Highly Sensitive Detection of Target ssDNA Based on SERS Liquid Chip Using Suspended Magnetic Nanospheres as Capturing Substrates

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Supporting Information

ABSTRACT: A new approach for sensitive detection of a specific ssDNA (single-stranded DNA) sequence based on the surface enhanced Raman spectroscopy (SERS) liquid chip is demonstrated. In this method, the probe DNA (targeting to one part of target ssDNA) was attached to the nano-SERS-tags (poly(styrene-co-acrylic acid)/(silver nanoparticles)/silica composite nanospheres), and the capture DNA (targeting to the other part of target ssDNA) was attached to the Fe₃O₄/poly(acrylic acid) core/shell nanospheres. The nano-SERS-tags with probe DNA were first allowed to undergo hybridization with the target ssDNA in solution to achieve the best efficiency. Subsequently, the magnetic composite nanospheres with capture DNA were added as the capturing substrates of the target ssDNA combined with the nano-SERS-tags. Upon attraction with an external magnet, the nanospheres (including the nano-SERS-tags) were deposited together due to the hybridization, and the deposit sediment was then analyzed by SERS. Quantitative detection of target ssDNA was achieved based on the well-defined linear correlation between the SERS signal intensity and the target ssDNA quantity in the range of 10 nM to 10 pM, and the limit of detection was approximately 10 pM. Multiplexed detection of up to three different ssDNA targets in one sample was demonstrated using three different types of nano-SERS-tags under a single excitation laser. The experimental results indicated that the liquid-phase DNA sequencing method, thus named the SERS liquid chip (SLC) method, holds significant promises for specific detection of trace targets of organisms.

INTRODUCTION

In recent years, intensive efforts have been made to develop the nucleic acid biosensing technologies with the aim to achieve a high-level multiplexing detection and thus to fulfill the demands of high-throughput gene sequencing. To date, some great achievements have been attained in genetic analysis, including greener agriculture, better pathogen detection and clinical diagnosis, faster drug discovery, and even personalized medicine.1,2 Among the various approaches, the fluorescence detection is currently the most widely utilized method owing to its high sensitivity and the well-developed technologies. Whereas the fluorescence approach exhibits many advantages,3 it is inherently limited by the broad electronic emission spectra of the fluorophores in the condensed-phase experiments. For high-throughput systems, this phenomenon leads to complex overlap of the emission peaks, which is difficult to deconvolute and, consequently, has low dynamic range for multiplexed analyses.

Surface enhanced Raman spectroscopy (SERS) has long been recognized as a major complement to the fluorescence methods due to its well-known hyperfine resolution ability of the vibrational states for most of the analytes. For instance, through the vibrational state analysis, SERS can provide abundant chemical bonding information that is highly structure and functional group specific, that is the fingerprints of the analytes. The Raman peaks, with a typical bandwidth of ∼20 cm⁻¹, are spread out over 3000 cm⁻¹ and suitable for selecting baseline-resolved SERS tags for multiplexed detection.4–8 In addition to the information with rich spectroscopic features, SERS is a promising for biological application due to its compatibility with water and much better depth penetration of biological samples. Up until now, SERS-based assays have been

Received: February 23, 2013
Revised: April 22, 2013
Published: April 23, 2013

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extensively demonstrated in immunoassay, tumor imaging, cancer therapeutics, and nucleic acid detection systems.

