Supporting Information

Amplified chemiluminescence aptasensor based on bi-resonance energy transfer on the gold nanoparticles and exonuclease III-catalyzed target recycling

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Experimental Section

Materials: All DNA oligonucleotides were synthesized and HPLC-purified by Sangon Biotechnology Inc. (Shanghai, China). The aptamer hairpin sequence was 5'-CACCAACCAACAAGAAAGCCAAACCTG ATGTT*GGTTGGTGTGGTTGG*-3', where stems are underlined, and aptamer nucleotides for thrombin (Tb) are italicized. A DNA sequence that is partly complementry to the hairpin was 5'-SH-TTTTTTTTAATTCTT GTTGGTTGGTG-FAM-3'. Tb, IgG, human serum albumin (HSA), α-fetoprotein (AFP), and carcinoembryonic antigen (CEA) were purchased from Sigma-Aldrich. Exonuclease III (Exo III) was purchased from New England Biolabs. Other chemicals were of analytical grade and used without further purification. The water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work. AuNPs (15 nm diameter) were synthesized by citrate reduction of $HAuCl_4$ as previously reported.[1] Chemiluminescence (CL) spectra and fluorescence spectra were measured with a LS-55 luminescence spectrometer (Perkin-Elmer, USA).

Preparation of DNA-functionalized AuNPs : The DNA-functionalized AuNPs were prepared based on the previously reported protocol.[2] Briefly, the FAM-labeled DNA was first added to a AuNPs solution (10 nM) to reach a final concentration of 1 μM DNA. After being incubated overnight, the mixture was diluted with 20 mM phosphate buffer solution (PBS; 50 mM NaCl, 20 mM phosphate, pH 7.4) and allowed to stand for 24 h at room temperature. Unconjugated oligonucleotides were removed by centrifugation at 12000 rpm for 20 min at 4 ℃ and by washing three times with 20 mM PBS. The DNA-functionalized AuNPs obtained was redispersed in a tris(hydroxymethyl)aminomethane (Tris) buffer solution (pH 7.4) containing 50 mM NaCl, 20 mM Tris-HCl, 10 mM $MgCl_2$ and 5% BSA.

Detection of analyte with CRET aptasensor: In a typical experiment, 500 μL of assay buffer solution (20 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, and 0.8 mM luminol, pH 7.4) that contained 10 nM of the functionalized AuNPs, 40 units of Exo III, 10 nM of the hairpin probe and varying concentrations of Tb or other proteins was incubated at 37 ℃ for 2 h. The resultant mixture was transfered into a 1 cm path length quartz cell. Then, 500 μL of CL buffer solution (30 mM Na_2CO_3, 3 mM H_2O_2, 1 μM HRP, pH 10.0) was poured to the quartz cell, and CL spectrum was recorded immediately with a LS-55 luminescence spectrometer. The luminescence intensity of FAM at 520 nm was used for quantification of the analyte.

Target assisted exonuclease cleavage assay by fluorescence measurement:

The samples for fluorescence assays were obtained as the following: The target Tb (40 pM) was added into a solution (1.2 mL) containing FAM-labeled DNA-functionalized AuNPs (10 nM), aptamer hairpin (10 nM) and Exo III (40 units) in assay buffer, and the mixture was incubated for 2 h at 37 ℃. A blank sample was prepared similar to the procedure mentioned above except no addition of Tb. The fluorescence measurements were performed on a LS-55 luminescence spectrometer. The exciting wavelength was 494 nm, and slits for both the excitation and the emission were set at 5 nm. The fluorescence spectra were recorded in the range of 510 nm to 600 nm. As shown in Figure S1, FAM-labeled DNA-functionalized AuNPs probes only exhibited small background fluorescence in the presence of Exo III due to the ultrahigh quenching ability of AuNPs. When only the target Tb was present (without Exo III), the fluorescence of the probes remained almost unchanged. However, upon incubation with both the target Tb and Exo III, the fluorescence increased significantly, which indicated that the cleavage of DNA associated with AuNPs by Exo III in the presence of Tb have occurred.

