Supporting Information

Mixed DNA-functionalized Nanoparticle Probes for Surface Enhanced Raman Scattering-based Multiplex DNA Detection

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1. Materials and methods

1.1. Materials

Silver nitrate, 4-aminothiophenol, 6-mercaptonicotinic acid and 2-mercaptopyrimidine, and sodium citrate tribasic dihydrate were purchased from Aldrich. 1,2-dithiane-4-O-dimethoxytrityl-5-[(2-cyanoethyl)-N,N-diisopropyl]-phosphoramidite (DTPA) was synthesized according to the literature procedures.[S1] The oligonucleotides used were purchased from TaKaRa Biotech (Dalian, China) and used as received. Silver colloids with mean diameters of 30 nm were synthesized by the citrate method.[S2] All buffers were prepared with ultra-pure MilliQ water (resistance > 18 MΩ cm^{-1}).

DNA oligonucleotides (DTPA represent the cyclic disulfide-containing phosphate derivative) :

a1: 5'-AGA TTA CTG ACC GAT $(DTPA)_3$ -3'

b1: 5'- AGT AAC GAA GTC ATA $(DTPA)_3$-3'

c1: 5'- CGT ATC TTC ATT TGG $(DTPA)_3$-3'

a2: 5'-$(DTPA)_3$ TTG TGT TAT TCG TGA-3'

b2: 5'-$(DTPA)_3$ GAT TCA TCA TCA CTG-3'

c2: 5'-$(DTPA)_3$ ACC TGT CGT TTG CTA-3'

Ta: 5'- ATC GGT CAG TAA TCT TCA CGA ATA ACA CAA -3'

Tb: 5'- TAT GAC TTC GTT ACT CAG TGA TGA TGA ATC -3'

Tc: 5'- CCA AAT GAA GAT ACG TAG CAA ACG ACA GGT -3'

Control sequence: C_T: 5'-AAT TGA TAT GTC ACG AAT AAC ACA AAT CGG -3'

Thiol-capped DNA sequence: 5'- AGT AAC GAA GTC ATA $(CH_2)_6$SH-3'

1.2. Apparatus

Transmission electron microscopy (TEM) images were obtained using a Philips CM 200 kV electron microscope. UV-Vis spectra were collected using a Hitachi U-4100 spectrophotometer. Raman spectra were taken on a Renishaw invia Raman Microscope using the 532 nm laser excitation

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2. Experimental details

2.1 The Synthesis of DTPA Molecule

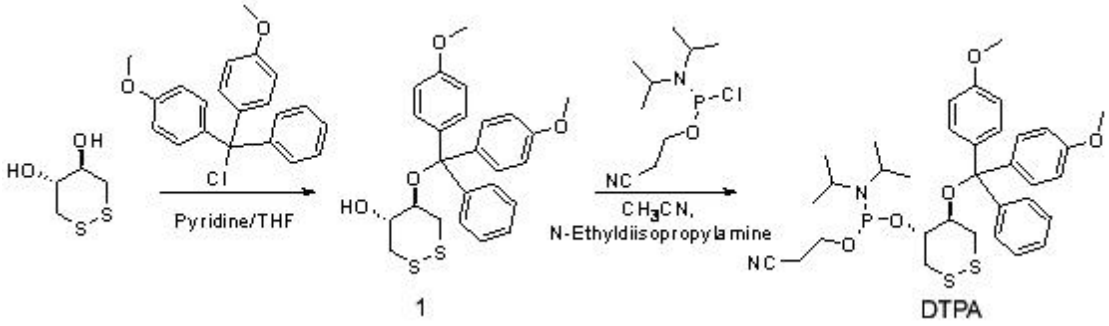

Fig. S1. Synthetic approach to molecule DTPA.

Target molecule DTPA was synthesized as previously described by P. Liepold et al.[S1] Chemicals were purchased from Aldrich and used as received. All air and water sensitive reactions were performed under nitrogen atmosphere. Toluene and tetrahydrofuran (THF) were distilled from sodium. Acetonitrile and pyridine were distilled from CaH_2. ^{1}H NMR spectra were recorded on a Mercury plus 300 MHz using $CDCl_3$ as solvent unless otherwise noted. All chemical shifts were reported in parts per million (ppm), ^{1}H NMR chemical shifts were referenced to TMS (0 ppm) or residual $CHCl_3$ (7.26 ppm). ESI mass spectra were recorded on a Bruker Apex IV FTMS.

