Electronic Supplementary Information

Dimerizing DNA-AgNCs *via* C-Ag^{+}-C structure for fluorescence sensing with dual-output signals

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Experimental section

1.1. Materials and regents

The DNA were purchased from Sangon Biotechnology (Shanghai, China) and could be used without practicing further purification. The DNA we used were listed in Table S1. Silver nitrate ($AgNO_3$, 99.8%), sodium borohydride ($NaBH_4$, 98%), Cysteine (Cys, 99%), glutathione (GSH, 98%), different amino acids, fructose, sucrose, glucose, ascorbic acid and urea were supplied by Aladdin Bio-chem technology (Shanghai, China). Other metal ions were purchased from Chemical Reagent Company (China). All solutions were prepared using Millipore Milli-Q water (18.2 $M\Omega \cdot cm^{-1}$).

1.2. Apparatus

The DNA-AgNCs were characterized by high-resolution transmission electron microscope (HRTEM, JEOL, Japan) and Zeta sizer Nano-ZSE (Malvern Instruments, UK). UV-Vis absorption spectra were obtained on a Shimadzu UV-2450. Fluorescence measurements were carried out on a Hitachi F-7100. The samples for fluorescence measurements were excited from 460 nm to 770 nm with the emission wavelength range from 500 nm to 900 nm, and the fluorescence spectra were acquired with the slit widths (Ex/Em) of 5 nm/ 5 nm, unless otherwise noted. Circular dichroism (CD) spectra were acquired using MOS-500 equipped with CD cell holder. The samples containing DNA (DNA only, DNA-AgNCs, DNA-AgNCs / Cys, DNA-AgNCs / Ag^+) were diluted to a final volume of 200 μL. Quartz Hellma (QS) cuvettes were used to measure the samples.

1.3. Preparation of fluorescence DNA-AgNCs

According to typical methods, DNA-AgNCs were prepared with appropriate modification[1], and the following were the specific steps. Firstly, 20 μL of DNA solution (100 μM) was mixed with 70 μL of HOAc-NaOAc (200 mM, pH 5.6) and 5 μL of $AgNO_3$ solution (5 mM) was introduced into the above solution and incubated at 4℃ for 20 min. Subsequently, 5 μL of $NaBH_4$ (2.5 mM) freshly prepared was added. After being mixed with violent shaking, the solution was then incubated in darkness at 4℃ for 24 h. When the value of [Ag^+/C] changed, [DNA] kept 20 μM and [$NaBH_4$] was half of [Ag^+]. Finally, the prepared DNA-AgNCs solution was stored at 4 ℃ until use.

1.4. Detection of Cys

Cys aqueous solutions used were freshly prepared. For detection, 50 μL of dimerized DNA2-AgNCs (1 μM) and Cys with equivalent volume were mixed and fluorescence (FL) spectrum was recorded immediately in the wavelength range from 770 to 900 nm at room temperature under 750 nm excitation. To study the selectivity of DNA2-AgNCs to Cys over other interfering substrates. The following substrates were used: glutathione (GSH), lysine (Lys), l-glutamine (Gln), l-glutamic acid (Glu), l-glycine (Gly), l-histidine (His), l-leucine (Leu), l-phenylalanine (Phe), fructose, sucrose, glucose, ascorbic acid and urea. The interfering substances with 20-fold concentration Cys

were mixed with DNA2-AgNCs in ddH_2O, and the FL spectra was recorded immediately (Ex = 750 nm).

1.5. Procedures for Ag^+ detection

The method for synthesizing DNA3-AgNCs followed above steps in 1.3. Different concentrations of Ag^+ (50 μL) were mixed with 10 μM DNA3-AgNCs (50 μL), and the FL spectra were recorded for the detection of Ag^+. To study the selectivity of DNA3-AgNCs to Ag^+ over other interfering substrates. The following substrates were used: $Cd(NO_3)_2$, $Al_2(SO_4)_3$, $Cu(OAc)_2$, $Hg(NO_3)_2$, $Mg(OAc)_2$, $Ca(OAc)_2$, $Zn(OAc)_2$, $CoCl_2$.