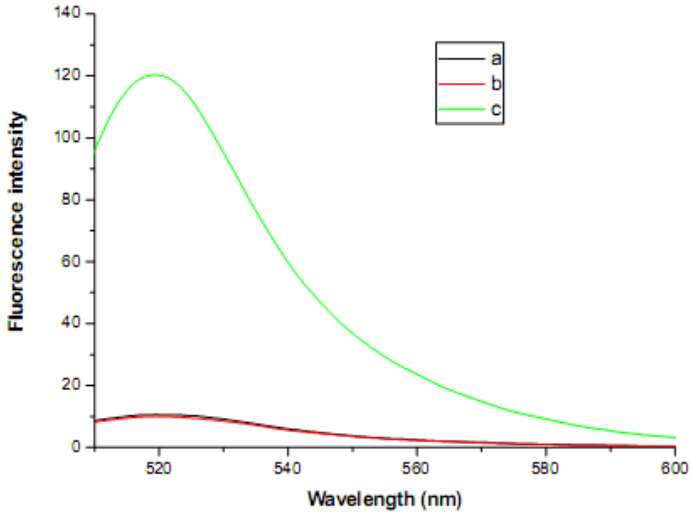

Figure S1. Fluorescence spectra obtained for different conditions: (a) FAM-labeled DNA-functionalized AuNPs; (b) FAM-labeled DNA-functionalized AuNPs+ExoIII; (c) FAM-labeled DNA-functionalized AuNPs+ExoIII+Tb.

Target assisted exonuclease cleavage assay by microchip electrophoresis:

The samples for microchip electrophoresis (MCE) assays were obtained as the following: The target Tb (40 pM) were added into a solution (500 μL) containing FAM-labeled DNA-functionalized AuNPs (10 nM), aptamer hairpin (10 nM) and Exo III (40 units) in assay buffer, and the mixture was incubated for 2 h at 37 °C. The mixture was centrifuged at 14,000 rpm for 30 min at 4 °C. The supernatant was used for MCE assay. A blank sample was prepared similar to the procedure mentioned above except no addition of Tb. MCE assays with laser induced fluorescence (LIF) detection were perform on a simple cross microchip as the procedure described in our previous work.[3] Obtained electropherograms were shown in Figure S2.

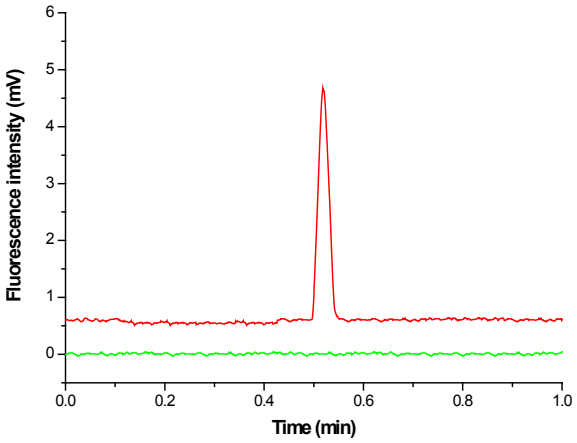

Figure S2. Electropherogram obtained from the samples before (a) and after (b) target assisted the cleavage of DNA associated with AuNPs by Exo III. MCE conditions: 30 mM Tris-HCl running buffer (pH 8.0). Voltages used in MCE-LIF detection were as described in Ref. 2 in the Supporting Information.

