Supporting Information

One-step Engineering Silver Nanocluster Aptamer Assembly as Luminescent Label of Target Tumor cells

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Experimental Section:

Materials: All the DNA sequences used in this study were purchased from Sangon Biotechnology Co., Ltd. (shanghai, China). Sodium borohydride (>96%), Magnesium chloride hexahydrate (\geq 98%), Sodium chloride (\geq 99.5%) and Silver nitrate (\geq 99.8%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Unless otherwise noted, all reagent-grade chemicals were used as received without further purification. Deionized water was prepared by the Milli-Q ultrapure water system (18.2MQ • cm⁻¹, Millipore System Inc.).

Synthesis of AgNCs by templated DNA: In this work, all the DNA-templated AgNCs were prepared using the method developed by Dickson.^{1c} DNA strand was first dissolved in deionized water. DNA and AgNO₃ were mixed by vortexing in deionized water, and kept on ice in the dark. After 1 hour, 100 μ M of freshly prepared NaBH₄ was added with vigorous shaking, and then stored at 4°C in the dark for 24 h. The molar rate of DNA, AgNO₃ and NaBH₄ was 1:6:6. Final concentrations were 16.7 μ M of DNA strand, 100 μ M of AgNO₃ and 100 μ M of NaBH₄ in water, respectively.

The stability assays of AgNCs-aptamer assembly: The DNA-templated AgNCs were prepared in water. And 50 μ L of the AgNCs was mixed in 200 μ L with 5mM MgCl₂, 1mg/mL BSA (Sigma) and 4.5g/L glucose in Dulbecco's PBS. The results

were performed by using fluorescence spectrometer with excitation was at 488 nm after 12h.

Characterization: The fluorescence measurements were all achieved by Hitachi F-7000 fluorescence spectrometer (Hitachi. Ltd., Japan). A quartz fluorescence cell was used. The excitation was at 488 nm, and the recording emission range was 510-800 nm. All excitation and emission slits were set at 5 nm. All these detections were recorded at room temperature. High Response Transmission Electron Microscopy (HRTEM) was performed on a JEOL-3010 microscope operating at an accelerating voltage of 200 kV.

Cell lines and Buffer: CCRF-CEM cells (CCL-119, T lymphoblast, human acute lymphoblastic leukemia) and Ramos cells (CRL-1596, Blymphocyte, human Burkitt's lymphoma) were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences. The cells were cultured in RPMI 1640 medium supplemented with 12% fetal bovine serum (FBS, heat inactivated) and 100 IU/mL penicillin-streptomycin. The same buffer was used to wash and incubate. It contained 5mM MgCl₂, 1mg/mL BSA (Sigma) and 4.5g/L glucose in Dulbecco's PBS (Sigma). The cell density was determined by using a hemocytometer, and this was performed prior to any experiments. Cells were dispersed in this buffer, centrifuged at 2000 rpm for 5 min, and redispersed in the buffer for incubation with Aptamer–Ag NCs. During all experiments, the cells were kept in Thermostated Mixing Block (MB-102, BIOER) bath at steady 4 °C.

Flow Cytometry Analyses: Fluorescence measurements were obtained by using FACScan cytometer (FACScalibur, BD Bioscience, USA) in order to demonstrate the capabilitiy of AgNCs aptamer assembly toward target cells. A detailed procedure of the binding experiments as follow: the cells were obtained from the culture medium and then centrifuged at 2000 rpm for 5 min, and redispersed in the buffer, and about 3×10^5 cells were dispersed in 200 µL with Ag NCs aptamer assembly, and the mixture was incubated at 4 in thermostated Mixing Block for 40 min. The mean

fluorescence was determined by counting 10,000 events. AgNCs without aptamer were used as a negative control. AgNCs with the other cells' aptamer were used as a positive control. The signal was monitored in channel 2 for AgNCs.

Cell Imaging: After cells incubation with AgNCs aptamer assembly, the fluorescence images of them were taken by depositing 30 μ L of the incubated suspensions onto a thin clean glass above a 40×4 objective on the confocal microscope. The fluorescence images were conducted by a laser scanning confocal microscope. (Fluorescence Ag NCs aptamer assembly channel: EX 488nm, EM 535-565 nm band pass.)

Figure Legend:

Name	Sequence (5'-3')
C12	CCCCCCCCCCC
A2-C12	AACCCCCCCCCCC
A4-C12	AAAACCCCCCCCCCC
A6-C12	AAAAAACCCCCCCCCCC
A8-C12	AAAAAAACCCCCCCCCCC
A10-C12	AAAAAAAAACCCCCCCCCCC
T2-C12	TTCCCCCCCCCC
T4-C12	TTTTCCCCCCCCCC
T6-C12	TTTTTTCCCCCCCCCC
T8-C12	TTTTTTTCCCCCCCCCC
T10-C12	TTTTTTTTTCCCCCCCCCC
G2-C12	GGCCCCCCCCCC
G4-C12	GGGGCCCCCCCCCC
G6-C12	GGTGGGGCCCCCCCCCC
G8-C12	GGTGGTGGGGCCCCCCCCCC
G10-C12	GGTGGGGTGGGGCCCCCCCCCC
Sgc8c-A6-C12	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGAAAA
•	AAACCCCCCCCCCC
TD05-A6-C12	AACACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCG
	GTGAAAAACCCCCCCCCCC
Sgc8c-G8-C12	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGAGGT
	GGTGGGGCCCCCCCCCC
TD05-G8-C12	AACACCGGGAGGATAGTTCGGTGGCTGTTCAGGGTCTCCTCCCG
	GTGGGTGGTGGGGCCCCCCCCCC

Table S1. Names and sequences of the oligonucleotides. All the sequences were used in this

work.



Figure S1. (A) The Fluorescence emission spectra of sgc8c-AgNCs assembly in water (solid line) and binding buffer (dotted line) after 12h. (B) The time-dependent fluorescence spectra of sgc8c-AgNCs assembly and TD05-AgNCs assembly in binding buffer for 3600s. ($\lambda \text{ ex}$ = 480 nm, $\lambda \text{ em}$ =560 nm, slit width: 5 nm)



Figure S2. Specific recognition of Ramos cells with the TD05-AgNCs assembly. (A) The result of flow cytometry assays by counting 10,000 events. (B) The confocal laser scanning microscopy images of the TD05-AgNCs assembly incubated Ramos cells and CCRF-CEM cells. (a) and (b) represented the fluorescent image and merged image of TD05-AgNCs assembly incubated Ramos cells; (c) and (d) represented the fluorescent image and merged image of TD05-AgNCs assembly incubated CCRF-CEM cells. Scale bar is 20 μm.