SERS-based DNA detection methods at present are usually based on the principles of hybridization of complementary DNA sequences, using SERS-tagged probe DNA to hybridize with target ssDNA (single-stranded DNA), and subsequently detecting the Raman signals as reporters for the presence of the targets. The sensitivity and specificity are highly dependent on properties of the Raman tags and the capturing substrates in this type of sandwich hybridization assay. Recently, we successfully developed a new type of Raman tags, that is SERS-encoded poly(styrene-co-acrylic acid)/silver/silica core/shell nanospheres (denoted as nano-SERS-tags). Experimental results revealed that these silica-coated nano-SERS-tags are stable and inert under the biological conditions, and they exhibited a great deal of potential for multiplexed SERS signal processing and quantitative detection of multiple analytes simultaneously. The sandwich hybridization assays can be performed by immobilizing the capture DNA strands on the surface of a solid substrate, such as a planar chip or a planar film. Because of the large specific surface areas, various nanoparticles were subject to intensive research for DNA enrichment and purification. For example, magnetic nanoparticles (NPs) were introduced to act as capturing substrates to develop the NP-based DNA detection. In contrast to hybridization reaction of the solid chip method, the hybridization reaction of NP-based DNA assay can be conducted in solution and thus performed more rapidly and effectively.

For a long time, we have explored magnetic nanoparticles for bioanalytical applications, such as biosensors, protein purification, targeted drug delivery, and disease diagnosis. A variety of magnetic nanomaterials, including magnetic nanocrystals, magnetic colloidal nanocrystal clusters (MCNCs), and magnetic core/shell nanospheres, have been prepared and investigated. More recently, we designed and prepared MCNCs/poly(acrylic acid) core/shell nanospheres, which possessed high saturation magnetization value (∼50 emu/g) and excellent biocompatibility, thus ensuring the rapid and easy biological reaction and magnetic separation.

Motivated by these early successes, we explored the SERS liquid chip (SLC) approach for effectively detecting DNA oligonucleotides. The SLC method utilized biocompatible magnetic composite nanospheres as the capturing substrates and stable silica-coated nano-SERS-tags as the specific Raman reporters. The benefits of using the magnetic composite nanospheres as the capturing substrates are manifold: (1) Because the nanospheres possess much larger surface areas than those of the solid chip substrates, they can capture more targets and ensure higher sensitivity; (2) the target hybrids can be easily separated from a reaction mixture using an external magnetic field; (3) upon the application of an external magnetic field, the hybrids can be compacted in a small spot allowing the best SERS sampling, and hence greatly amplified the detection signals. Our experimental results indicated that this method had a limit of detection (LOD) below 10 pM for specific ssDNA. Moreover, multiple ssDNA oligonucleotides were successfully identified simultaneously in a single SERS experiment using the easily deconvoluted signals from specific nano-SERS-tags.

**EXPERIMENTAL SECTION**

**Materials.** N,N′-Methylene bisacrylamide (MBA) was bought from Fluka and recrystallized from acetone. 2,2-Azobisobutyronitrile (AIBN) was supported by Sinopharm Chemical Reagents Company and recrystallized from methanol. Acrylic acid (AA), silver nitrate (AgNO₃ > 99.8%), tetraethoxysilane (TEOS > 98%), iron(iii) chloride hexahydrate (FeCl₃·6H₂O), ammonium acetate (NH₄Ac), ethylene glycol (EG), trisodium citrate dihydrate, acetonitrile, 4-aminothiophenol (4-ABT > 98%), 4-chlorothiophenol (4- CBT > 98%), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB > 98%), 3-aminopropylthiioxysilane (APTES), succinic anhydride (SA), N,N-dimethylformamide (DMF), anhydrous ethanol, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 98.5%), and aqueous ammonia solution (NH₄H₂O, 28%) were purchased from Shanghai Chemical Reagent Co., Ltd. γ-Methacryloyloxypropyltrimethoxysilane (MPTES) was obtained from Jiangsu Chengguang Silane Coupling Reagent Co., Ltd. Highly pure water (Millipore) of resistivity greater than 18.0 MΩ·cm was used in all experiments. The oligonucleotides were purchased from Sango Biotech (Shanghai) Co., Ltd. and purified using high-performance liquid chromatography, and the detailed information was listed in Table 1.

