Electronic Supplementary Information

Target-triggered autonomous assembly of DNA polymer chain through catalyzed hairpin assembly and its application for enzyme-free and signal amplified colorimetric nucleic acids detection

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Experimental section

Materials, equipment, and measurement Trishydroxymethylaminomethane hydrochloride (Tris-HCl), sodium hydroxide, sodium chloride, magnesium chloride, sodium citrate and Tris-EDTA buffer solution (100 ×) were purchased from Sigma (St. Louis, MO). Hydrogen tetrachloroaurate (III) ($HAuCl_4$) was purchased from Shanghai Chemical Reagent Research Institute Co. Ltd. Oligonucleotides designed in this study were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and purified by HPLC. The DNA sequences are listed in Table S1. Other chemicals were used as received without further purification. AuNPs of ~13 nm were synthesized by the citrate reduction of $HAuCl_4$ as previously reported.[1] The water (≥ 18.2 MΩ) was purified using a Millipore filtration system and used in all experiments.

DNA concentration was measured by Qubit® 2.0 Fluorometer (Thermo Fisher Scientific Inc.). UV-vis absorption spectra were obtained using a UV-1100 spectrophotometer (Techcomp (China) Ltd.). Photographs were taken using a Sony DSC-WX150 digital camera. Atomic force microscopy (AFM) images were recorded using a

Nanoscope 3A controller (Digital Instruments/Veeco Probes/USA) with NSC 15 AFM tips (Mikromasch, Germany) using the tapping mode at their resonant frequency.

Table S1 DNA sequences [a]

DNA name	DNA sequence (5' to 3')
H1	TTGAAGTAGTGATTGAGCGTGATGAATGTCACTACTTCAACTC GCATTCATCACGCTCAATCTAGTTGCGAG
H2	GCGTGATGAATGCGAGTTGAAGTAGTGACATTCATCACGCTCA ATCACTACTTCAACTCGCAACTAGATTGA
Perfectly matched Target	GACATTCATCACGCTCAATCACTACTT
Deleted Target	GACATTCATCACG_TCAATCACTACTT
Inserted Target	GACATTCATCACGTCTCAATCACTACTT
Mismatched Target	GACATTCATCACGTTCAATCACTACTT

[a] The design of the hairpin probes was adapted from the literature[2]. The underlined letters indicate the predesigned complementary sequences for the DNA polymer chain formation.

DNA polymer chain preparation and AuNPs-based colorimetric DNA assay

H1 and H2 were heated to 95 °C for 5 min and then allowed to cool to room temperature for 3 h before use. Then different concentrations of target DNA were mixed with H1 (200 nM) and H2 (200 nM) in 50 mM Tris-HCl (pH 8.0) buffer solution containing 5 mM MgCl2, and then incubated for 3 h at room temperature. Subsequently, 5 μL of the reaction solution was added to the 100 μL of 2.7 nM AuNPs colloidal solution. After incubation for 2 min, 10 μL of 150 mM NaCl solution was added into the mixed solution, followed by either visual observation or UV/Vis characterization.

AFM imaging

H1 and H2 were heated to 95 °C for 5 min and then allowed to cool to room temperature for 3 h before use. 5 nM target DNA was mixed with H1 (200 nM) and H2 (200 nM) in the above-mentioned buffer solution, and then the DNA sample was deposited on a freshly cleaved mica surface (Structure Probe Inc., West Chester, PA), dried in air, and

gently washed with double-distilled water. The excess water was removed with filter paper, the mica was dried in air, and then the AFM images were recorded. AFM image of the system consisting of only H1 and H2 also was recorded as control.

Native PAGE analysis

H1 and H2 were heated to 95 °C for 5 min and then allowed to cool to room temperature for 3 h before use. Target DNA samples were mixed with H1 (1 μM) and H2 (1 μM) in the above-mentioned buffer solution. The gel was run in 10% acrylamide solution (Acr : Bis = 29 : 1) with 1 × TBE buffer, at 100 V constant voltage for 1 h. All the gels were run at room temperature and were stained 18 h using Stains-All (Sigma-Aldrich) to image the position of DNA. Photographic images were obtained under visible light with a digital camera.

