Electronic Supplementary Information (ESI)

One-step conjugation chemistry of DNA with highly scattered silver nanoparticles for sandwich detection of DNA

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Experimental section

1. Apparatus

The absorption spectra of AgNPs and AuNPs colloid solution were measured with U-3010 UV-Vis Spectrophotometer (Hitachi, Japan), and the scattering spectra of AgNPs were measured by F-4500 Fluorescence Spectrophotometer (Hitachi, Japan). S-4800 scanning electron microscope (SEM) (Hitachi, Japan) was used for imaging the size and shape of AgNPs and AuNPs. Fluolog-3 Spectrophotometer (Jobin Yvon, France) was used for single nanoparticles counting. Zetasizer Nano ZS (Malvern, America) was used to measure the hydrodynamic diameter and Zeta potential. Dark-field imaging was carried out through BX51 optical microscope (Olympus, Japan) equipped with a high numerical dark-field condenser (U-DCW, 1.2-1.4). The scattering light was collected by a 100× object lens and images were taken by DP72 single chip truecolor CCD camera (Olympus, Japan) controlled by IPE software.

2. Reagents and materials

Zonyl® FSN-100 and DTT were purchased from Sigma-Aldrich (Missouri, U.S.A.). Chloroauric acid tetrahyrate ($HAuCl_4 \cdot 4H_2O$) was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Sodium citrate, silver nitrate ($AgNO_3$), sodium chloride (NaCl),

cetyltrimethylammonium bromide (CTAB), dodecyl sulfate sodium salt (SDS), polyvinyl pyrrolidone (PVP), and Tween-20 are analytical reagent grade for use without further purification. The used DNA sequences listed in Table S1 were synthesized by Sangon Biotech (Shanghai, China) Co., Ltd. The underlined bases are mismatched. 10 mmol/L PBS buffer was used for DNA hybridization. Milli-Q purified water (18.2 MΩ) was used for all the experiments.

Table S1. DNA sequences used in this contribution.

Oligonucleotides	Sequences
DNA_1	5′-SH-$(CH_2)_6$-A_{10}-ATG TGG AAA AT-3′
DNA_2	5′-CTC TAG CAG T-A_{10}-$(CH_2)_3$-SH-3′
HIV DNA	5′-ACT GCT AGA GAT TTT CCA CAT-3′
One mismatched	5′-ACT GCT AGA **T**AT TTT CCA CAT-3′
Two mismatched	5′-ACT **T**CT AGA **T**AT TTT CCA CAT-3′

3. Synthesis of AgNPs and AuNPs

AgNPs were synthesized by reducing $AgNO_3$ with citrate trisodium according to previous work (P. C. Lee and D. Meisel, *J. Phys. Chem.*, 1982, **86**, 3391). To a clean cone-shaped bottle, 50 mL of $AgNO_3$ solution (1.0 m mol/L) was added and brought to boiling. Shortly, 0.5 mL of citrate sodium (w/v, 5%) was added under vigorous stirring. The mixture was kept boiling and refluxed for 15 minutes. Then the colloid solution was cool down to room temperature with continuous stirring. For the synthesis of AuNPs with about the same particle size, the procedure was almost the same as AgNPs with 50 mL of $HAuCl_4$ (0.25 mmol/L) and 0.1 mL of citrate trisodium (w/v, 5%). The concentrations of AgNPs and AuNPs were measured by Lambert-beer's law ($A = \varepsilon bc$). The molar extinction coefficient (ε) of AgNPs and AuNPs were calculated according to previous work (J. Yguerabide and E. E. Yguerabide, *Anal. Biochem.*, 1998, **262**, 157). The calculated ε for our prepared AgNPs is 5.426×10^{10} $M^{-1} \cdot cm^{-1}$, and 4.658×10^{10} $M^{-1} \cdot cm^{-1}$ for AuNPs.

4. Preparation of DNA-AgNPs conjugates

The SH-DNAs (DNA_1 and DNA_2) were treated with 0.17 mol/L PBS buffer containing 0.1 mol/L DTT for about 3 hours, then purified using a NAP-5 column. DNA-AgNPs conjugates

were prepared through one-step incubation of AgNPs with SH-DNA. Typically, 1 mL of 10 mmol/L PB buffer (pH 7.4) solution containing ~80 pmol/L AgNPs, ~1.6 μmol/L SH-DNA, 0.1% FSN, and 1.0 mol/L NaCl was incubated at 30°C for about 4 hours. The mixture was then centrifuged at 10000 rpm for 10 min and suspended in purified water. This process was repeated four times to completely wash off the unreacted SH-DNA and the DNA-AgNPs conjugates was finally suspended in 0.1% FSN containing 0.2 mol/L NaCl for further use.

