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Quantitative SERS-based DNA detection assisted by magnetic microspheres

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We report a quantitative SERS measurement scheme based on the magnetic microsphere-Ag nanoparticles to detect target DNA. The quantitative SERS measurements can quantify the target DNA concentration down to 10 nM.

Detection of specific DNA strands in nucleic acids is widely used in a variety of applications such as forensic investigations, diagnosis of genetically inherited diseases, and environmental monitoring. There are hundreds of genetic tests currently in uses and more are being developed. Fluorescence-based methods of DNA testing are the most widely used ones among these tests [1-8]. However, fluorescence-based methods have some limitations, such as cross-talk due to broad emission bands and photobleaching of the fluorophores [9]. On the other hand, because of the weak signal, Raman scattering has not been widely used in biosensing until the discovery of surface-enhanced Raman scattering (SERS). SERS-based detections have the inherently high specificity, thanks to the "fingerprint" of molecules. SERS could be performed by excitation at different wavelengths, providing flexibility to avoid spectral cross-talk. Because of its scattering nature, SERS signals do not suffer from photobleaching, resulting in good photostability [10].

There has been a number of SERS-based DNA detection strategies reported over the last decade [11-18]. For instance, one study used thiol-modified DNA oligonucleotides to bring the oligonucleotides close to Au nanoshells, and observed that SERS spectra of different DNA oligonucleotides to be extremely similar, regardless of their sequences [13]. The method relied on the "fingerprint" of DNA bases themselves, which appear to be dominated by the Stokes lines of adenine. Other studies include the detection of chemical modifications in DNA either with or without Raman reporters [14-17]. Yet another recent report introduced a scheme where an electromagnetic "hot spot" was created by assembling a DNA probe-attached Ag nanoparticle onto the Raman reporter-labelled Ag film in the presence of the complementary target DNA strand, resulting in the enormous increase of Raman intensity of the reporter [18].

In this paper, we report a quantitative SERS measurement scheme, using separately prepared DNA-conjugated Ag nanoparticles (AgNPs) and DNA-conjugated magnetic microspheres to detect target DNA. Magnetic microspheres, as compared to the planar film, can be used in solution and facilitate rapid and effective sample isolation [19-22]. The SERS-based method demonstrated a detection limit of 10 nM of matched

target DNA, with the capability of differentiating single-base mismatched DNA strand.

The design of the magnetic microsphere-AgNPs detection scheme is illustrated in Figure 1. A DNA probe complementary to a segment of the intended target DNA is first conjugated onto the surface of the magnetic microspheres through the widely-used EDC-NHS crosslink chemistry [23] between the carboxyl groups on the surface of magnetic microspheres and the amino groups on the amine-functionalized DNA strands. Separately, AgNPs are treated with a linker molecule with thiol and carboxyl groups at both ends, such as 4-mercaptobenzoic acid (MBA) used in this study. The thiol group of MBA brings it to the AgNPs surface while its carboxyl group makes the surface of AgNPs full of carboxyl groups to be subsequently conjugated to a second DNA probe, whose sequence is complementary to a segment of the intended target DNA adjacent to that to the first probe. MBA serves as not only a linker molecule between AgNPs and the second DNA probe but also as a Raman reporter in the detection scheme. In the presence of target DNA, the DNA-conjugated AgNPs are drawn to the surface of the DNA-conjugated magnetic microspheres, which can be isolated by a magnet. The detection of the target DNA is achieved by measuring the SERS signal of MBA on the magnetic microsphere surface.

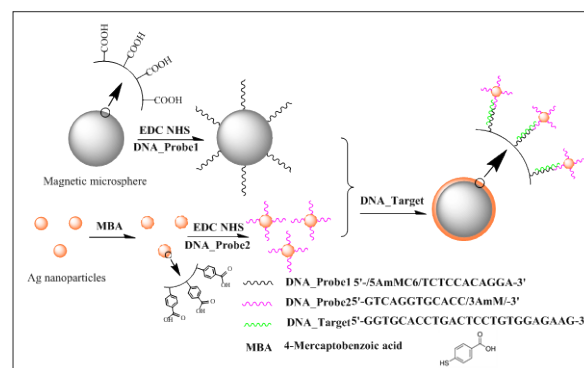


Figure 1. Illustration of the detection scheme assisted by magnetic microspheres and MBA-functionalized AgNPs.

