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Resonance energy transfer and electron-hole annihilation induced chemiluminescence of quantum dots for amplified immunoassay

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Experimental section

Materials and reagents. The biotin functional strand (5'-biotin-TTTTTTTTTT-$(CH_2)_6$-NH_2-3') was synthesized and purified by Sangon Biotech Shanghai Co., Ltd. (China). Capture antibodies (Ab1) of α-fetoprotein (AFP) (from mouse, clone No. 102k7) and carcinoembryonic antigen (CEA) (from mouse, clone No. Z-2011), biotinylated detection antibodies (Ab2) of AFP (clone No. 9k5) and CEA (clone No. Z-2012), standard solutions of CEA, AFP, carcinoma antigen 125 (CA 125) and carbohydrate antigen 199 (CA 199) were purchased from Beijing Key-Bio Biotech Co., Ltd. (China). Bis(2,4,6-trichlorophenyl) oxalate (TCPO), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), 2,2,6,6-tetramethylpiperidine (TEMP) were obtained from Tokyo Chemical Industry Co., Ltd (Japan). Monodispersed carboxyl polystyrene (PS) microspheres (diameter: 50 nm) were supplied by Nanjing Olive Twigs Biotechnology Co., Ltd. (China). Ascorbic acid was purchased from Aladdin Chemical Co., Ltd. (China). Mercapto propionic acid (MPA), N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), bovine serum albumin (BSA) and avidin were all obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO). Cadmium chloride ($CdCl_2$) and tellurium (Te) were from Alfa Aesar China Ltd. Sodium borohydride ($NaBH_4$) was purchased from Nanjing Chemical Reagent Co., Ltd. The clinical serum samples were from Jiangsu Cancer Hospital. The ECL immunoassay

reagent kits for reference detection were provided by Roche Diagnostics GmbH (Germany). The aldehyde-modified glass slides for preparing protein chip were purchased from Shanghai Baio Technology Co., Ltd. (China).

Immunoreaction buffer was 0.01 M phosphate buffered saline (PBS) (pH 7.4). Blocking buffer was 0.01 M PBS (pH 7.4) containing 5% BSA. Washing buffer was 0.01 M PBS (pH 7.4) spiked with 0.05% Tween-20. MES buffer (pH 6.0) was used to dissolve EDC and NHS for connection of the amino group and the carboxyl group. Ultrapure water from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all experiments. All other reagents were of analytical grade and used without further purification.

Apparatus. X-ray photoelectron spectra (XPS) was acquired using PHI 5000 VersaProbe X-ray photoelectron spectrometer (ULVAC-PHI, Japan). UV-Vis absorption spectra were recorded on a Shimadzu UV-3600 UV-Vis-NIR spectrometer (Shimadzu Co., Japan). Electron paramagnetic resonance (EPR) spectra were measured on an EMX-10/12 spectrometer (Bruker, Germany). Transmission electron microscope (TEM) image was recorded by JEM-2100 instrument (JEOL, Japan). Statistical map of the particle size distribution in TEM image was identified with analyzing software Nano Measurer v1.2.5. An IFFM-E luminescent analyzer (Remax, China) was used to collect the CL signal. A F97XP fluorospectophotometer (Lengguang Tech., China) was used to record the FL signal. The control levels of CEA in sera were obtained with an automated ECL analyzer (Elecsys 2010, Roche).

Preparation of MPA-capped CdTe QDs. CdTe QDs were synthesized according to the previous protocol.[S1] Briefly, 0.125 mmol of Te was dissolved in 15 mL of deionized water, and excess $NaBH_4$ was added into the solution with N_2 bubbled, until the solution turned to transparent purple, indicating the formation of NaHTe. Simultaneously, 0.375 mM of $CdCl_2$ and 0.94 mM of MPA were added to 135 mL of deionized water with N_2 bubbled for 30 min. The mixture solution was adjusted with 1.0 M NaOH to be transparent. Then, the fresh NaHTe solution was injected into $CdCl_2$ under stirring. The molar ratios of Cd^{2+}/NaHTe/MPA were 4:1:10. The as-prepared solution was subsequently heated to 110 °C with N_2 bubbled under stirring and refluxed for some time to obtain the QDs. Before usage, the QDs were purified by ultrafiltration using a 100 KD Millipore and resuspended in ultrapure water and stored at 4 °C in the dark. The concentration of the CdTe QDs were estimated to be 1.77 μM.[S2]

Preparation of CdTe-biotin. 70 μL 1.77 μM of CdTe QDs were suspended in 1 mL MES buffer containing EDC (40 mM) and NHS (10 mM) for 30 min, followed by centrifugation at 10 000 rpm for 30 min and resuspended in 70 μL of PBS. The CdTe-biotin was prepared by mixing 70 μL of the biotin functional strand (100 μM) and the activated CdTe QDs overnight, followed by centrifugation at 10 000 rpm for 45 min, removal of supernatant and resuspension in 70 μL of ultrapure water.