1.6. Detection of Cys and Ag^+ in real sample

Milk samples were purchased from the local supermarket and the real water was obtained from Xiangjiang and Taozi Lake (Changsha, China). The milk samples were degreased under the condition of centrifugation at 2000 rpm for 15 min and 3000 rpm for 30 min at 4 ℃. Then the content of Cys was then diluted to 1.92 μM and stored in 4 ℃. As described in above, the diluted milk with total Cys concentration from 1.0 μM to 4.0 μM were detected using dimerized DNA2-AgNCs-based fluorescence sensor. The real water with a total spiked Ag^+ concentration of 10 μM and 25 μM were detected using monomer DNA3-AgNCs sensor, finally the concentration of Cys and Ag^+ was calculated.

1.7. Preparation of fluorescent AgAuNCs

DNA2-AgAuNCs were synthesized by one-step method according to previously reported methods with a slight modification, and the following provides the specific steps[2]. Firstly, 20 μL of DNA solution (100 μM) was mixed with 70 μL of NH_4OAc (10 mM, pH 7.0). A volume of 5 μL of $AgNO_3$ solution (5 mM) and a volume of 2.5 μL of $HAuCl_4$ (5 mM) were then added to the mixture and incubated at 4℃ for 20 min. Subsequently, 5 μL of $NaBH_4$ (2.5 mM) prepared with iced water was added. After being mixed with violent shaking, the mixture was then incubated in darkness at 4 °C for 4 h. Finally, the prepared DNA_4-AgAuNCs solution was stored at 4 °C until use.