Optimization of the CL reaction condition:

To maximize the detection sensitivity, the parameters affecting the CRET detection, including the concentrations of luminol, H_2O_2, and HRP, and the pH value of CL reaction solution were examined and optimized. For luminol, the CRET-stimulated FAM emission was intensified as the concentration of luminol was higher. And the highest was obtained at 0.8 mM luminol (Figure S3). For H_2O_2, the CRET-stimulated FAM emission was also intensified with H_2O_2 concentration increasing, and the maximal intensity was obtained when 3 mM H_2O_2 was used (Figure S4). Hence, 0.8 mM luminol and 3 mM H_2O_2 were selected for further experiments. As an catalyst, HRP can significantly enhanced the CL of luminol-H_2O_2 reaction. The effect of the concentration of HRP on the CRET-stimulated FAM emission was investigated from 0.2 μM to 1.4 μM. As shown in Figure S5, the CRET-stimulated FAM emission increased with increasing HRP concentration, and remain almost unchanged after 1.0 μM. Thus, 1.0 μM HRP was selected. The effect of pH values of CL buffer solution was also studied and the results are shown in Figure S6. As the pH value increased, the CRET-stimulated FAM emission increased

gradually, and reached the maximum at pH 10.0. Further increasing the pH value, the CRET-stimulated FAM emission decreased. So, a CL buffer solution at pH 10.0 was chosen.

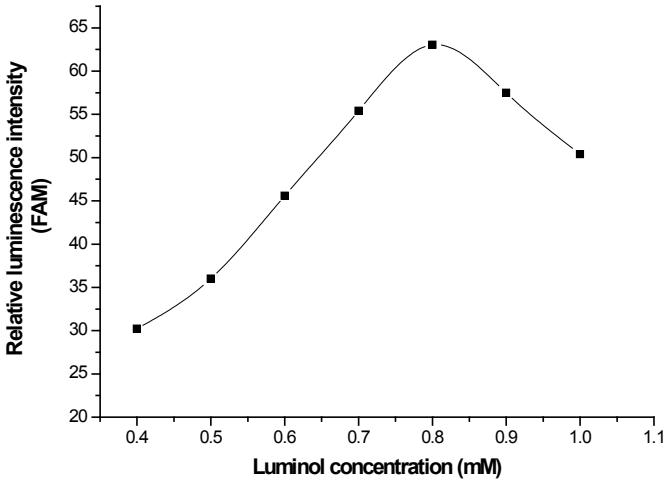

Figure S3. Effect of luminol concentration on the CRET-stimulated FAM emissions. The experiments were completed in a CL buffer solution (pH 10.0) containing different concentrations of luminol, 3 mM H_2O_2 and 1.0 μM HRP.

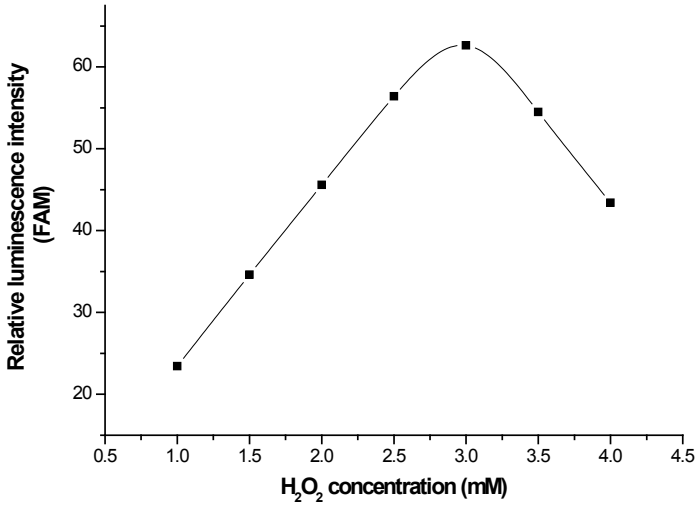

Figure S4. Effect of H_2O_2 concentration on the CRET-stimulated FAM emissions. The experiments were completed in a CL buffer solution (pH 10.0) containing different concentrations of H_2O_2, 0.8 mM luminol and 1.0 μM HRP.