5. Calculation of DNA loading

The calculation of loading density and number was carried out through a subtraction method. As stated in previous work (*Anal. Chem.*, **2009**, 81, 8523), FSN capping layer can inhibit the nonspecific adsorption of DNA on AgNPs surfaces. In addition, FSN can adsorb on a variety of substrates (*WO Pat.*, 2010110749, 2010), and thus, in our opinion, inhibit the adsorption of DNA on container (glass) surfaces. Thus, it is reasonable to regard the loss of DNA after incubation of AgNPs with DNA as the total DNA loading on AgNPs surfaces. In such case, the loading number (σ, strands/particle) and density (ρ, pmol/cm^2) can be calculated using the following equation:

$$\sigma = \frac{\Delta A_{DNA}}{\varepsilon b} \Big/ c_{AgNPs}$$

$$\rho = \sigma / \left(4NA\pi r^2\right).$$

Where, ΔA_{DNA} is the absorbance change of DNA after incubation; ε is the molar absorption coefficient of DNA; b is the light path of 1 cm; c_{AgNPs} is the concentration of AgNPs; NA is the Avogadro's constant; r is the average radius of AgNPs.

6. Detection of HIV DNA

Generally, 40 μL mixtures containing 17 pmol/L DNA_1-AgNPs, 17 pmol/L DNA_2-AgNPs, 10 mmol/L PB buffer, 0.3 mol/L NaCl, 0.1% FSN, and appropriate HIV DNA were mixed thoroughly and incubated in a water bath at 37°C for 45 min. Then, the mixture was diluted to 400 μL and transferred for scattering measurements by F-4500 Fluorescence Spectrophotometer with voltage of 400 V and slits of 2.5 nm.

Additional Figures

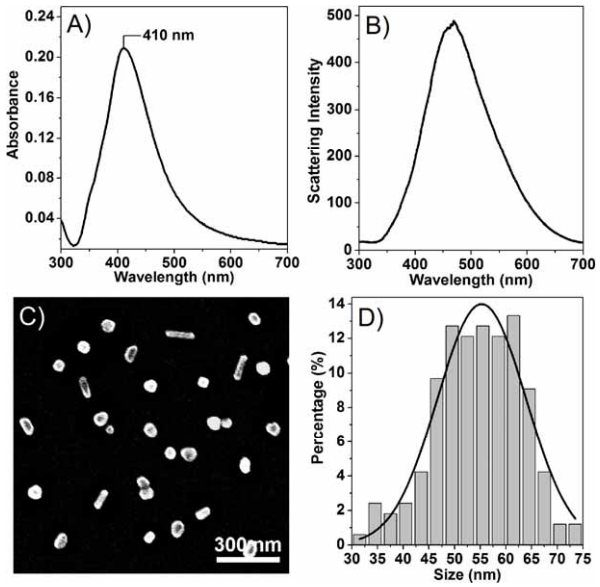

Fig. S1 (A) Absorption spectrum, (B) scattering spectrum, (C) SEM image, (D) size statistics of AgNPs. As the figures show, AgNPs have characterized absorption and scattering at 410 nm and 470 nm, respectively. AgNPs are in different shapes, main spheres with a few rods. And the statistics displays AgNPs have an average diameter of 54.4 nm.

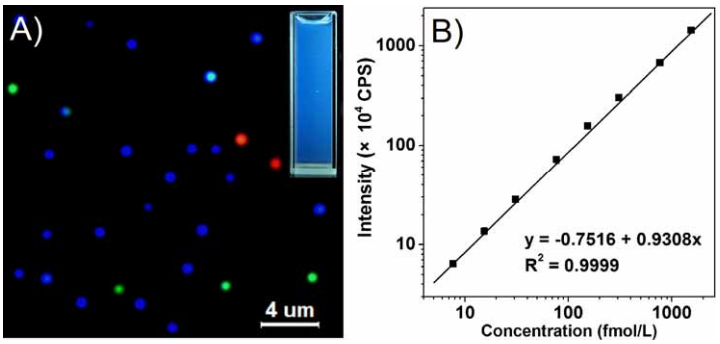

Fig. S2 (A) A dark-field scattering image of AgNPs. The insert is a picture of AgNPs colloid solution (3.85 pmol/L) irradiating with a common LED torch. Note that the dark-field image was taken with light source of a halogen lamp and a common LED torch for the inserted picture, suggesting AgNPs have highly scattered property. (B) Plot depicting the linear relationship between the AgNPs concentration and the scattering intensity. All data were collected from three measurements. The detection limit was calculated based on three folds of signal-to-noise.

