Electronic Supplementary Information

Targeted DNA-driven catalytic assembly lighting-up ratiometric fluorescence of biemissive silver nanoclusters for amplified biosensing

Jiayang He, Yuxuan Zhang, Zehui Chen, Chong Li, Ruo Yuan*, and Wenju Xu*

Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

*Corresponding authors. Tel.: +86 23 68252277; fax: +86 23 68253172.
E-mail address: xwju@swu.edu.cn (W.J. Xu), yuanruo@swu.edu.cn (R. Yuan).

Experimental section

Reagents and chemicals.

Together with target HIV-related DNA (hDNA), all the synthetic oligonucleotides purified by high performance liquid chromatography were provided by Sangon Biotech Co., Ltd (Shanghai, China). Analytical grade silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Kelong Chemical Company (Chengdu, China). The stock solutions of all oligonucleotides (100 μM) were prepared by using phosphate buffer saline (PBS, 20 mM, pH 7.4) containing 20 mM KH_2PO_4 and Na_2HPO_4, and 5 mM $Mg(NO_3)_2$ and stored at 4 °C for subsequent use. Rhodamine 101 was got from Aladdin (Shanghai, China). Ultra GelRed (Polypropylene gel electrophoresis stain) was ordered from Vazyme Biotech Co., Ltd (Nanjing, China). Human serum samples were obtained from the Xinqiao Hospital, Army Medical University (Chongqing, China). Ultrapure water was prepared by an ATsrese Ultra-pure water system with 18.2 MΩ of electrical resistance.

Table S1 lists the base sequences of the oligonucleotides used in this work. Among them, hDNA (21-nt) is totally complementary to the bolded bases of recognition hairpin (RH), the underlined letters of which is complementary to those of catalytic hairpin (CH). The merged italicized bases (26-nt) of RH and CH can hybridize with those of hairpin beacon (HB) that is especially designed to encode the G-AgNCs sequence template (12-nt) in 5'-end. By ligating the G-AgNCs bases, all the underlined letters (22-nt) in HB are acted as the template to wrap R-AgNCs. As highlighted in bolded letter(s), hDNA with one-, two- or three-mismatched base(s) is

designated as M1, M2 and M3, respectively.

Table S1 Base sequences of oligonucleotides used in this work.

Names	Sequence (5'→3')
hDNA	**ACTGCTAGAGATTTTCCACAT**
Recognition hairpin (RH)	TTCCACATCTTCATCTTCATC**ATGTGGAA*AAT CTCTAGCAGT***
Catalytic hairpin (CH)	*CTTCATCTTCATC*TTCCACATGATGAAGATGAA GATGTGG
Hairpin beacon (HB)	**TTCCCACCCACC**CCGGCCCGTT*ACTGCTAGA GATTGATGAAGATGAAG*AACGGGCCGG
One mismatch (M1)	ACTGCTAG**C**GATTTTCCACAT
Two mismatch (M2)	ACTGCTAG**C**GAT**G**TTCCACAT
Three mismatch (M3)	ACTGCTAG**C**GAT**G**TTC**A**ACAT

Apparatus.

By setting 900 V of photomultiplier tube voltage and 5 nm excitation slits, Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) was used to measure the fluorescence emission. With a quartz cuvette (100 μL) of 10 mm light path, UV-2450 spectrophotometer (Tokyo, Japan) was utilized to collect the UV-Vis absorption spectra by setting an interval of 0.5 nm in the wavelength range of 400 nm to 700 nm. The transmission electron microscope (TEM) and atomic force microscopy (AFM) images of AgNCs were obtained on FEI Tecnai G2 F20 S-Twin (America) and Bruker NanoWizard 4 BioScience Atomic Force Microscope (Karlsruhe, Germany), respectively. Native polyacrylamide gel electrophoresis (PAGE) photograph was got from Bio-Rad ChemiDoc XPS (California, America).

Synthesis of HB-bearing AgNCs.

