Cation exchange in aptamer-conjugated CdSe nanoclusters: a novel fluorescence signal amplification for cancer cell detection

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Experimental Section

1 Reagents

TE02 and TD05 aptamers were purchased from Sangon Biotech (Shanghai) Co., Ltd. Dynabeads M-280 streptavidin (2.8 μm) and Rhod-5N were purchased from Invitrogen. Tris(2-carboxyethyl)phos phosphane Hydrochloride, $CdCl_2 \cdot 2.5H_2O$, Na_2SeO_3, Reduced Glutathione (GSH), $NaBH_4$, $AgNO_3$ and Sulfo-NHS Biotin were purchased from Sigma-Aldrich. All the reagents were analytical grade and were used without further purification. Ultrapure water was used throughout (Millipore, 18.25 MΩ·cm).

3'-biotion-TE02 aptamer sequence:

TAGGCAGTGGTTTGACGTCCGCATGTTGGGAATAGCCACGCCTTTTTTTTTTT

3'-biotion-TD05 aptamer sequence:

AACACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCGGTGTTTTTTTTTT

2 Apparatus

High-resolution transmission electron microscopy (HRTEM) images were acquired using a JEM-2010 FEF transmission electron microscope operating at an accelerating voltage of 200 kV. UV-visible (UV-Vis) absorption spectra were obtained with a PerkinElmer Lambda 25 UV-Vis spectrophotometer. The fluorescence (FL) spectra were obtained by a fluorescent spectrometer (F900, Edinburgh Instruments Ltd.) (λ_{ex}=530 nm, λ_{em}= 576 nm). Dynamic light scattering (DLS) was measured on Malvern Zetasizer Nanoseries using 633 nm laser at 25 °C. Laser scanning confocal microscopy was carried out with a Leica SP5 confocal microscope (Leica Microsystems, Heidelberg, Germany).

3 Cell culture

Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma), CCRF-CEM cells (CCL-119 T-cell,

human acute lymphoblastic leukemia), K562 cells (CCL-243, chronic myelogenous leukemia) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin-streptomycin. The washing buffer contained 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS (Sigma). Binding buffer was prepared by adding yeast tRNA (0.1 mg/mL) (Sigma) and BSA (1 mg/mL) (Fisher) into the washing buffer to reduce background binding. The cell density was determined using a hemocytometer, and it was calculated prior to any experiments. Cells were dispersed in washing buffer, centrifuged at 1000 rpm for 5 min, and redispersed in binding buffer for incubation with nanomaterials. During all experiments, the cells were kept in an ice bath at 4 °C.

4 Synthesis of GSH-capped CdSe QDs

GSH-capped CdSe QDs were prepared using our previously reported method with slight modifications.[1] $CdCl_2 \cdot 2.5H_2O$ (0.0566 g) was dissolved in 50 mL ultrapure water, and GSH (0.0922 g), Na_2TeO_3 (0.01g) and $NaBH_4$ (0.1g) were added with vigorous stirring. After heating 1.5 h, GSH-capped CdSe QDs were obtained. The UV-Vis absorption, FL spectra and digital photos of the prepared CdSe QD solution were shown in Fig S1.