All sequences of DNA oligonucleotides used in this study are listed in Table 1. The amount of DNA_Probe1 conjugated on the surface of the magnetic microspheres was determined experimentally by a method reported previously [24]. SYBR

Green I, an asymmetrical cyanine dye used as a nucleic acid stain in molecular biology, is barely fluorescent by itself. When mixed with single-strand DNA, SYBR Green I becomes weakly fluorescent and can be used to quantify the amount of the DNA. A standardization curve of DNA_Probe1 from 625 nM to 5 μ M in 5 mM SYBR Green I solution was first obtained (Figure S2a), showing a linear relationship between the fluorescence intensity of SYBR Green I at 522 nm and the concentration of DNA_Probe1 (Figure S2b). The fluorescence intensity of 5 mM SYBR Green I solution in the presence of DNA_Probe1-conjugated magnetic microspheres was then measured under the same experimental conditions. By comparison to the standardization curve, we calculated that the concentration of DNA_Probe1 conjugated to the magnetic microspheres was 2.4 μ M, resulting in a conjugation yield of ~24% between the magnetic microspheres and DNA_Probe1.

Table 1. Sequences of oligonucleotides used in this study.

Strand name	Sequence (5'-3')
DNA_Probe1	/5AmMC6/TCT CCA CAG GA
DNA_Probe2	GTC AGG TGC ACC/3AmMC6/
DNA_Target	GGT GCA CCT GAC TCC TGT GGA GAA G
DNA_Mismatch	GGT GCA CCT GAC TCC TGA GGA GAA G

The synthesis of AgNPs was similar to that reported in the literature [25], using ethylene glycol as the reducing agent and polyvinylpyrrolidone as the stabilizing agent. Figure 2 shows the UV absorption of the AgNPs with a plasmon peak at ~424 nm, which is in line with the result from the TEM measurement, showing the size of Ag nanoparticles to be ~75 nm. Subsequently, the AgNPs were treated with MBA to functionalize the surface of AgNPs with carboxyl groups. Non-binding MBA molecules were washed off by centrifuging the AgNPs. SERS signals of MBA were readily observed from the resulting MBA-functionalized AgNPs (Figure S1). DNA_Probe2 was then conjugated to the carboxyl groups of MBA on the surface of AgNPs through the EDC-NHS crosslink.

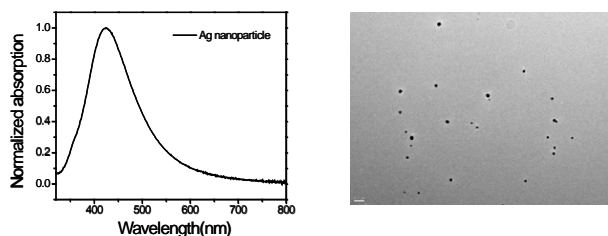


Figure 2. (Left) UV-Vis spectrum of the Ag nanoparticles. (Right) TEM image of the Ag nanoparticles. Scale bar is 200 nm.

DNA_Target, a 25-mer oligonucleotide, was used as a model target strand. DNA_Target solutions of different concentrations (from 10 to 500 nM) were prepared in Tris buffer, and used in the study. The detection of the target DNA was performed by the mixing of solutions of DNA_Probe1-conjugated magnetic microspheres, DNA_Probe2-conjugated AgNPs and DNA_Target. The DNA_Probe1-conjugated magnetic

microspheres would capture DNA_Target and DNA_Probe2-conjugated AgNPs through sequence-specific hybridization, forming the magnetic microspheres-AgNPs. The magnetic microspheres-AgNPs were subsequently isolated from the mixture by a magnet and rinsed by fresh buffer multiple times to remove any possible free AgNPs, ensuring that all SERS signals in the SERS measurements were from magnetic microspheres-AgNPs, not from free MBA-functionalized AgNPs. Finally, the magnetic microspheres-AgNPs were dispersed in Tris buffer for Raman measurements. TEM image in Figure 3 shows that DNA_Probe2-conjugated AgNPs are indeed attached to the surface of the magnetic microspheres.