In order to ensure the formation of hairpin secondary structures within DNA molecules, the stock solutions of RH, CH and HB were initially pretreated by heating to 95 °C for 5 min and cooling down to room temperature. After that, each 10 μL of RH, CH and HB with the same concentration (10 μM) was mixed with 10 μL of hDNA of different concentrations. By vigorously shaking for 30 s, the resultant solution was reacted at room temperature for 1 h, followed by the addition of 10 μL of freshly prepared $AgNO_3$ (60 μM). When incubating in dark environment at 4 °C for 0.5 h, 10 μL of fresh $NaBH_4$ (60 μM) was introduced to reduce Ag^+ ions, allowing R-AgNCs nucleation. Meanwhile, G-AgNCs were formed by locating in the overhang tail of free HB adopting hairpin-shaped configuration. After kept in the dark at 4 °C for 0.5 h, the final solution was diluted to 100 μL with PBS (pH 7.4) for fluorescence measurements of G-AgNCs and R-AgNCs. For control experiments, the same processes were performed in the absence of hDNA (Blank), RH, CH or HB.

Fluorescence measurement.

To study the 3D fluorescence contour maps of the prepared G-AgNCs and R-AgNCs in our ratiometric system, the excitation wavelength and the emission wavelength from 450 nm to 700 nm were implemented using 10 nm increment steps. After excited at 467 nm and 572 nm, their fluorescence emission spectra were collected in the wavelength of 530 nm-620 nm, and 600 nm-680 nm, respectively. The corresponding fluorescence intensity of G-AgNCs at 550 nm (F_G) and R-AgNCs at 620 nm (F_R) was used to obtain the ratio (F_R/F_G) for the quantification of hDNA.

Moreover, the fluorescence quantum yield of R-AgNCs (Q_U) acted as unknown probe in our detection system was estimated using Rhodamine 101 (Q_S) as control standard. To this end, we recorded their UV-Vis absorption spectra from 400 to 670 nm and fluorescence emission spectra within 580 nm to 700 nm of R-AgNCs and Rhodamine 101, respectively, where these parameters could be obtained: F_U and F_S, the integrals in fluorescent emission; A_U and A_S, the maximum absorption in UV-Vis spectra. Q_U could be calculated by using this equation, $Q_U = Q_S \times \frac{F_U}{F_S} \times \frac{A_S}{A_U}$.[1,2]

Native polyacrylamide gel electrophoresis.

After different oligonucleotide samples (10 μL, 2 μM) were mixed and incubated at 37 °C for 2 h, 2.5 μL 6×loading buffer was added. The native polyacrylamide gel electrophoresis (PAGE) was run in 1×TBE buffer at 120 V for 90 min, followed by staining with 10 μL of Ultra GelRed solution for 25 min. Under the irritation of UV light, the gel picture was taken by Bio-Rad ChemiDoc XPS.

Results and discussion

Characterizations.

To verify the reliability of ratiometric detection for hDNA, firstly we carried out the native polyacrylamide gel electrophoresis (PAGE) of different oligonucleotide samples. From Fig. S1A, the pure hDNA, RH, CH and HB exhibited their own characteristic mobility (lane 1, 2, 3 and 4). In contrast, lowered electrophoretic movement was observed for the mixture of hDNA and RH (lane 5), as well as those after successively adding CH (lane 6) and HB (lane 7), revealing the successful

hybridization reactions of hDNA, RH, CH and HB. However, simply mixing RH and CH only gave two separate bands that were significantly different from lane 5, suggesting the absence of hDNA in the assay system disabled hDNA-mediated isothermal assembly of RH and CH. As such, R-AgNCs could not be nucleated because of the unavailability of corresponding capping scaffold without unfolded HB.