**Table 1. Oligonucleotide Sequences of Capture/Target/Probe DNA Strands Utilized in the Multiplexed Experiments**

<table>
<thead>
<tr>
<th>code</th>
<th>DNA pair and tag</th>
<th>Capture DNA,target ssDNA/Probe DNA/tag</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5′NH₄(A₁₀)TCTATATAACCTTTATT/AGATTTTGGAATACATGACCTGGATGCAG/ GTACCTGACCTACGT(A₁₀)NH₄³⁻/Nano-SERS-tag 4⁺ABT</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5′NH₄(A₁₀)ACACCAGAATCAATA/TTGCCCTTCAAGTATAGGATGCTGTGTA/ TACCTTACACCAT(A₁₀)NH₄³⁻/Nano-SERS-tag 4⁻ABT</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5′NH₄(A₁₀)AAATCCTCAAGACCT/TTAGAGTGGATGATGTGGTA/ AATCGGAAAGAGA(A₁₀)NH₄³⁻/Nano-SERS-tag DTNB</td>
<td></td>
</tr>
</tbody>
</table>

**Synthesis of SERS-Encoded Core/Shell Nanospheres (Nano-SERS-Tags).** SERS-encoded nanospheres with core/shell structure were prepared according to our previously reported method. First, polymer substrates, poly(styrene-co-acrylic acid) (PSA) nanospheres were prepared via surfactant-free emulsion copolymerization of styrene and acrylic acid. Second, silver nanoparticles (Ag−NPs) were deposited onto the surface of PSA nanospheres to obtain PSA/Ag−NPs composite nanospheres by in situ reduction with the use of PVP as a reduction agent and stabilizer. Finally, the preparation of nano-SERS-tags was described below: Raman reporters were adsorbed onto PSA/Ag−NPs composite nanospheres, and then the resulting nanospheres were dispersed in a mixture of 9 mL of water, 40 mL of ethanol, 0.1 g of TEOS, and 1 mL of ammonia solution by sonication for 10 min. After the reaction was performed at 0 °C for 40 min under sonication, 0.05 g of APTES was added for another 20 min sonication to obtain the nano-SERS-tags functionalized with amino groups. Obtained products were washed for three times with ethanol and water to eliminate excess reagent.

**Synthesis of Poly(Acrylic Acid) Shell-Coated Magnetic Composite Nanospheres (MCNs).** The magnetite colloidal
nanocrystal clusters (MCNCs) were initially prepared through a solvothermal reaction described in our previous report. Typically, 1.350 g of FeCl$_3$·6H$_2$O, 3.854 g of NH$_4$Ac, and 0.400 g of sodium citrate were dissolved in 70 mL of ethylene glycol. The mixture were stirred vigorously for 1 h at 160 °C under the N$_2$ atmosphere to form a homogeneous black-red solution and then transferred into a Teflon-lined stainless-steel autoclave (100 mL capacity). The autoclave was heated at 200 °C and maintained for 16.5 h. Then, it was cooled to room temperature. The black product was washed with ethanol and collected with the help of a magnet. The cycle of washing and magnetic separation was repeated for several times. The final product was dispersed in about 10 mL ethanol for further use. Before coating MCNCs with poly(acrylic acid) (PAA), the MCNCs were modified with MPTES, and then the MCNCs/PAA core/shell nanospheres were synthesized by distillation—precipitation polymerization of AA with MBA as the cross-linker and AIBN as the initiator in acetonitrile. Typically, about 25 mg of MCNCs/MPTES nanospheres were dispersed in 20 mL acetonitrile in a dried 50 mL single-necked flask with the aid of ultrasonication for 10 min. Then, a mixture of 100 mg of AA, 25 mg of MBA, and 2.5 mg of AIBN were added to the flask to initiate the polymerization. The flask submerged in a heating oil bath was attached with a fractionating column, Liebig condenser, and a receiver. The reaction mixture was heated from ambient temperature to the boiling state within 30 min and the reaction was ended after about a 10 mL of acetonitrile was distilled from the reaction mixture within 1 h. The obtained MCNCs/PAA nanospheres were collected by magnetic separation and washed with ethanol to eliminate excess reactants and few generated polymer nanospheres.