Results and Discussion

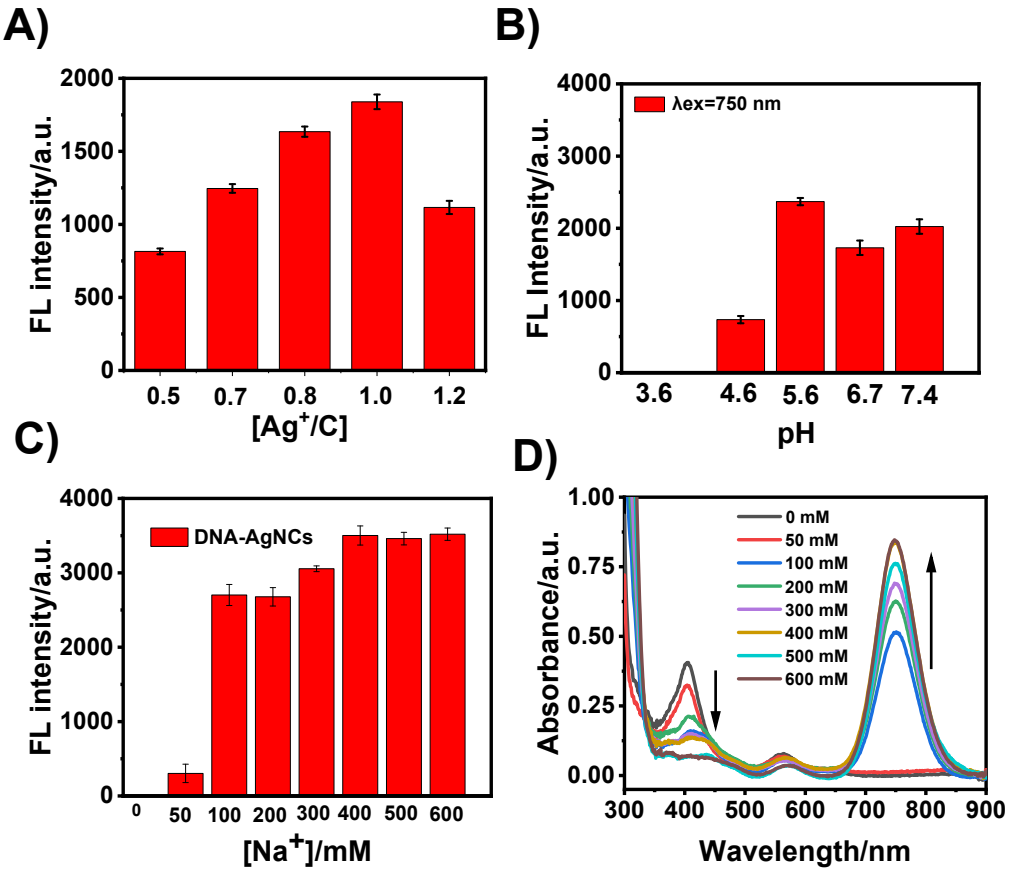

Fig. S1 Optimal synthesis of DNA2-AgNCs. (A) FL intensity of the DNA2-AgNCs changed with the [Ag^+/C] at 0.5, 0.7, 0.8, 1.0 and 1.2 (pH=5.6 and [Na^+] 100 mM). (B) FL intensity of the DNA2-AgNCs with pH at 3.6, 4.6, 5.6, 6.7 and 7.4 ([Ag^+/C] at 1.0 and [Na^+] 100 mM). (C) FL intensity and (D) UV-Vis absorption spectra of the DNA2-AgNCs with the [Ag^+/C] at 1.0 changed with the [Na^+] at 0, 50, 100, 200, 300, 400, 500 and 600 mM. Excitation wavelength: 750 nm.

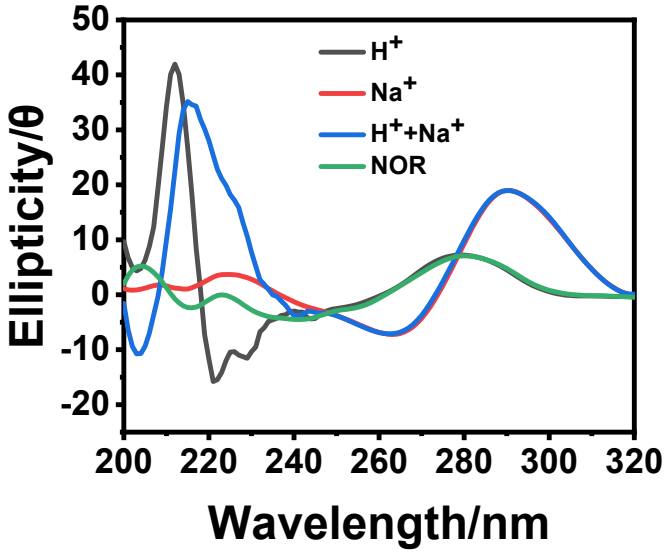

Fig. S2 CD spectra of 10 μM DNA2 alone at four different buffers. (H^+ meant pH=5.6, Na^+ meant pH=7.4 and Na^+, H^++Na^+ meant pH=5.6 and Na^+ and NOR meant pH=7.4).

