Supporting Information

Ultrasensitive and feasibly achieved protein detection based on the integration of three signal amplification reactions via sharing a DNA sequence

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Experimental Section

Reagents and Chemicals

Recombinant human TNF-α (>98%) and all the DNA sequences used in this study were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The DNA sequences are as follows: HP1 (5'-TGAGGGTTGTCGCCGAGCTTTTTTGGTGGA TGGCGCAGTCGGCGACAA-PO_3-3'), HP2 (5'-GGGGGGGGGGGGCCTCAGCT CGGCGACAACCCTCAGCAAAAATCGGTTGTCGCCGATTTTT-3'). All the DNAs were purified by high-performance liquid chromatography (HPLC). Recombinant human Insulin (>98%) was purchased from Tocris (UK). Bovine serum albumin (BSA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The Nb.BbvCI endonuclease, the Klenow Fragment (exo^-) and the deoxynucleotide mixture (dNTPs) were purchased from New England Biolabs, Inc. (Ipswich, MA, USA). Other chemicals were all of analytical grade. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Barnstead, USA) to a specific resistance of > 18MΩ cm. Human venous blood samples from ovarian cancer patients were obtained from the local hospital and were approved by the ethical committees. The fresh blood samples were pretreated as follows. Venous blood samples were centrifugated at 2500 rpm for 10 minutes, and the supernatants were collected and dialyzed. The prepared supernatants were ready for assay.

Signal Amplification Reaction

The amplification reaction was carried out with reaction solution (10 mM

Tris-HNO_3, 50 mM $NaNO_3$, 10 mM $Mg(NO_3)_2$, 100 μg/ml BSA, PH 7.9) containing 50 nM HP1, 500 nM HP2, 0.5 mM dNTPs, 0.12 U/μL Klenow Fragment (exo^-), 0.4 U/μL Nb.BbvCI and different concentrations of target TNF-α. Before the reaction, the HP1 and HP2 solutions were separately heated at 95 ℃ for 5 min, followed by cooling down to room temperature over 30 min to make the probe perfectly fold into a hairpin structure. Then the hairpin probes were added to the mixture and incubated at 37 ℃ for 60 min.

Preparation of DNA-AgNCs and Fluorescence Measurements

Briefly, the resultant products of amplification reaction was mixed with 100 μM $AgNO_3$ in the PBS buffer (10 mM, pH 7.0) and centrifuged at 12000 rpm for 5 min at room temperature to collect the supernatant. The supernatant was incubated at 4 ℃ for 15 min in the dark. Then, 100 μM freshly prepared $NaBH_4$ was added under vigorously shaking and the reaction mixture was incubated at 4 ℃ for 5 h in the dark. Finally, the as-prepared fluorescent DNA-AgNCs were ready for fluorescence measurements. The fluorescence measurements were all achieved by Hitachi F-2500 fluorescence spectrometer (Hitachi. Ltd., Japan). The excitation was at 580 nm, and the recording emission range was 600-700 nm. All excitation and emission slits were set at 5 nm with a PMT voltage of 700 V. All these detections were recorded at room temperature.

Characterization

Transmission Electron Microscopy (TEM) was performed on a Jeol JEM-2100 microscope with an accelerating voltage of 200 kV. The TEM specimens were

prepared by dispersing the DNA-AgNCs on 400 mesh size copper grids. Ultraviolet-visible (UV-vis) spectra were recorded by a Shimadzu UV-2450 spectrophotometer.

Electrophoresis Demonstration

Gel electrophoresis analysis of 4% agarose gel was employed for the characterization of the amplification reaction. Electrophoresis was carried out at room temperature in TBE buffer (9 mM Tris-HCl, 9 mM boric acid, 0.2 mM EDTA, pH 7.9) with a 110 V constant voltage for 50 min. After separation, the gel was stained with GelRed and imaged using the fluorescence gel imaging system.