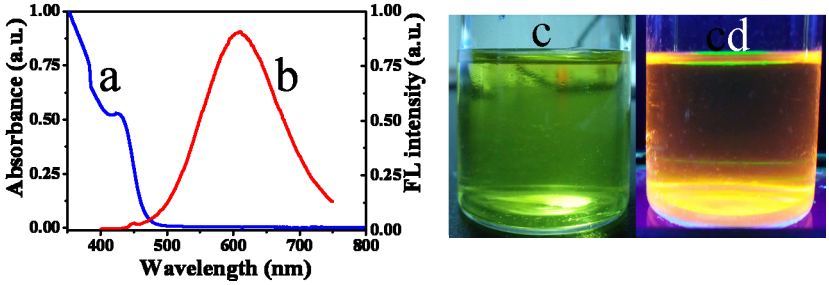
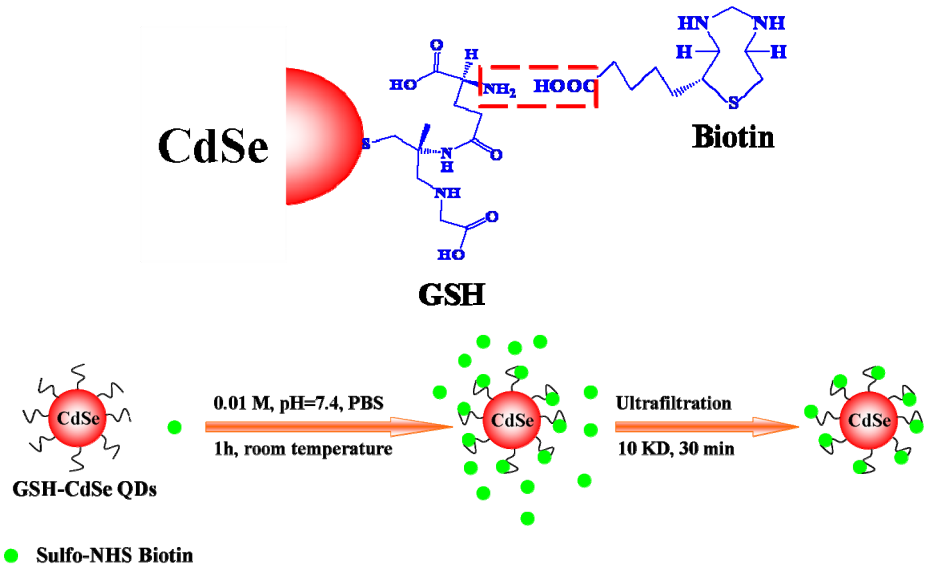


Fig S1 The UV-Vis absorption (a), FL (b) and digital photos of the prepared CdSe QDs (c: visible light, d: 365 nm UV light).

5 Synthesis of biotin-conjugated CdSe QDs

The preparation process of biotin-conjugated CdSe QDs was shown in Scheme S1. The CdSe QDs (1.0×10^{-7} M) was mixed with Sulfo-NHS Biotin stock solution (20 mg/mL, 100 μL) for 60 minutes at room temperature. After purification (10 KD, 500 μL, Millipore Ultra-filtration tube, 8000 rpm/min, 15 min), the biotin-conjugated CdSe QDs were obtained.



Scheme S1 The preparation process of biotin-conjugated CdSe QDs.

6 Synthesis of avidin-conjugated CdSe NCs

The biotin-conjugated CdSe QDs (1 mL, 1.0×10^{-7} M) was reacted with avidin (1.0×10^{-5} g/mL, 50 μL) in PBS buffer for 30 min. After centrifugal separation (4 ℃, 12000 rpm/min, 20 min), the precipitation (avidin-conjugated CdSe NCs) was collected and dispersed in PBS buffer.

7 Synthesis of TD05-aptamer conjugated CdSe NCs

TD05 aptamer-conjugated CdSe NCs were prepared through interaction between avidin and biotin. 3'-biotin-TD05 aptamer (50μL, 1.0×10^{-5} M) was mixed with avidin-conjugated CdSe NCs (500μL, 1.0×10^{-7} M) at room temperature for 30 min. After purification (50 KD, 500 μL, Millipore Ultra-filtration tube, 8000 rmb/min, 15 min), the TD05-aptamer conjugated CdSe NCs were obtained.

8 Detection of Ramos cells with signal amplification via cation exchange reaction

TD05-aptamer conjugated CdSe NCs (50μL, 1.0×10^{-7} M) and TE02-aptamer conjugated Dynal Magentic Beads (10μL, 1 mg/mL) were added into Ramos cells. After reaction 45 min, the target cells were separated with a separator having a high intensity magnetic field. Then, the 100 μL detection solution (0.1 M KAc, pH 7.4, 0.05% Tween 20, 500 μM $AgNO_3$, and 4 μM Rhod-5N) was added. The fluorescence was measured after the removal of magnetic particles. Optimization of the incubation time, concentration of Rhod-5N and the combination manner of aptamer pairs was performed by holding all other experimental conditions constant as described above and varying the corresponding duration only.

