Controllable and reproducible construction of SERS substrate and its sensing applications†

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1. Materials and methods

1.1. Materials

Silver nitrate, 4-merpaptopridine, para-aminothiophenol (p-ATP), 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 (Liepold et al, 2008). 5-ethylthiotetrazole, controlled pore glass (CPG) and reagents for automated DNA syntheses were purchased from Glen research. Microcon® size-exclusion centrifugal filter devices were purchased from Millipore. All buffers were prepared with ultra-pure MilliQ water (resistance > 18 MΩ cm^{-1}).

1.2. Apparatus

Standard automated oligonucleotide solid-phase synthesis was performed on a Bioautomation Mermade 4 DNA synthesizer. UV-Vis spectra were measured on a Varian Cary 300 biospectrophotometer. High-performance liquid chromatography (HPLC) was performed using a Techcomp LC2000 series HPLC. Gel electrophoresis experiments were carried out on an acrylamide 20 × 20 cm vertical DYCZ24 electrophoresis unit, and an agarose 8.5 × 8 cm horizontal DYCZ31 separation minigel system. 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 with a 50 × objective. The Raman light was dispersed by a diffraction grating with 1800 lines/mm. Data acquisition time: 10 s. Circular dichroism (CD) spectra were measured using a JASCO J-815 circular dichroism spectrometer.

1.3 DNA sequences

DNA switch: 5'-TTCCCCCCTTTTT$(DTPA)_3$ (DTPA represent the cyclic disulfide-containing phosphate derivative);

P_a: 5'- CGT ATC TTC ATT TGG (DTPA)3-3';

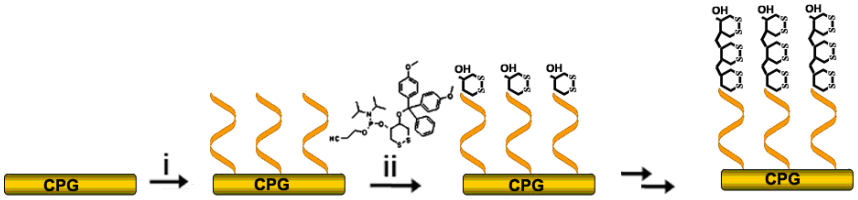
P_b: 5'-(DTPA)3 ACC TGT CGT TTG CTA-3';

T_{ab}: 5'-CCA AAT GAA GAT ACG TAG CAA ACG ACA GGT-3';

control sequence: 5'-CGT ATC CTA TAG CAA AAT GTA AGT AGT CAT-3'.

2. Experimental details

2.1 Synthesis of oligonucleotides



Scheme S1. DNA sequence of the appropriate length is (i) synthesized on CPG with a standard automated oligonucleotide synthetic protocols, which is then followed by (ii) the incorporation of a dithiolphosphoamidite molecule using a modified protocol (extended coupling/deprotection time of 6/2 min) More dithiolphosphoamidite molecules can be incorporated into the same strand by following the step ii) procedures.

The oligonucleotides were constructed on CPG supports using conventional phosphoramidite chemistry. For the sequences modified by one or more DTPA at the end of the DNA sequence,[S2] the appropriate sequence was initially grown on the solid support using standard automated solid phase oligonucleotide synthetic protocols. One or more dithiolphosphoamidite was then incorporated using a modified protocol (Scheme 1). Products were cleaved from the support by treatment with concentrated NH_4OH for 16 h at 55°C. The NH_4OH solution was decanted and dried down to yield the crude DNA mixture. The crude mixture obtained was purified by preparative reverse-phase HPLC with 0.03 M triethylammonium acetate (TEAA), pH 7 and a 1%/min gradient of 95% CH_3CN/5% 0.03 M TEAA at a flow rate of 1 mL/min. Quantification was estimated based on UV-Vis absorbance at 260 nm.

2.2 Synthesis of silver nanoparticles

The silver nanoparticles used in the experiment were synthesized by a straightforward, one-phase reaction according to the literature with some modification.[S1] In a typical synthesis process of silver nanoparticles, silver acetate (0.51 g) and 1-hexadecylamine (1.82 g) in toluene (20 mL) were firstly heated at 60 °C with stirring until silver acetate and 1-hexadecylamine were completely dissolved. A solution of phenylhydrazine (0.31 g) in toluene (10 mL) was slowly added into the resulting solution with continuous stirring and reacted at 60 °C for 1 h.

Subsequently, before the above solution down to 30°C, acetone (30 mL) was added into to precipitate the silver nanoparticles. The silver nanoparticles were isolated by centrifugation at 5000 rpm, washed twice with acetone (30 mL), and vacuum-dried at room temperature to obtain a blue-black solid. The TEM images of synthesized silver nanoparticles (Fig. S1) indicated that this procedure gave silver nanoparticles with the size of about 30 nm.

