Supporting Information

Versatile Electrochemiluminescence Assays for Cancer Cells Based on Dendrimer/CdSe-ZnS-Quantum Dot Nanoclusters

Guifen Jie, Lei Wang, Jinxin Yuan, Shusheng Zhang*

State Key Laboratory Base of Eco-chemical Engineering, Ministry of Education; College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

***Corresponding author: shushzhang@126.com**

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DNA Sequences Used in This Study

Table S1. The Sequences of the DNA in Scheme 1

DNA	Sequences
C-aptamer:	5’- SH-TAC AGA ACA CCG GGA GGA TAG TTC GGT GGC TGT TCA GGG T CTC CTC CCG GTG-3’
random DNA：	5'- SH-ATG TCT TGT GGC CCT CCT ATC AAGCCA CCG ACA AGT CCC ACTC CTC CCG GTG-3'
probe DNA (p-DNA):	5’-NH_2 CCT AAT GTG CAC CGG GAG GAG-3’
bio-bar-code DNA (bbc-DNA)：	5’-NH_2 CCT AAT TCG CGC TAG ACC GCC-3’

Table S2. The Sequences of the DNA in Scheme 2

DNA	Sequence
C-aptamer:	5’- NH_2-TAC AGA ACA CCG GGA GGA TAG TTC GGT GGC TGT TCA GGG T CTC CTC CCG GTG-3’
Complementary DNA: (c-DNA1)	5’-TTTTTTCCG CCT CA GC CA C CGG GAG GAG -3’
probe DNA (p-DNA):	5’-SH- TTT GAG GAG GGT T TG GC TG AGG TTT TTT TTT TTT TTT TTT- NH_2-3’
bio-bar-code DNA (bbc-DNA)：	5’- TTT GAG GAG GGT T CC GC CC CCC CCC CCC - NH_2-3’
capture DNA (c-DNA2):	5’-AAA AAA AAA CCT CA TTT TTT- SH-3’

Table S3. The Sequences of the DNA in Scheme S1

DNA	Sequence
C-aptamer:	5'- SH-TAC AGA ACA CCG GGA GGA TAG TTC GGT GGC TGT TCA GGG T CTC CTC CCG GTG-3'
random DNA：	5'- SH-ATG TCT TGT GGC CCT CCT ATC AAGCCA CCG ACA AGT CCC ACTC CTC CCG GTG-3'
probe DNA (p2-DNA):	5'-NH_2 CCT AAT GTG CAC CGG GAG GAG-3'
bio-bar-code DNA (bbc-DNA)：	5'-NH_2 CCT AAT TCG CGC TAG ACC GCC-3'

Synthesis of CdSe-ZnS QDs. According to the literature,[S1] 0.0514g of CdO, 0.90 g of zinc acetate, 17.6 mmol of oleic acid, and 20 mL of 1-octadecne were placed in a 250 mL round flask. The mixture was heated to 150 ^{o}C, degassed with N_2 gas, and further heated to 300 ^{o}C. At this temperature, 0.0315 g of Se powder and 0.128 g of S powder were dissolved in 3 mL of TOP and then quickly injected into the reaction flask. After the injection, the temperature of the reaction flask was set to 300 ^{o}C and proceeded for 4~5 min, then it was cooled to room temperature to stop the growth. QDs were purified by adding 20 mL of chloroform and an excess amount of acetone (3 times), they were then redispersed in toluene.

According to the literature,[S1] the OA attached to the surface of QDs was replaced with mercaptopropionic acid (MPA) as follows: 10 mL of QDs dispersed in chloroform was placed in a 100 mL round flask, and 2 mL of MPA was added to the solution. The resulting reaction mixture was heated up to 60 ^{o}C for 1 h and cooled to room temperature. The MPA-capped QDs were extracted by centrifugation at 6000 rpm, purified twice with chloroform, and finally dissolved into 10 mL water of pH 8. The water-soluble QDs were obtained.

Optimization Conditions in Preparation of the PAMAM dendrimer NCs/QD-DNA probe. To control the size of the dendrimer nanocluster (NCs), the molar ratio between PAMAM dendrimers and NHS containing $BS(PEG)_5$ cross-linker was optimized. It was found that a molar ratio of 50:1 [NH_2]:[NHS] was appropriate to obtain the NCs with an average diameter of about 150 nm as determined by SEM images (Figure S1A). Thus 0.40 mg dendrimers and 70 μL of the crosslinking

agent NHS-PEG-NHS (250 μM in DMSO) were used in the experiment.

