Electronic Supplementary Material (ESI) for Chemical Communications

Electrochemiluminescence resonance energy transfer between emitter electrochemically generated by luminol as donor and luminescent quantum dot as acceptor and its biological application

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Supplementary Information

Chemical structure of ABEIL. The structure of ABEIL is shown in Figure S1.

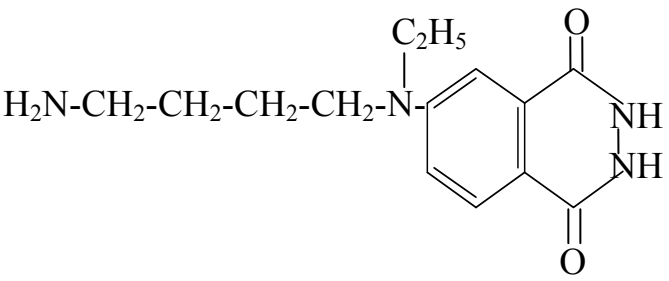

Figure S1. Chemical structure of ABEIL.

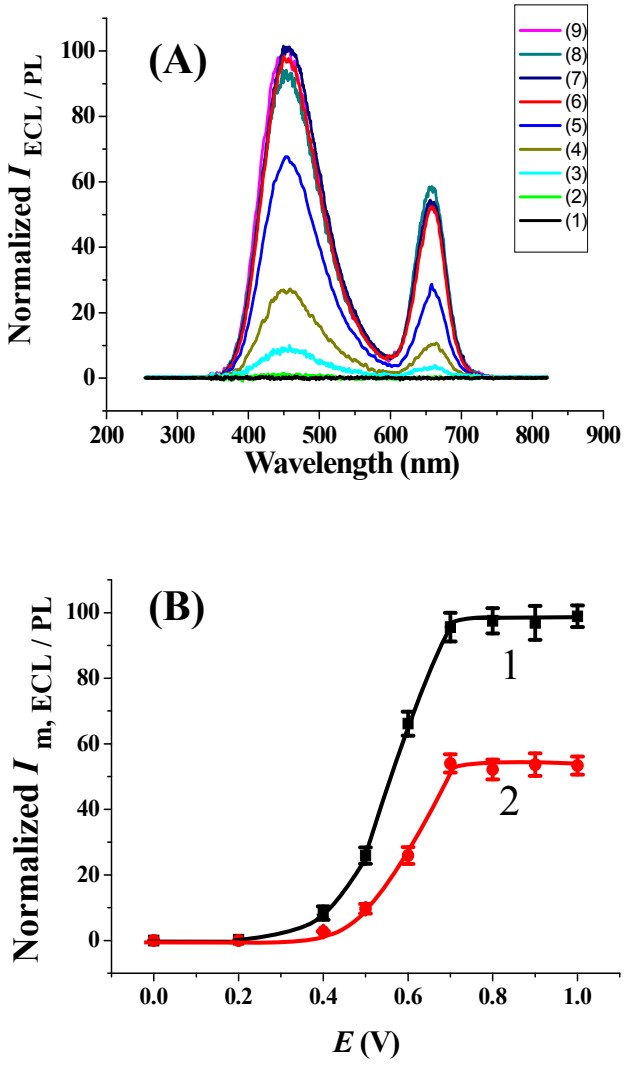

Figure S2. (A) Spectra of luminol-QD conjugates on the Au electrode held at different potentials *E* for 5 s. *E* (V): 0 (1); 0.2 (2); 0.4 (3); 0.5 (4); 0.6 (5); 0.7 (6); 0.8 (7); 0.9 (8) and 1.0 (9), and (B) the relationships between maximum intensity of luminol ECL ($I_{m,ECL}$) and *E* (1), and between maximum intensity of QD PL ($I_{m,PL}$) via ECRET and *E* (2).