Compound 1:

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ 7.50-7.47 (d, *J* = 7.8 Hz, 2H), 7.40-7.16 (m, 7H), 6.86 -6.83 (d, J = 9.0 Hz, 4H), 3.80 (s, 6H), 3.44 (m, 2H), 2.90-2.73 (m, 4H). EI-MS: Calcd for $C_{25}H_{26}O_4S_2$: 454.1. Found: 454 (M^+).

Compound DTPA:

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ 7.56-7.53 (dd, *J* = 8.1 Hz, *J* = 2.7 Hz, 2H), 7.43-7.39 (m,

4H), 7.33-7.15 (m, 3H), 6.86-6.81 (m, 4H), 3.80 (d, *J* = 1.5 Hz, 6H), 3.74-3.64 (m, 2H), 3.60-3.45 (m, 2H), 3.11-3.07 (m, 2H), 2.75-2.68 (m, 4H), 2.65-2.54 (t, *J* = 6.6 Hz, 1H), 2.35-2.29 (t, *J* = 6.6 Hz, 1H), 1.18-0.96 (m, 12H). ESI-MS: Calcd for $C_{34}H_{43}N_2O_5PS_2$: 654.2. Found: 655.2 ($M+H^+$), 677.2 ($M+Na^+$).

2.2 Preparation of AgNPs

SERS intensity of adsorbates on the surface of metal nanoparticles is dependent on the size and shape of the particles of interest, which had been reported in some literatures.[S2] In our experiment, silver colloids with mean diameters of 30 nm were synthesized by the citrate method.[S3] Briefly, $AgNO_3$ (16 mg) was dissolved in 100 mL of H_2O and brought to boiling. A solution of 1% sodium citrate (4.8mL) was added. The solution was kept on boiling for 1 h. The Ag sols prepared by this procedure was greenish yellow and had absorption maximum at 410 nm.

2.3 Preparation of DNA-AgNPs conjugates

For preparation of conjugate 1, equal mole (2.0 nmoles) triple cyclic disulfide-derivated single-stranded oligonucleotide (P_{a1}, P_{b1}, P_{c1}) were added to the 30 nm AgNPs (1 mL, 5 nM) in 100 mM phosphate buffer (pH 7.4). Then the NaCl concentration was gradually increased to 0.3 M by adding 1 M NaCl over 48 h. The mixture was centrifuged at 13000 rpm for 30 min to remove the excess reagents. The precipitates containing DNA-AgNP conjugates were washed with 10 mM phosphate buffer containing 0.3 M NaCl twice and finally redispersed in the same buffer. For preparation of conjugate 2, DNA P_{a2}, P_{b2}, P_{c2} were grafted on surface of AgNPs respectively as the above method. After that, freshly prepared reporter solutions (3 mM in methanol) were added dropwise to the solution of DNA-AgNP conjugates, respectively, incubated and separated by centrifugation.

2.4 SERS analysis

Conjugates 1 and 2 were dispersed in 100 μL PBS buffer at concentrations of about 1 nM. An amount of 20 μL of 10 nM target DNA were then added. Raman spectra were taken on a Renishaw invia Raman Microscope using the 532 nm laser excitation with a 50 × objective.

The Raman light was dispersed by a diffraction grating with 1800 lines/mm. Data acquisition time: 10 s. All SERS spectra were baseline-corrected and normalized.

2.5 Stability studies of thiol-modified DNA conjugated AgNPs

The stability of the conjugated AgNPs with thiol-modified DNA was evaluated by assessing the formation of AgNP aggregates in 50 mM phosphate buffer at different salt concentration at 40 ºC, which was monitored by measuring the change in the plasmon band absorbance at 410 nm using UV-Vis spectrophotometer at different time.

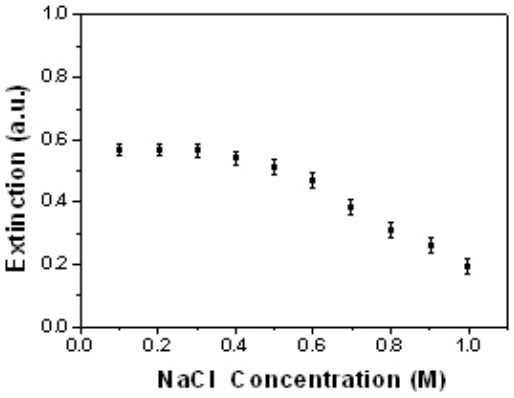

Fig. S2. UV-Vis extinction spectra changes of thiol-modified DNA conjugated AgNPs in 50 mM phosphate buffer at various salt concentrations: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 M. All the data were obtained after mixing the AgNPs in the salt solution for 48 hours.

