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Spiky gold shells on magnetic particles for DNA biosensors

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ABSTRACT

Combined separation and detection of biomolecules has the potential to speed up and improve the sensitivity of disease detection, environmental testing, and biomolecular analysis. In this work, we synthesized magnetic particles coated with spiky nanostructured gold shells and used them to magnetically separate out and detect oligonucleotides using SERS. The distance dependence of the SERS signal was then harnessed to detect DNA hybridization using a Raman label bound to a hairpin probe. The distance of the Raman label from the surface increased upon complementary DNA hybridization, leading to a decrease in signal intensity. This work demonstrates the use of the particles for combined separation and detection of oligonucleotides without the use of an extrinsic tag or secondary hybridization step.

1. Introduction

Nanotechnology has led to faster, more sensitive, and more selective methods of biomolecule detection [1,2]. In addition, it has offered the possibility of miniaturization and the ability to combine the steps required for detection into a single platform. By combining multiple steps (concentration, purification, detection) into a single platform, we can envisage new applications in point-of-care diagnostics, environmental testing, and biomolecular analysis.

Magnetic particles are commonly used for separating and concentrating specific biomolecules [3–7]. The benefits of magnetic particles for biomolecule concentration and separation include a simple and inexpensive set-up (typically requiring only the particles themselves and a magnet), no extensive technical training, and result in highly sensitive and selective separation [8]. Superparamagnetic particles are considered optimal since they will remain dispersed when added to a sample, allowing the analyte to bind to their surface, but will be separated when a magnet is used [9,10]. A challenge with using superparamagnetic particles is that separation times are often prohibitively long [11,12]. One method that has been used to overcome this is to use controlled aggregates (150–300 nm) of superparamagnetic nanoparticles (~ 20 nm) to increases the total magnetic moment while still maintaining the superparamagnetic properties of the particles.

In most cases where magnetic particles are used, the purified biomolecule is dissociated from the magnetic particles for subsequent analysis. Since the biomolecule is already pre-concentrated and specifically bound to the surface, an interesting possibility is to directly detect it while still attached using a surface-sensitive detection method. Surface-enhanced Raman spectroscopy (SERS) relies on the electromagnetic enhancement induced by nanostructured metal surfaces (typically gold or silver) to greatly increase the Raman signal (enhancements on the order of 10^4–10^7) [13]. SERS also offers fingerprint specificity, which leads to the possibilities of highly specific detection and multiplexing. Coating magnetic particles with a nanostructured metal can therefore enable combined magnetic separation and SERS detection of molecules bound to the particle surface.

Several other researchers have investigated forming nanostructured gold shells on various core particles [14]. Gold nanostars with small iron oxide cores (gold nanostars: ~ 100 nm, iron oxide cores: < 30 nm) have been synthesized for use in gyromagnetic imaging [15,16], as recyclable catalysts [17], for protein separation and SERS detection [18], and for enhanced electromagnetic properties [19,20]. Others have formed nanostructured gold shells on cores of various materials including polymer beads [21,22], block copolymer assemblies [21], gold nanowires [23], gold nanorods [24], and other metallic particles [25–27].

Label-free methods of detection using SERS are of interest because they can reduce the number of steps required for detection and because of the fingerprint specificity obtainable by SERS. Label-free detection of nucleic acids using SERS has been used to quantitatively analyze ssDNA [28], identify single-base mismatches [29,30], and to differentiate between ssDNA and dsDNA [31–33]. This latter task can still be challenging, especially at the low concentrations seen with hybridization detection, since the same four bases are usually present in both probe and target. In addition, when this involves selective capture, a capture probe must first be bound to the surface, leading to an orientation-

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dependent signal [31,34] that can increase the challenge of hybridization detection.

Another approach is to harness the distance dependence of the signal by using a Raman tag bound to the probe; the probe is designed so that the proximity of the tag relative to the surface changes upon target binding, resulting in a signal change [35–40]. While this approach loses the direct correlation between the molecule and the signal (and thus potential information on the nature of the binding interaction), it still has the benefit of not requiring an extrinsic tag and has the potential for quantitative measurement of binding, both being beneficial features in a DNA detection method. It also offers the potential for multiplexed detection, as different tags providing different Raman signals can easily be used [41,42].

In this work, we synthesized magnetic particles coated with a nanostructured gold shell that acts as a SERS substrate. To show the effectiveness of the particles in biosensing, we used them to detect oligonucleotide hybridization. This is, to our knowledge, a first example of combined magnetic separation and SERS detection of oligonucleotides without the use of an extrinsic tag.

2. Material and methods

2.1. Materials

Gold(III) chloride hydrate ($HAuCl_4{\cdot}xH_2O$), cetyl trimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), and ascorbic acid, were purchased from Sigma Aldrich. Millipore water and reagent grade ethanol were used. Silica-coated iron oxide particles were synthesized previously at the University of Waterloo [43].

All oligonucleotides were purchased from IDT (Integrated DNA Technologies, Coralville, Iowa). DNA stock solutions were prepared in Millipore water then diluted in buffer purchased as a disulfide and reduced to a thiol using tris(2-carboxyethyl)phosphine (TCEP), purchased from Sigma Aldrich. For the oligonucleotide ruler experiment, the sequences used are listed in Table 1. For the experiment investigating the signal differences between ssDNA and dsDNA, the sequence of the untagged, non-hairpin probe was –S-$(CH_3)_6$ – 5′-GIGGTTGGTTGTGTTGGGTGTTGT-GTCCAACCCC-3′, the complementary target sequence was 5′-ACACAAC ACCCAACACAACCAACCCC-3′, and the non-complementary target sequence was 5′-ACACACACACACACACACACACACCC-3′. The sequence of the tagged hairpin probe, also used for the hybridization experiments, was Cy5-5′-TTTTTCGCTCCCTGGTGCCGTAGATGAGCGTTTTT-3′-$(CH_3)_3$-S- (purchased as a disulfide), the complementary target sequence was 5′-ATCTACGGCACCAGG-3′, and the non-complementary target sequence was 5′-TCACACGGAGGCTAC-3′. Both 3-mercapto-1-propanol (MCP) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma Aldrich.