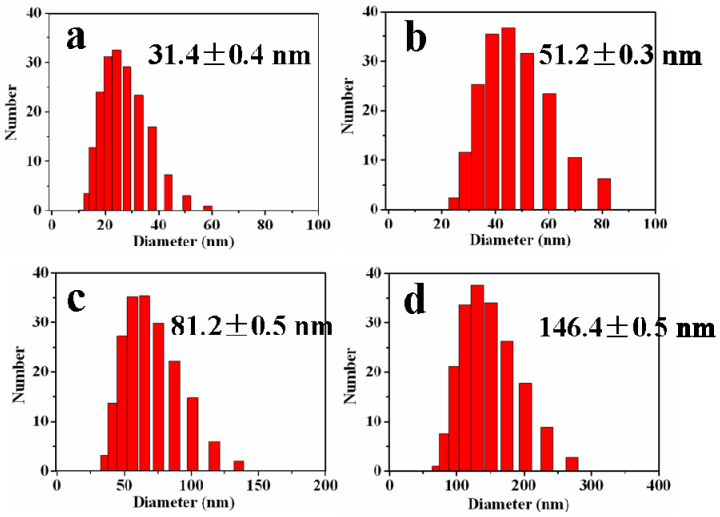


Fig. S2 DLS measurements of CdSe NCs using different volume of avidin (1.0×10^{-5} g/mL avidin, a: 10μL, b: 20μL, c: 50μL, d: 100μL .CdSe QDs: 1 mL, 1.0×10^{-7} M).

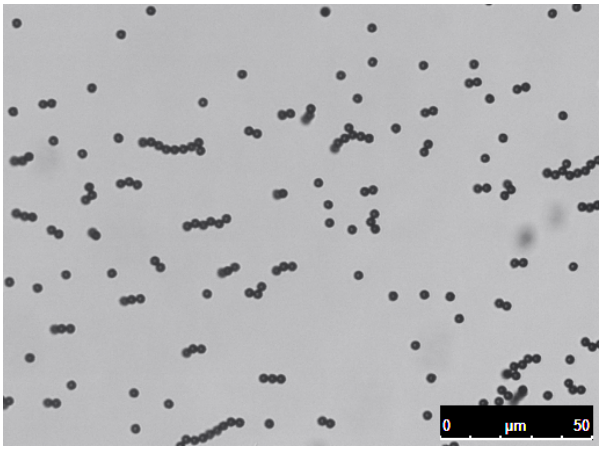


Fig. S3 The optical micrograph of streptavidin conjugated Dynal Magentic Beads. The average particle size is 2.8 μm.

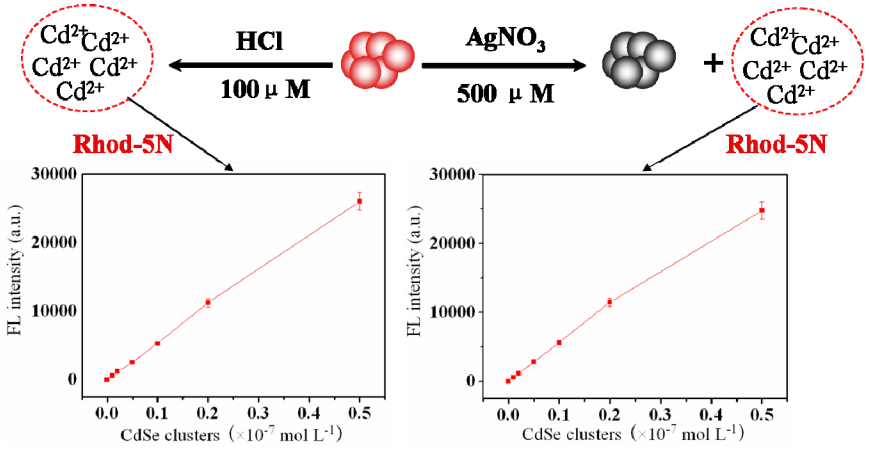


Fig. S4 The comparison effects of acid dissolution and cation exchange reaction on the fluorescence of Rhod-5N.

