Electronic Supplementary Information (ESI)

In situ fluorescence activation of DNA-silver nanoclusters as a label-free and general strategy for cell nucleus imaging

Duo Li,‡ Zhenzhen Qiao,‡ Yanru Yu, Jinlu Tang, Xiaoxiao He, Hui Shi,* Xiaosheng Ye, Yanli Lei and Kemin Wang*

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Hunan University, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Changsha, Hunan 410082, China.

* E-mail: kmwang@hnu.edu.cn. Phone/Fax: +86-731-88821566.

* E-mail: huishi_2009@hnu.edu.cn. Phone/Fax: +86-731-88821566.

‡ These authors contributed equally to this work.

Contents

EXPERIMENTAL SECTION

Chemicals and Materials. All DNA probes used in this study were custom-designed and then synthesized by Sangon Biotechnology Company, Ltd. (Shanghai, China). Sequences of the oligos were listed in **Table S1**. Sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) and sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$) were purchased from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). Hoechst 33342 was obtained from Sigma. Unless otherwise noted, all the chemicals were used as received without further purification. Deionized water was prepared by the Milli-Q ultrapure water system (18.2 MΩ·cm, Millipore System Inc.).

Cell Culture. SMMC-7721 cells (human hepatocellular cancer cell line), Hela cells (human cervical carcinoma cell line), HBL-100 cells (human breast cell line) and CCRF-CEM cells (T lymphoblast, human acute lymphoblastic leukemia cell line) were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in RPMI 1640 medium supplemented with 15% fetal calf serum (FBS, heat inactivated) and 100 IU/mL penicillin–streptomycin, and incubated at 37 ℃ in a humidified incubator containing 5% wt/vol CO_2.

Preparation of DNA-AgNCs. In this work, all the fluorescent DNA-templated AgNCs were synthesized using the method developed by Dickson.[1,2] Briefly, 10 μL of DNA templates (100 μM) was firstly mixed with 10 μL of phosphate buffer (pH7.0, 200 mM). Then, 10 μL of $AgNO_3$ (900 μM) was added to provide a Ag^+-to-DNA molar ratio of 6:1. After vortexing and resting on ice in the dark for 30 min, 10 μL of freshly prepared $NaBH_4$ (900 μM) was added with vigorous shaking to form AgNCs, which were then stored at 4 ℃ in the dark before use.

Characterization of DNA-AgNCs. In this work, fluorescence measurements of AgNCs were routinely carried out using a Hitachi F-7000 fluorescence spectrometer (Hitachi. Ltd., Japan). To inspect the fluorescence enhancement effect of AgNCs in

buffer, AgNCs (templated by NC-a), G-rich sequences (b-15G, b-T1, b-T2, b-T3, b-T4 or b-T5) and C-ab were mixed in phosphate buffer at a ratio of 1:1:1 and incubated at 37 ℃ for 30 min. Then, the fluorescence spectra were measured at 37 ℃ using a 0.2 ×1 cm^2 quartz fluorescence cell with a bandwidth of 5 nm. Control groups were treated through the same procedure without addition of G-rich sequences and/or C-ab.

The morphology of DNA-AgNCs was studied using high resolution transmission electron microscopy (HRTEM). Samples were made by spin coating 10 μL of as-prepared AgNCs templated by NC-a onto carbon-coated copper grid substrates. After drying naturally overnight under dark condition, the samples were measured by Tecnai G^2 F20 S-TWIN TEM.

Cell Fixation. Cells were fixed using a methanol-acetate (3:1) mixture as cell fixative. Briefly, cells in culture medium were collected through centrifugation at 2000 rpm for 5 min, and re-dispersed in the cell fixative for 8 min at room temperature. Then, the fixed cells were collected through centrifugation at 3000 rpm for 8 min and dispersed in phosphate buffer for subsequent experiments.