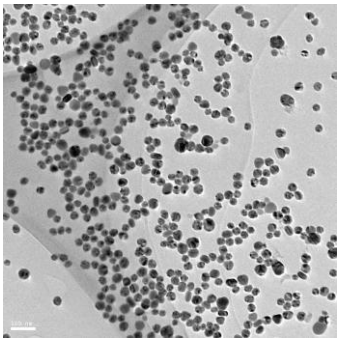


Fig. S1 The typical TEM image of AgNPs. The scale bar is 100 nm.

2.3 Preparation of labeled DNA-AgNPs conjugates

30 nm AgNPs (1 mL, 0.5 nM) was mixed with purified DNA strands P_a (10 nmoles). The mixture was incubated in 50 mM phosphate buffer. 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 20 min. The supernatant containing free oligonucleotides was removed. The precipitates containing DNA-AgNP conjugates were washed with phosphate buffer containing 0.3 M NaCl twice and finally redispersed in the same buffer. A freshly prepared reporter (4-merpaptopridine or para-aminothiophenol (p-ATP)) solution (3 mM in methanol) was added dropwise to a solution of DNA-AgNP conjugates. The mixture was gently stirred for 24 h. The solution was then centrifuged for 20 min at 13000 rpm, and the supernatant was removed.

2.4 Preparation of DNA-modified Au film

A 0.3×0.3 cm n-type Si_{100} wafer was cleaned by ultrasonication in acetone, isopropanol, and deionized water for 5 min each. The wafer was dried with N_2. Gold film of about 50 nm in thickness was deposited in a physical vapor deposition (PVD) at a rate of 3 Å s^{-1}. The silicon wafer was dipped into 0.1 M PBS buffered 100 nM DNA probe (P_b) solution for 12 h. The resultant silicon wafer was then washed with PBS buffer solution and dried with a gentle flow of nitrogen.

2.5. Confirmation of the DNA nanomachine

The pH-induced transformation of the DNA nanomachine used in our system was confirmed by CD spectroscopy. As shown in Fig. S2, when the system is in its initial state where the pH value is around 8.0, the CD spectrum shows positive and negative bands at 274 nm and 247 nm, respectively (red curve), indicating that the structure of DNA is random coil. When the pH of the solution was changed to slightly acidic conditions (pH 5.0), the CD spectrum shows a positive band at 286 nm, a crossover at around 271 nm, and a negative band centered at 256 nm (black curve), which is the characteristic spectrum of i-motif structures as reported previously.[S3]

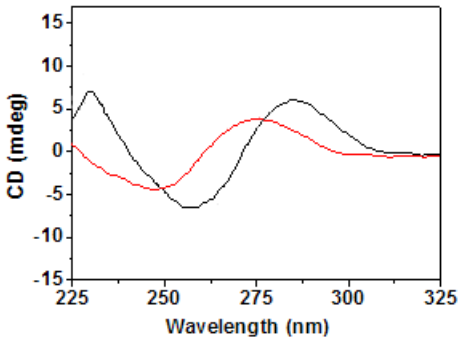


Fig. S2. CD spectra of i-motif DNA measured in 10 mM phosphate buffer containing 0.3 M NaCl at pH 8.0 (red curve) and the random coil conformation at pH 5.0 (black curve). DNA sequence: 5'-TTCCCCCCTTTTT(DTPA)$_3$

2.6 DNA detection by using SERS

The modified silicon wafer and AgNPs-DNA probes (100 μL, 100 nM) were hybridized with the target DNA in a sealed box overnight. The detections were operated for different concentration in the range of 0.1 pM-10 nM. The resultant silicon wafer was washed thoroughly with Milli-Q water to remove the unhybridized AgNP-DNA conjugates and the aggregated AgNPs, and then dried with a gentle flow of nitrogen for SERS detection.

References

S1. B. S. Ong, Y. Li, Y. Wu, *J. Am. Chem. Soc.* 2006, **128**, 4202-4203.

S2. P. Liepold, T. Kratzmüller, N. Persike, M. Bandilla, M. Hinz, H. Wieder, H. Hillebrandt, E. Ferrer, G. Hartwich, *Anal. Bioanal. Chem.* 2008, **391**, 1759-1772.

S3. H. J. Liu, Y. Xu, F. Y. Li, Y. Yang, W. X. Wang, Y. L. Song and D. S. Liu, *Angew. Chem. Int.* Ed. 2007, **46**, 2515.