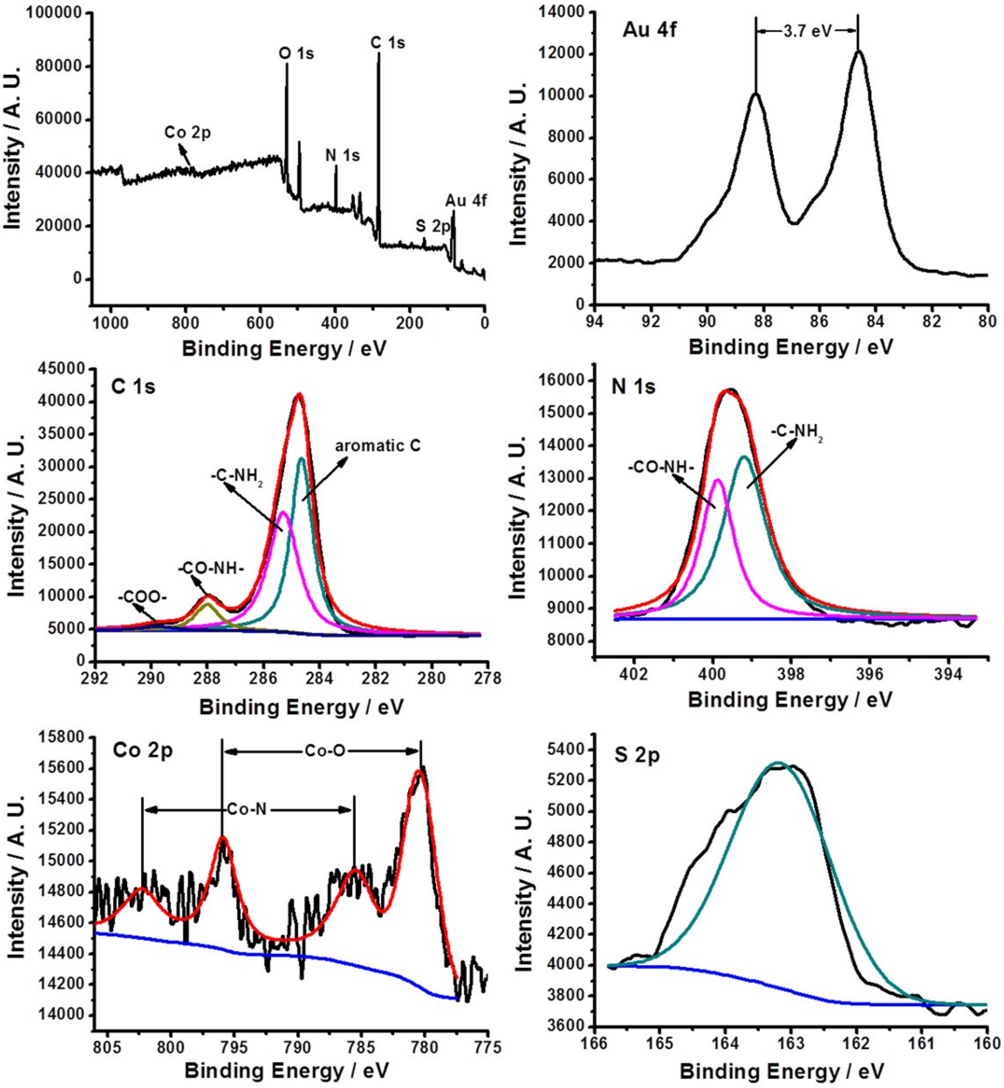

Fig. S4. X-ray photoelectron spectra of Co^{2+}/DTDTPA/isoluminol-GNPs

Fig. S4 shows the survey spectrum and the Au 4f, C 1s, N 1s, Co 2p, S 2p X-ray photoelectron spectra of Co^{2+}/DTDTPA/isoluminol-GNPs after further treatments. All binding energies (BEs) were calibrated with respect to the C 1s BE at 284.6 eV. The spin-orbit splitting of doublet components for Au $4f_{7/2}$ and Au $4f_{5/2}$ were measured to be 3.7 eV, which is in good agreement with previous results. This finding is supporting evidence for the presence of Au_0 in as-prepared AuNPs. As can be seen from Fig. S3, C 1s spectrum of Co^{2+}/DTDTPA/isoluminol-GNPs was curve-fitted into four components at 284.6, 285.4, 287.8 and 288.7 eV. The maximal peak centered at

