Supporting Information

Experimental Section

Materials

All chemicals were purchased from Sigma and were used without further purification. DNA oligonucleotides were synthesized and purified by Genotech (South Korea). The following sequences of DNA oligonucleotides were used in this work: Peroxidase mimicking DNAzyme: 5’-Thiol-(T)$_{15}$ TTG TGG GTA GGG CGG GTT GGG-3’; Capture Probe 1: 5’-AGT ACA AAC GCC TAG (T)$_{10}$-Thiol-3’; Capture Probe 2: 5’-Biotin-(T)$_{9}$ TGC TTC GAG CAA CCG C-3’

Preparation of AuNPs modified with both peroxidase mimicking DNAzymes and capture DNA

Approximately 30 nm diameter Au particles were prepared by the citrate reduction of HAuCl$_4$.\textsuperscript{[1]} The concentration of Au nanoparticles was determined by measuring the absorbance at $\lambda = 528$ nm. The gold nanoparticle solution (1 mL, 300 $\mu$m) was mixed with the thiolated peroxidase mimicking DNAzyme (104.8 $\mu$L, 31.3 $\mu$M) and the thiolated capture probe 1 (2.4 $\mu$L, 13.6 $\mu$M) (Table 1) [the ratio of the peroxidase mimicking DNAzyme to the capture probe 1 was 100:1]. The suspension was incubated for 16 h. A phosphate buffer solution (100 mM pH 7.4) and NaCl (2 M) were added to the resulting AuNP solution to achieve a concentration of 0.1 M for NaCl in phosphate buffer solution (10 mM pH 7.4). After additional 40 h incubation, the gold nanoparticles were separated by using centrifugation (13 000 rpm, 15 min) and washed with 10 mM phosphate buffer solution (0.1 M NaCl, pH 7.4). The final
centrifugate was resuspended in the buffer solution (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100 and 1% DMSO; pH 7.4) including 1 μM hemin and incubated at room temperature for 3 h.

**Preparation of MNPs modified with capture DNA**

The magnetic nanoparticles were modified with the second generation (G2) PAMAM dendrimer according to the literature procedure.[2] The PAMAM-MNP was dispersed into 10% glutaraldehyde in phosphate buffer solution (0.05 M, pH 7.4), stirred for 3 h, and then washed with phosphate buffer solution (0.05 M, pH 7.4) 3 times by using magnetic separation. 85 μL streptavidin (2 mg mL⁻¹) was added to 1 mL PAMAM-MNP solution (0.5 mg mL⁻¹). The mixture was incubated for 10 h with stirring and subsequently washed 3 times with PBS solution (10 mM, pH 7.4). 50 μL biotinylated capture probe 2 (50 μM) (Table 1) was added to 1 mL STA-PAMAM-MNP (0.5 mg ml⁻¹). After 3 h incubation, the mixture was washed and suspended in bovine serum albumin (BSA) solution (1% w/v) for 30 min. The mixture was then washed again and stored in PBS solution for further use.

**Preparation of PCR-amplified Chlamydia DNA**

PCR amplification of *Chlamydia trachomatis* gene was carried out using a Perkin-Elmer 9200 thermo-cycler (Perkin-Elmer, Norwalk, CT) in 50 μL total reaction volume using 1X PCR reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂), 0.25 mM of each primer [5’CTAGGCGTTTGTACTCCGTCA3’ (forward), 5’TCCTCAGAAGTTTATGCACT3’ (reverse)], 0.2mM dNTPs, and 0.7U Taq DNA polymerase (Takara, Japan). The thermal circling conditions were 1 cycle of 94°C for
5 min, 35 cycles of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, and followed by a final 5 min extension step at 72°C. After the amplification reaction, the PCR products were purified with GeneAll PCR SV kit (General Biosystem, Korea). The concentration of the amplicon was calculated by using a Cary 100 UV–visible spectrophotometer (Varian, Palo Alto, CA) by measuring absorbance at 260 nm.

**Detection of sequence-specific DNA**

The PCR products were denatured at 95°C for 5 min and cooled on ice for 5 min. After a serial dilution, the sample of PCR-amplified target DNAs with different concentration were allowed to hybridize with 50 μL of the MNPs (0.5 mg mL⁻¹) and 50 μL AuNPs for 1 h at 37°C. The resulting complexes were magnetically collected and washed several times with PBS buffer (10 mM, pH 7.4).

**Absorbance measurements**

The separated complexes were dispersed in ABTS solution (5.4×10⁻⁴ M). Catalytic reactions were initiated by the addition of 1 μL H₂O₂ (0.3% w/v). After the magnetic separation, absorption spectra of the supernatant were recorded by using a Cary 100 UV–Visible spectrophotometer (Varian, Palo Alto, CA).
Supporting Data

Elimination of the intrinsic peroxidase-like activity of magnetic nanoparticles

Yan and coworkers recently reported that magnetic nanoparticles (Fe₃O₄) possess intrinsic peroxidase-like activity.[³] In the current study, the MNPs also showed the peroxidase activity by oxidizing of the ABTS in the presence of H₂O₂ which causes a background signal. Bovine serum albumins (BSA) were employed to diminish the peroxidase activity of the MNPs (Figure S1) by protecting the MNPs from directly contacting with the substrates.

To efficiently eliminate the background signal induced by MNPs, the effects of reaction time and temperature on the peroxides-like activity of the both DNAzymes and the MNPs were examined. As shown in Figure S1 (A), the absorbance by the DNAzymes (1 μM) reached a maximum value within 8 min after which no further increase observed. In contrast, the absorbance by the MNPs increased continuously with the increase of reaction time in the studied range. Therefore, a relatively short reaction time (4 min) was chosen in the further experiments to reduce the background signals. Likewise, the effect of temperature was also investigated. As shown in Figure S1 (B), the absorbance by the DNAzymes first increased with an increase in temperature in the range of 25°C and 30°C, and then decreased over 30°C. However, the absorbance by the MNPs continuously increased with the increase of the temperature. The ratio of the peroxidase activity of the DNAzymes to that of the MNPs reached the highest value at 25°C. Therefore all the experiments in this study were performed at 25°C.
Reference


Figure S1. (A) Time-dependent absorbance changes of the peroxidase mimicking DNAzymes and the MNPs with and without BSA blocking. (B) Temperature-dependent absorbance changes of the peroxidase mimicking DNAzymes and the MNPs with and without BSA blocking. Data were obtained from three independent measurements.
Figure S2. TEM images of (A) the gold nanoparticles; (B) the magnetic nanoparticles.