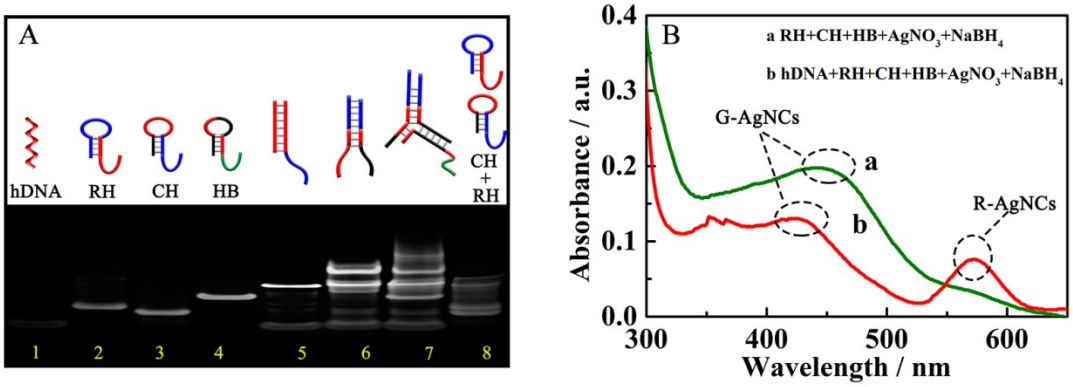

Fig. S1. (A) PAGE image of hDNA (lane 1), RH (lane 2), CH (lane 3), HB (lane 4), hDNA+RH (lane 5), hDNA+RH+CH (lane 6), hDNA+RH+CH+HB (lane 7) and RH+CH (lane 8). All the samples (each 2.0 μM) were incubated at 25 ℃ for 2 h. (B) UV-Vis absorption spectra of the detection system without (a) and with (b) hDNA in. 10 nM of hDNA and 1.0 μM of each oligomers were used.

On the other hand, the feasibility of ratiometric assay was also demonstrated by recording UV-Vis absorption spectra of HB-bearing AgNCs. As displayed in Fig. S1B, the biggest absorption of G-AgNCs occurred at about 430 nm. After adding hDNA, a new absorption peak at 572 nm appeared, and correspondingly the absorption at 467 nm weakened with a red transfer of 37 nm, indicating the successful formation of R-AgNCs and simultaneously decreased amount of G-AgNCs in the presence of hDNA.

Transmission electron microscope (TEM) and atomic force microscopy (AFM)

Fig. S2 illustrated the TEM and AFM images of G-AgNCs and R-AgNCs. Obviously, they were well distributed in the homogeneous detection system and displayed an average size of about 2.08 nm-2.21 nm (Fig. S2A and S2C) and 2.63 nm-2.75 nm (Fig. S2B and S2D), respectively. The size difference of molecule-like Ag clusters might be attributed to various template sequence and length, rationally bringing variable fluorescent brightness.[1,3-4] Basically, the similar results were also observed in other reported methodologies with predesigned DNA-scaffolding AgNCs as signal emitters.[5-8]

