Supplementary Information (SI)

Ag Nanocluster-based Label-Free Catalytic and Molecular Beacons for Amplified Biosensing

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1. Experimental details

Reagents and materials

Silver nitrate, sodium borohydride (powder, 98%), were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Lead acetate and L-histidine were of analytical reagent grade, purchased from Sigma-Aldrich Chemical Co. Sodium acetate, magnesium acetate, disodium hydrogen phosphate, sodium dihydrogen phosphate and tris(hydroxymethyl) aminomethane were purchased from Dingguo Changsheng Biotechnology Co. Ltd.. All reagents used without further purification. All solutions were prepared with Milli-Qwater from a Millipore system.

DNA oligonucleotide used in this work was purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and used without further purification. The DNA sequences are as follows:

MB:5'-
GAGAGAAGGTTTATATAGGTTAGTATACTCTrAGGAAGCATCAGTAATTTA
TCATTCCTTCTCTCTA GGGTGGGGTGGGGTGGGG-3'

Ag-1:5'-
CCCTTAATCCCCTAGAGAGAAGGAATGATAAATTACTTTTTTATCATTCC -3'

Ag-2:5'-
CCCTTAATCCCCTAGAGAGAAGGAATGATAAATTACTTTTTTATCATTC -3'

Ag-3:5'-
CCCTTAATCCCCTAGAGAGAAGGAATGATAAATTACTTTATTATCATTC-3'

Ag-4: 5'-CCCTTAATCCCCTAGAGAGAAGGAATGATACATAATATATCATTC -3'

GR-5: 5'-CTGATGCTGAAGTAGCGCCGCCGTAGAGTATAC-3'

L-hiszyme:5'-
CTGATGCTTAACGGGGCTGTGCGGCTAGGAAGTAAGAGTATAC-3'

Preparation of silver nanoclusters on DNA

A complete list of DNA used in this study for silver nanoclusters can be found in 2.1. AgNCs were prepared according to the literature method. [22] Briefly, 5 μL of 160 mM DNA template sequence was dissolved in buffer Ⅰ (20 mM Na_2HPO_4－NaH_2PO_4, pH 6.6); followed adding 9.6 μL of 1 mM $AgNO_3$ under stirring for 30s, after ice-water bath for 30 min, AgNCs were formed by reduction with 9.6 μL of 1 mM freshly prepares $NaBH_4$ accompanied with a color change from colorless to pale yellow, then the reaction was kept in the dark at room temperature for 2 hours, AgHS was formed and stored in a refrigerator at 4 ^oC before used.

Fluorescent DNA assay

The hybridization and restriction of Pb^{2+}-depended DNAzyme (GR-5) and its substrate sequence (GHS) performed in buffer Ⅱ (20 mM HEPES, 50 mM NaAc, 5 mM $Mg(Ac)_2$, PH 7.26). Firstly, GHS and GR-5 hybridized with the concentrations 600 nM and 300 nM for 10 min, and then add the corresponding concentration of Pb^{2+} for 20 min, Secondly, add the AgHS after joining the bufferⅢ (20 mM Na_2HPO_4－NaH_2PO_4, 300 mM $NaNO_3$, pH 6.6), and reaction for 45 min. The whole process is in progress at room temperature. Fluorescence was measured using HORiBA Fluoromax-4 fluorescence spectrometer.