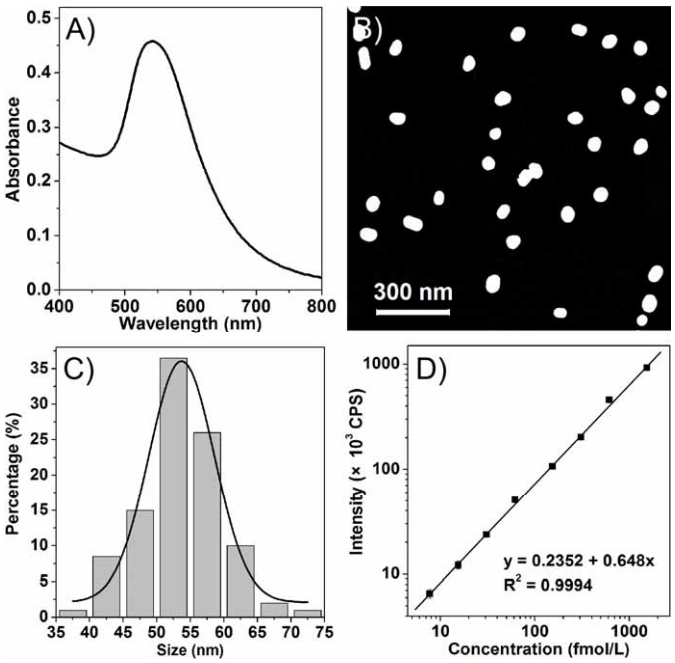

Fig. S3 (A) Absorption spectrum, (B) SEM image, and (C) size statistics of AuNPs, (D) Plots depicting the linear relationship between the AuNPs concentration and the scattering intensity. All data were collected from three measurements. AuNPs have characterized absorption at 542 nm, are in different shapes, and main spheres with a few short rods. The statistics displays AuNPs have an average diameter of 53.5 nm. The detection limit was 1.2 fmol/L calculated based on three folds of signal-to-noise.

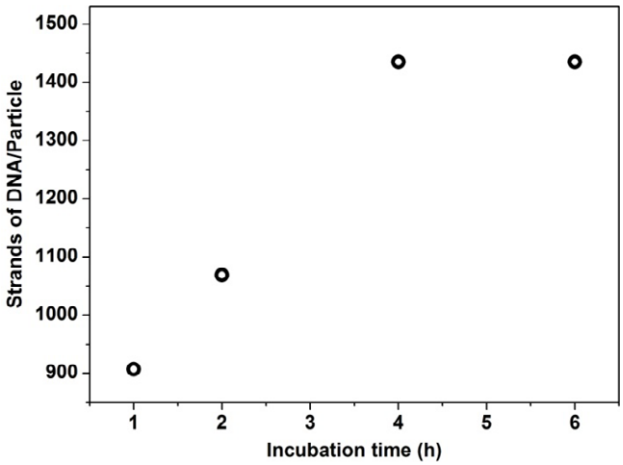

Fig. S4 Plots depicting the DNA loading as a function of incubation time. Within 4 hours, the DNA loading number increased as the incubation time prolonged. While, the DNA loading number changed hardly after incubation for another 2 hours. This result told that the conjugation was completed within 4 hours.