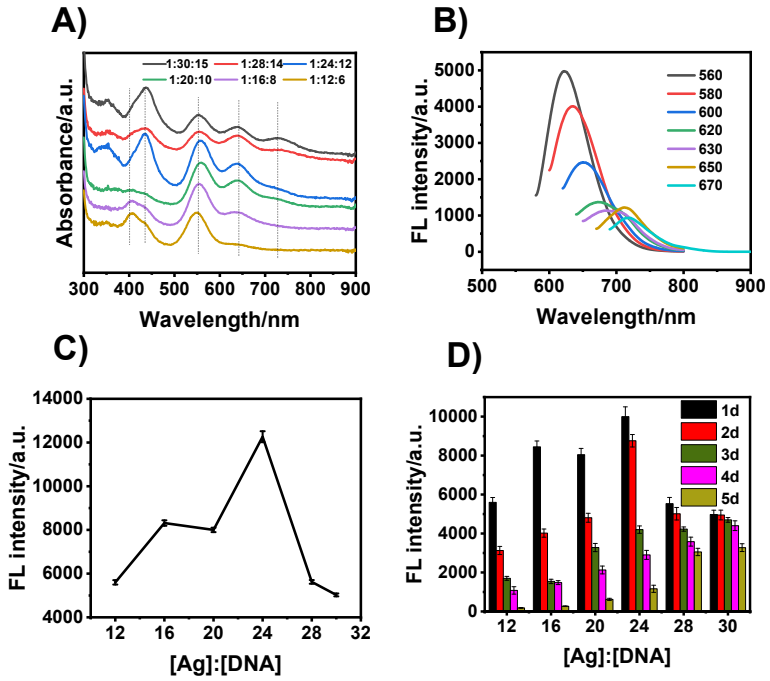

Fig. S3 Synthesis and properties of DNA2-AgNCs in the solution without Na^+. (A) and (C) was the UV-Vis and FL intensity of synthesized DNA2-AgNCs without Na^+ under the conditions of [Ag]/[DNA] 12, 16, 20, 24 and 28, respectively. (B) FL of DNA2-AgNCs at [Ag]/[DNA] 28 with different excitation wavelength. (D) Changes in fluorescence intensity of DNA2-AgNCs within 5 days under different [Ag]/[DNA] conditions (Ex=560 nm/Em=620 nm).

It can be seen from Fig. S3A that DNA2-AgNCs was synthesized without Na^+ by adjusting [Ag]/[DNA] from 12 to 28. With the increase of [Ag]/[DNA], the UV-VIS absorption spectrum red-shift from 550 nm to 750 nm, indicating that the particles of

DNA2-AgNCs gradually grew larger. As shown in Fig. S3B, when [Ag]/[DNA] was 28, the red emission of DNA2-AgNCs (λex/λem=560/620 nm) still occupied a dominant position, indicating that the structure of DNA2-AgNCs has not undergone major changes, and as [Ag]/[DNA] was 24, the red emission of DNA2-AgNCs remained unchanged. When the fluorescence intensity reached the highest, the [Ag]/[DNA] at 24 ([Ag]/[C] ratio of 1:1) was the most favorable for the synthesis of DNA2-AgNCs. As [Ag]/[DNA] exceeded 24 ([Ag]/[C] ratio exceeded 1:1), the fluorescence decreased significantly, showing that excessive Ag^+ content was unfavorable to the synthesis of AgNCs (Fig. S3C). Then, the solutions were placed at room temperature to characterize their stability through fluorescence changes within a week. As shown in Fig. S3D, DNA2-AgNCs with a high [Ag]/[DNA] ratio showed the most obvious fluorescence stability within a week.