2. Supplementary Figures and Table

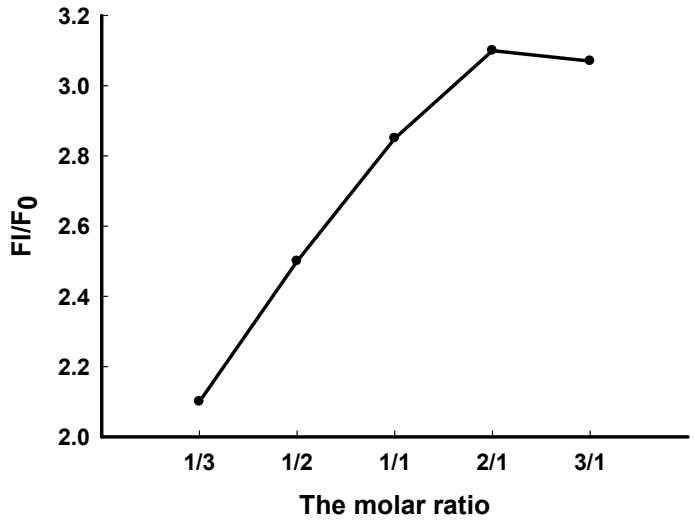

Fig. S1. The effect of the molar ratio of GHS to GR-5 on the fluorescence response of the sensing system. The concentrations of GHS and GR-5 were both fixed at 1uM. FI and F_0 are the fluorescence intensity of the sensing system in the presence and absence of Pb^{2+} whose concentration was 1 μM.

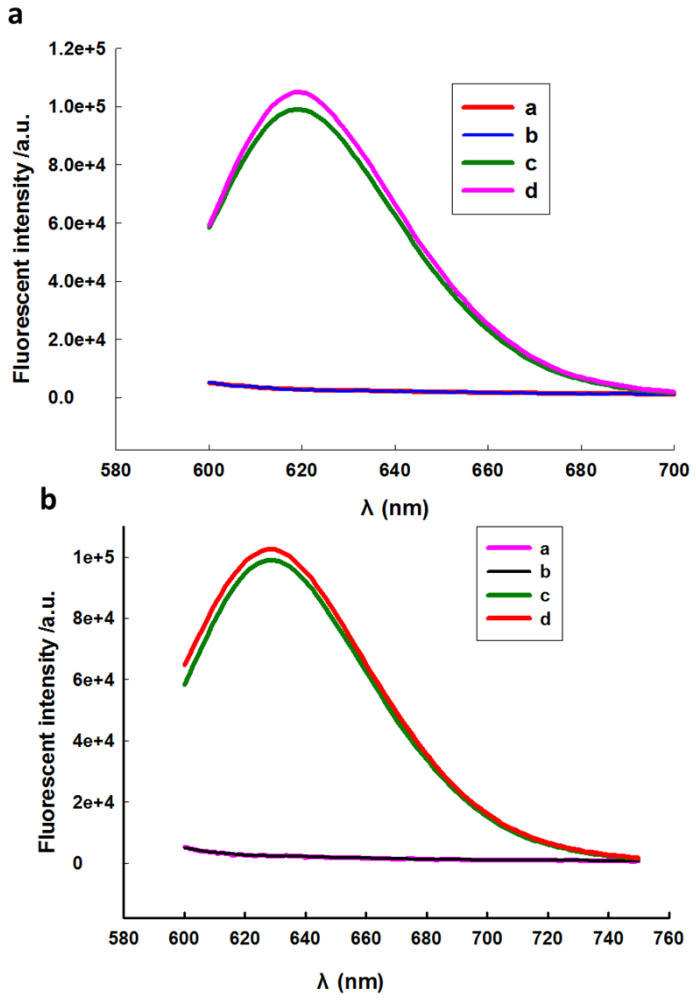

Fig. S2. Feasibility verifies of the sensing system. (a)The fluorescence spectra of the AgHS under different conditions: a. AgHS; b. AgHS+Pb^{2+}; c. AgHS +G-rich sequences; d. AgHS +G-rich sequences+Pb^{2+}; The AgHS /G-rich sequences/Pb^{2+} concentration: 1 μm. (b)The fluorescence spectra of the AgHS under different conditions: a. AgHS; b. AgHS + L-histidine ; c. AgHS +G-rich sequences; d. AgHS + G-rich sequences + L-histidine ; The AgHS /G-rich sequences concentration: 1μm, The L-histidine concentration: 10 mM.