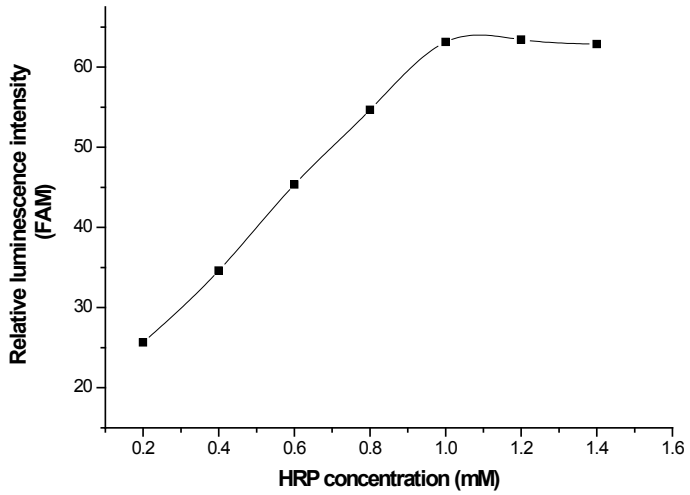

Figure S5. Effect of HRP concentration on the CRET-stimulated FAM emissions. The experiments were completed in a CL buffer solution (pH 10.0) containing different concentrations of HRP, 0.8 mM luminol and 3 mM H_2O_2.

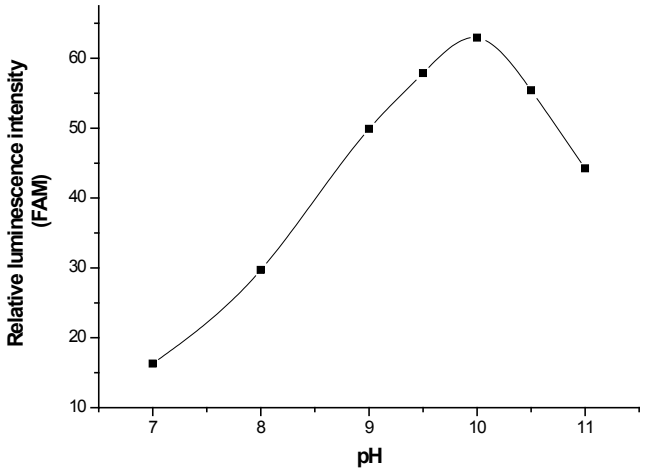

Figure S6. Effect of pH value of the CL buffer solution on the CRET-stimulated FAM emissions. The experiments were completed by varying the pH value of the CL buffer solution containing 3 mM H_2O_2, 0.8 mM luminol and 1.0 μM HRP.

Calibration curve for Tb analysis:

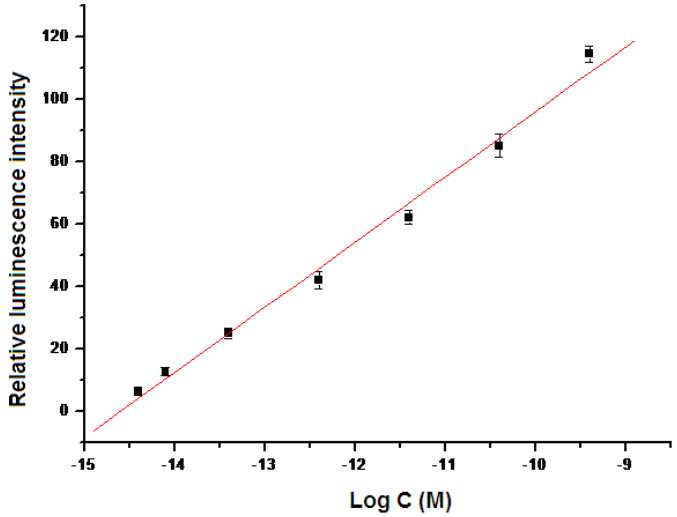

Figure S7. Calibration curve corresponding to the luminescence intensity increase of FAM at λ=520 nm upon analyzing different concentrations of Tb after a fixed time-interval of 2 h. The luminol concentration was 0.8 mM. The CL reaction buffer (pH 10.0) contained 3 mM H_2O_2 and 1 μM HRP.