Target detection in 1:9 dilution of saliva sample.

The saliva samples were obtained from laboratory personnel, which were centrifuged before use, then the treated saliva was diluted 10 times using 50 mM Tris-HCl (pH 8.0) buffer solution containing 5 mM $MgCl_2$. The 1:9 dilution of saliva samples were spiked with DNA target three concentrations (100, 300, and 500 pM) and measured.

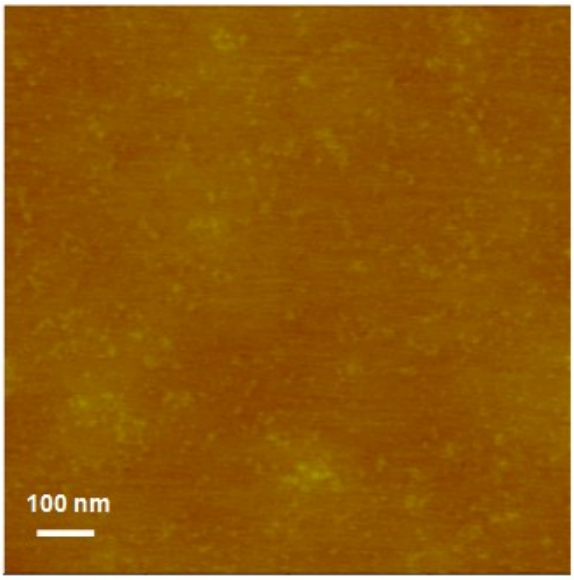

Figure S1 AFM image of the system consisting of only H1 and H2. H1 and H2 concentration: 200 nM.

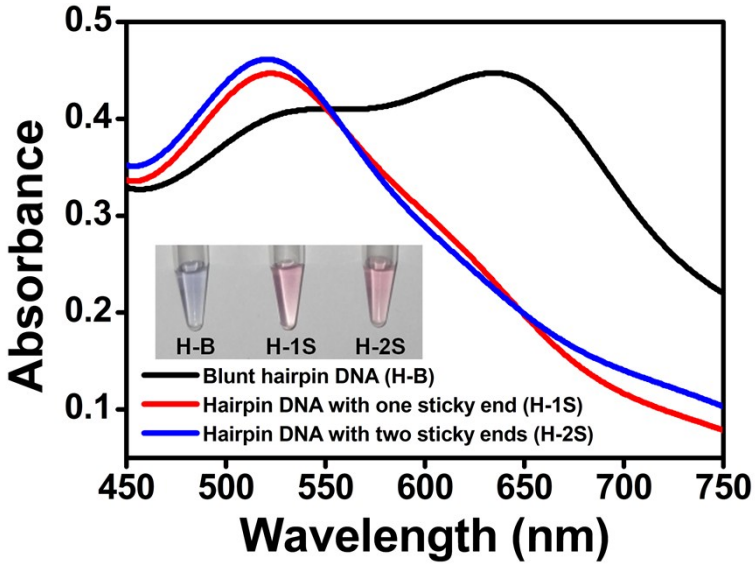

Figure S2. Absorption spectra of AuNPs in the presence of blunt hairpin DNA (H-B), hairpin DNA with one sticky end (H-1S), and hairpin DNA with two sticky ends (H-2S). Hairpin concentration: 1 μM. Inset: Photographs of AuNPs solutions in the presence of different hairpin DNAs.

