Electronic Supplementary Information

A new strategy for DNA assay based on target triggered isothermal exponential degradation reaction

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EXPERIMENTAL SECTION

Chemicals

Hydrogen tetrachloroaurate trihydrate (HAuCl₄·3H₂O), Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP), sodium citrate and other salts were obtained from Sigma. Acrylamide, N,N,N’,N’-tetramethylethylenediamine were supplied from SBS (Beijing, China). The Bst DNA polymerase, Large Fragment and BsaBI endonuclease were purchased from New England Biolabs Inc. Other chemicals were of analytical grade and were used without further purification. For all experiments, Milli-Q water (18.0 MΩ) was used, purified by a Milli-Q Plus 185 ultrapure water system (Millipore purification pack).

DNA oligonucleotides were synthesized by Sangon (Shanghai, China) and used without further purification. The DNA sequences described in paper are as follows.

Target DNA: 5’-CAGTCGTAGG-3’

Linker: 5’-CCTACGACTGGATGACGATCCCTACGACTGCTCGCCTCCCCC-3’

Helper DNA: 5’-CGTCATCCAGTCGAAAACTCGCCTCCCCC-3’

Probe DNA: 5’-SH-AAAAAGGGGGAGGCGAG-3’

Mismatched DNA: 5’-CAGTCCTAGG-3’

TT-isoTexpDR Protocol

TT-isoTexpDR was carried out in a solution containing 0.5 unit/μL BsaBI endonuclease, 0.1 unit/μL Bst polymerase, 250 μM dNTPs, 0.5 × NE buffer 2 (50 mM NaCl, 10 mM Tris-HCl (pH 7.9), 10 mM MgCl₂), 1 × Thermopol buffer (10 mM KCl, 10 mM (NH₄)₂SO₄, 20 mM Tris-HCl (pH 8.8), 0.1% Triton X-100). 100 nM Linker and desired
amount of target DNA were added into the solution before the reaction. The resulting mixtures were incubated at 60 °C for 8.5 min, and the enzymes were then denatured to stop reaction. It is noted to state that the template and polymerase are separately heated to 60 °C before the incubation.

**Preparation of probe DNA-functionalized gold Nanoparticles**

The 13 nm gold Nanoparticles (Au NPs) were firstly prepared using a standard citrate method.\(^1\) Briefly, trisodium citrate (10 mL, 38.8 mM) was added to a boiling solution of HAuCl\(_4\) (100 mL, 1 mM). Within several minutes, the color of the solution was changed from pale yellow to deep red. The mixture was allowed to heat under reflux for another 30 min to ensure complete reduction and was slowly cooled down to room temperature.

Au NPs were then functionalized with thiol-modified probe DNA according to the previously reported method.\(^2\) After activated by treatment with TCEP at room temperature, the deprotected probe DNA (9 μl, 1 mM) was added into 3 ml citrate-stabilized nanoparticles solution (17.5 nM). The resulting solution was stored in a drawer at room temperature for at least 16 h. After that, 1 M NaCl solution was gradually added to enhance the NaCl concentration of the solution to 100 mM, and the solution was incubated for another day at room temperature. The probe functionalized Au NPs was purified three times by centrifugation (16,600 g for 15 min) in Tris-HCl (10 mM, 0.1 M NaCl, pH 7.5), and the solution was stored in the same buffer at 4 °C.

**Gel Electrophoresis**
The reaction mixture was monitored by using denaturing polyacrylamide gel electrophoresis (20% acrylamide, acrylamide/bisacrylamide=19:1, 7 M Urea). Electrophoresis was carried out in 1 x TBE (pH 8.0) at 150 V constant voltages for 1.5 h. After stained by SYBR Green, the gel was recorded by a GelDoc XR+ System (Bio-Rad).

**Spot Test**

Spot test solution contained 20 μL Probe-AuNPs (17.5 nM), 10 μL helper (1 μM) and 30 μL TT-isoTexpDR reaction products. Hybridization of DNA was facilitated by freezing and thawing of the solutions, and the color change of the solution was recorded by a Canon Camera.

**References**

**Fig. S1** The obtained fitting curve for the degradation of the linker with different reaction time (minutes). The data was quantified by the Quantity-One software (Bio-Rad), and the amount of linker at the reaction time of 2.5 min was assumed as 1.
**Fig. S2** Polyacrylamide gel assays of the degradation of the linker with different reaction time. The reaction time is 8.5min (Lane 1-3), 9.5min (Lane 4-6) and 10.5min (Lane 7-9), separately. For each time, the lanes from left to right indicate the degradation of the linker when no target, 100 pM target and 100 pM mismatched DNA are added in the TT-isoTexpDR system.