Potential application:

To evaluate the application potential of the proposed CRET aptasensor for real sample analysis, we applied the CRET aptasensor to detect Tb in human healthy serum samples. Because human healthy serum sample does not contain thrombin,[4,5] we performed spike experiments. The 10-fold diluted serum sample was spiked with 40 fM, 400 fM, 40 pM Tb, and then analyzed. The results were compared with the same concentrations prepared in buffer. As shown in Figure S8, The the luminescence intensity of FAM Tb spiked serum samples were slightly lower (< 4.5%) than that obtained in buffer solutions. These results indicated the potentiality of the proposed CRET aptasensor for real biological sample analysis.

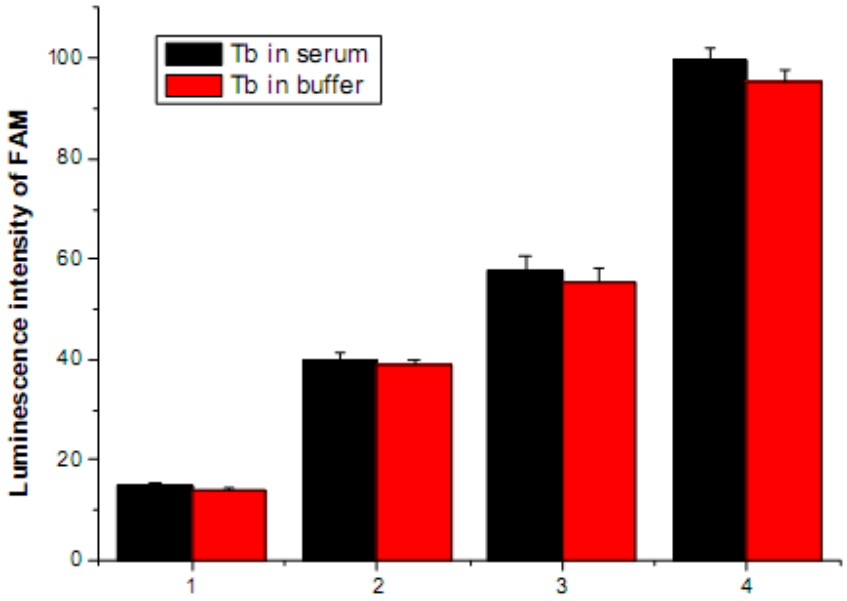

Figure S8. Luminescence intensity of FAM upon analyzing different concentrations of Tb in human serum and buffer solution. 1: 0 fM; 2: 40 fM; 3: 400 fM; 4: 40 pM Tb.

Methods comparison:

Table S1 summarizes the analytical performance of different aptamer-based homogeneous sensing platforms for the detection of Tb. It was very clear that the detection sensitivity of the proposed CRET aptasensor were much higher than that of traditional homogeneous aptasensors,[6-10] and also better than other amplification methods.[11-14] Moreover, the proposed CRET aptasensors also exhibited a wide dynamic range (Tb concentration from 4 fM to 400 pM). These results clearly demonstrate the good analytical performance of the proposed method.

Table S1. Performance comparison between homogeneous optical aptasensors.

Type	Sensitivity	Dynamic range	Reference
Carbon nanotube-based aptasensor	1.8 nM	4.0 nM-150 nM	[6]
Graphene oxide-based aptasensor	2.0 nM	5 nM-100 nM	[7]
Quantum dot-based aptasensor	30 pM	0.1 nM-10 nM	[8]
FP aptasensor	250 pM	250 pM-8 nM	[9]
Graphene oxide-based CRET aptasensor	1 pM	1 pM-100 nM	[10]
Exonuclease III amplification	89 pM	360 pM-14 nM	[11]
Nicking enzyme amplification	2 pM	5 pM-50 nM	[12]
Exonuclease III amplified CRET aptasensor	2 fM	4 fM-400 pM	This work

Reference

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