Flow Cytometry Assays. A FACScan flow cytometer (FAC Scalibur, BD Bioscience) was used to explore the feasibility of intracellular fluorescence activation of AgNCs and the related influence factors. A typical procedure for flow cytometry assays was described as below. About 3×10^5 cells were incubated with AgNCs (360 nM) in 200 μL of phosphate buffer on ice for 60 min. Then, without washing and separation steps, the cell samples were analyzed directly on the flow cytometer by counting 10000 events. The fluorescence of AgNCs was detected in channel 3 with an excitation of 488 nm and an emission over 650 nm. At special notes, the working conditions varied.

Confocal Fluorescence Microscopy Imaging. A typical procedure was described as below. Cells were firstly incubated with AgNCs (30 μM) at 37 ℃ for 2 h in the dark and then dispersed in phosphate buffer. Next, 5 μg/mL Hoechst 33342 (blue light emission with UV light excitation) was used to stain cell nucleus at 37 ℃ for 10 min.

Finally, cells were observed with oil immersion lens on a laser scanning confocal microscope (AgNCs channel: EX 488 nm, EM 560 nm long-pass).

Specially for investigation of the nuclear targeting ability of AS1411, fixed 7721 cells were firstly incubated with AgNCs (30 μM) at 37 ℃ for 2 h in the dark, and then incubated with 100 nM FITC-AS1411 (or FITC-Control) on ice for 30 min. Hoechst 33342 (5 μg/mL) was used as a contrast agent for cell nucleus staining 5 min before imaging.

Table S1. All of the oligonucleotides used in this work. [α]

Name	Sequence (5'-3')
NC-a	CCCTTAATCCCCATCTAACTGCTGCGCCGCCGGGAAA
b-15G	TACTGTACGGTTAGATGGGTGGGGTGGGGTGGGG
C-ab	TTTTCCCGGCGGCGCAGCAGTTAGATATCTAACCGTACAGTA
NC-R0	CCCTTAATCCCC
NC-R2	CCCTTAATCCCCNN
NC-R3	CCCTTAATCCCCNNN
NC-R4	CCCTTAATCCCCNNNN
NC-R5	CCCTTAATCCCCNNNNN
NC-R10	CCCTTAATCCCCNNNNNNNNNN
NC-R15	CCCTTAATCCCCNNNNNNNNNNNNNNN
NC-R30	CCCTTAATCCCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NC-R45	CCCTTAATCCCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NC-30	CCCTTAATCCCCTATTTCAAGCCGGAAATAGCAATAAGACAG
b-T1	TACTGTACGGTTAGATTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG
b-T2	TACTGTACGGTTAGATTTAGGGTTAGGGTTAGGG
b-T3	TACTGTACGGTTAGATGGGATTGGGATTGGGATT
b-T4	TACTGTACGGTTAGATGGGATTGGGATTGGGATTGGGATTGGGATT
b-T5	TACTGTACGGTTAGATGGGATTGGGGGGATTATT
Cy5.5-NC-R10	(Cy5.5)-CCCTTAATCCCCNNNNNNNNNN
FITC-AS1411	(FITC)-GGTGGTGGTGGTTGTGGTGGTGGTGG
FITC-Control	(FITC)-NNNNNNNNNNNNNNNNNNNNNNNNN

[α] The template sequence for AgNCs synthesis is showed in orange. The G-rich sequence for AgNCs activation is showed in purple.