Preparation of avidin-PS. Avidin-PS microspheres were synthesized using a slightly modified procedure.[S3] 2.5 μL 2.5% w/v of PS microspheres were suspended in 1 mL MES buffer containing EDC (40 mM) and NHS (10 mM) and mixed for 1 h, followed by centrifugation at 6000 rpm for 5 min and dissolved in 1 mL PBS. Next, 200 μL of 0.2 mg/mL avidin was added and incubated with the microspheres for 1 h under stirring. The resulting avidin-PS microspheres were purified by centrifugation at 8000 rpm for 10 min and resuspended in 200 μL of PBS.

CL Spectrum. CL emission distributed in different wavelength ranges was measured using a series of cutoff filters from 420 to 650 nm, and a fitting curve was obtained as the CL spectrum.[S4,S5]

EPR analysis. To capture and detect the $OH^{\bullet}$, 50 μL of 1.6 M DMPO, 450 μL of 1.77 μM CdTe QDs, 150 μL of 17 mM TCPO and 150 μL of 50 mM H_2O_2 containing 7.5 mM imidazole were mixed in a capillary. The analysis of $^1O_2$ was performed similarly with TEMP instead of DMPO. During the studies on ground-state properties of CdTe QDs, no DMPO or TEMP was added in the solution.

Fabrication of disposable protein chip. The home-made protein chip contains 48 sensing cells.[S6] Briefly, 6 μL of 10 μg/mL Ab1 was dropped on the cell to incubate overnight at 4 °C. The 48 cells were then thoroughly washed with washing buffer, and the unreacted aldehyde groups were blocked with blocking buffer for 1 h. After washing, the disposable protein chip was obtained and stored in 0.01 M pH 7.4 PBS at 4 °C for immunoassay.

QDs CL-based CL immunoassay. To obtain the calibration curve for CEA or AFP, 6.0 μL of different standard antigen solutions at known concentrations were dropped into the sensing sites and incubated for 15 min. After washing and drying, 6.0 μL of biotin-Ab2 was added to the sensing sites for 15 min, followed by washing and drying. Then, 6.0 μL of avidin-PS and 6.0 μL of CdTe-biotin were sequentially dropped on the sensing cells to incubate with the sandwich immunocomplex for 15 and 20 min, respectively. After washing and drying, 3 μL of 17 mM TCPO and 3 μL of 50 mM H_2O_2 containing 7.5

mM imidazole were added into the sensing cells respectively. The CL signals were recorded at gain 2 and 950 V.

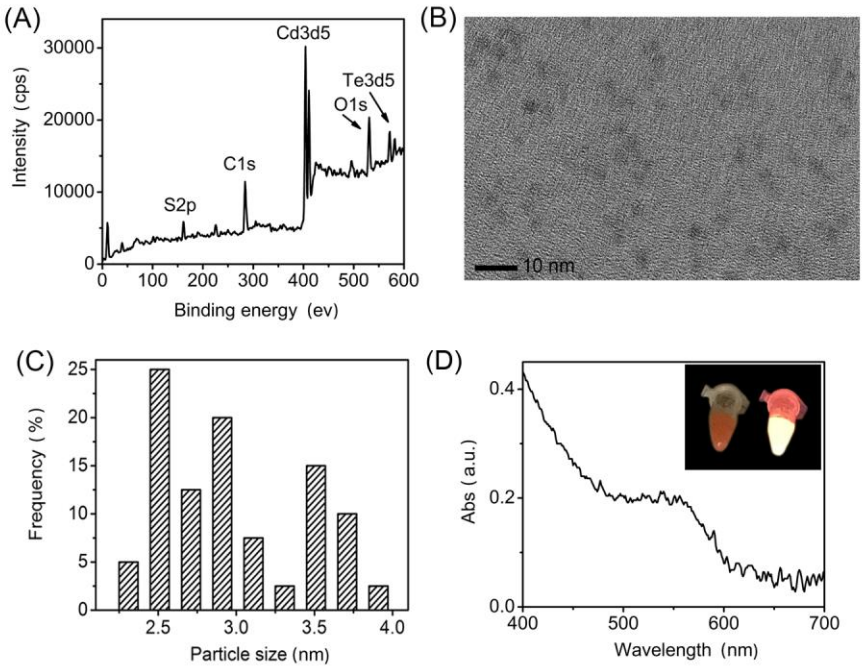

Fig. S1 (A) XPS, (B) TEM image, (C) statistical map of the particle size distribution and (D) UV-vis spectrum of CdTe QDs. The inset in D is the photograph of CdTe QDs under visible light (left) and under 365 nm (right).

