Electronic Supplementary Information (ESI)

An Enzyme-Free and Amplified Colorimetric Detection Strategy via Target-Aptamer Binding Triggered Catalyzed Hairpin Assembly

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Experimental Section

1. DNA sequences

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

(1) Aptamer-Trigger (**Apt-T**): Trigger is red and aptamer is blue.

CGACATCTAACCTAGCTCACTGACACCTGGGGGAGTATTGCGGAGGAAGGT

(2) Inhibitor 1 (**Inh 1**):

CAGGTGTCAGTGAG

(3) Inhibitor 2 (**Inh 2**):

AGGTGTCAGTGA

(4) Inhibitor 3 (**Inh 3**):

CAGGTGTCAGTG

(5) Inhibitor 4 (**Inh 4**):

GGTGTCAGTGAG

(6) Hairpin 1 (**H1**): a b c d c* b*

*TTTTTTTTTTTTTTT*GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCT AGC

(7) Hairpin 2 (**H2**): c d* c* b* d

*TTTTTTTTTTTTTTT*AGATGTCGTCTACACATGGCGACATCT AACCT AGCCCATGTGTAGA

(8) Trigger (**T**):

CGACATCTAACCTAGCTCACTGAC

(9) Linker (**L***):

AAAAAAAAAAAAAAAAAAAAAAAA-SH

Note: Fragment a, b, c, d is complementary to a*, b*, c*, d*, respectively.

2. Preparation of AuNPs and DNA-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. 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 [2]: The thiol modified DNA was first activated by 10mM TCEP for 1 h. Then AuNPs were functionalized by mixing deprotected alkanethiol oligonucleotides with aqueous nanoparticle solution (particle concentration ~10 nM) to a final oligonucleotide concentration of 3.5 μM. After ~16 h, the colloidal solution was brought to pH 7.4 of 10 mM of PB buffer by adding 0.1M 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 0.1M and allowed to age for another 6-8 h, were then salted to 0.2M 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) 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.

3. Gel Electrophoresis

H1 and H2 were heated to 90°C for 5min and then allowed to cool to room temperature before use. T (80 nM) or Apt-T（500 nM）/Inh（2 μM）/Ade（2 mM）complex 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. Analytical Protocol

All samples were prepared in TNaK buffer (20mM Tris-HCl , 140 mM NaCl , 5mM KCl, pH 7.5). Each hairpin structure was heated to 90 °C for 5 min and then allowed to cool to room temperature before use. For the detection of adenosine, the detailed procedure was as follows: Apt-T (10 μL, 1.25 μM) and Inh (10 μL, 5 μM) were firstly mixed and heated to 90 °C for 5 min and then allowed to slowly cool to 25 °C, and then mixed with equal volumes of different concentrations of adenosine (or other nucleosides) for 2 h at 37 °C. Some 5 μL of this mixture was mixed with H1 (10 μL, 625 nM), H2 (10 μL, 625 nM) in a total volume of 25 μL. To achieve high sensitivity, the above assay mixture (H1/H2/T or H1/H2/Apt-T/Inh/ade) was incubated for 9 h. 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 37°C, at last the above mixed solution was diluted to 300 μL and detected by UV−vis spectrophotometer.

6. 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 [3], then washed with PBS buffer(10 mM phosphate, 137 mM NaCl, and 2.7 mM KCl, pH 7.4) 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 adenosine assays.

References

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

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

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

Supporting Figures

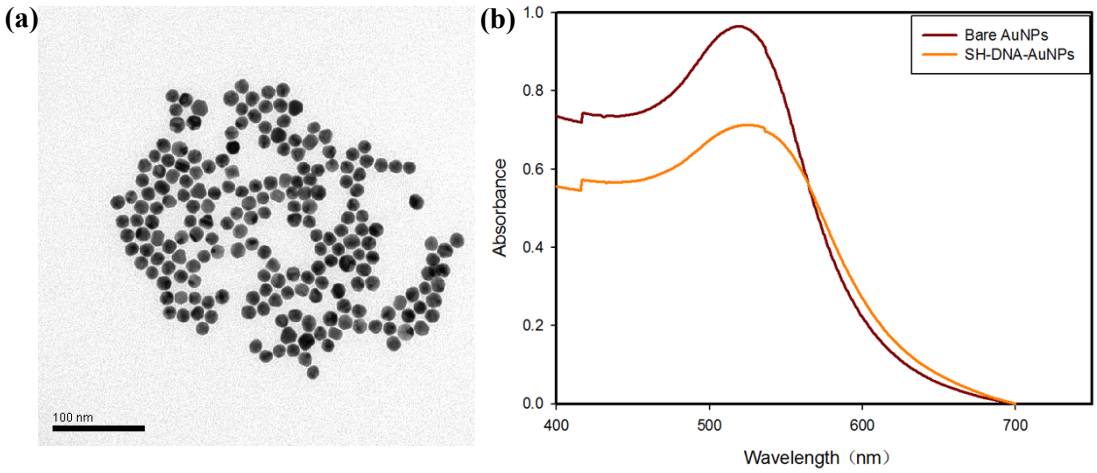

Figure S1. (a) TEM micrograph of AuNPs; (b) Absorption spectra of AuNPs (λ_{max} = 519 nm) and DNA functionalized AuNPs (λ_{max} = 524 nm).