Calculation of Förster distance. Förster distance (or radius) R_0, at which the RET efficiency is 50%, represents a characteristic parameter for a given donor-acceptor couple. It is defined as

$$R_0 = (9000\ (\ln 10)/N_A 128\pi^5)\kappa^2 n_m^{-4} \Phi_{lum} J(\lambda) = 8.8 \times 10^{-25} \kappa^2 n_m^{-4} \Phi_{lum} J(\lambda) \qquad \text{(S1)}$$

where N_A is Avogadro's number, κ^2 depends on the relative orientation of the donor and acceptor dipoles and is 2/3 for randomly oriented dipoles, n_m is the refractive

index of the medium (1.34 in aqueous solutions), Φ_{lum} is the quantum yield of the donor ABEI-luminol and $J(\lambda)$ is the spectral overlap integral, which is a function of the ECL intensity of the donor ABEI-luminol, $I_{lum}(\lambda)$, and molar absorptivity of the acceptor QD, $\varepsilon_{QD}(\lambda)$, as a function of wavelength, λ, normalized against the total donor emission. $J(\lambda)$ can be calculated from eq. S2:

$$J(\lambda) = \Sigma I_{lum}(\lambda)\, \varepsilon_{QD}(\lambda)\, \lambda^4\, \Delta\lambda / \Sigma I_{lum}(\lambda)\, \Delta\lambda \qquad \text{(S2)}$$

Φ_{lum} can be determined relative to rhodamine 6G dye in ethanol with a quantum yield of 0.95.[1] $J(\lambda)$ can be obtained from the ECL emission spectrum of ABEI-luminol and absorption spectra of QD (in the text, Figure 1B). R_0 was calculated to be 7.2 nm from eqs. S1 and S2.

Experimental Sections

Materials. Carboxyl group-coated QDs (COOH-QDs, 15-20 nm, 8 μmol/L) with a maximum emission at 655 nm, goat anti-human IgG-coated QDs (QD-anti-IgG, 1 μmol/L) with a maximum emission at 655 nm from Invitrogen (Eugene, OR, USA), human IgG; from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China), the carcinoembryonic antigen (CEA, 400 ng/mL) from Shanghai Linc-Bio Science Co., Ltd. (Shanghai China), purified human plasma fibronectin (Fn, 95%) from Millipore Trading Co., Ltd. (Billerica, MA, USA), N-(4-aminobutyl)-N-ethylisoluminol (ABEI-luminol, 97%) from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), *N*-hydroxysuccinimide (NHS) from Huifeng Chemical Industry Ltd. (Weinan, China) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) from Shanghai Medpep Co., Ltd. (Shanghai, China), guanidine hydrochloride (GdnHCl) and 2-(N-morpholino)ethanesulfonic acid (MES) from Shanghai Sango Biological Engineering Technology & Services Co., Ltd. (Shanghai, China) were used in this work. Other chemicals (analytical grade) were obtained from standard reagent suppliers. The preparation of QD-anti-IgG, CEA, human IgG and Fn solutions was performed in a clean bench. A 1.0×10^{-2} mol/L stock solution of ABEI-luminol was prepared by dissolving an appropriate amount of ABEI-luminol in 0.1 mol/L NaOH

without purification and stored for a week at 4 ^{o}C in the dark. The dilution solutions were obtained by a serial dilution with 0.1 mol/L NaOH.

The physiological buffer saline (PBS) consisted of 0.15 mol/L NaCl, 7.6×10^{-3} mol/L NaH_2PO_4 and 2.4×10^{-3} mol/L Na_2HPO_4 (pH 7.4). To prevent possible contamination from repeated sampling, the commercial QD-anti-IgG, human IgG, CEA and Fn were divided into several small packs in disinfected plastic vessels. Before use, phosphate that reduced reactivity of carbodiimides in protein solutions was removed. To do so, 500 μL of the human IgG or CEA or Fn solution was ultrafiltered to remove phosphate. Then, 500 μL of MES buffer was added and the solution was ultrafiltered. The process was repeated. Finally, MES buffer was added with a total solution volume of 500 μL. All aqueous solutions were prepared with doubly distilled water, passed through a 0.22 μm filter and stored at 4 ^{o}C. All buffers, disposable plastic wares and disposable micro-pipet tips were disinfected under the pressure of 1.4 kg/cm^2 for 20 min in electrothermal-pressure vessel before use, in order to prevent the growth of microorganisms. All solutions were prepared in disposable plastic wares using disposable micro-pipet tips. In this work, ultraultrafiltering was performed using Nanosep centrifugal devices (Ann Arbor, MI, USA).