The molar ratio between the dendrimer NCs and QDs was also optimized. According to the SEM image, the diameter of dendrimer NC/QD Probe was about 200 nm, and the QD number assembled on each dendrimer NC was about 900, which was calculated as follows:

Dendrimer NC/QD Probe diameter = 200 nm

Probe Volume = $4/3\pi r^3 = 4.19 \times 10^{-15}$ cm^3

Dendrimer NC diameter = 150 nm

Dendrimer NC Volume = $4/3\pi r^3 = 4.2 \times 10^{-16}$ cm^3

Total QD Volume =4.19×10^{-15}- 4.2×10^{-16} = 3.77×10^{-15} (cm^3)

QD diameter = 10 nm, QD Volume = $4/3\pi r^3 = 4.19 \times 10^{-18}$ cm^3

QD number on each dendrimer NC = $3.77 \times 10^{-15}/4.19 \times 10^{-18} = 900$

In addition, the molar ratio between the DNA and dendrimer NCs/QDs was optimized. Different volume of 1.0×10^{-5} M probe-DNA was added to 100 μL of the resulting dendrimer NCs/QDs nanostructure, and incubated at 37 ^{o}C overnight with gentle mixing. Then the supernatant was taken for UV-vis absorbance detection. It was found that the absorbance markedly increased when the volume of the probe-DNA was more than 550 μL, indicating that the reaction of DNA with dendrimer NCs/QDs was saturated after 550 μL. Thus, 500 μL of 1.0×10^{-5} M biobarcode (bbc)-DNA and 50 μL of 1.0×10^{-5} M probe-DNA were the optimized values.

SEM Characterization of the dendrimer NCs and dendrimers NCs/QDs-DNA probe.

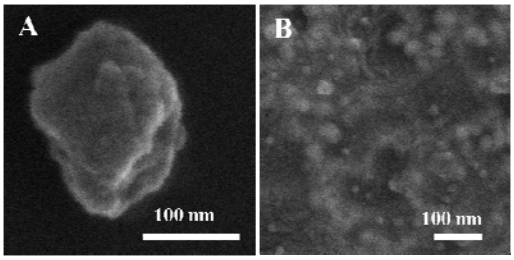
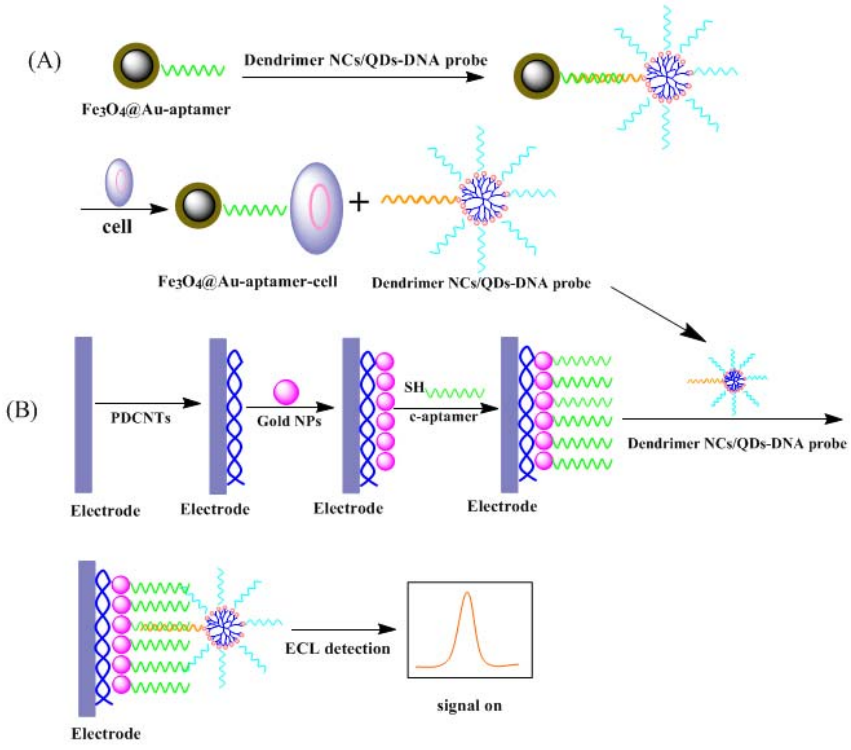


Figure S1. SEM images of A) dendrimer NCs and B) dendrimer NCs/QDs-DNA probe.