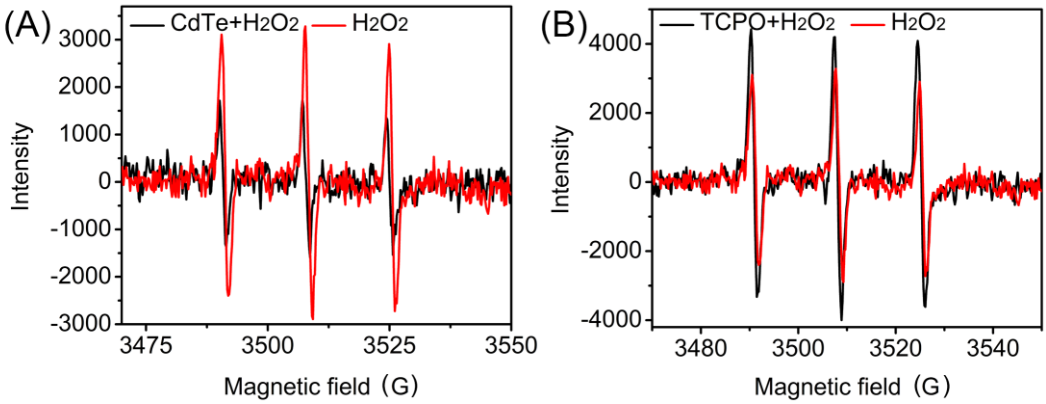

Fig. S2 EPR spectra of $^1O_2$ radicals in (A) H_2O_2 and CdTe-H_2O_2 system, (B) H_2O_2 and TCPO-H_2O_2 system. Experimental conditions: 100 mM TEMP, 1 μM CdTe QDs, 3.2 mM TCPO, 9.4 mM H_2O_2 containing 1.4 mM imidazole.

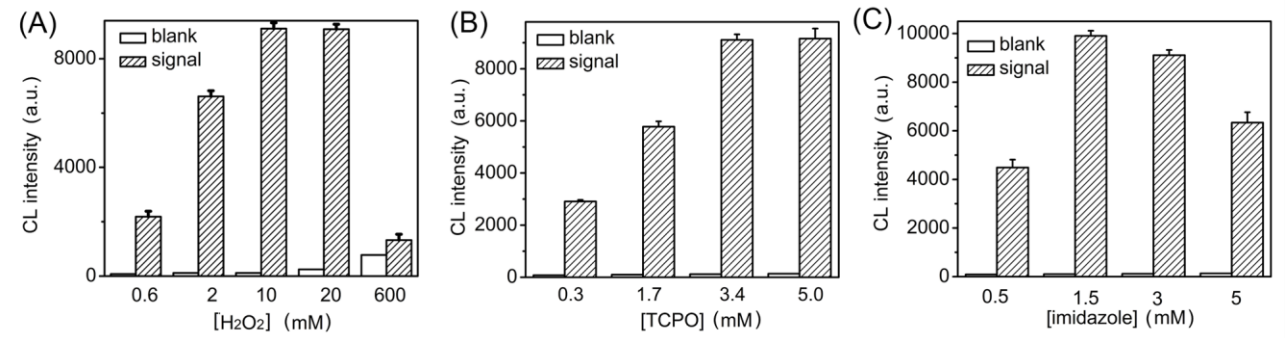

Fig. S3 Optimization of (A) H_2O_2, (B) TCPO, and (C) imidazole concentrations. Blank: TCPO-H_2O_2 CL system, signal: CdTe-TCPO-H_2O_2 CL system, CdTe QDs: 600 nM.

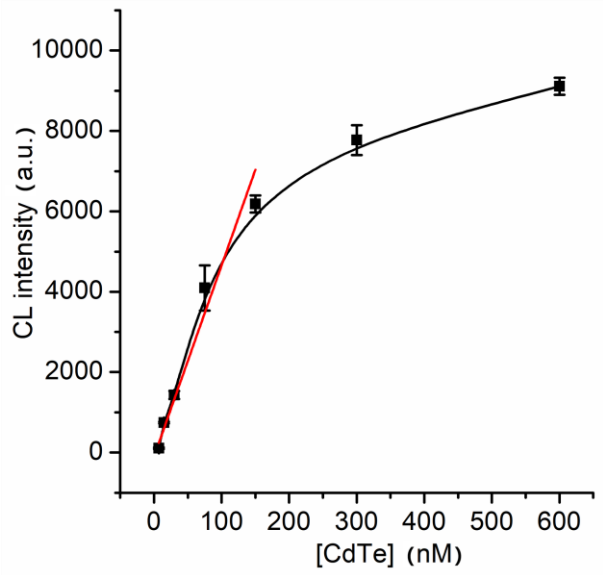

Fig. S4 Dependence of CL intensity on CdTe QDs concentration in CdTe-TCPO-H_2O_2 system.