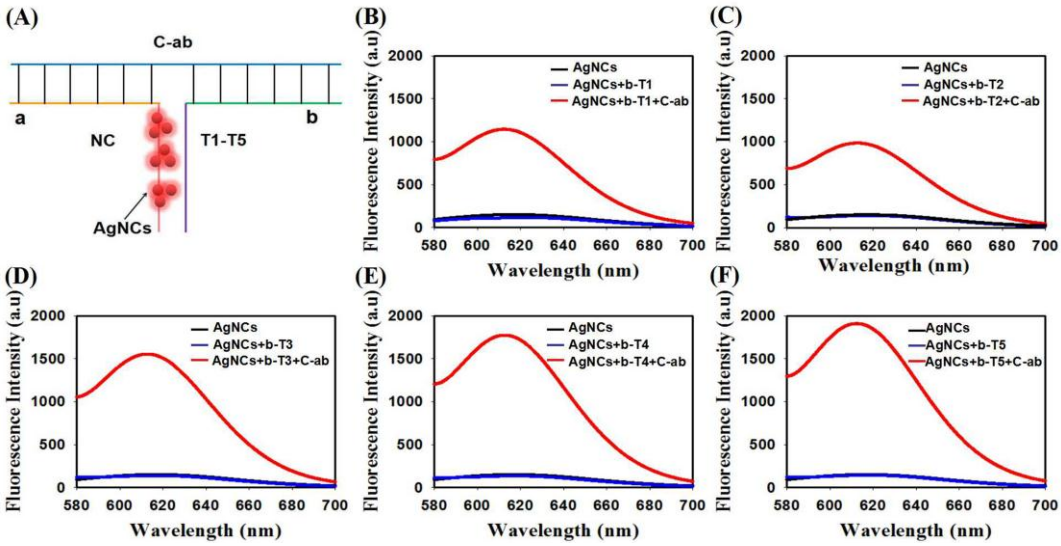

Figure S1. Fluorescence activation feasibility investigation of DNA-AgNCs induced by different G-rich sequences. (A) The schematic diagram of the proximity-induced fluorescence enhancement strategy, in which AgNCs and G-rich sequences are pulled close to each other by C-ab DNA probe through complementary hybridization. (B-F) Fluorescence emission spectra of AgNCs templated by NC-a (10 μM), before and after proximity to (B) T1, (C) T2, (D) T3, (E) T4 and (F) T5, respectively. The DNA hybridization process was carried out for 30 min at 37 ℃ in phosphate buffer. The concentration of G-rich sequences and C-ab was 10 μM.

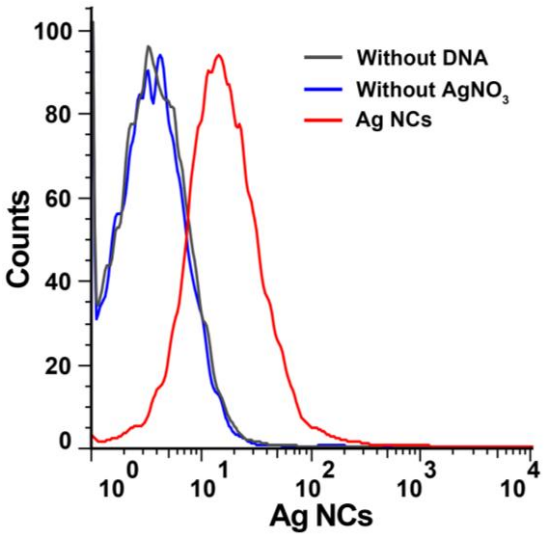

Figure S2. Verification of substances causing intracellular fluorescence enhancement. Flow cytometry assay results of fixed CEM cells after incubation with different samples on ice for 60 min. AgNCs were synthesized using NC-a as template through reduction of $AgNO_3$ by $NaBH_4$. Control (without DNA) group and control (without $AgNO_3$) group were treated through the same procedure without addition of NC-a and $AgNO_3$, respectively.

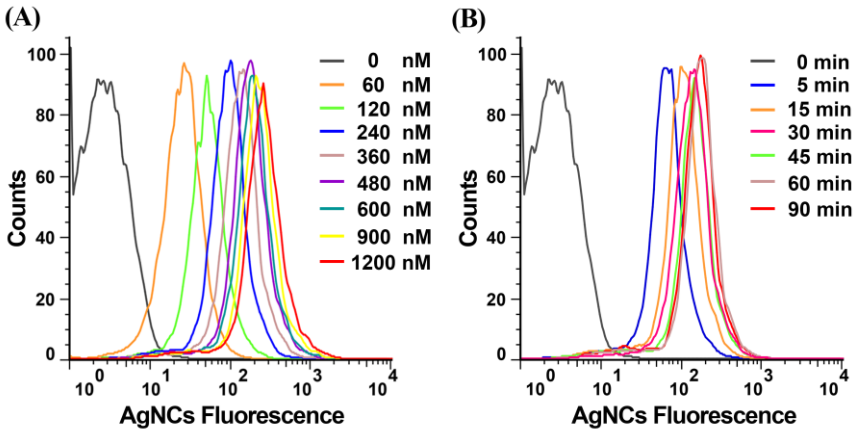

Figure S3. Investigation of factors affecting intracellular fluorescence enhancement of AgNCs. (A) Flow cytometry assay results of fixed CEM cells after incubation with different concentrations of AgNCs (templated by NC-a) on ice for 60 min. (B) Flow cytometry assay results of fixed CEM cells after incubation with 360 nM AgNCs (templated by NC-a) on ice for different time.