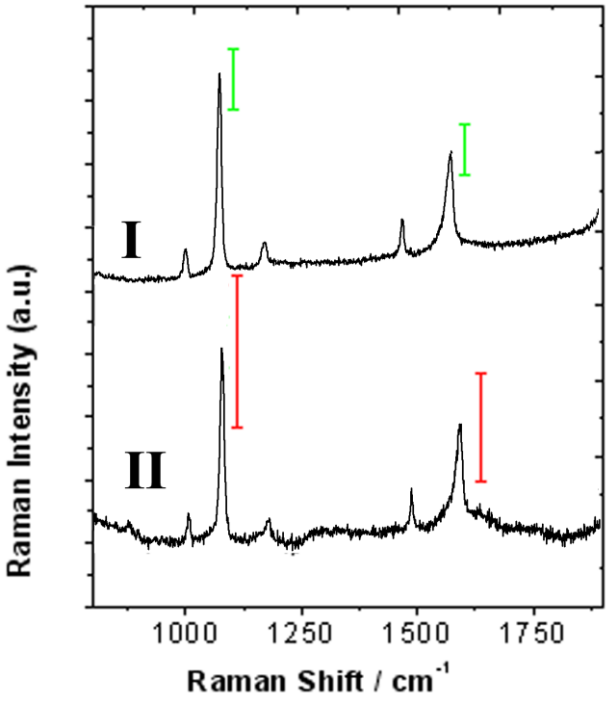

Fig. S3. SERS spectra of 4-aminothiophenol molecules obtained from triple cyclic disulfide (I) and thiol modified-DNA based detection system (II). Error bars (green and red markers) represent the standard deviation of 5 replicate SERRS analyses. The comparation of the error bars indicates that the system we proposed in this paper display a better SERS signal reproducibility.

2.6. Confirmation of the detection mechanism

The controlled aggregation of dye-coded AgNPs by the addition of target DNA was confirmed by UV-Vis spectra and TEM images (Fig. S3). At the initial stage, the UV-Vis spectrum displays a plasmon resonance at 410 nm (Fig. S3, curve I), indicating a nonaggregated state. After the addition of target DNA and incubation for 24 h, the AgNPs plasmon resonance broadened and redshifted (Fig. S3, curve II), indicating AgNPs were brought much closer to each other due to the formation of interparticle double strand DNA structure. The disperse and aggregated states of AgNPs can be clearly observed by the corresponding TEM images (see the inset in Fig. S3).

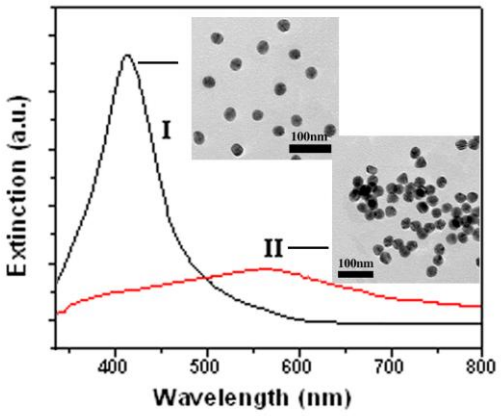

Fig. S4. Typical extinction spectra of Raman dye and DNA-functionalized AgNPs solutions at initial and the detection stages. The inset shows the typical TEM image of AgNPs accordingly.

To demonstrate that the observed AgNP assembly is specific to the target DNA, control experiment was performed. The experimental results indicates the used ligonucleotide do not

aggregate under identical experimental conditions. No significant changes in extinction spectra and TEM images were observed. This not only confirmed that the color change and spectral shift observed for the probe DNA-modified AgNPs was specific to the target DNAs, but also indicated that the AgNPs functionalized with triple cyclic disulfide-modified DNA were quite stable in the experimental conditions.

References

S1. P. Liepold, T. Kratzmüller, N. Persike, M. Bandilla, M. Hinz, H. Wieder, H. Hillebrandt, E. Ferrer, G. Hartwich, *Anal. Bioanal. Chem.* 2008, **391**, 1759.

S2. (a) M-L. Cheng, J. Yang, J. Raman Spectrosc. 2010, 41, 167-174; (b) K. G. Stamplecoskie, J. C. Scaiano, J. Phys. Chem. C, 2011, 115, 1403-1409.

S3. P. C. Lee and D. Meisel, *J. Phys. Chem.* 1982, **86**, 3391.