Electronic Supplementary Information (ESI)

An Enzyme-Free and Amplified Colorimetric Detection Strategy: Assembly of Gold Nanoparticles through Target-Catalytic Circuits

Ke Quan[+], Jin Huang[+], Xiaohai Yang, Yanjing Yang, Le Ying, He Wang, Kemin Wang*

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

[+] K. Quan and J. Huang contributed equally to this work.

* Email: kmwang@hnu.edu.cn

Experimental Section

1. Buffer solution

Phosphate Buffer **(PB)**: 10 mM Na_2HPO_4 , 10 mM NaH_2PO_4, pH 7.4.

HCR Buffer (**SPSC**): 50 mM Na_2HPO_4, 0.4 M NaCl, pH 7.5.

CHA Buffer (**TNaK**): 20 mM Tris-HCl , 140 mM NaCl , 5 mM KCl, pH 7.5.

Electrophoresis Buffer (**1×TAE**): 40 mM Tris AcOH, 2 mM Na_2EDTA, pH 8.5.

Phosphate Buffer (**PBS**): 10 mM PB, 137 mM NaCl, and 2.7 mM KCl, pH 7.4.

2. DNA sequences

Oligonucleotide (L*) was obtained from Takara Biotechnology Co.Ltd. (Dalian, China), the other oligonucleotides were synthesized by Sangon Biotechnology Co.Ltd. (Shanghai, China), All the sequences are listed as below (from 5′ to 3′):

Strands	Sequences (5'-3')
H1	TTTTTTTTTTTTTTTTTTTTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGCGTG
H2	TTTTTTTTTTTTTTTTTTTAGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTT
T	AGTCTAGGATTCGGCGTGGGTTAA
T'	AGTCTAGGATTCAGCGTGGGTTAA
H1*	TTTTTTTTTTTTTTGTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTCGATCCAA
H2*	TTTTTTTTTTTTTTTAGATGTCGTCTACACATGGCGACATCT AACCT AGCCCATGTGTAGA
T*	CGACATCTAACCTAGC TCACTGAC
T*'	CGACATCTAACCTAGCAAACTTAC
L*	AAAAAAAAAAAAAAAAAAAAAAAAA-SH

3. Gel Electrophoresis

HCR experiment: H1 and H2 were heated to 90°C for 5min and then allowed to cool to room temperature before use. Then 2 μM H1 and H2 were incubated with 400 nM T. The 1% agarose

gel was prepared using a TAE buffer. The gel was run at 55V for 2h in TAE buffer and finally scanned using the gel image analysis system.

CHA experiment: H1* and H2* were heated to 90°C for 5min and then allowed to cool to room temperature before use. 80 nM Target DNA T* was incubated with 2 μM each of H1* and H2* at 37°C overnight. A 2% agarose gel was prepared using a TAE buffer. The SYBR Gold was used as an oligonucleotide dye and mixed with samples. The gel was run at 100 V for 40 min in a TAE buffer.

4. Transmission Electron Microscopy (TEM)

The samples for TEM characterization were prepared by pipetting 10 μL of colloid solution onto a carbon-coated copper grid. After the evaporation of solvent, the grid was dried overnight. All of the images were bright-field images.

5. Preparation of AuNPs and DNA-Modified AuNPs

AuNPs with an average diameter of 13 nm were prepared as previously described.[1] In brief, an aqueous solution of $HAuCl_4$ (1 mM, 100 mL) was brought to reflux while stirring. Then 10 mL of trisodium citrate solution (38.8 mM) was quickly added to the vortex of the solution, which resulted in a color change from pale yellow to deep red. Boiling was continued for 15 min. AuNPs with an average diameter of 20 nm and 40 nm were prepared as follows[2]: An aqueous solution of $HAuCl_4$ (0.01%, 50 mL) was brought to reflux while stirring. Then 1 mL (for 25 nm) or 0.75 mL (for 40 nm) of trisodium citrate solution (1%) was quickly added to the vortex of the solution, which resulted in a color change from pale yellow to Red (20 nm) or pinkish red (40 nm). Boiling was continued for 15 min. After the solution reached room temperature, it was filtered and stored in a refrigerator at 4°C before use. The DNA-modified AuNPs were prepared as follows[3]: The thiol modified DNAs were first activated by 10 mM TCEP for 1 h. Then AuNPs were functionalized by mixing deprotected alkanethiol oligonucleotides with aqueous nanoparticle solution. After ~16 h, the colloidal solution was brought to pH 7.4 of 10 mM of PB buffer by adding 0.1 M concentrated buffer. In the subsequent salt aging process, colloids were first brought to 0.05 M of NaCl by dropwise addition of 2 M NaCl solution and allowed to stand for 6-8 h, were next salted to 0.1 M and allowed to age for another 6-8 h, were then salted to 0.2 M for standing 6-8 h, and were finally salted to 0.3 M NaCl. To remove excess thiol-DNA, the solution was centrifuged (13000 rpm, 30 min for 13 nm, 10000 rpm, 20 min for 20 nm, 8000 rpm, 20 min for