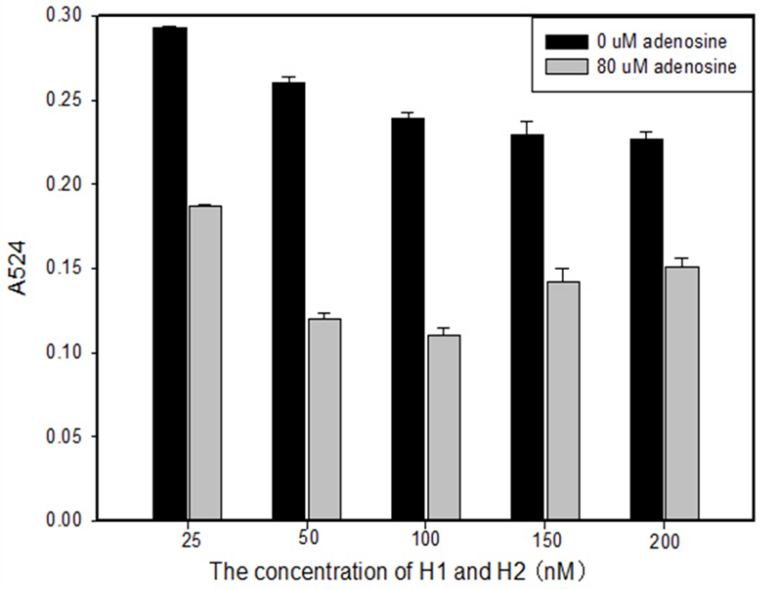

Figure S2. Optimization of the H1 and H2 concentrations. The plot shows various amounts of H1 and H2 vs the absorption peak of AuNPs at 524 nm with (gray histogram) or without (black histogram) 80 μM adenosine. [Apt-T] = 12.5 nM, [Inh]=50 nM, [AuNPs] = 3 nM.

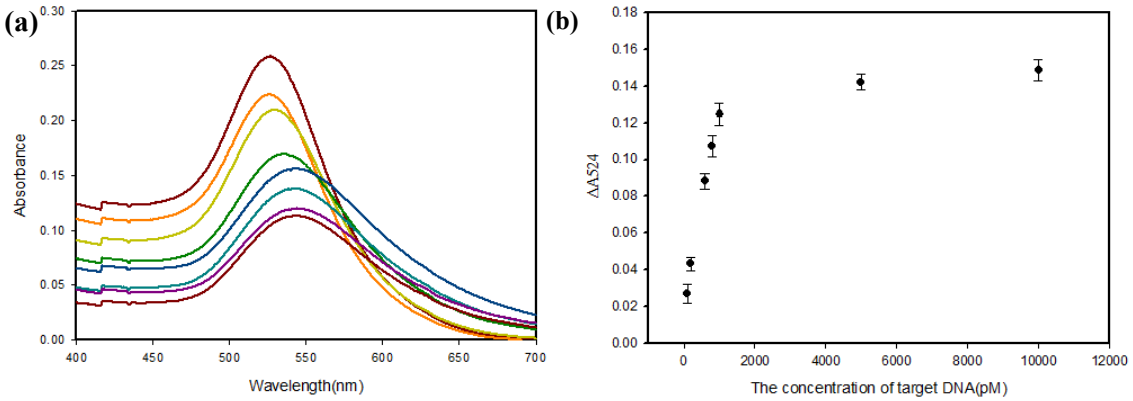

Figure S3. (a) UV-vis spectra detection based on CHA for different concentrations of T. (b) UV-vis absorption peak change at 524 nm as a function of the concentration of T.