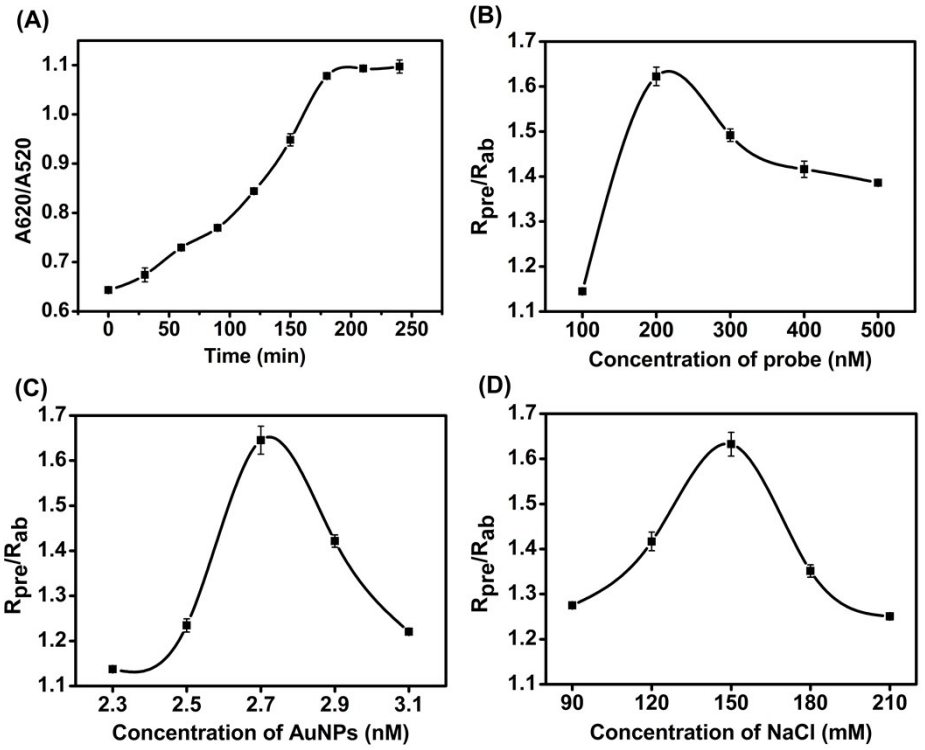

Figure S3. Condition optimization for nucleic acids detection: (A) reaction time, concentrations of (B) hairpin probes, (C) AuNPs and (D) NaCl.

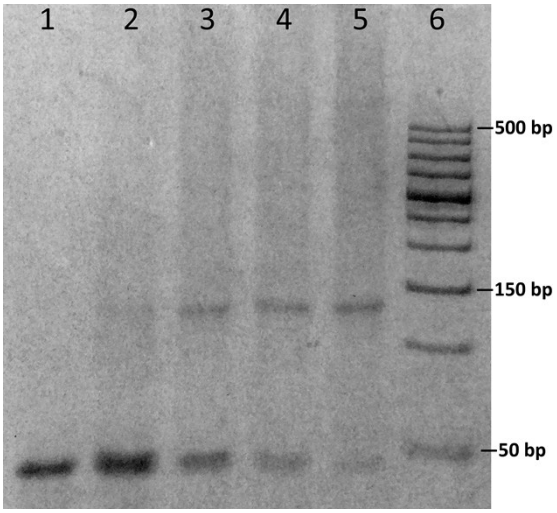

Figure S4. The gel electrophoresis image for the DNA polymer chain triggered by target DNA in 1:9 dilution of saliva. Lane 1: H1 only; Lanes 2─5, four different concentrations of target DNA with 200 nM of H1 and H2 in 1:9 dilution of saliva (from left to right: 0, 0.1, 1, and 10 nM); Lane 6: DNA ladder.

Table S2. Detection of the target DNA from 1:9 dilution of saliva

Samples	Added (pM)	Detected[a] (pM)	Recovery (%)	RSD (%) (n=3)
1	100	95.7	95.7	1.1
2	300	342.6	114.2	5.2
3	500	535.3	107.1	2.0

[a] Average of three samples.

References

(1) J. W. Liu and Y. Lu, *Nat. Protoc.*, 2006, **1**, 246.

(2) J. H. Huang, X. F. Su and Z. G. Li, *Anal. Chem.*, 2012, **84**, 5939.