40 nm) and the supernatant was carefully removed. The precipitate was washed by a equal volume of 0.3 M PBS (10 mM PB, 0.3 M NaCl, pH 7.4) and recentrifuged twice. Finally, the functionalized AuNPs were redispersed in 0.3 M PBS and stored at 4°C for further use.

6. Analytical Protocol and Measurement Procedure

Each hairpin structure was heated to 90 °C for 5 min and then allowed to cool to room temperature before use.

HCR experiment: All samples were prepared in SPSC buffer. Different concentrations of the target DNA T were incubated with annealed H1 (10 μL, 625 nM), H2 (10 μL, 625 nM) in a total volume of 25 μL at 37°C overnight. 25 μL of the reaction mixture (H1/H2/T) was added into a AuNP (100 μL，4 nM) colloidal solution in SPSC buffer and incubated for 1.5 h. At last the above mixed solution was diluted to 300 μL and detected by UV−vis spectrophotometer.

CHA Experiment: All samples were prepared in TNaK buffer. Different concentrations of the target DNA T* were incubated with H1* (10 μL, 625 nM), H2* (10 μL, 625 nM) in a total volume of 25 μL at 37°C overnight. Subsequently, 25 μL of the reaction mixture was added into a AuNP (100 μL，3 nM) colloidal solution in TNaK buffer and incubated for 1.5 h, At last the above mixed solution was diluted to 300 μL and detected by UV−vis spectrophotometer.

7. Preparation of Cell Lysate

Hela cell lines were established in our lab and were grown in RPMI 1640 cell medium with 10% inactivated fetal bovine serum (Hyclone, USA) at 37℃ in 5% CO2. Vigorous growth cells were collected after trypsin digestion. The cell density was determined using a hemocytometer, and this was performed prior to each experiment. A suspension of 5×10^5 cells was centrifuged at 1000 rpm for 3 min [4], then washed with PBS buffer three times and at last suspended in TNak buffer. Finally, the cells were disrupted by sonication for 20 min at 0 °C. To remove the homogenate of cell debris, the lysate was centrifuged at 18000 rpm for 20 min at 4 ° C. The supernatant was ready for CHA assays.

References

[1] T. Songa and H. Liang, *J. Am. Chem. Soc*., 2012, **134,** 10803

[2] Y. Chen，M. B. O'Donoghue，Y. F. Huang，H. Z. Kang，J. A. Phillips，X. L. Chen，M. C. Estevez and W. H. Tan, *J. Am. Chem. Soc.*, 2010, **132**, 16559

[3] R. Jin, G. Wu, Z. Li, C. A. Mirkin, and G. C. Schatz, *J. Am. Chem. Soc.*, 2003, **125**, 1643

[4] J. Li, H. Fu, L. Wu, A. Zheng, G. Chen, and H. Yang , *Anal. Chem.*, 2012, **84,** 5309