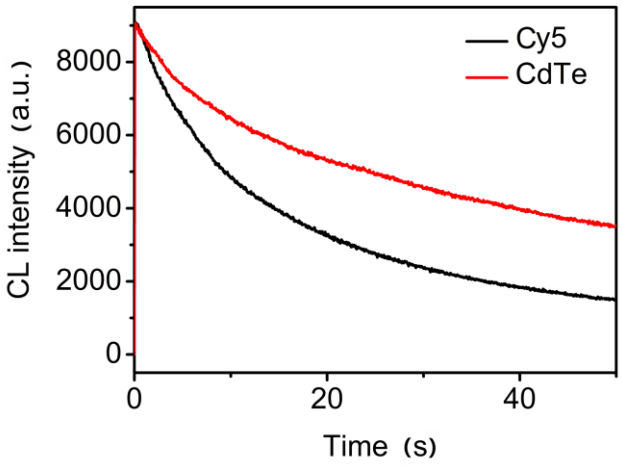

Fig. S5 CL kinetic curves of CdTe QDs-TCPO-H_2O_2 and TCPO-H_2O_2-Cy5 CL systems. Experimental conditions: 3.4 mM TCPO, 10 mM H_2O_2, 1.5 mM imidazole, 600 nM CdTe QDs or Cy5.

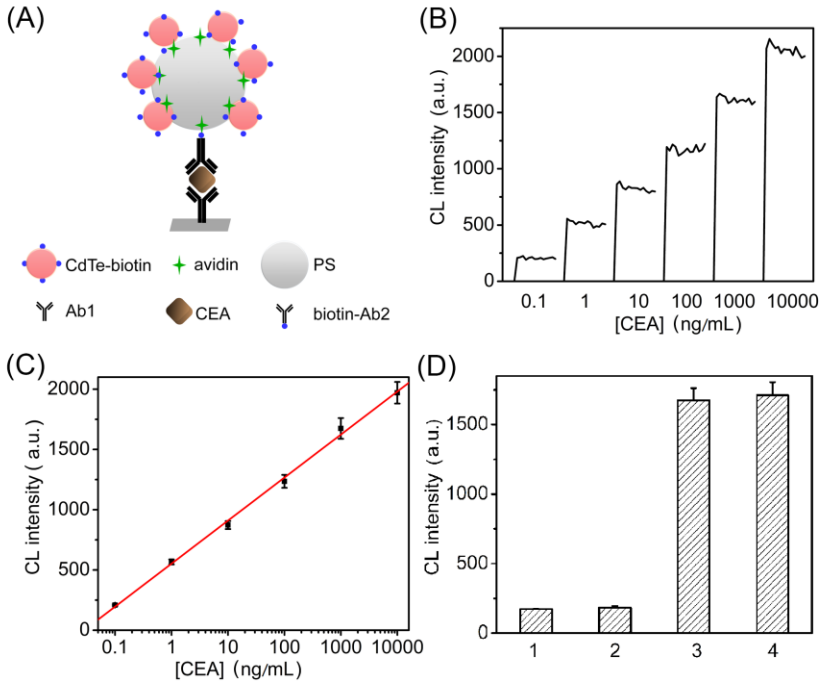

Fig. S6 (A) Sandwich immunoassay of CEA with PS assisted CdTe QDs amplification. (B) CL responses to CEA at 0.1, 1, 10, 100, 1000, and 10000 ng/mL. (C) Calibration curve for CEA sensing. (D) Selectivity evaluation: (1) blank solution, (2) 1 KU/mL CA 199 + 1 KU/mL CA 125 + 1 μg/mL AFP, (3) 1 μg/mL CEA , (4) 1 KU/mL CA 199 + 1 KU/mL CA 125 + 1 μg/mL AFP + 1 μg/mL CEA.