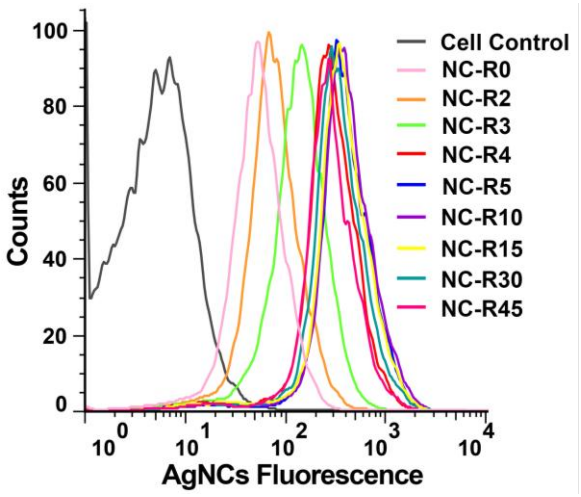

Figure S4. Influence of the number of random bases at the end of synthetic template (NC) on the fluorescence enhancement of AgNCs in CEM cells. Flow cytometry assay results of fixed CEM cells after incubation with AgNCs (368 nM) prepared using different templates for 60 min on ice, corresponding to **Figure 2B**.

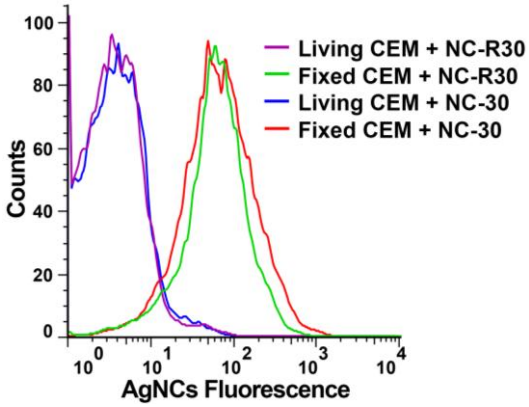

Figure S5. Influence of the tailing sequences of the synthetic template (NC) on the fluorescence enhancement of AgNCs in CEM cells. Flow cytometry assay results of fixed or living CEM cells incubated with AgNCs (368 nM) prepared using NC-R30 or NC-30 for 60 min on ice.

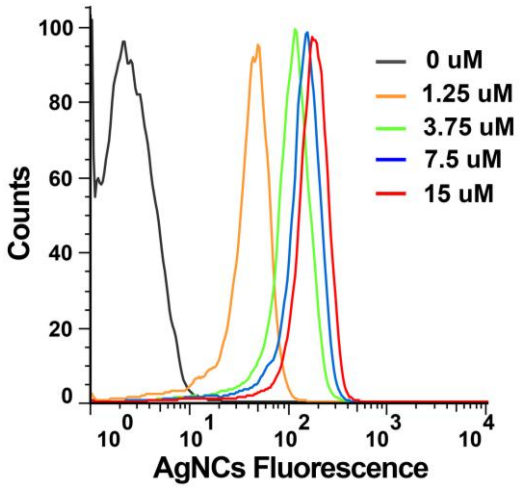

Figure S6. Applicability investigation of the DNA-AgNCs based strategy for nucleus imaging of living cells. Flow cytometry assay results of living CEM cells incubated with different concentrations of AgNCs (templated by NC-R10) for 60 min on ice.