284.6 eV was attributed to the aromatic carbon atoms in the isoluminol molecules. The component at 285.4 eV was associated with the carbon atom in the aromatic ring linked to the amine group (C-NH_2). The component at 287.8 eV was due to the carbon atom in the amide group (-CO-NH-). The component centered at 288.7 eV was attributed to the carbon atom in the car-boxylic group (-COO-). The N 1s spectrum of Co^{2+}/DTDTPA/isoluminol-GNPs was curved-fitted into two components at 398.8 and 399.8 eV attributed to the nitrogen atoms in the amine group (-NH_2) and amide group (-CO-NH-), respectively. The Co 2p spectrum of Co^{2+}/DTDTPA/isoluminol-GNPs was curved-fitted into four components. The components at 802.2 and 786.3 eV attributed to the cobalt atom in the Co-N group and 796.2 and 780.4 eV attributed to the cobalt atom in the Co-O group, respectively. The spin-orbit splitting of doublet components for Co $2p_{1/2}$ and Co $2p_{3/2}$ were measured to be 15 eV, which is in good agreement with literature results. The S 2p spectrum of Co^{2+}/DTDTPA/isoluminol-GNPs at 163.1 eV indicated the existence of S element. These results strongly supported the notion that isoluminol and Co^{2+}/DTDTPA coexisted on the surface of the GNPs and DTDTPA were grafted on the surface of GNPs through the Au-S bond.

ECL measurements: ECL measurements were performed with a homemade ECL/electrochemical cell system, including a model CHI760B electrochemical workstation (Chenhua, China), an H-type electrochemical cell (self-designed), a model CR-105 photomultiplier tube (PMT) (Beijing, China), a model RFL-1 luminometer (XiNan, China), and a computer. [5, 6] An ITO electrode served as the working electrode, a platinum wire as the counter electrode, and a silver wire as the quasi-reference electrode (AgQRE). Although the potential of the AgQRE was essentially stable during an experiment, the measurements of $\Delta E = E_{Ag/Ag^+} - E_{SCE}$ in different solutions were taken for potential calibrations. A solution containing 0.4 mL BF-GNPs solution and 4 mL 0.02 M PB was used as working solution for the ECL measurements. During measurements, a 4.4 mL portion of the working solution and the blank solution without BF-GNPs were added to the working compartment and the auxiliary compartment of the ECL cell, respectively. When the suitable potential was applied to the working electrode, an ECL signal was generated. The curves of current vs. applied potential (i-E) and the curves of ECL intensity vs. applied potential (I_{ECL}-E) were recorded simultaneously. Under a nitrogen or an oxygen atmosphere, nitrogen or oxygen was bubbled through the solutions for 15 min in both compartments of the cell, and the flow was maintained over the solution during experiments. Unless otherwise stated, all experiments were carried out at ambient room temperature.

The ECL spectrum of ECL-1 was measured by inserting filters at wavelengths of 360, 380, 400, 420, 450, 490, 510 nm (light cannot pass at wavelengths lower than these), as described previously[6] due to weak ECL intensity. $\Delta I_{\mathrm{f}\lambda}$ (the difference between ECL intensity with the filter at λ_{l} and ECL intensity with the filter at λ_{h}; λ_{l} is a lower wavelength, and λ_{h} is a higher wavelength) was calculated as shown: e.g., $\Delta I_{\mathrm{f}360} = I_{\mathrm{blank}}$(without filter) - I_{360}, $\Delta I_{\mathrm{f}380} = I_{360} - I_{380}$, $\Delta I_{\mathrm{f}400} = I_{380} - I_{400}$, etc. The curves of $\Delta I_{\mathrm{f}\lambda}$ versus λ are consistent with CL spectra.

The ECL spectra of ECL-2 and ABEI were measured by an F-7000 spectrofluorophotometer (Hitachi, Japan) with the excitation source off.