Trigger Aptamer

CGACATCTAACCTAGCTCACTGACACCTGGGGGAGTATTGCGGAGGAAGGT

Inh 1 **GAGTGACTGTGGAC**

Inh 2 **AGTGACTGTGGA**

Inh 3 **GTGACTGTGGAC**

Inh 4 **GAGTGACTGTGG**

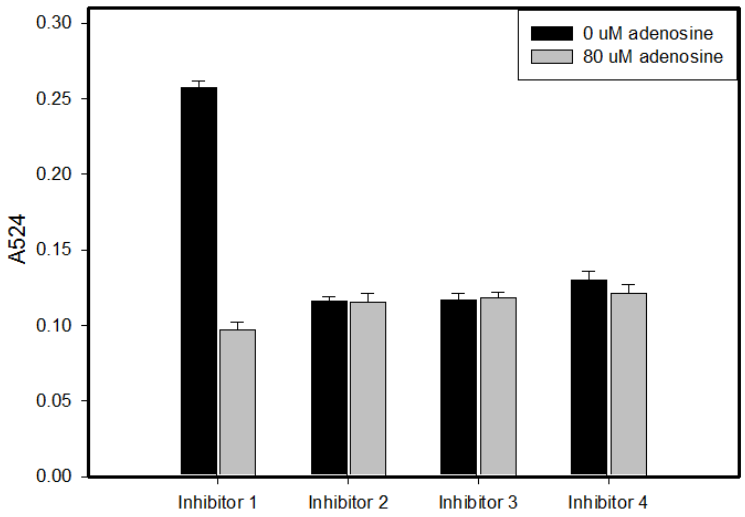

Figure S4. Optimization of the inhibitor lengths. The plot shows the relationship between different inhibitor sequence and absorption peak of AuNPs at 524 nm in the absence (black histogram) or presence (gray histogram) of 80 μM adenosine. All the inhibitor probes were of the same concentration (50 nM). [Apt-T] = 12.5 nM, [H1] = [H2] = 50 nM, [AuNPs] = 3 nM.

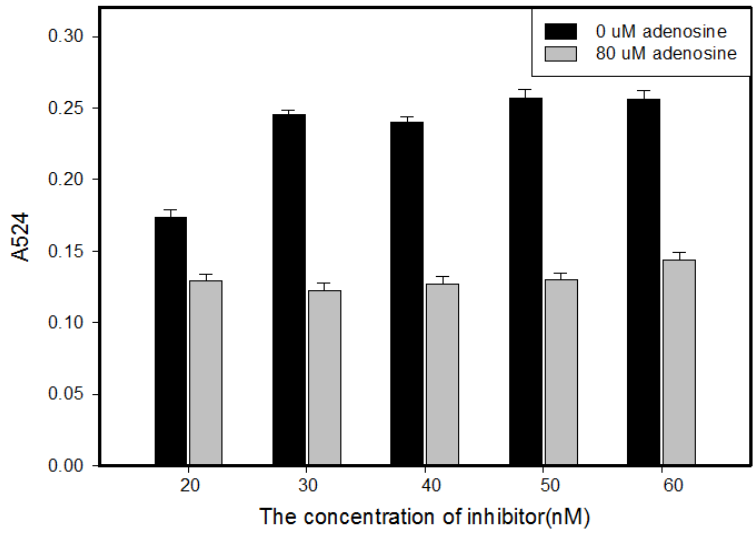

Figure S5. Optimization of the inhibitor concentrations. The plot shows various amounts of inhibitor vs the absorption peak of AuNPs at 524 nm with (gray histogram) or without (black histogram) 80 μM adenosine. [Apt-T] = 12.5 nM, [H1] = [H2] = 50 nM, [AuNPs] = 3 nM.

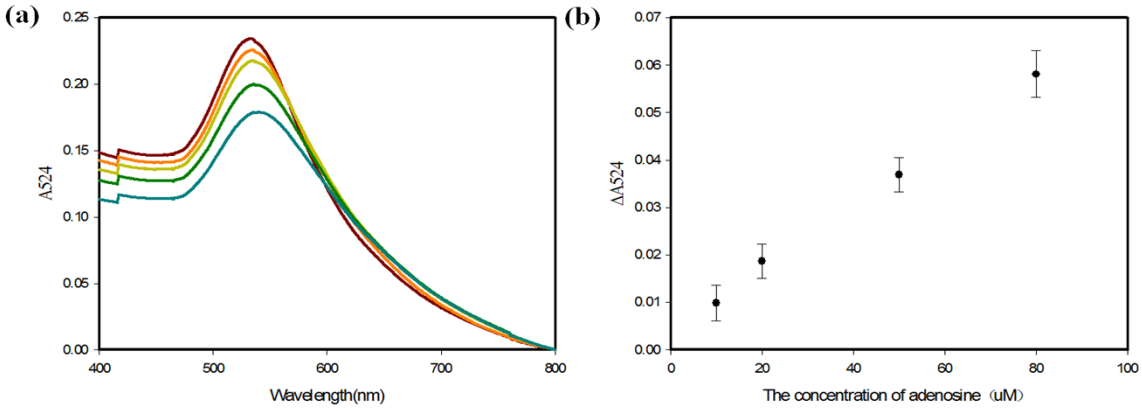

Figure S6. (a) UV-vis spectra detection based on CHA for different concentrations of adenosine in 20% cell lysate. (b) UV-vis absorption peak change at 524 nm as a function of the various concentrations of adenosine. [Apt-T] = 12.5 nM, [Inh] = 50 nM, [H1] = [H2] = 50 nM, [AuNPs] = 3 nM