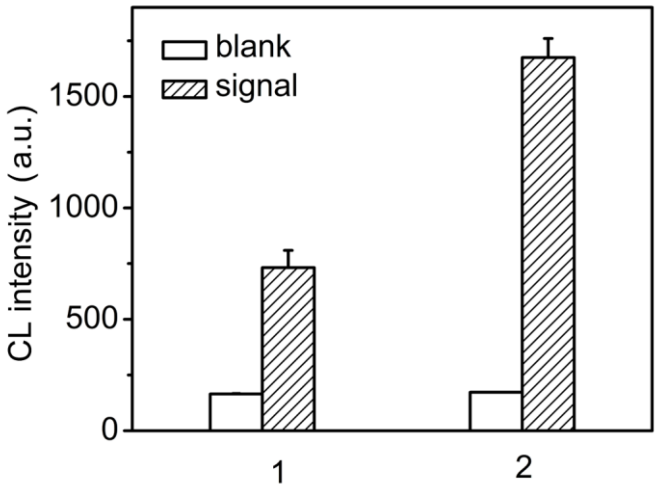

Fig. S7 CL intensities of sandwich immunoassay of 1000 ng/mL CEA without (1) and with (2) PS assisted CdTe QDs amplification.

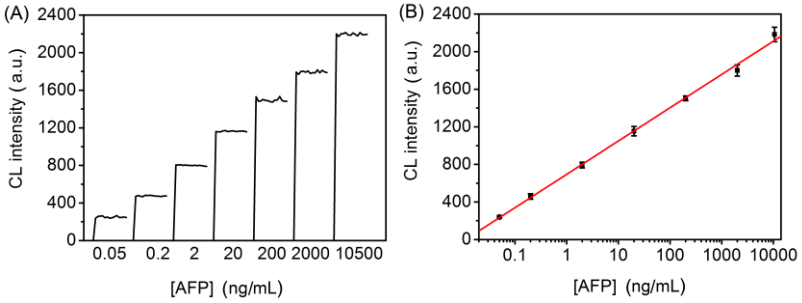

Fig. S8 Sandwich immunoassay of AFP with PS assisted CdTe QDs amplification. (A) CL responses to AFP at 0.05, 0.2, 2, 20, 200, 2000 and 10500 ng/mL. (B) Calibration curve for AFP sensing.

Table S1. Effects of radical scavengers, common ions and surfactant on CdTe QDs-TCPO-H_2O_2 system. Experimental conditions: 600 nM CdTe QDs, 3.4 mM TCPO, 10 mM H_2O_2 containing 1.5 mM imidazole.

Species added	Concentration (mol/L)	CL intensity
/	/	9110
ascorbic acid	5×10^{-4}	469
NaN_3	1×10^{-3}	8977
Mg^{2+}	6×10^{-6}	8859
Ca^{2+}	6×10^{-6}	8931
Fe^{3+}	6×10^{-6}	8993
K^+	6×10^{-4}	9102
Na^+	6×10^{-4}	9115
SO_4^{2-}	6×10^{-4}	9105
Cl^-	6×10^{-4}	9050
NO_3^-	6×10^{-4}	8594
SO_3^{2-}	6×10^{-4}	8198
CTAB	6×10^{-4}	8568

Table S2. An overview of CL immunoassays of CEA and other analytes using different CL systems.

CL system	Analytes	Signal amplification	Detection range	Detection limit	References
CdTe QDs-TCPO-H_2O_2	CEA	CRET/EHA & 1:n amplification	0.1 ng/mL-10 μg/mL	0.092 ng/ mL (~0.3 pM)	this work
luminol-H_2O_2-DNAzyme	CEA	proximity-dependent DNAzyme catalysis	0.16 ng/mL - 1.6 μg/mL	0.15 ng/mL	S6
luminol-H_2O_2-HRP	AFP	microchip electrophoresis & HRP catalysis	10-150 ng/mL	7.2 ng/mL	S7
luminol-H_2O_2-DNAzyme	thrombin	target-catalyzed hairpin assembly and Exo III-assisted amplification	1-1000 pM	0.92 pM	S8
luminol-H_2O_2-MOFs	glucose	MIL-53(Fe) MOF-mediated catalysis	0.1-10 μM	0.05 μM	S9
luminol-H_2O_2-DNAzyme	thrombin	CRET & catalytic he-min/G-Quadruplexes	1.43-86 nM	200 pM	S10

Table S3. Assay results of CEA in clinical serum samples using the proposed and reference methods.

Sample No.	1	2	3	4	5	6	7
Proposed method (ng/mL)	23.02	1.28	133.9	438.9	48.65	0.759	0.909
Reference method (ng/mL)	23.84	1.23	131.9	412.8	49.91	0.764	0.870
Relative error (%)	-3.4	4.1	1.5	6.3	-2.5	-0.7	4.5

Supporting references

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