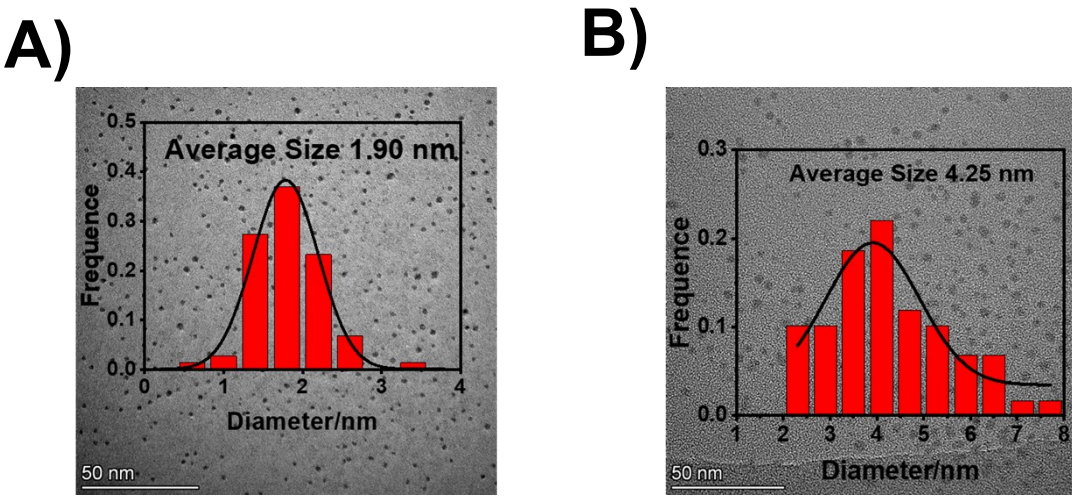

Fig. S4 TEM images of synthesized DNA2-AgNCs with [Ag^+/C] 0.5 (A) and 1.0 (B). Scale bar is 50 nm.

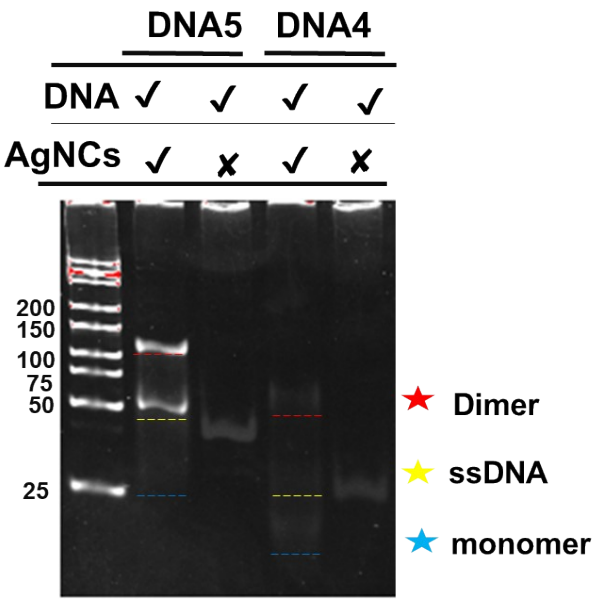

Fig. S5 PAGE proved the possibility of dimerization. The versatility of this method to form dimerized DNA5-AgNCs and DNA4-AgNCs.