Conjugation of Nano-SERS-Tags with Probe DNA Strands (Nano-SERS-Probes). To bind with probe DNA strands, the amino groups on the surface of nano-SERS-tags were derived as carboxylic groups as described below. Twenty milligrams of amino-modified nano-SERS-tags, 20 mg of succinic anhydride, and 20 mL of DMF were mixed in a 50 mL single-necked flask, and then the mixture was stirred for 10 h with a magnetic bar at room temperature. The resulting products were washed several times with ethanol and water to obtain carboxylated nano-SERS-tags. Subsequently, the carboxylated nano-SERS-tags and probe DNA strands with amino groups at the 3′ end were covalently reacted in the presence of EDC to achieve nano-SERS probes. Specifically, 1 mg of carboxylated nano-SERS-tags was dispersed in 2 mL of EDC aqueous solution (10 mg mL$^{-1}$), afterward, 1 mL of probe DNA strands (1 μM) in phosphate buffer solution (PBS, 10 mM, pH 7.4) was added, and the mixture was incubated under continuous shaking for 12 h at room temperature. The resultant nano-SERS-probes were washed by centrifugation and redispersion in 10 mM PBS (pH 7.4) several times, and then dispersed in 2 mL of 0.6 M NaCl 10 mM PBS (pH 7.4) and stored at 4 °C prior to use. Similarly, three types of nano-SERS-probes corresponding to three kinds of target ssDNA strands were obtained, respectively.

Conjugation of Magnetic Composite Nanospheres with Capture DNA Strands (Nano-Mag-Catchers). Magnetic composite nanospheres containing carboxylic groups and capture DNA strands with amino groups at the 5′ end were covalently reacted using the carbodiimide method to achieve nano-Mag-catchers. Specifically, 1 mg of carboxylated magnetic composite nanospheres was dispersed in 2 mL of EDC aqueous solution (10 mg mL$^{-1}$), and then 1 mL of capture DNA strands (1 μM) in 10 mM PBS (pH 7.4) was added and the mixture was incubated under continuous shaking for 12 h at room temperature. The resultant nano-Mag-catchers were washed by 10 mM PBS (pH 7.4) several times and then dispersed in 2 mL of 0.6 M NaCl 10 mM PBS (pH 7.4) and stored at 4 °C prior to use. Similarly, multiplexed nano-Mag-catchers were produced using 1.5 mL of a mixture of three kinds of capture DNA strands with equal mole (0.5 nanomoles).

Procedure of Sandwich Hybridization Assays for Analysis of Target DNA Strands. For quantitative analysis, sandwich hybridization assays that detect target ssDNA strands were performed. First, 10 μL of target ssDNA solution of a certain concentration (10$^{-8}$, 10$^{-9}$, 10$^{-10}$, 10$^{-11}$, 10$^{-12}$ M) prepared with 0.6 M NaCl 10 mM PBS (pH 7.4), 100 μL nano-
Mag-catchers (0.5 mg mL<sup>−1</sup>) and 100 μL nano-SERS-probes (0.5 mg mL<sup>−1</sup>) were mixed and incubated at 25 °C for 4 h under shaking. Afterward, the sandwich complexes were collected by magnetic separation and washed with 10 mM PBS (pH 7.4) three times to remove physically adsorbed nano-SERS-probes. The resultant sandwich complexes were stored at 4 °C prior to Raman measurement. Hybridization reactions for multiplex detection were conducted using multiplexed nano-Mag-catchers as capture substrates. Typically, a mixture solution composed of multiple 10 μL of target ssDNA strands (10 nM), 100 μL of multiplex nano-Mag-catchers (0.5 mg mL<sup>−1</sup>), and a mixed solution consisting of the corresponding multiple 100 μL of nano-SERS-probes (0.5 mg mL<sup>−1</sup>) were mixed and incubated at 25 °C for 4 h under shaking. The unbound nano-SERS-probes and target ssDNA strands were removed by magnetic separation.