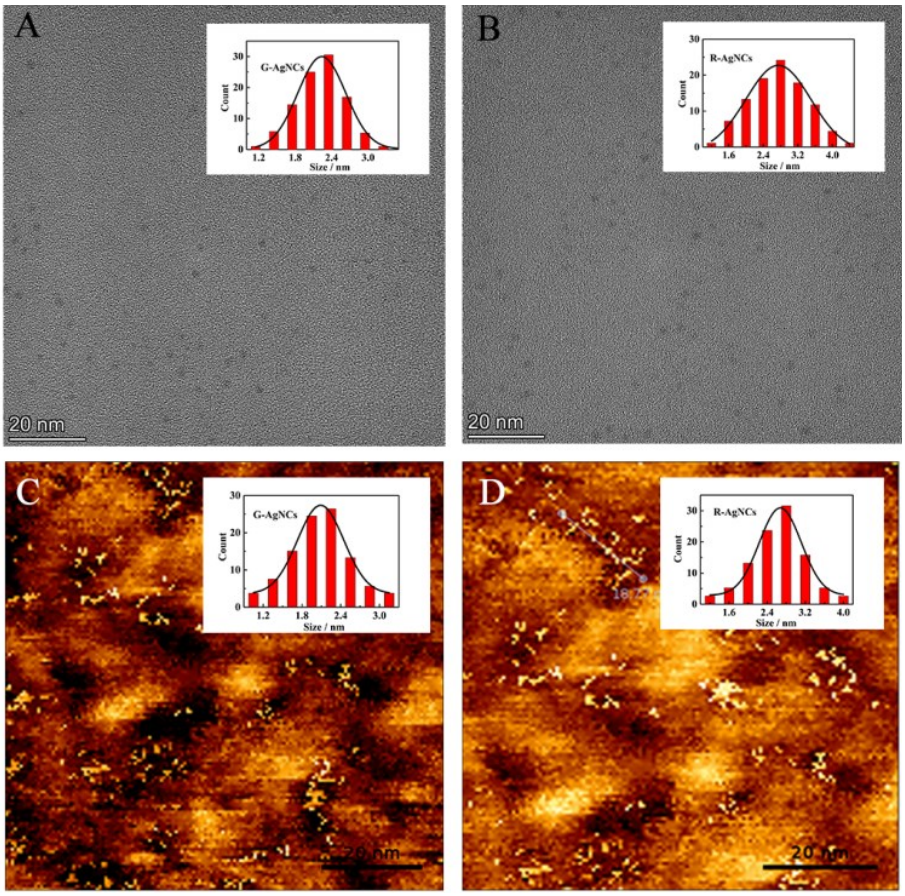

Fig. S2. TEM (A and B) and AFM (C and D) images of G-AgNCs (A and C) and R-AgNCs (B and D), together with the size distribution shown in the insets.

Demonstration of amplified ratiometric fluorescence.

In our design, hDNA-driven isothermal assembly of RH and CH is crucial to amplify duplex outputs, thereby enabling the open of hairpin-shaped HB for R-AgNCs formation and achieving ratiometric signal readout. Firstly we measured the quantum yield (Q_U) of R-AgNCs by recording UV-Vis absorption and fluorescence emission of R-AgNCs and Rhodamine 101 (Fig. S3). Based on these observations, the Q_U was calculated to be 93.83%, which was similar to that reported in the literature,[1] demonstrating R-AgNCs acted as hDNA-responsible emissive probes for ratiometric fluorescence was feasible.

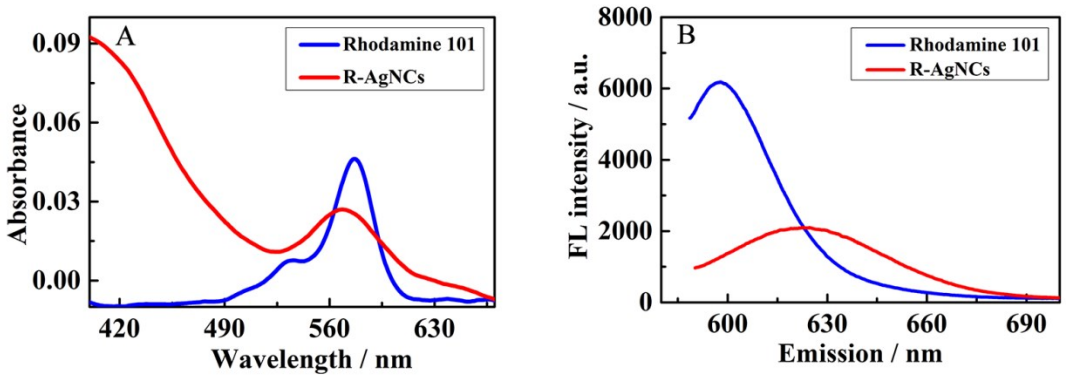

Fig. S3 (A) UV-Vis absorption spectra of Rhodamine 101 and R-AgNCs. (B) Fluorescence emission spectra of Rhodamine 101 and R-AgNCs.