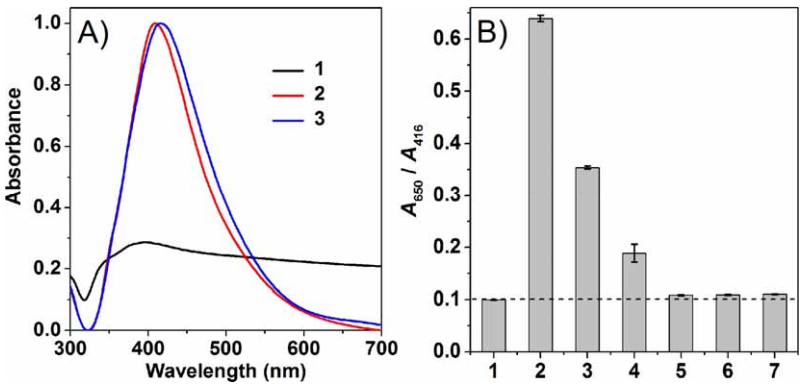

Fig.S5 (A) Absorption spectra of bare AgNPs in 50 mmol/L NaCl (1), bare AgNPs in water (2), and in 500 mmol/L NaCl with the addition of 0.1% FSN (3). (B) The absorbance ratio ($A_{650\ nm}/A_{416\ nm}$) of AgNPs in 1, water and in 500 mmol/L NaCl with the addition of 2, 0.02%; 3, 0.05%; 4, 0.075%; 5, 0.1%; 6, 0.2%; 7, 0.3% FSN. All data were collected from three measurements. Due to the increase of surface intension, bare AgNPs aggregated dramatically in the presence 50 mmol/L NaCl. However, no aggregation of AgNPs was observed with the addition of 0.1% FSN, suggesting that FSN can protect AgNPs well. Further find was that FSN lower than 0.1% hardly protects AgNPs, and no obvious difference in stability of AgNPs was observed when FSN was higher than 0.1%. Without indicated, 0.1% FSN was used to stabilize AgNPs in our experiments.

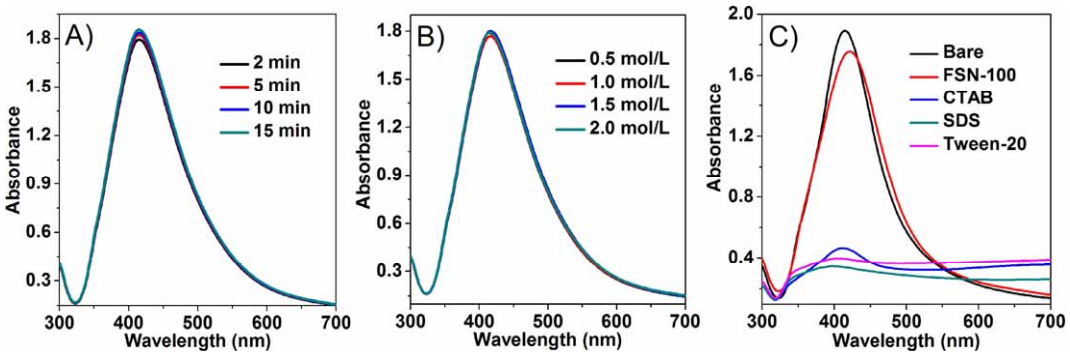

Fig. S6 Absorption spectra of AgNPs colloid solution (A) incubating with 0.1% FSN for 2, 5, 10, 15 min followed by exposure to 0.5 mol/L NaCl; (B) incubating with 0.1% FSN followed by exposure to 0.5, 1.0, 1.5, 2.0 mol/L NaCl; (C) incubating with 0.1% FSN, 0.3% CTAB, 0.3% SDS, 0.3% Tween-20 followed by exposure to 0.5 mol/L NaCl. No obvious difference in stability of AgNPs was observed with different incubation time (A) and in different concentration of NaCl (B). 0.1% FSN can stabilize AgNPs well, while 0.3% CTAB, SDS, and Tween-20 cannot stabilize AgNPs.

