Electronic Supplementary Material (ESI) for ChemComm.

Quantitative SERS-based DNA detection assisted by magnetic microspheres

Jinnan Zhang, Padmanabh Joshi, Yan Zhou, Rui Ding and Peng Zhang*

Supporting Information

Chemicals

Silver nitrate, ethylene glycol, polyvinylpyrrolidone (PVP, MW 55,000), and 4-mercaptobenzoic acid (MBA) were purchased from Sigma-Aldrich. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), tris(hydroxymethyl)aminomethane, sodium chloride and magnesium chloride were purchased from Fisher Scientific. All DNA strands, as listed in Table 1, were obtained from Integrated DNA Technologies. Carboxyl-functionalized magnetic microspheres (ProMag^{TM} HP carboxyl) were purchased from Polysciences, Inc. All chemicals were used as received.

Conjugation of DNA strands to magnetic microspheres

In a typical run, 122 mg magnetic microspheres with carboxyl functional group were dispersed in 10 ml of DI water. 100 µl of 1 mM EDC and 100 µl of 2 mM NHS were added quickly and stirred for 15 min. Next, 100 µl of 100 µM DNA_Probe1 was added under continuous stirring for 6 h. The microspheres were then collected by placing a

magnet on the side of the vial to attract the DNA-conjugated magnetic microspheres before the supernatant was removed by a pipette. The collected microspheres were subsequently dispersed in DI water, and washed 3 more times by the same procedures. The final product was dispersed in 1 ml DI water.

Synthesis of DNA-conjugated AgNPs

The synthesis of Ag nanoparticles was similar to that reported in the literature [S1]. Briefly, 0.1 g of PVP and 25 mg of $AgNO_3$ were dispersed in 10 ml of ethylene glycol. The solution was kept under 160 °C and continuous stirring for 1 h. The color of solution slowly changed from colorless to dark brown. After cool down to room temperature, the products were dispersed into 40 ml of acetone, and centrifuged at 4000 rpm for 15 min. The centrifugation procedure was repeated several times until the supernatant became colorless, and the washed Ag nanoparticles were finally dispersed in 10 ml of DI water as the stock solution. Subsequently, 1 ml of the Ag nanoparticle stock solution was diluted by 9 ml of DI water. Then 10 μl of 1 mM MBA aqueous solution was added slowly and stirred for 1 h. The concentration of MBA in the mixture should be kept under 1 μM to avoid the aggregation of AgNPs. After purified 3 times by centrifugation and washed by DI water, the products were dispersed in 10 ml of DI water. Next, 100 μl of 1 mM EDC and 100 μl of 2 mM NHS were added quickly and stirred for 15 min. Finally, 100 μl of 100 μM DNA_Probe2 was added and stirred for 6 h. The final products were purified 3 times by centrifugation and washed by DI water before dispersed in 1 ml of DI water.

Detection of target DNA

500 μl of DNA-conjugated magnetic microspheres and 500 μl of DNA-conjugated Ag nanoparticles were added into 10 ml of 20 mM Tris buffer, which includes 100 mM sodium chloride and 4 mM magnesium chloride, and stirred for 15 min. Then DNA_Target or DNA_Mismatch were added, respectively, under stirring. The final products were purified 3 times by placing a magnet on the side of the vial to attract the microspheres and removing the supernatant. The collected products were dispersed in Tris buffer and washed 3 times by the same procedures and dispersed in 1 ml of Tris buffer for measurement. The final products are well dispersed in Tris buffer. Since the average size of the magnetic microspheres is 3 μm, the magnetic microspheres-AgNPs would settle down after overnight. However, the re-dispersed magnetic microspheres-AgNPs could be stable for hours in Tris buffer.

TEM measurement

TEM was used to observe the size and morphology of the magnetic microsphere-AgNPs. A drop of magnetic microsphere-AgNPs dispersion was dried on a carbon-coated copper grid at room temperature. TEM images were taken using a Phillips Biotwin 12 transmission electron microscope.

UV-vis spectra measurements

UV-vis spectra were recorded by an Ocean Optics USB 4000 spectrophotometer using a 1-cm path-length quartz cell at room temperature.

Raman measurements

Magnetic microsphere-AgNPs were dispersed thoroughly in Tris buffer. A small amount of the solution was transferred to a quartz capillary tube (I.D: 1 mm). The tube was then placed on the stage of a Renishaw InVia microscope with a 10× objective (NA 0.25) for Raman measurement. Laser intensity at the sample was ~11 mW from a 785 nm diode laser for all measurements. Samples at each concentration were measured 5 times. Each measurement was the average of 5 scans. The exposure time for each scan was 20 s.

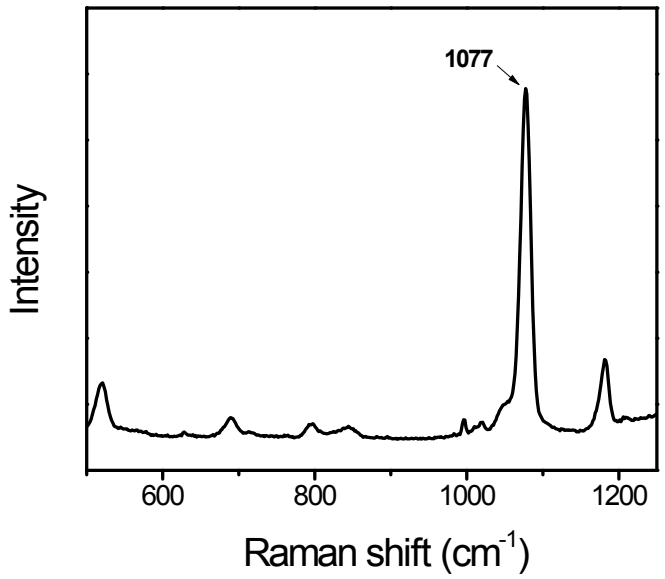

Figure S1. SERS spectra of MBA-functionalized AgNPs.

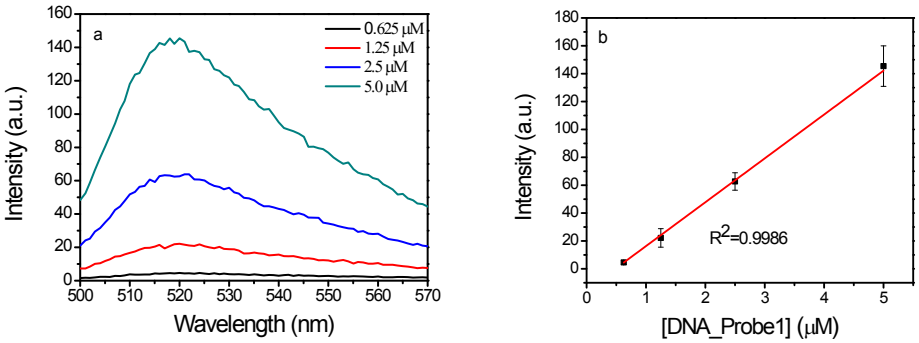

Figure S2. a) SYBR Green I fluorescence spectra excited at 480 nm with different concentrations of DNA_Probe1. b) Plot of I_{522} vs. [DNA_Probe1]. The error bars are based on the results of 5 measurements for each data point.

REFERENCES

[S1] Sun, Y.; Yin, Y.; Mayers, B. T.; Herricks, T.; Xia, Y. *Chem. Mater.* **2002**, 14, 4736-4745.