To further verify the effectiveness of hDNA-mediated amplification, we studied the fluorescence spectra of G-AgNCs and R-AgNCs in different systems without introducing hDNA (Blank), RH, CH and/or HB. From Fig. S4A and S4B, the absence of individual RH, CH or HB led to almost unchanged absolute fluorescence intensity (FL) that was the same as Blank. Whereas, upon introducing hDNA, decreasing of FL for G-AgNCs and increasing for R-AgNCs were obtained, respectively, revealing that

the lowered amount of G-AgNCs ('off' fluorescence) and risen R-AgNCs ('on' fluorescence) were sensitive to hDNA. This was because of hDNA-driven duplex outputs for successfully and significantly amplifying the ratiometric signal.

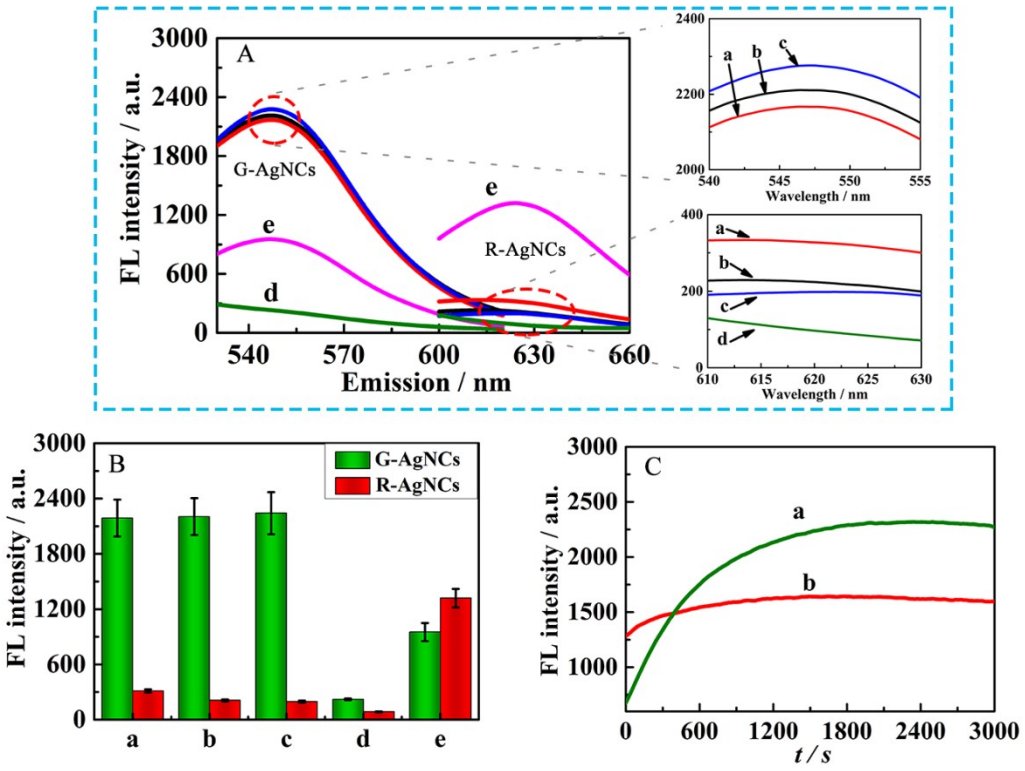

Fig. S4 Fluorescence emission spectra (A and B) in different condition without (a) hDNA (Blank), (b) RH, (c) CH or (d) HB in comparison with (e) our detection system. (C) Real time fluorescence of (a) G-AgNCs and (b) R-AgNCs. Error bars: standard deviation (*s*), *n*=5.

Besides, the time-dependence of fluorescence response for R-AgNCs was also investigated within 3000 s. As shown in Fig. S4C, the fluorescent emission was gradually brighter with duration of up to 1800 s. This might well-support the long-term stability and rapid detection of R-AgNCs as hDNA-sensitive ratiometric signal probe.

Optimization of reaction time.