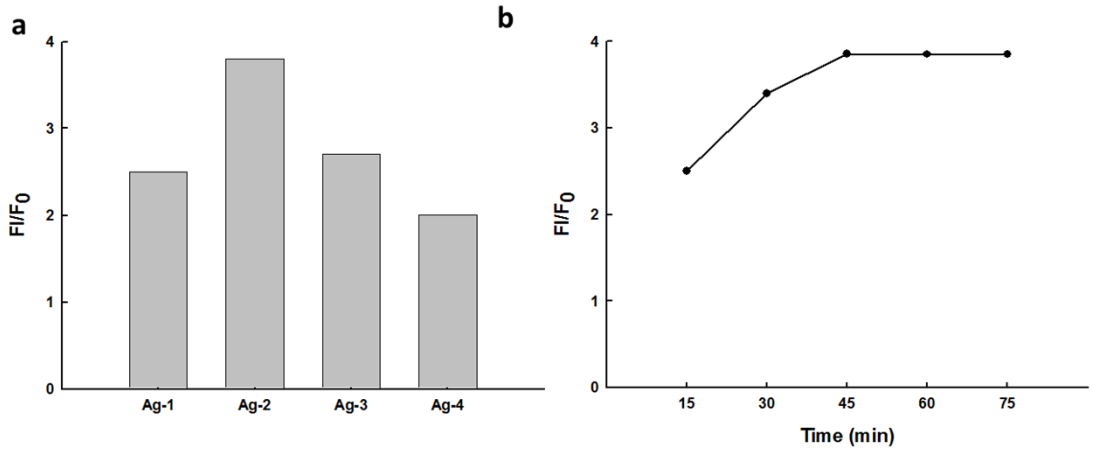

Fig. S3 Optimization of experimental conditions. (a) The effect of different stem length base pairs of AgHS on DNAzyme-AgNCs based biosensor, The Ag-1, Ag-2, Ag-3, and Ag-4 denote stem DNA sequence containing 11, 10, 9 and 8 bases, respectively; (b) impact of incubation time of target cofactor on the fluorescence of the biosensor.

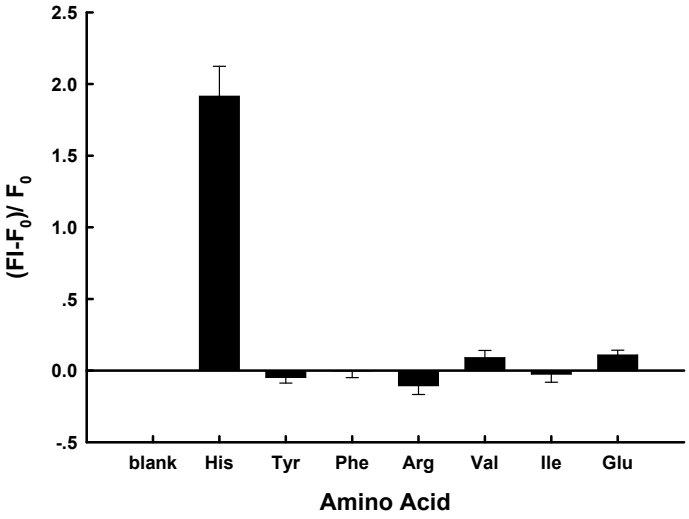

Fig. S4. Selectivity of L-histidine-dependent DNAzyme/AgNCs based sensing system. The degree of signal enhancement is defined as $(FI-F_0)/F_0$, where F_0 and FI are the fluorescence intensities at 635 nm in the absence and presence of L-histidine and other kinds amino with the concentration of 1mM, respectively.

Table S1. Recovery experiments of Pb^{2+} in river water samples

River water	Added (nM)	Found(mean ± S.D, nM)	Recovery(%)
1	10	10.58 ± 0. 16	105.80
2	30	31.00 ± 0.35	103.34
3	50	49.06 ± 0.84	98.13