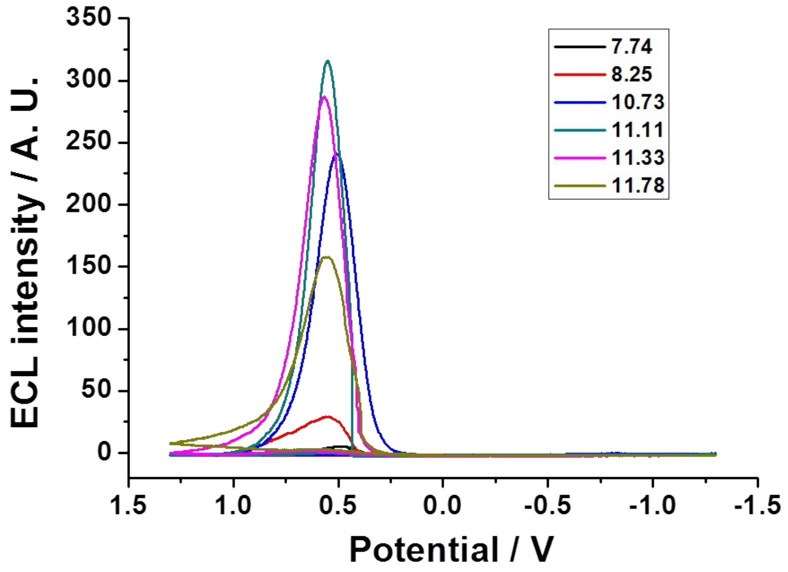

Fig. S5 Effect of pH on I_{ECL}–E curves of Co^{2+}/DTDTPA/ABEI-GNPs with a high voltage of -500 V. Initial scan direction: negative.

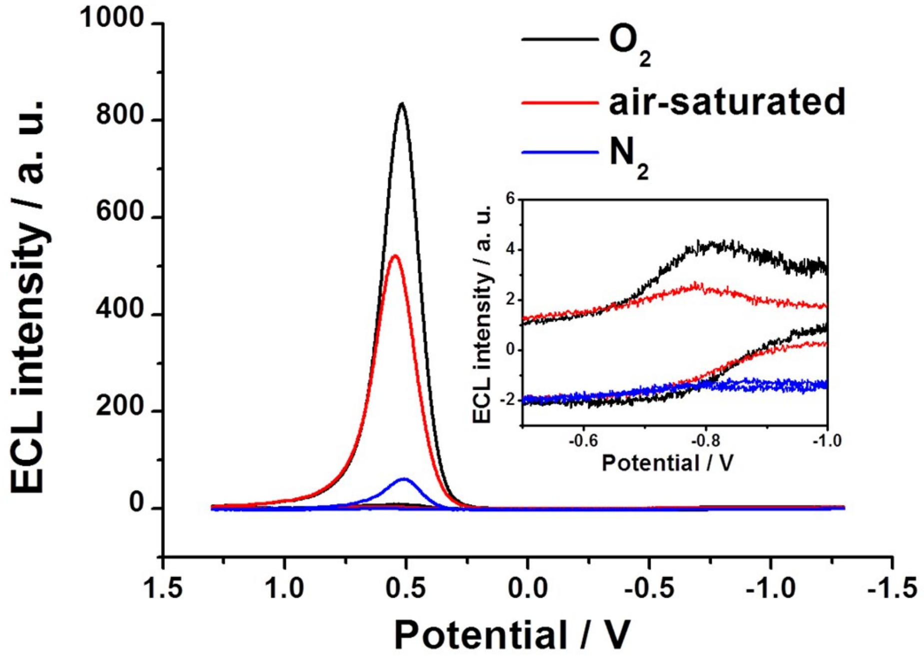

Fig. S6 I_{ECL}-E curves of Co^{2+}/DTDTPA/ABEI-GNPs with negative initial scan direction under O_2, air-saturated and N_2 atmospheres. The inset shows the enlarge I_{ECL}-E curves from -0.5 to -1.0 V. Photomultiplier tube voltage: -500 V.

References

1 D. Y. Tian, H. L. Zhang, Y. Chai and H. Cui, *Chem. Commun.*, 2011, **47**, 4959-4961.

2 H. Cui, W. Wang, C. F. Duan, Y. P. Dong and J. Z. Guo, *Chem.-Eur. J.*, 2007, **13**, 6975-6984.

3 F. Li, D. Tian and H. Cui, *Luminescence*, 2013, **28**, 7-15.

4 M. X. Liu, H. L. Zhang, J. N. Shu, X. Y. Liu, F. Li and H. Cui, *Anal. Chem.*, 2014, **86**, 2857-2861.

5 H. Cui, Z. F. Zhang, G. Z. Zou and X. Q. Lin, *J Electroanal Chem*, 2004, **566**, 305-313.

6 H. Cui, G. Z. Zou and X. Q. Lin, *Anal. Chem.*, 2003, **75**, 324-331.