**Characterization.** High-resolution transmission electron microscopy (HRTEM) images were taken on a JEM-2100F transmission electron microscope at an accelerating voltage of 200 kV. Samples dispersed at an appropriate concentration were casted onto a carbon-coated copper grid. UV−vis spectra were measured on a Shimadzu UV-3150 spectrometer. Raman spectra were recorded using the Invia Reflex by Renishaw with 632.8 nm laser excitation and a 50× long objective. The data

**Figure 1.** (a) HRTEM image and (b) particle size distribution of nano-SERS-tags containing 4-ABT measured by dynamic light scattering.

**Figure 2.** (a) HRTEM image, (b) FESEM image, (c) plot of dynamic light scattering, and (d) magnetic hysteresis curve of MCNCs/PAA core/shell nanospheres.
aminothiophenol (4-ABT), 4-chlorothiophenol (4-CBT), and Nanospheres. Three types of nano-SERS-tags containing 4-

composites, we demonstrated the formation of sandwich hybridization complexes, we first conducted two hybridization assays in the presence of target ssDNA. Nano-SERS-probes with 4-ABT, nano-Mag-catchers (capture DNA conjugated MCNs), and target ssDNA (excess amount) were mixed together and incubated for 4 h at room temperature, and then an external magnetic field was applied to separate the magnetic nanospheres. Only 30 s, the nano-Mag-catchers were completely

acquisition time was usually 20 s and the peak intensities of samples were normalized with respect to that of the silicon wafer at 520 cm\(^{-1}\). Magnetic characterization was carried out with a VSM on a Model 6000 physical property measurement system (Quantum, USA) at 300 K. Zeta potential measurements were conducted by dynamic light scattering (DLS) with a ZEN3600 (Malvern, UK) Nano ZS instrument using He–Ne laser at a wavelength of 632.8 nm. Fourier transform infrared spectra (FTIR) were determined on a NEXUS-470 FTIR spectrometer. Spectra were scanned over the range of 400–4000 cm\(^{-1}\). All of the dried samples were mixed with KBr and then compressed to form pellets.

## RESULTS AND DISCUSSION

The strategy for SERS liquid chip (SLC) based on DNA hybridization in solution is illustrated in Scheme 1. The probe DNA-modified nano-SERS-tags act as the SERS probes (nano-SERS-probes) and the capture DNA modified magnetic composite nanospheres serve as suspension substrates (nano-Mag-catchers).

### Preparation and Characterization of the Candidate Nanospheres

Three types of nano-SERS-tags containing 4-aminothiophenol (4-ABT), 4-chlorothiophenol (4-CBT), and S,S′-dithiobiis (2-nitrobenzoic acid) (DTNB) as Raman reporters were fabricated, respectively. Take nano-SERS-tags containing 4-ABT as an example, their HRTEM image is shown in part a of Figure 1 indicating the nano-SERS-tags are highly monodisperse with the silica shell of ca. 43 nm. Part b of Figure 1 displays the dynamic size distribution of the nano-SERS-tags, which suggests that they are uniform with a polydispersity index of 0.108. The characterization of other two types of nano-SERS-tags containing 4-CBT and DTNB showed similar experimental results as well.

The MCNCs/PAA core/shell nanospheres, that is magnetic composite nanospheres (MCNs), were synthesized through the distillation–precipitation polymerization as it was reported earlier.\(^ {31} \) The size and morphology of the MCNs were characterized by HRTEM, FESEN, and DLS. The HRTEM image (part a of Figure 2) showed that the mean diameter of MCNs core and the thickness of PAA shell were 230 and 25 nm, respectively. The FESEM image and DLS data displayed that the MCNs were uniform (part b of Figure 2) and then dispersed in water (part c of Figure 2). Furthermore, the MCNs possessed good magnetic response with a saturation magnetization value of approximately 50 emu/g (part d of Figure 2).