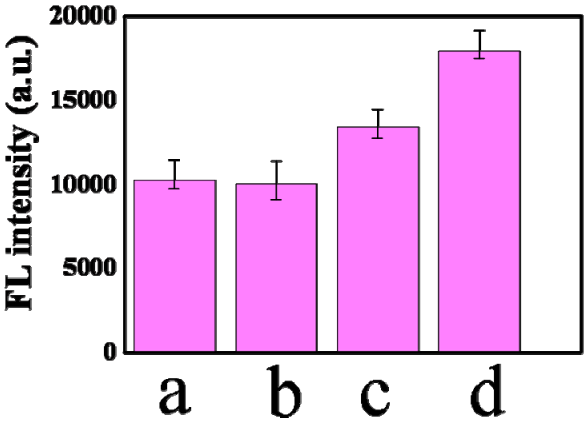


Fig S5 Responses of different aptamer pair-based cation-exchange reaction in CdSe nanoclusters. (a: TE02-TE02, b: TD05-TD05, c: TE02-TD05, d: TD05-TE02, Ramos cells: 1000 cells/mL, Rhod-5N: 4μM, CdSe NCs: 1.0×10^{-7} M).

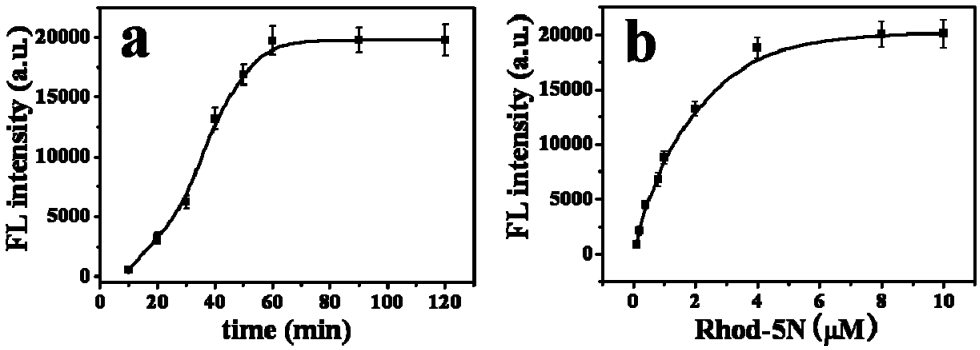


Fig. S6 Effects of incubation time and concentration of Rhod-5N dye on the fluorescent intensity of Rhod-5N (Ramos cells: 1000 cells/mL).

Table S1 Comparison between the proposed method and other reported techniques for detection of Ramos cells

Detection method	Detection system	Detection range	Detection limint[a]	Refs
Circular strand-displacement polymerization	FL	1.0×10^{2}-1.0×10^{4}	100	2
Carbohydrate-functionalized CdS nanocomposite	ECL	1.0×10^{2}-3.0×10^{4}	58	3
Signal probe encapsulated by DNA	CL	2.0×10^{2}-3.0×10^{4}	126	4
Strip biosensor	Abs	8.0×10^{3}-4.0×10^{5}	800	5
The proposed method	FL	5.0×10-1.0×10^{3}	50	

ECL: electrogenerated chemiluminescence, CL: chemluminescence, Abs: absorption spectra.
a: in cells/mL.

Compared with the ECL method, our proposed fluorescence signal amplification for cancer cell detection has some advantages. First, the detection process was completed in homogeneous solution without complex surface modifications. Second, our proposed method has a high

selectivity, and can be used to detect mixed cancer cell samples using magnetic separation technology.

Reference

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