Preparation of PDDA-CNTs (PDCNTs). CNTs were chemically shortened by ultrasonic agitation in a mixture of sulfuric acid and nitric acid (3:1) for about 3 h. The resulting CNTs were separated and washed repeatedly with distilled water by centrifugation until pH was 7.0. Then 0.5 mg/mL CNTs were dispersed into a 0.25% PDDA aqueous solution containing 0.5 M NaCl and the resulting dispersion was sonicated for 30 min to give a homogeneous black suspension.

Residual PDDA polymer was removed by centrifugation, and the complex was rinsed with water. The collected complex was redispersed in water with mild sonicating.

Preparation for Signal-on ECL Detection of cancer cells based on Fe_3O_4@Au-aptamer and dendrimer NCs/QDs probe. The fabrication principle for signal-on ECL detection of cancer cells based on Fe_3O_4@Au-aptamer and dendrimer NCs/QDs probe was shown in Scheme S1. First, 40 μL of Fe_3O_4@Au MBs were transferred into a 1.5 mL Eppendorf tube and were washed three times with 500 μL of PBS buffer. Second, the MBs were resuspended in pH 7.4 PBS buffer and 50 μL of 1.0×10^{-6} M thiol-modified aptamer were added. Thiol-modified aptamer was activated with TCEP (10 mM) for 1 h before attaching to Fe_3O_4@Au. After shaking gently for 16 h at room temperature, the aptamer-Fe_3O_4@Au conjugates were "aged" in the solution (0.3 M NaCl, 10 mM Tris-acetate, pH 8.2) for another 24 h. Excess reagents were removed by magnetic force, followed by adding 100 μL of the above dendrimers-QDs-DNA probe solution and incubating at 37 ^{o}C for 2 h to obtain the MB-probe biocomplex.



Scheme S1. Schematic representation for A) Strategy of cell assay based on the Fe_3O_4@Au-aptamer biocomplex and dendrimer NCs/QDs-DNA probe, B) ECL detection of cancer cells based on the biocomplex.

The MB-probe biocomplex were incubated with 100 μL PBS containing different number of

cells at room temperature for 50 min. Then the dendrimer NCs/QDs-probe released from the MB-probe biocomplex were separated from the solution with a magnetic field.

The cleaned Au electrode was firstly immersed into 100 μL 1.0 × 10^{-6} M thiolated capture DNA 2 for 12 h, followed by immersing in 1 mM MCH for 30 min to eliminate nonspecific adsorption and washed carefully with 10 mM PBS. Then, the modified electrode was incubated in the dendrimer NCs/QDs-probe solution at 37 °C for 1 h to form ds-DNA. The electrodes were sequentially washed with 10 mM PBS to remove the unbinding ECL probe and performed the ECL measurements.

Bar graph comparison for the applications of the ECL assays.

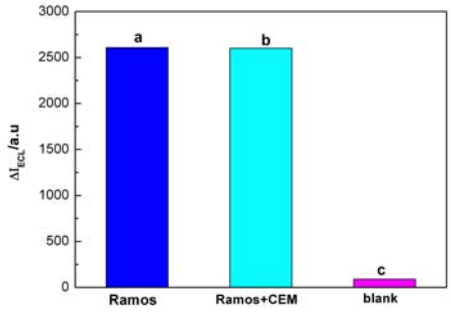


Figure S2. Bar graph showing the ECL responses to a) the target cells at 1000 cell mL^{-1}; b) the mixture of both the target cells and control cells at 1000 cell mL^{-1}; c) The blank PBS without cells.

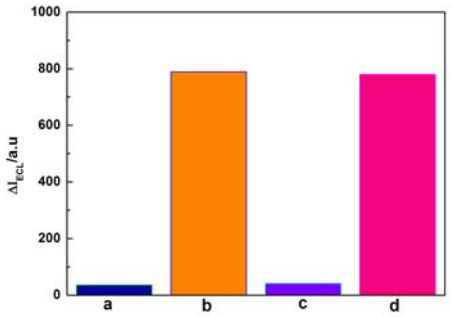


Figure S3. Bar graph showing the ECL responses to a) the control cells in cell media; b) the target cells in cell media; c) the control cells in serum; d) the target cells in serum. The concentrations of both the control cells and target cells were 1000 cells mL^{-1}.