After the successful preparation of the nano-SERS-tags, the following steps were taken to bind the probe DNA to their surface. At first, the nano-SERS-tags were sequentially allowed to react with APTES (a coupling silane agent with amino group) and succinic anhydride to form the carboxyl-rich surface. Zeta-potential characterization in PBS (10 mM, pH 7.4) was employed to confirm the successful surface modification of the nano-SERS-tags. The zeta-potential values of the original nano-SERS-tags and amino-modified nano-SERS-tags were −31 and −4 mV, respectively. The carboxylated nano-SERS-tags showed a lower zeta-potential value, that is −38 mV, resulting from the presence of a large number of negatively charged carboxylic groups. Next, the probe DNA strands (for recognizing one part of the target ssDNA strands) with amino group were bound to the nano-SERS-tags activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and then the nano-SERS-probes were prepared. Similarly, the magnetic nanospheres with a PAA shell, presenting a zeta-potential value of −39 mV, were conjugated with the corresponding capture DNA strands (for recognizing the other part of the target ssDNA strands) with amino group using EDC activation, and then the nano-Mag-catchers were obtained. The successful reactions were also confirmed by the results of FTIR measurement (Figure S1 of the Supporting Information). It is worth noting that the massively negatively charged surfaces provided high stability for nano-SERS-tags and magnetic composite nanospheres in aqueous solution and thus guaranteed the SLC method to be applied for detection of target ssDNA in aqueous solution.

### Detection of Target ssDNA Strands with SLC Method

To demonstrate the formation of sandwich hybridization complexes, we first conducted two hybridization assays in the presence of target ssDNA. Nano-SERS-probes with 4-ABT, nano-Mag-catchers (capture DNA conjugated MCNs), and target ssDNA (excess amount) were mixed together and incubated for 4 h at room temperature, and then an external magnetic field was applied to separate the magnetic nanospheres. Only 30 s, the nano-Mag-catchers were completely

![Figure 3. SERS spectra and photographs (inset) obtained from hybridization assays in the presence of (a) target ssDNA and (b) none target ssDNA. M represents a magnet. In this experiment, capture DNA is 5′-NH2-(A10) TC TAT AAA CCT TATT, probe DNA is GTA CTG GAC CTA CGT (A10) NH2-3′, target ssDNA is AGA TAT TTG GAA TAA CAT GAC CTG GAT GCA.](image)
collected onto the wall of the vial and the color of the residual solution turned colorless (inset in part a of Figure 3). This result is due to the formation of the sandwich hybridization complex, and all nano-SERS-probes were bound to the surface of the nano-Mag-catchers and were collected together with the nano-Mag-catchers onto the wall of the vial. Additionally, the SERS spectrum of the collected sediments showed strong spectroscopic signals and the major peaks of 4-ABT located at positions 1077, 1143, 1390, 1437, and 1579 cm\(^{-1}\). Nevertheless, in the absence of target ssDNA after the incubation sample was isolated by an external magnetic field, the color of the residual incubation solution remained a brown color (inset in part b of Figure 3) because the nano-SERS-probes were not collected with the magnetic sediments in the absent hybridization. SERS spectrum of collected sediment also did not show any spectroscopic signals of 4-ABT (part b of Figure 3), for all of the nano-SERS-probes were washed out and only the nano-Mag-catchers were left. These results illustrated that this method is specific to the complementary hybridizations of nano-SERS-probes and nano-Mag-catchers with target ssDNA strands, and thus more sensitive than typical fluorescence based sandwich immunoassay. As a result, the SLC method can be utilized for the detection of specific biomolecules in solution.