2.2. Particle synthesis

Particle synthesis was done in several steps: magnetite sphere synthesis, silica coating and functionalization, gold seed binding, then growth of the gold seeds into spikes (Fig. 1).

Table 1
Oligonucleotide sequences used as “rulers”.

	Position of AAA	Oligonucleotide Sequence
i	1	5′-CCCCCCCCCCCCAAA-3′-$(CH_3)_3$-S-
ii	2	5′-CCCCCCCCCCCAAAC-3′-$(CH_3)_3$-S-
iii	4	5′-CCCCCCCCCAAACCC-3′-$(CH_3)_3$-S-
iv	5	5′-CCCCCCCCAAACCCC-3′-$(CH_3)_3$-S-
v	7	5′-CCCCCCAAACCCCCC-3′-$(CH_3)_3$-S-
vi	10	5′-CCCAAACCCCCCCCC-3′-$(CH_3)_3$-S-
vii	13	5′-AAACCCCCCCCCCCC-3′-$(CH_3)_3$-S-
viii	–	5′-CCCCCCCCCCCCCCC-3′-$(CH_3)_3$-S-

2.2.1. Synthesis of magnetite spheres

Magnetite spheres were synthesized using a previously developed hydrothermal synthesis [43–45]. In brief, sodium citrate dihydrate, polyacrylamide (PAM), and $FeCl_3{\cdot}6H_2O$ were mixed and dissolved in Millipore water. A small amount of ammonium hydroxide was then added to the solution under vigorous stirring. This mixture was poured into a 125 mL PTFE-lined stainless steel pressure vessel (Parr Instrument Company) and heated at 200 °C for 12 h. The product was recovered magnetically and washed with deionized water and ethanol by magnetic decantation, then dried under nitrogen.

Magnetic separation of samples in all steps was performed using either a small neodymium magnet or a rare earth homogenous magnetic separator (Sepmag Lab 2142, inner bore diameter 31 mm, radial magnetic field gradient 45 T/m). The samples were placed in or near the magnet and left to separate for 1–2 min. The supernatant was then gently removed using a pipette.

2.2.2. Silica coating and functionalization

Silica coating was done in a second step [43]. In brief, the magnetite particle powder was dispersed into a solution of EtOH and Millipore deionized water by probe sonication. Ammonium hydroxide was then added to the dispersion, followed by the slow dropwise addition of TEOS in EtOH solution over 1 h under vigorous mechanical stirring. This mixture was then stirred at room temperature for 18 h, after which the product was recovered magnetically and washed with EtOH by magnetic decantation, then dried under nitrogen.

Silica-coated magnetite particles were functionalized with thiol groups by first functionalizing the surface with amine groups using (3-aminopropyl)triethoxysilane (APTES), then by linking 11-mercaptoundecanoic acid (MUA) to these groups. The particles were dispersed in 2:1 ethanol: Millipore water for a final concentration of 5 mg/mL by bath sonication. While mechanically stirring the particles in a 50 °C water bath, 20% v/v APTES solution was quickly added so that the final APTES concentration was 2% v/v. After 24 h of stirring at 50 °C, the particles in solution were magnetically separated and decanted, washed, and dried. To bind the MUA to the amine groups, the carboxylic acid groups were activated by adding 12 mM of NHS and 12 mM of EDC to 10 mM MUA in ethanol and left for 15 min. The particles were dispersed in the activated MUA solution and mechanically mixed for 90 min. The particles were then magnetically decanted and washed three times in ethanol by magnetic separation.

2.2.3. Gold seed binding

Gold seeds were prepared by warming 5 mL of 0.2 M CTAB in Millipore water to 30 °C using a water bath, then by adding 0.125 mL of 0.01 M $HAuCl_4{\cdot}xH_2O$ to the vial while magnetically stirring. The bright yellowish-orange solution was stirred for 5 min. While still under magnetic stirring in the water bath, 0.06 mL of 0.05 M $NaBH_4$ in Millipore water was added. The light brown solution was stirred for 10 min.

Silica-coated magnetite spheres were dispersed in ethanol at 2 mg/mL using a sonic bath. These were combined with equal parts Millipore water and gold seeds (1 mL of each) and mixed by gentle shaking. The mixture was placed on a gentle rotating mixer for 1 h. The particles were then magnetically decanted, washed three times using magnetic separation in 1 mM CTAB in water, and redispersed in the same volume of 1 mM CTAB as the ethanol that was first used to disperse particles at 2 mg/mL.

2.2.4. Gold shell growth

Growth solution (20 mL) was prepared by adding 1 mL of 0.01 M $HAuCl_4{\cdot}xH_2O$ and 0.2 mL of 0.01 M $AgNO_3$ to 100 mM CTAB, then partially reducing the metal salts using 0.16 mL of 0.1 M ascorbic acid (final concentration of 0.5 mM $HAuCl_4$, 0.1 mM $AgNO_3$, and 0.8 mM ascorbic acid). The solution was warmed to 30 °C using a water bath and magnetically stirred for 10 min. Following this, 400 µL seeded