Supporting Figures

Optimization of HP2 Concentration

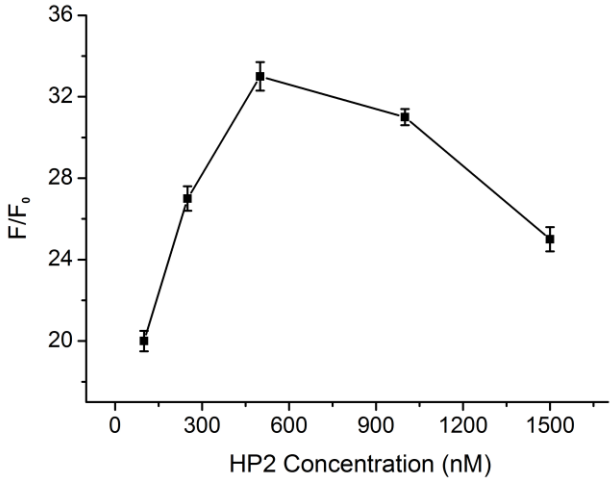

Fig. S1 Variance of fluorescence ratio of F/F_0 with the concentration of HP2. The F and F_0 represent the fluorescence intensity in the presence and absence of TNF-α, respectively. The error bars show the standard derivation of three independent experiments.

Optimization of Enzyme Concentration

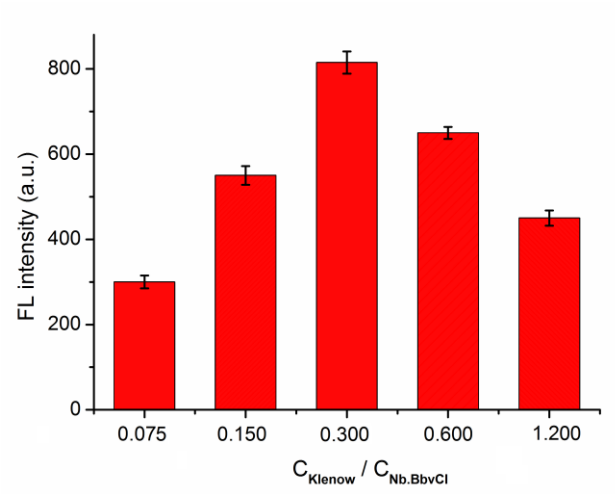

Fig. S2 Variance of the fluorescence (FL) intensity with the ratio of C_{Klenow} and $C_{Nb.BbvCI}$. C_{Klenow} is the concentration of Klenow Fragment (exo$^-$), and $C_{Nb.BbvCI}$ is the concentration of Nb.BbvCI nickase. Note: the concentration of Nb.BbvCI nickase is set at 0.4 U/μL in the experiments. The error bars show the standard derivation of three independent experiments.

Optimization of Amplification Reaction Time

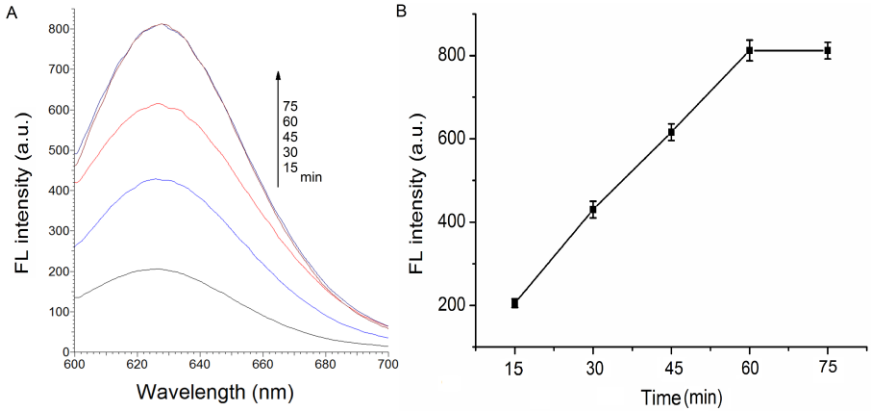

Fig. S3 (A) Variance of the fluorescence intensity with the amplification reaction time. Concentration of TNF-α is 1000 pg/ml. (B) Relationship between the fluorescence intensity and the amplification reaction time. The error bars show the standard derivation of three independent experiments.