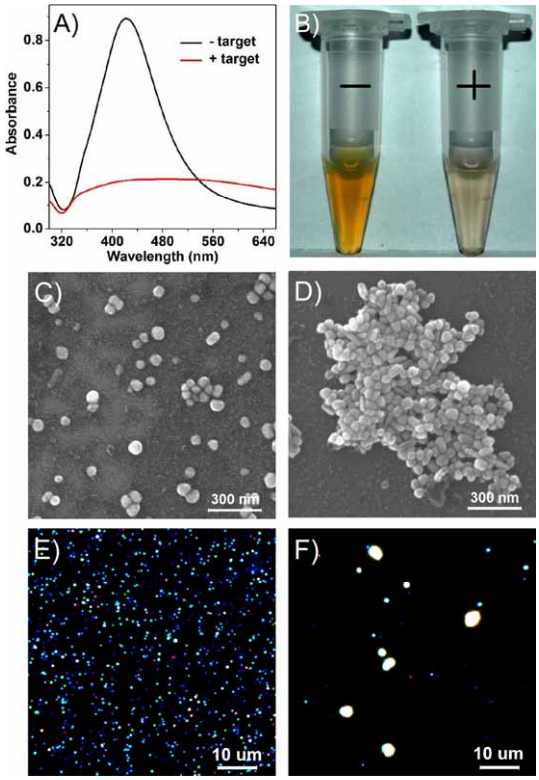

Fig. S7 (A) Absorption spectra, (B) a digital photograph, (C, D) SEM and (E, F) dark-field images of the prepared DNA-AgNPs conjugates before (C, E) and after (D, F) hybridization with target DNA**.** The absorption spectra (A) showed that the absorption peak at 420 nm broadened and decreased to a maximum at about 600 nm. At the same time, the color of AgNPs colloid solution changed from brown-yellow to colorless (B). These results indicated that AgNPs were aggregated, which were directly observed by SEM imaging (C and D). After hybridization, dispersed AgNPs aggregated. This can also be seen from dark-field images. Before hybridization, AgNPs were dispersed with blue scattering light. While, the scattering light intensity of a particle increased and the scattering color changed from blue to white due to strongly plasmon coupling between aggregated nanoparticles.

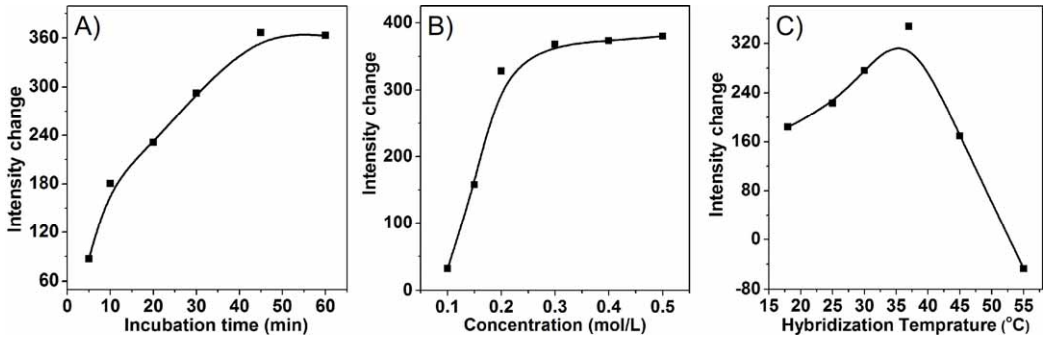

Fig. S8 The scattering intensity change of DNA_1-AgNPs conjugates (17 pmol/L) and DNA_2-AgNPs conjugates (17 pmol/L) after hybridizing with HIV DNA (5.0 nmol/L) (A) for different incubation time at 37°C with the addition of 0.2 mol/L NaCl, (B) in the presence of different concentration of NaCl at 37°C for 45 min, (C) at different hybridization temperature for 45 min with the addition of 0.3 mol/L NaCl. The hybridization buffer was 0.01 mol/L PB buffer. As one can see, the maximum intensity change of DNA-AgNPs conjugates was occurred with an incubation time of about 45 min at 37°C in the presence of 0.3 mol/L NaCl.

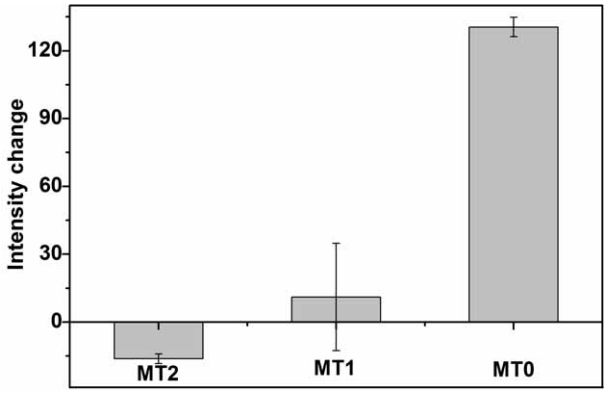

Fig. S9 Column diagram of the scattering intensity change (ΔI) after the prepared conjugates hybridized with 1.0 nmol/L target DNA (MT0), one base-pair mismatched target DNA (MT1), and two base-pair mismatched target DNA (MT2).