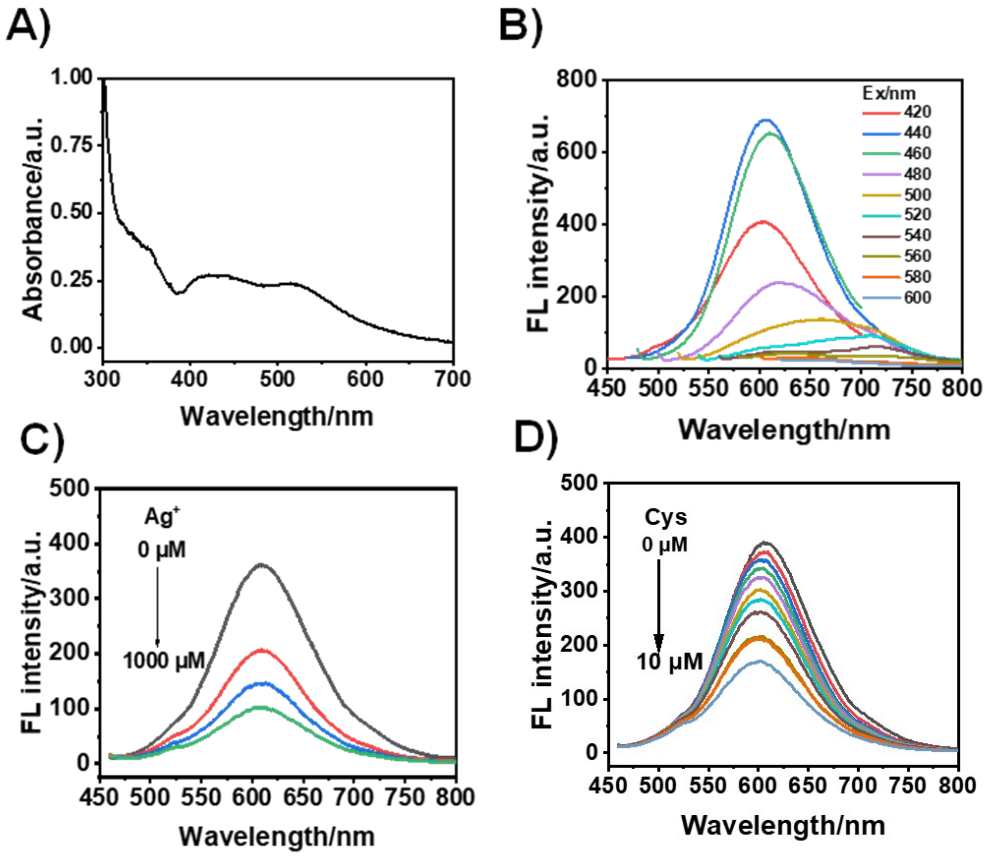

Fig. S6 The properties of DNA2-AgAuNCs. (A) The UV-Vis absorption spectra of DNA2-AgAuNCs with the ratio of [Ag]:[Au]:[NaBH4] 1:0.5:0.5 and (B) the FL spectra of DNA2-AgAuNCs at different excitation wavelength. (C) and (D) was FL spectra of DNA2-AgAuNCs with different concentrations of Ag^+ and Cys, respectively. Ex=440 nm.

Fig.S6 demonstrated that UV-Vis absorption spectrum showed obvious characteristic absorption peak in the range of 400-600 nm and the maximum FL emission wavelength is 600 nm.

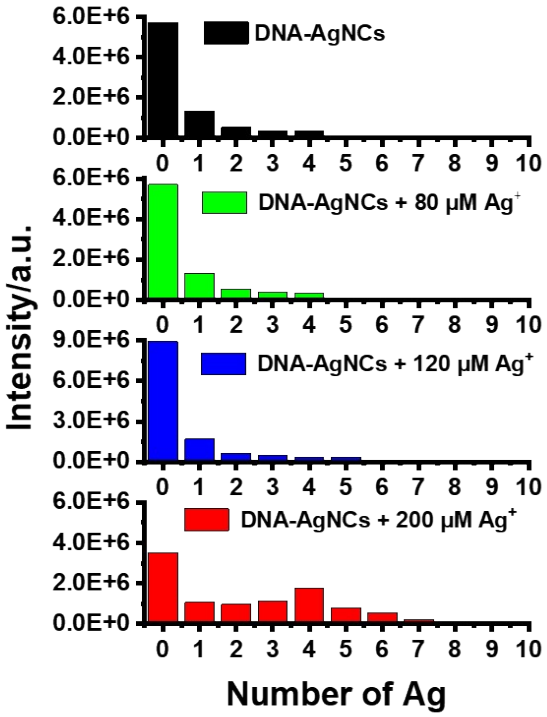

Fig. S7 The trend of growing number of Ag atom in DNA3-AgNCs when the input of extra Ag^+ to the monomer DNA3-AgNCs increased from 0 to 200 μM in the MS data. In the figure, the chief component were Ag_1NCs, Ag_2NCs, Ag_3NCs, Ag_4NCs. After addition of $AgNO_3$ (200 μM), a large proportion of AgNCs, containing Ag_3NCs, Ag_4NCs grew and Ag_5NCs, Ag_6NCs appeared. It was deduced that the DNA3-AgNCs dimer consists of two strands of DNA3 bridged by 1~4 Ag^+, hence the total number of Ag was 7.