The detection sensitivity for target ssDNA was investigated according to the previous method. In the experiment, nano-SERS-tags containing 4-ABT were employed as the reporting agents in a series of hybridization assays at varied concentrations of target ssDNA ranging from \(10^{-12}\) to \(10^{-8}\) M. The concentration-dependent SERS spectra are shown in part a of Figure 4. It can be observed that the intensity of SERS peaks from 4-ABT molecules increases with the increase of the concentrations of the target ssDNA. For a more quantitative investigation, the results were analyzed by plotting the SERS

Figure 4. (a) SERS spectra obtained with different concentrations of target ssDNA strands in the sandwich hybridization assay: (i) a control solution, (ii) non complementary DNA, (iii) \(10^{-12}\) M, (iv) \(10^{-11}\) M, (v) \(10^{-10}\) M, (vi) \(10^{-9}\) M, (vii) \(10^{-8}\) M; and (b) the linear correlation between the logarithm of the target ssDNA concentration and intensity of the SERS band at 1143 cm\(^{-1}\) (\(n = 3\)). In this experiment, capture DNA is 5’NH\(_2\)-(A10) TC TAT AAA CCT TATT, probe DNA is GTA CTG GAC CTA CGT (A10)-NH\(_2\), target ssDNA is AGA TAT TTG GAA TAA CAT GAC CTG GAT GCA, noncomplementary DNA is TTA GAG TTG CAT GGA TTA ACT CCT TCT.

Scheme 2. Schematic Illustration of the Multiplex Sandwich Hybridization Assays for Triple-Target ssDNA Detection
signal intensity of 4-ABT located at 1143 cm$^{-1}$ as a function of the logarithmic concentration of target ssDNA strands generating the corresponding curve in part b of Figure 4. The error bars indicate standard deviations from three measurements. As shown in part b of Figure 4, a good linear response was achieved in the concentration range of 10 nM (spectrum vii) to 10 pM (spectrum iv), and the correlation coefficient ($R^2$) is 0.977. The control spectrum (spectrum ii) was obtained by replacing the target ssDNA with non-complementary DNA strands ($10^{-9}$ M), and a very weak feature peaks of 4-ABT can be observed indicating that there existed some nonspecific binding between mismatch DNA strands. The limit of detection (LOD) was conservatively estimated to be 10 pM because the SERS signal intensity acquired from 1 pM target ssDNA was nearly as low as that obtained from the mismatch experiment (spectrum ii). Currently, the reported LOD of NPs-based methods was usually in the range of $10^{-9}$ to $10^{-9}$ M.25-27 and our method can greatly improve the LOD of the DNA detection based on the colloidal nanospheres. These experimental results demonstrated that the SLC method is a very useful analytical technique for the highly sensitive and selective detection of biological molecules. Furthermore, the hybridizing and magnetic steps are simple and quick due to the application of magnetic composite nanospheres with fast magnetic response.

**Multiplex Detection of ssDNA Targets.** The simultaneous detection of multiple analytes in one sample is a critical demand for the development of more effective and more facile biological detection. The detection capacity of the SLC method for multiple ssDNA targets was tested using different nano-SERS-tags as reporting agents. In this study, three types of nano-SERS-tags were respectively conjugated with corresponding probe DNA strands, being sequence complementary to specific target ssDNA strands to prepare nano-SERS-probes, and magnetic composite nanospheres were bound with a mixed DNA strands composed of three kinds of capture DNA strands to form multiplexed suspension capturing substrates. Subsequently, the multiplexed substrates were incubated with a mixture of multiple target ssDNA strands and corresponding nano-SERS-probes, and then magnetic extraction was applied for further SERS characterization (Scheme 2).