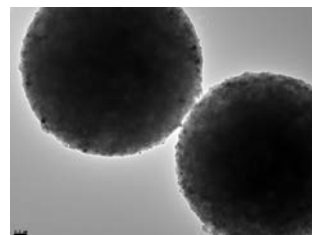


Figure 3. TEM image of the magnetic microspheres after MBA-functionalized AgNPs are drawn to the surface by the target DNA. Scale bar is 200 nm.

A series of measurements have been done to determine the detection limit of DNA_Target. Figure 4A shows the SERS spectra of the same amounts of DNA_Probe1-conjugated magnetic microspheres and DNA_Probe2-conjugated AgNPs but different concentrations of DNA_Target. Most of the pronounced Raman peaks can be attributed to MBA (Figure S1). It is observed that, as the DNA_Target concentration increases, the Raman intensity of MBA peaks also increases. In Figure 4B, the intensity of the 1077 cm^{-1} peak of MBA on the magnetic microspheres-AgNPs is plotted against the concentration of DNA_Target added in the mixture. The excellent linear relationship allows to quantify the DNA_Target concentration to as low as 10 nM, which is comparable to some fluorescence-based methods [8]. While there are other methods reporting higher detection sensitivity [26], this proof-of-concept study demonstrates the potential of the detection scheme, which can be further improved by optimizing some parameters, such as the sizes of the AgNPs and the magnetic microspheres.

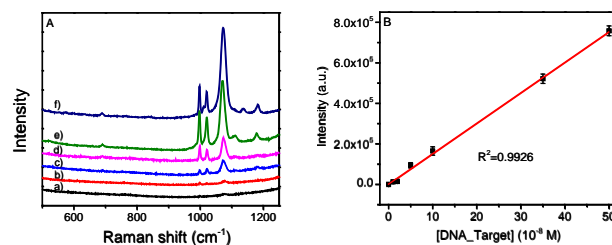


Figure 4. A) SERS spectra of mixture containing the same amounts of DNA_Probe1-conjugated magnetic microspheres and DNA_Probe2-conjugated AgNPs, and a) 10 nM; b) 20 nM; c) 50 nM; d) 100 nM; e) 350 nM; and f) 500 nM, DNA_Target. Excitation at 785 nm. B) Plot of intensity of 1077 cm^{-1} peak vs. [DNA_Target]. Error bars are based on the results of 5 measurements for each data point.

Control experiments with no target or with single-base mismatched target have also been performed to check out the specificity of the detection scheme. Figure 5 shows the SERS spectra of the magnetic microspheres-AgNPs with 100 nM DNA_Target, 100 nM DNA_Mismatch, and no DNA target at all. Note that the two DNA strands, DNA_Target and DNA_Mismatch, have only one-base difference in the sequences. The results show that the detection scheme readily differentiates the perfectly matched target from the single-base mismatched target.

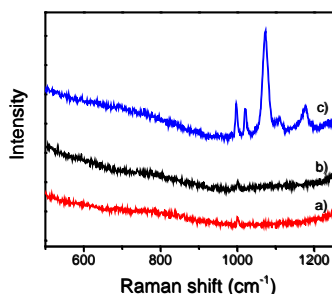


Figure 5. SERS spectra of the same amount of DNA_Probe1-conjugated magnetic microspheres and DNA_Probe2-conjugated AgNPs with a) no DNA_Target; b) 100 nM DNA_Mismatch, and c) 100 nM DNA_Target.

We should note that the detection scheme is rather flexible. For instance, the magnetic microspheres and the AgNPs can be of other sizes, or have other functional groups, as long as the capturing oligonucleotides can be conjugated to their surface. Even the linker molecule between AgNPs and oligonucleotides (MBA in this case) can be replaced with a different one, so long as it can give relatively strong SERS signals. It is likely that the optimization of such parameters could further improve the detection sensitivity.

In summary, we report a quantitative SERS measurement scheme based on the magnetic microspheres and AgNPs to detect target DNA. This detection displays both high sensitivity (down to 10 nM) and high specificity (differentiating single-base mismatched targets). The use of magnetic microspheres facilitates rapid, efficient and reproducible sample preparation. The results demonstrate great potential of using SERS for quantitative DNA detection.

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Notes and references

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