Supporting Figures

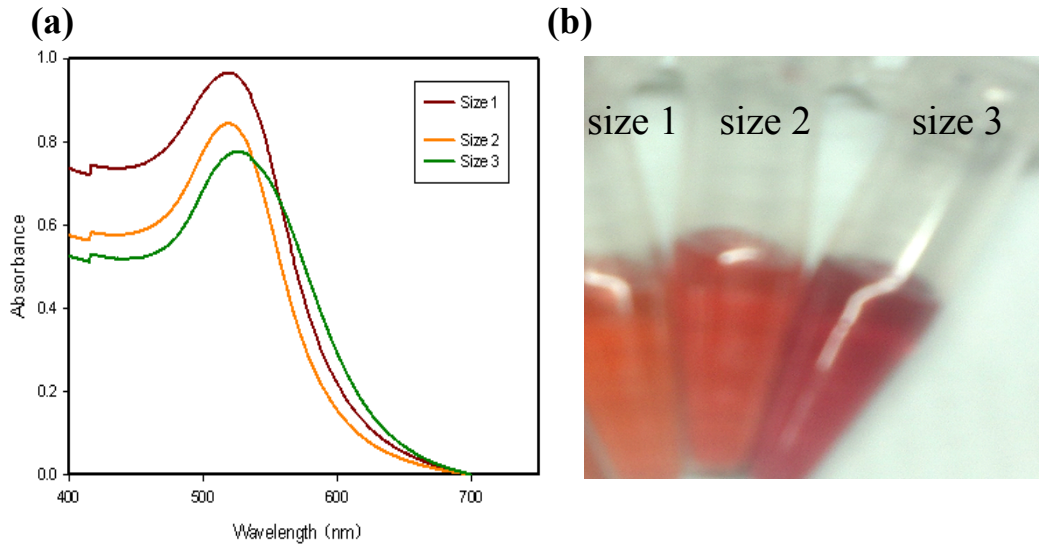

Figure S1. Characterization of different sized AuNPs. (a) UV sbsorption spectra of different sized AuNPs. (b) The visual colors of different size AuNPs.

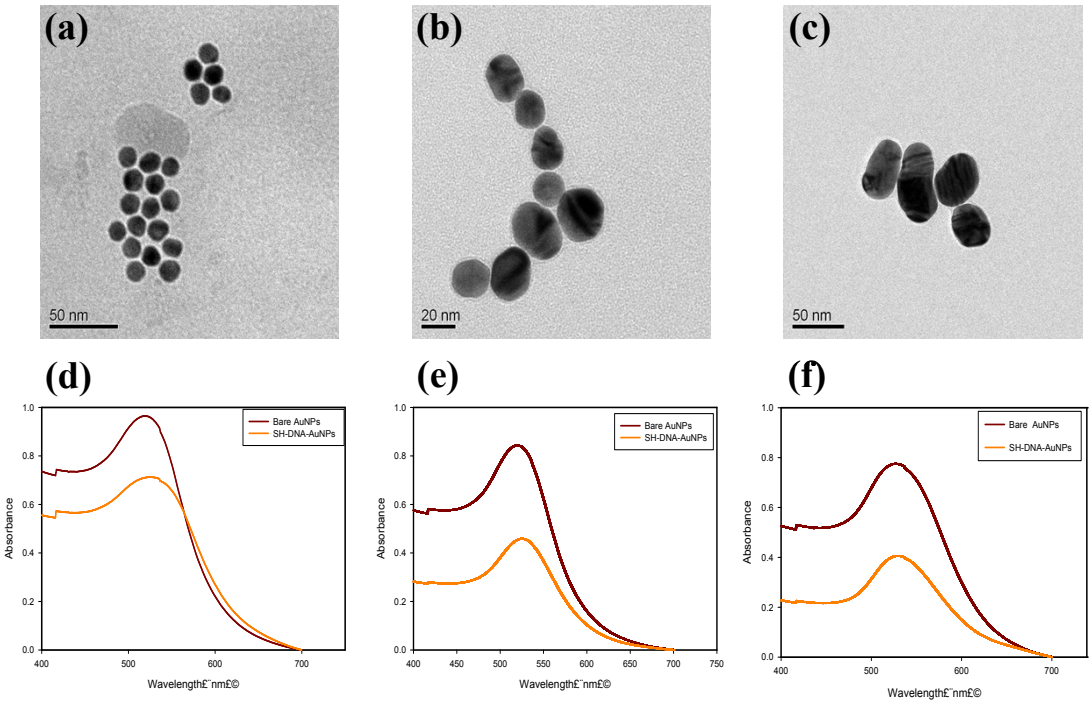

Figure S2. TEM images of different sizes of AuNPs: (a) size 1: 13±1 nm; (b) size 2; 20±3 nm; (c) size 3: 40±5 nm. Absorption spectra of AuNPs (black line) and DNA functionalized AuNPs (red line) of (d) size 1, (e) size 2, and (f) size 3.

Table S1. Summary of properties for different sizes of AuNPs.