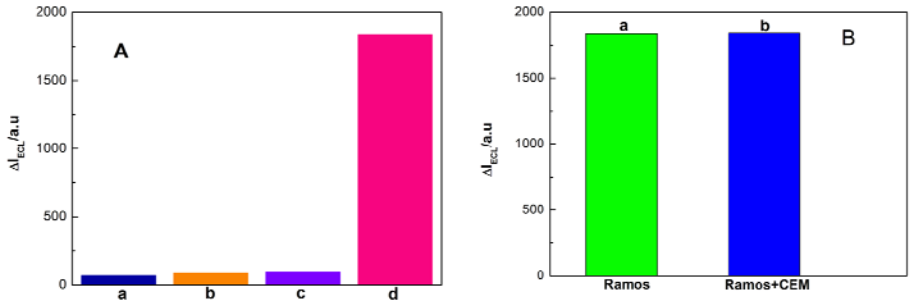


Figure S4. A) Bar graph showing the change of intensity between a) the blank PBS without cell; b) the PBS with the control CEM cells; c) the PBS with random DNA; d) the PBS with the target Ramos cells. The numbers of cells is 2000 cells mL^{-1}. B) Bar graph showing the change in ECL intensity between a) the pure target cells at 2000 cell mL^{-1}; b) the mixture of both the target cells and control cells at the same concentration of 2000 cell mL^{-1}.

Optimization for ECL Performance based on device cycle amplifying technique. The nicking endonuclease activity is very important to the cleave-substrate efficiency which is related to ECL intensity. The temperature is one of the key elements which determine the nicking endonuclease activity. The effect of temperature was examined from 20 ºC to 50 ºC (Figure S5A), and the maximum ECL intensity was at 37 ºC. In addition, the effect of nicking endonuclease incubation time was investigated. Figure S5B shows the influence of the incubation time of nicking endonuclease used in the DNA device. The ECL intensity increased from 40 min to 70 min and changed very little after 70 min. Therefore, the time of 70 min was chosen for nicking endonuclease.

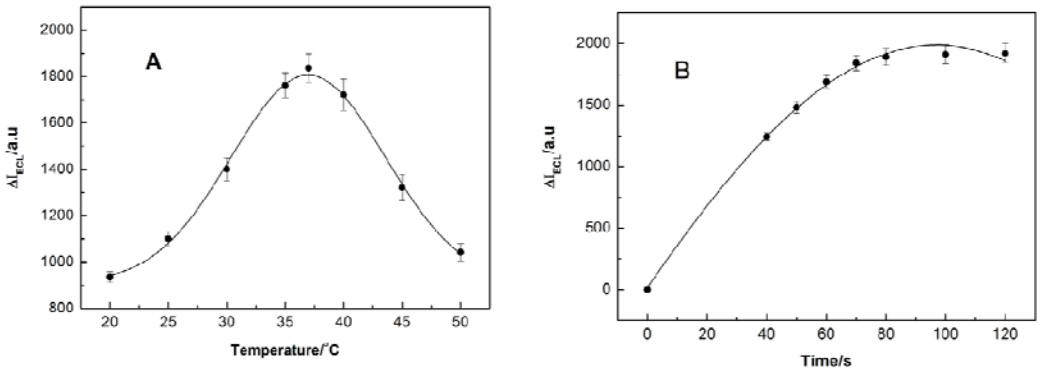


Figure S5 Optimization of (A) the nicking endonuclease reaction temperature and (B) the nicking endonuclease reaction time. All the ECL signals were detected in the presence of 1×10^{-7} mol mL^{-1}.

References

(S1) (a) Bae, W. K.; Char, K.; Hur, H.; Lee, S. *Chem. Mater.* **2008**, *20*, 531–539, (b) Bunge, S. D.; Krueger, K. M.; Boyle, T. J.; Rodriguez, M. A.; Headley, T. J.; Colvin, V. L. *J. Mater. Chem.,* **2003**, *13*, 1705–1709.