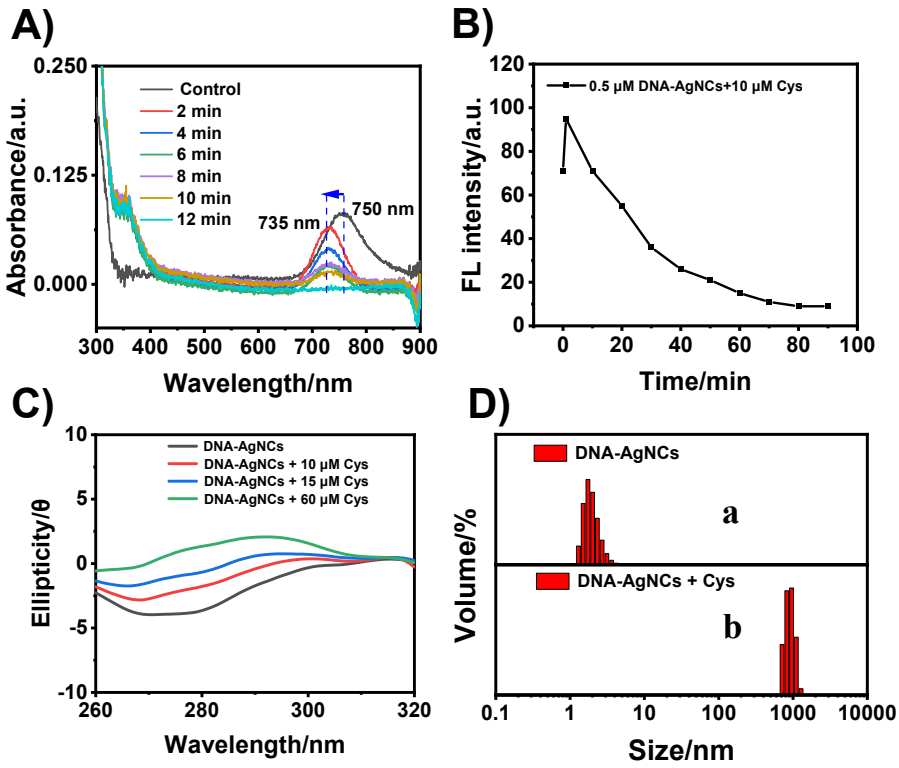

Fig. S8 (A) The UV-Vis absorption spectra of dimerized DNA2-AgNCs before and after addition of Cys. (B) Impact of reaction time on the fluorescence quenching efficiency with Cys (Ex/Em=750 /820 nm). (C) CD spectra of DNA2-AgNCs alone (0.5 μM) and incubation with different concentration of Cys at 10 μM, 15 μM and 60 μM. (D) Dynamic light scattering (DLS) data of DNA2 -AgNCs alone (a) and DNA2-AgNCs / Cys (b).

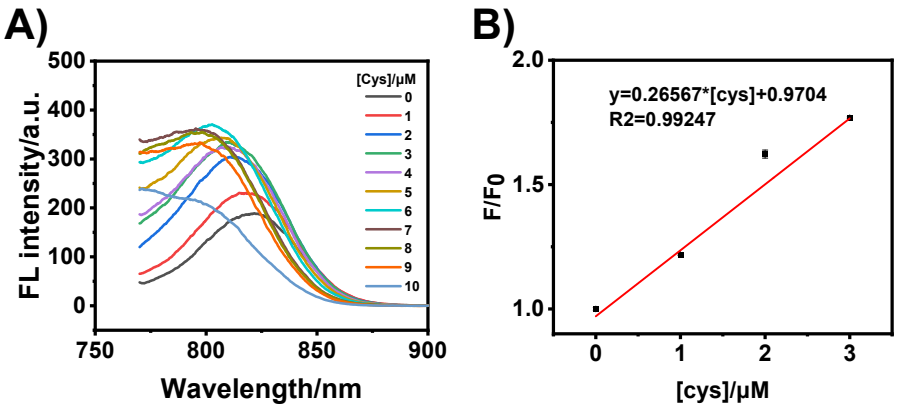

Fig. S9 (A) FL spectrum of 0.5 μM dimerized DNA3-AgNCs ([Ag^+/C] =1.0) reacting with different concentrations of Cys. (B) The F/F_0 of 0.5 μM DNA3-AgNCs versus different concentrations of Cys (0, 1.0, 2.0 and 3.0 μM, n = 3) under 750 nm excitation.