	Average diameter	λ max of bare AuNPs	λ max of DNA-AuNPs	△A of CHA	△A of HCR
Size 1	13±1nm	518nm	524nm	0.3046	0.1554
Size 2	20±3nm	520nm	525nm	0.119	0.0997
Size 3	40±5nm	526nm	530nm	0.0508	0.0473

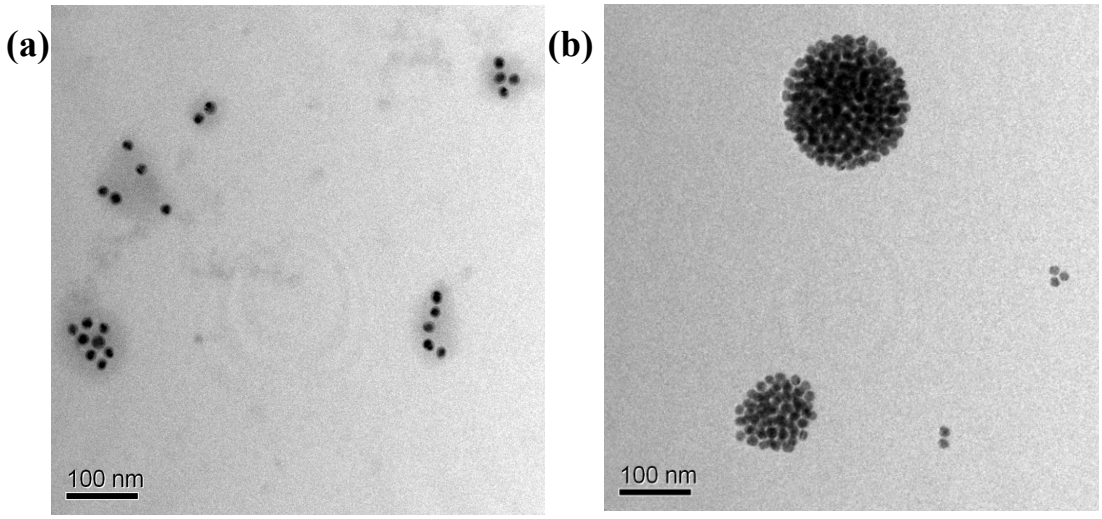

Figure S3. TEM images of AuNPs in HCR system without (a) or with 5 nM target DNA.

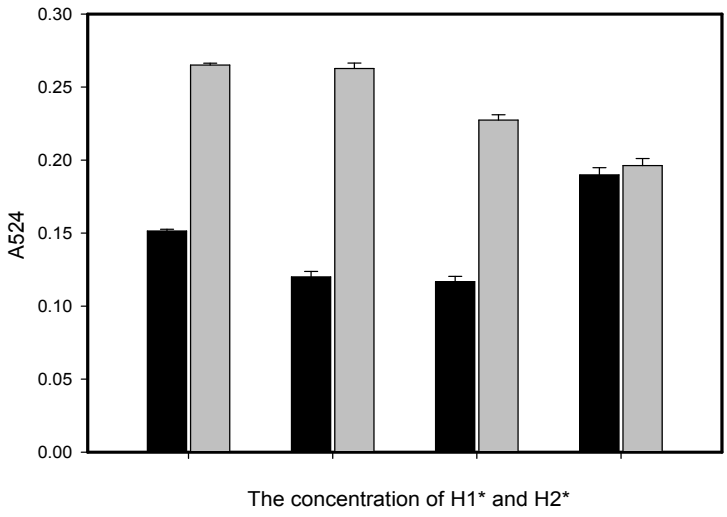

Figure S4. Optimization of the H1* and H2* concentrations. The plot shows various amounts of H1* and H2* vs the absorption peak of AuNPs at 524 nm with (black histogram) or without (gray histogram) 5 nM Target DNA T*. [AuNPs] = 3 nM.

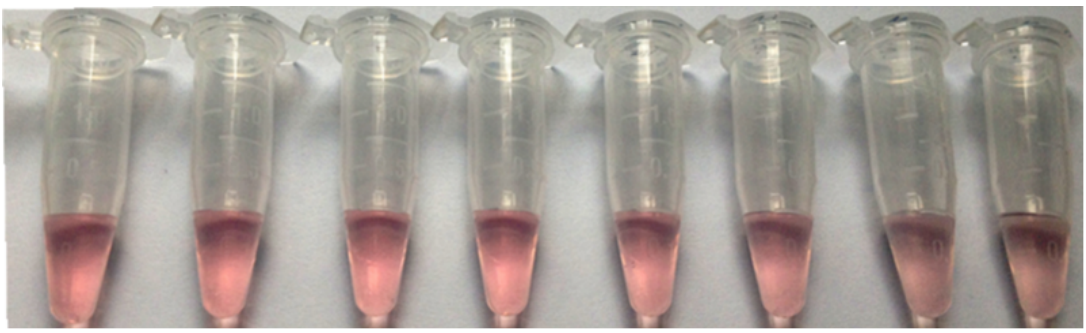

Figure S5. The color appearance of the AuNPs mixed with the test solution containing various concentrations of target DNA, respectively.