Apparatus. The experimental setup for ECRET is shown in Figure S3. A CHI832 electrochemical analyzer (CH Instruments, Austin, TX, USA) coupled with an imaging spectrograph (Shamrock SR-303i-A, Andor Tech. Ltd., UK.) and an ICCD camera (iStar DH740-18F-03, Andor Tech. Ltd., UK.), the slit of which was adjusted to 2.5 mm, was used to record the ECRET spectra. A cuboid electrochemical cell fabricated using glass plates with three electrodes (an Au working electrode, a saturated calomel reference electrode and a Pt auxiliary electrode) was placed in front of the observation window of the imaging spectrograph with the working electrode faced the observation window. The three electrodes were connected to the electrochemical analyzer to apply the potential to the working electrode.

In order to fabricate the Au electrode, an Au plate of was glued to a microscope

glass slide with epoxy resin. A coiled end of a 0.5-mm-diameter, ~6-cm-long copper lead was glued to one end of the Au plate with silver epoxy. The ensemble was cured for 20 min at 120 ^{o}C in an oven. Epoxy resin was added to the junction between the Au electrode and the copper lead in order to protect the electrical junction. The ensemble was cured for 12 h at room temperature.

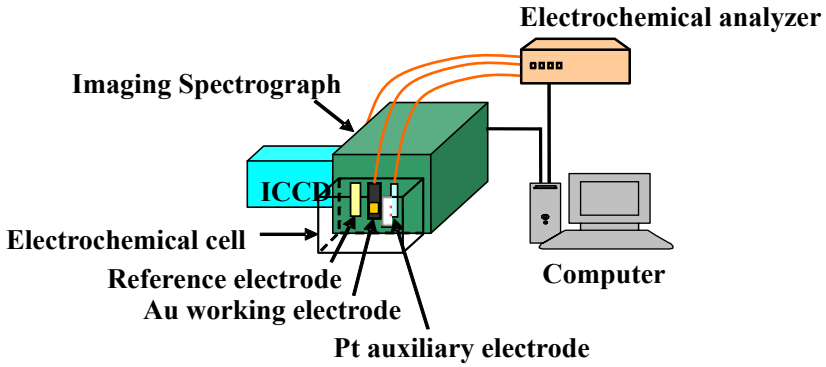

Figure S3. Schematic diagram of the instrumental setup for ECRET.

Preparation of luminol-QD conjugates. A solution consisting of 5 μL of COOH-group coated QD (8×10^{-6} mol/L), 8 μL of EDC (1×10^{-4} mol/L), 8 μL of NHS (1×10^{-4} mol/L) and 200 μL of borate buffer (0.2 mol/L, pH 7.4) was incubated for 1 h. Then, 8 μL of ABEI-luminol solution (1.0×10^{-4} mol/L) was added and the solution was incubated for 2 h. The resulting luminol-QD conjugates were washed thrice with PBS buffer by ultrafiltering. The luminol-QD conjugates were diluted to 100 μL of PBS buffer and stored at 4 °C. In order to prepare the luminol-QD conjugates using the quenched ABEI-luminol, the ABEI-luminol solution was replaced by the quenched ABEI-luminol solution that was prepared by illuminating the solution for 72 h using a 10-W ultraviolet lamp.

Preparation of luminol-IgG and luminol-CEA conjugates. Three μL of human IgG (6.6×10^{-6} mol/L) was mixed with 135 μL of 0.5 mol/L MES buffer (pH 6.1). After 4 μL of EDC (1×10^{-4} mol/L), 4 μL of NHS (1×10^{-4} mol/L) and 4 μL of ABEI-luminol (1×10^{-4} mol/L) were added, the solution was incubated for 2 h. Then,

the residual reagents (EDC, NHS and ABEI-luminol) were removed by ultrafiltering. The resulting conjugates were diluted to 100 μL with PBS buffer and the solution was stored at 4 ºC. The same procedure was used to prepare luminol-CEA conjugates using 10 μL of CEA (2×10^{-6} mol/L) and 128 μL of MES buffer.