Table S1. Sequences of DNA strands used in this work. (DNA regions to form AgNCs were colored in red; hybridization region at 3'-end of DNA were italicized.)

abbreviation	sequence
DNA1	5'-CCCACCCACCCTCCCA-3'
DNA2	5'-CCCACCCACCCTCCCACCCACCCACCCTCCCA-3'
DNA3	5'-CCCACCCACCCTCCCAACCCACCC-3'
DNA4	5'- CCCACCCACCCTCCCAACCCACCCTCCCACCC-3'
DNA5	5'-CCCACCCACCCTCCCACCCACCCACCCTCCCACCCACCCACCCTCCCA-3'

Table S2. Comparison between ours and previously reported fluorescence sensors for detecting Cys and Ag^+.

method	sensor	analyte	Output signal	Linear Range (μM)	LOD (μM)	Reference
Fluorescence	β-lg/AuAgNCs	Cys	FI	200-600	80	[3]
Fluorescence	DNA/AgNCs	Cys	FI	0.02-0.6	0.002	[4]
Fluorescence	GSH-AuNCs	Ag^+	FI	0.1-8	0.06	[5]
Fluorescence	Cys-AuNCs	Ag^+	FI	3-30	0.26	[6]
Fluorescence	GSH-AgNCs	Cys	FI	2-3000	0.51	[7]
Fluorescence	BSA-AuAgNCs	Cys	$\triangle\lambda_{em}$	2-100	1.1	[8]
Fluorescence	5'-DNA-FAM-3'	Ag^+ Cys	FI, $\triangle\lambda_{em}$	0.001-0.7 0.06-0.5	0.0035 0.04	[9]
Fluorescence	berberine	Ag^+ Cys	FI, r, τ	0-264 0-70	0.079 0.03	[10]
Colorimetric	CNF-AgNPs	Cys	UV-Vis	0-1000	0.049	[11]
Electrochemical	BiPr	Cys	-	1-2000	0.21	[12]
Electrochemical	SQDs	Ag^+	-	0.0001-38	3.2×10^{-5}	[13]
Colorimetric	QDs	Ag^+	UV-Vis	0-100	0.026	[14]
Colorimetric	PVP-PtNcb	Ag^+	UV-Vis	10^{-5}-0.1	8×10^{-5}	[15]
Fluorescence	DNA2-AgNCs	Cys	FI, $\triangle\lambda_{em}$	1-7	0.032	This work
Fluorescence	DNA3-AgNCs	Ag^+	FI, $\triangle\lambda_{em}$	5-30	0.119	This work

Table S3. Detection of Cys in milk (n=3) with the DNA2-AgNCs.

	concentration/μM	added/μM	found/μM	RSD/%(n=3)	recovery/%
Milk 1	1.92	1.04	2.76	1.45	93.7
		2.02	3.73	2.94	94.0
		3.00	5.54	0.96	113.0
Milk 2	1.92	1.04	2.77	1.50	94.0
		2.02	4.20	1.20	106.0
		3.00	5.07	2.40	103.0
Milk 3	1.92	2.00	3.68	2.10	93.9
		3.00	5.11	0.88	104.0
		3.92	5.42	0.67	92.8

Table S4. Detection of Ag^+ in real water (n = 3) with the DNA3-AgNCs.

	added/μM	found/μM	RSD/%(n=3)	recovery/%
Water 1	10	10.1	3.7	101.0
	25	20.0	1.4	80.9
Water 2	10	11.2	2.9	112.0
	25	24.0	1.2	96.0

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