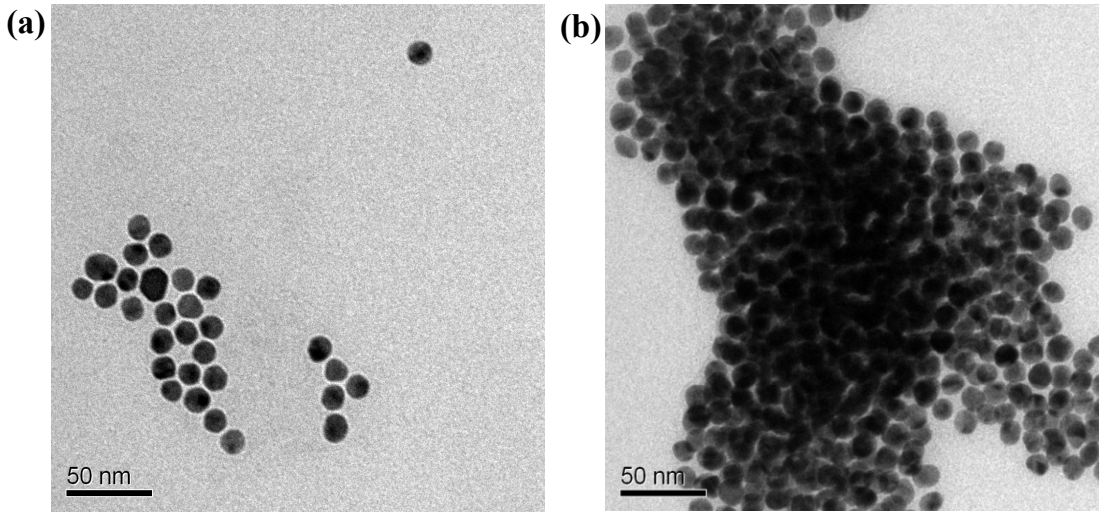

Figure S6. TEM images of AuNPs in CHA system without (a) or with 5 nM target DNA.

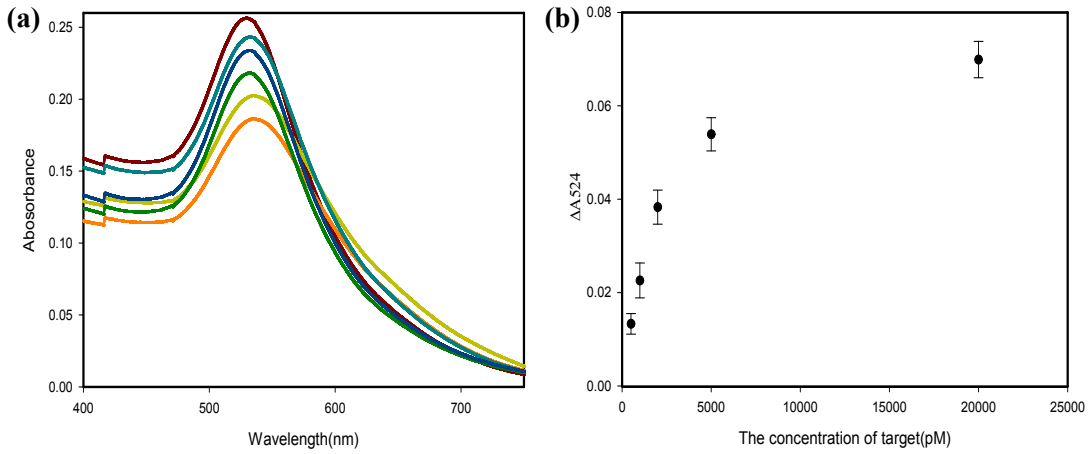

Figure S7. (a) UV-vis spectra detection based on CHA for different concentrations of Target T* in 10% cell lysate. (b) UV-vis absorption peak change at 524 nm as a function of the various concentrations of T*. [H1*] = [H2*] = 50 nM, [AuNPs] = 3 nM