Preparation of luminol-Fn-QD conjugates. First, Fn was bound to QDs to prepare Fn-QD conjugates. After 5 μL of COOH-QD (8×10^{-6} mol/L) was mixed with 50 μL of MES buffer, followed by adding 10 μL of NHS (100 mg/mL). Then, 10 μL of EDC (100 mg/mL) was added quickly with vigorous stirring, and the solution was incubated for 1 h under stirring. The resultant estered QDs were separated by centrifuging for 20 min at 13000 rpm and washed three times with MES buffer to remove residual EDC, NHS and byproduct urea. The estered QDs were dispersed in 50 μL of MES buffer. Then, 100 μL of 2.3×10^{-6} mol/L Fn in MES buffer was added. After incubation for 2 h, the resultant Fn-QD conjugates were collected by centrifuging for 20 min at 13000 rpm. The Fn-QD conjugates were washed thrice with MES buffer to remove unbound Fn. Then, the Fn-QD conjugates were dispersed in 10 μL of MES buffer. In order to prepare luminol-Fn-QD conjugates, 10 μL of Fn-QD conjugates, 10 μL of EDC (100 mg/mL), 10 μL of NHS (100 mg/mL) and 10 μL of ABEI-luminol (1×10^{-4} mol/L) were mixed and incubated for 2 h. The prepared luminol-Fn-QD conjugates were collected by centrifuging for 20 min at 13000 rpm. The luminol-Fn-QD conjugates were dispersed in 100 μL of PBS buffer.

Note: In the preparation procedures of the conjugates mentioned above, in order to avoid that luminol was quenched, all reactions were performed in the centrifuge tubes enveloped with black paper. In all incubation steps, the solutions were shaken gently using a shaker with a rate of 36 rpm to accelerate the reactions.

Methods

Measurements of ECRET Spectra. Before measurements, the Au electrodes were activated by performing repetitive cyclic scans between 0.0 and 1.5 V at 50 mV/s in 0.5 mol/L H_2SO_4 until stable voltammograms were obtained, followed by washing with doubly distilled water. In order to measure ECRET spectra, the constant

potential mode was used. A constant potential of 0.9 V was applied to the working electrode for 5 s and ECRET spectra were recorded. All measurements of ECRET spectra were carried out in 0.2 mol/L Na_2CO_3–$NaHCO_3$ buffer (pH=10) containing 1.0×10^{-2} mol/L H_2O_2. For the experiments of solution-phase ABEI-luminol, the ABEI-luminol solution was added into the electrochemical cell containing the buffer and H_2O_2, and then the three electrodes were put into the electrochemical cell to measure the ECL spectrum. For the experiments of the conjugates consisting of QDs and other components immobilized to the Au electrode, 5 μL of the solutions containing the conjugates was dropped onto the Au electrode and dried at 40 ^oC for 20 min in an oven. Then, the Au electrode with the conjugates was inserted into the electrochemical cell with the reference electrode and the auxiliary electrode containing the buffer and H_2O_2 to measure ECRET spectra.

Evaluation of interaction between proteins. First, 2 μL of QD-anti-IgG (1×10^{-6} mol/L) was added into 100 μL of luminol-IgG conjugates or luminol-CEA conjugates in PBS buffer prepared above. After incubation for 2 h, the solution was concentrated to 10 μL by ultrafiltering. Then, 10 μL of the solution containing the conjugates was used to measure ECRET spectrum.

Study on conformational change of proteins. To denature luminol-labeled Fn bound to QDs, the luminol-Fn-QD conjugates prepared above were dispersed to 40 μL of PBS buffer containing 2 or 8 mol/L GdnHCl and then denatured for 2 h. Five μL of the solution containing the conjugates was used to measure ECRET spectrum.

Note: In order to avoid that luminol was quenched, all reactions were performed in the centrifuge tubes enveloped with black paper, and in all incubation steps, the solutions were shaken gently using a shaker with a rate of 36 rpm to accelerate the reactions.

References

1 **I. L. Medintz, A. R. Clapp, H. Mattoussi, E. R. Goldman and J. M. Mauro, *Nat. Mater.*, 2003, 2, 630-638.**