Using this detection system, single and multitarget ssDNA detections were investigated. All detailed DNA pairs (capture DNA/target ssDNA/probe DNA) and DNA sequences are listed in Table 1. Part a of Figure 5 shows the SERS spectra for the three single-target ssDNA detections. The results indicated that the three types of nano-SERS-probes have unique SERS spectra, thus allowing the detected targets to be easily distinguished from each other. As shown in part a of Figure 5, the Raman spectra exhibited unique SERS signals at 1143, 1390, and 1436 cm$^{-1}$ for 4-ABT (curve I), 37 338, 536, and 1566 cm$^{-1}$ for 4-CBT (curve II),35 and 1335 cm$^{-1}$ for DTNB (curve III),38 respectively. Consequently, they can be regarded as the characteristic signals for the target ssDNA detections. Part b of Figure 5 shows the SERS spectra of the multiplexed detections including the double-target ssDNA and triple-target ssDNA detections. They all presented the characteristic signals of nano-SERS-probes on the basis of SERS bands. For example, in the case of the detection of a mixture of target ssDNA-1 and target ssDNA-2 (part b-iii of Figure 5), the representative SERS bands for 4-ABT located at 1143, 1390, and 1436 cm$^{-1}$ (nano-SERS-probe-1), and for 4-CBT at 341, 538, and 1569 cm$^{-1}$ (nano-SERS-probe-2) could be clearly observed and distinguished from each other. Similarly, other double-target ssDNA detections (parts b-ii and b-iv of Figure 5) and one triple-target ssDNA detection (part b-i of Figure 5) were carried out, and all characteristic SERS bands for each type of probes were distinctly distinguishable.

It can be seen from these intuitive symbols that the SERS signatures of the double- and triple-target ssDNA detections are the sums of the signatures from the corresponding single-target detection, and it is visually distinct for each type of nano-SERS-probes as well. The SERS bands observed in each of the tested system are listed in Table 2 and classified to the corresponding target ssDNA strands according to the unique fingerprints of nano-SERS-probes. The experimental results indicated that we could unambiguously identify each target ssDNA strand from the double-target ssDNA detection and the triple-target ssDNA.
Table 2. Unique SERS Bands of T1, T2, and T3 of the Multiplexed Detection in part b of Figure 5

<table>
<thead>
<tr>
<th>curve code</th>
<th>target ssDNA (ERS tag/ symbol)</th>
<th>observed Raman bands (\text{cm}^{-1})</th>
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<tbody>
<tr>
<td>i</td>
<td>T1 (4-ABT/*)</td>
<td>1143, 1390, 1436</td>
</tr>
<tr>
<td></td>
<td>T2 (4-CBT/*)</td>
<td>341, 538, 1569</td>
</tr>
<tr>
<td></td>
<td>T3 (DTNB/△)</td>
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<td>iv</td>
<td>T2 (4-CBT/*)</td>
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<td></td>
<td>T3 (DTNB/△)</td>
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This proof-in-concept study demonstrates the potential utility of the SLC method in multiplexed DNA detection. Henceforth, it can be expected that the biocompatible and multiplexed detection strategy will be applicable to high-throughput screening of a wide variety of biomolecules in biological and biomedical studies.

**CONCLUSIONS**

In summary, we have developed a novel approach, SERS liquid chip method, for target ssDNA detection based on SERS-encoded core/shell nanospheres as probes and magnetic composite nanospheres as suspension substrates. The quantitative detection of target ssDNA strands showed a well-defined linear correlation between SERS signal intensity and concentrations of target ssDNA strands, and the limit of detection was observed as low as 10 pM. Multiplexed detections were also successfully performed with simultaneous spectral identification of up to three different kinds of ssDNA targets in one sample on the basis of the unique fingerprints of each type of nano-SERS-probes. These results showed that the SLC method offered a combination of advantages in terms of the good sensitivity, excellent selectivity, and multiplexing capabilities, which might open up many opportunities toward practical biological detections.

**ASSOCIATED CONTENT**

4 Supporting Information

Complementary FTIR results of nano-SERS-tags and MCNs before and after conjugated with the DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was supported by National Science Foundation of China (Grant Nos. 21034003, 51073404, and 21128001) and National Science and Technology Key Project of China (2012AA0020204).

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