The reaction time of different species was optimized by measuring the

corresponding fluorescent emission. Firstly, we monitored the fluorescent intensity of G-AgNCs (F_R) and R-AgNCs (F_G) within 120 min for the hybridization of hDNA, RH, CH and HB (fixing clusters formation in 30 min). As shown in Fig. S5A, gradually increased F_R and decreased F_G were observed, and they leveled off at about 60 min. After reaction of hDNA, RH, CH and HB for 60 min, the emission intensity of G-AgNCs and R-AgNCs was also investigated in different binding time of Ag^+ ion from 0 to 60 min. Obviously, both F_R and F_G were increased and reached to the maximum at 30 min (Fig. S5B). As such, all the next experiments were carried out under the optimized conditions, unless otherwise noted.

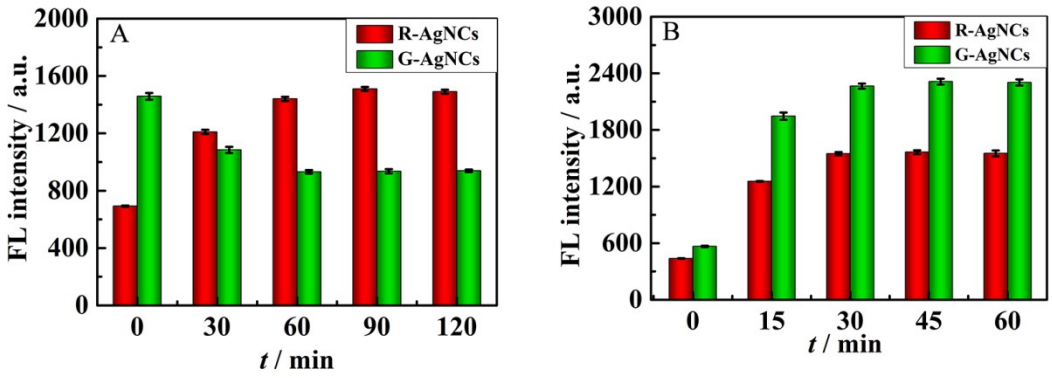

Fig. S5 The fluorescence response of the detection system in different reaction time: (A) hDNA, RH, CH and HB within 120 min by fixing 30 min for clusters formation, and (B) binding time of Ag^+ ion from 0 to 60 min after reaction of hDNA, RH, CH and HB for 60 min. The concentration of hDNA was (A) :50 nM, (B) :50 nM and 0 nM. Error bars: *s*, *n*=5.

Table S2 Strategies and analytical performance of diverse methodologies based on different DNA-scaffolding AgNCs as fluorescent emitters

Strategy	Targets	Linear range	LOD	Ref.
AgNCs molecular beacons	Pb^{2+}	5 nM to 50 nM	1 nM	3

Ratiometric fluorescent DNA Radar	DNA	0 to 2 and 2.5 to 5.5 μM	0.52 nM/ 0.92 nM	5
DNA-AgNCs as ECL emitters	RNA	1 aM to 104 fM	0.96 aM	9
MOF enhance fluorescence of AgNCs	RNA	10 fM to 100 nM	5.0 fM	10
PDAN@AgNC for fluorescence detection	AFP /CEA	10 to 100 nM	2.4 nM/ 5.6 nM	11
Metal nanoclusters as fluorescent probes	Cyt c	0 μM to 1 μM	15 nM	12
Ratiometric fluorescence of hairpin beacon-templated AgNCs	DNA	0.01 to 50 nM	4.0 pM	Our work

ECL: electrochemiluminescence, MOF: Metal-organic frameworks, PDAN@AgNC: polydopamine nanosphere@silver nanocluster, AFP: alpha-fetoprotein, CEA: carcinoembryonic antigen, Cyt c: cytochrome c.

Table S3 Recovery of hDNA in human serum samples using our ratiometric method

Samples	Spiked (nM)	Measured (nM)	Recovery (%)	RSD (%)
1	0.10	0.0963	96.3	0.84
2	1.0	1.06	106	3.6
3	10	9.30	93.0	7.5

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