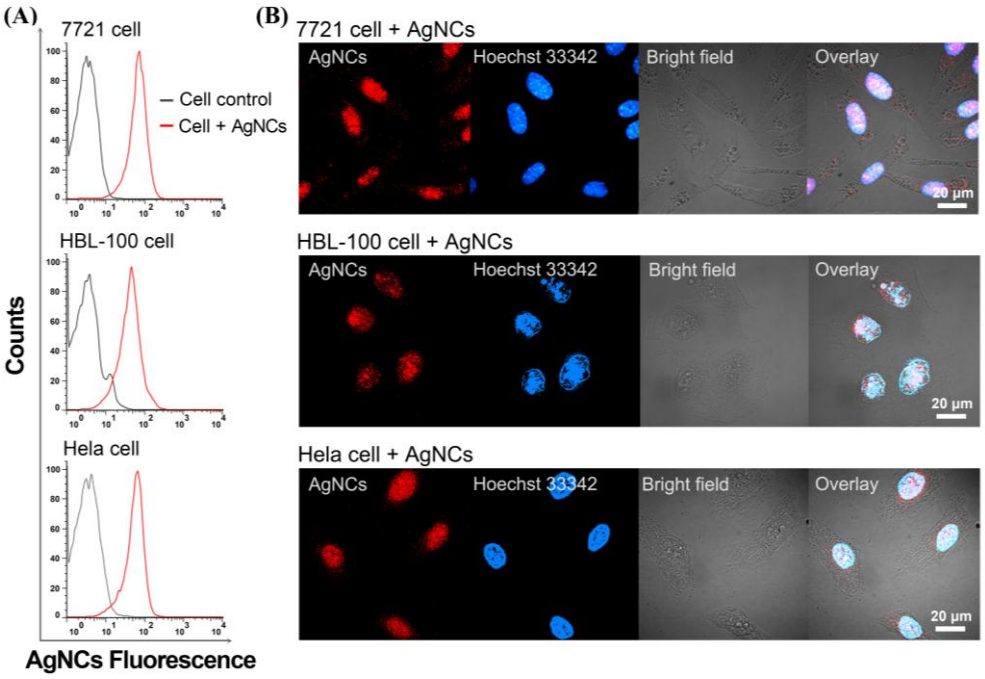

Figure S7. Applicability investigation of the DNA-AgNCs based strategy for nucleus imaging of different kinds of cell lines. (A) Flow cytometry assay results of different fixed cells after incubation with 368 nM Ag NCs (templated by NC-R10) for 60 min on ice. (B) Confocal fluorescence microscopy imaging of different fixed cells co-stained by AgNCs (templated by NC-R10) and Hoechst 33342.

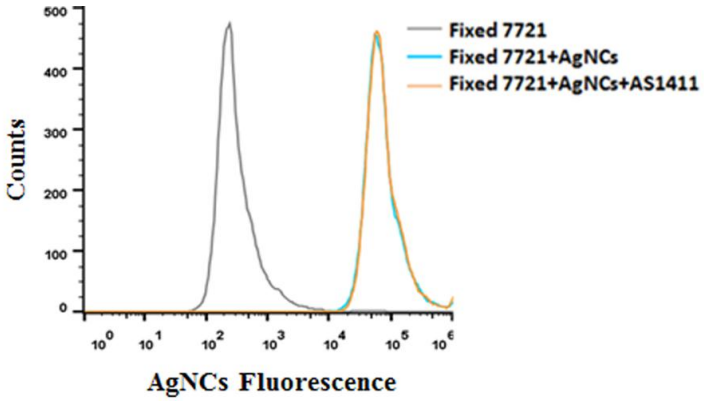

Figure S8. Investigation of the influence of AS1411 on the fluorescence of AgNCs in cells. Flow cytometry analysis of AgNCs-stained 7721 cells (fixed), before and after incubation with AS1411 (100 nM) on ice for 30 min. The cell staining was conducted using AgNCs (30 μM, templated by NC-R10) through incubation at 37 ℃ for 2 h.

References：

1. Petty, J. T.; Zhang, J.; Hud, N. V.; Dickson, R. M. *J. Am. Chem. Soc*. **2004**, *126*, 5207-5212.

2. Yu, J. H.; Choi, S.; Dickson, R. M. *Angew. Chem. Int. Ed*. **2009**, *48*, 318-320.