

DNA Recognition Using Metal Fluorescence Interactions on Two-Dimensionally Assembled Gold Nanoparticles

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ABSTRACT

We developed a novel DNA detection system using a probe DNA with a fluorophore immobilized on two-dimensionally assembled gold nanoparticles. This system enables quantitative and selective detection of DNAs without a labeling process. In the absence of a target DNA, fluorescence from cyanine dyes labeled at the distal end of the probe is quenched via energy transfer to the nanoparticles on which the dye moiety is adsorbed. However, once a complementary target DNA is hybridized with the probe, rigidity of the double stranded DNAs allows the dyes to desorb from the particles, and fluorescence is enhanced by the strong local electromagnetic field in the vicinity of the gold nanoparticles. The relative increase in fluorescence intensity after hybridization was maximized on gold nanoparticles of 10 nm where both quenching and enhancement factors were optimal.

Keywords: fluorescence enhancement, fluorescence quenching, DNA detection, DNA microarray, gold nanoparticles

1 INTRODUCTION

Sensors for recognition of specific biomolecules play a pivotal role in molecular biology and biodiagnostics. DNA microarrays are a widely accepted tool for parallel gene expression profiling and genotyping. In addition, the unique optical properties of oligonucleotide-metal nanoparticle composites have led to the development of sensors that can detect single-mismatch DNA [1-2] and are effective in sensitive DNA assays [3]. Although this hybrid material can provide high specificity and sensitivity [1], a quantitative DNA detection system that is simple to operate and has high parallelization has been still challenging to develop.

Here, we report a system for quantitatively detecting oligonucleotides without labeling. The system involves an array containing a two-dimensional assembly of gold nanoparticles. Single-stranded oligonucleotides used for probing target DNAs are tethered on the gold nanoparticles, and a fluorophore is labeled at the distal end of the tethered oligonucleotide as shown in Figure 1 (a). The nanoparticles, probe oligonucleotide, and fluorophore are assembled into a molecular recognition system. In the absence of a target DNA, the fluorophore connected with the conformationally

flexible chain of the probe oligonucleotide can adsorb on the gold nanoparticles to minimize the free energy of the ensemble. As a result, fluorescence from the fluorophore can be quenched by the nanoparticles because of energy transfer [4-5]. When the probe oligonucleotide is hybridized with the complementary target DNA, the fluorophore of the probes is separated from the nanoparticle surface due to rigidity of the newly formed double-stranded DNA, similar to the case of molecular beacons [6] (Figure 1(b)). Consequently, the fluorophore is liberated from the quenching region, and the fluorescence can be restored and even enhanced [7-11]. The advantages of the new array include: (1) an inherent signal transduction that does not require labeling of target DNAs, (2) the ability to detect target DNAs without intensive washing of non-hybridized targets after hybridization, and (3) higher sensitivity through utilization of a fluorescence enhancement mechanism. DNA detection without labeling and washing steps allows simple and reproducible analysis with high parallelization, and provides new opportunities in DNA detection assays. In this report, we describe the characteristics of the DNA recognition system with nanoscopic surface structures composed of fluorescently labeled probes tethered on two-dimensionally assembled gold nanoparticles.

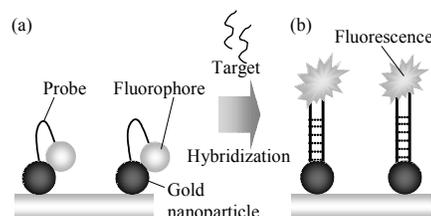


Figure 1. Schematic of the structure of the system. (a) A single-stranded probe oligonucleotide with a fluorophore immobilized on a two-dimensional assembly of gold nanoparticles. (b) A double-stranded DNA formed after the complementary target DNA is hybridized.

2 EXPERIMENTAL SECTION

2.1 Immobilization of Gold Nanoparticles

The fabrication scheme of the DNA recognition system is as follows. All substrates were cleaned by sequential

immersion in 0.1 wt% NaCl and ethanol, followed by rinsing in deionized water (>18 MΩcm) and baking at 80°C for 2 h prior to silanization. The silanization of the surface was performed using 3% 3-aminopropyltrimethoxysilane (APTMS) in methanol for 5 min at room temperature. The substrates were rinsed with methanol and baked at 180°C for 2 h. Then, citrate-stabilized gold nanoparticle (5 nm, 10 nm, 15 nm, 30 nm, or 50 nm diameter) solution was spotted on the entire surface of the silanized substrate. The substrates were incubated in a chamber under saturated humidity at 25°C for 20 h, and then rinsed with deionized water. The immobilized gold nanoparticles were visualized using scanning electron microscopy (SEM) (S4700, Hitachi).

2.2 Immobilization of Probe Oligonucleotides

The remaining amine groups were blocked with 4 mM N-hydroxysuccinimide ester of carboxyl-polyethyleneglycol (PEG-NHS) in 100 mM triethanolamine (TEA) to hinder non-specific adsorption of the oligonucleotides on the surface. After the blocking process, the thiolated probe oligonucleotides with Cy3 or Cy5 (Table 1) were immobilized on the gold nanoparticles. The probe oligonucleotides were diluted to a final concentration of 10 μM in 1 M potassium phosphate buffer adjusted to pH 6.7 [12]. A 1 μL portion of the probe oligonucleotide solution was spotted in a grid format on the surface containing the immobilized gold nanoparticles. The substrates were incubated in a chamber under saturated humidity at 25°C for 3 h, and then rinsed with 5 SSC solution to remove the non-specifically bound oligonucleotides.

The amount of the immobilized probe oligonucleotides was quantified using surface plasmon resonance (SPR) spectroscopy. Molecular density of the probes was calculated from the change in resonance angle before and after immobilization of the probes on the SPR sensor surface containing immobilized gold nanoparticles and blocking molecules.

To compare the fluorescence intensity from probes with and without gold nanoparticles, probes without gold nanoparticles were also prepared. For these, thiolated probe oligonucleotides were immobilized directly onto the thiolated surface via disulfide bonds. The thiolated surface was produced with a 3-mercaptopropyltrimethoxysilane (MPTES) coating. The cleaned substrates were immersed in 1% MPTES toluene solution for 0.5 h at room temperature, rinsed with toluene, and annealed at 115 °C for 0.5 h. The thiolated probe oligonucleotides were immobilized on the surface using the same immobilization procedure applied to the surface with gold nanoparticles.

2.3 Hybridization Assays

Hybridization experiments were performed using complementary target oligonucleotides and random

sequence oligonucleotides as shown in Table 1. After the target oligonucleotides were dissolved in HBS buffer (Biacore), a solution with a concentration range of 1 nM - 1 μM was spotted on the substrates containing the immobilized probe oligonucleotides. Hybridization was performed at 42°C for 12 hr under saturated humidity.

Table 1. Sequences of the probe and target oligonucleotides

Probe -50 mer	●S-(CH ₂) ₃ -AGTCGAGCGGTAGCACAGAGACTT GCTCTCGGGTGACGAGCGGGGACG-Cy3 (or Cy5)
Target -50 mer (Complementary)	5'-CGTCCCGCTCGTCACCCGAGAGCAAGCTC TCTGTGTACCCTCGACT-3'
Target -50 mer (Random)	5'-TGAGTTTTTTAACCCTGATTGTACTGCAA CAAGTGAATCATCTACT-3'

2.4 Evaluation of Fluorescence Intensities on Gold Nanoparticles

Fluorescence intensity from the immobilized probes on gold nanoparticles was measured using a confocal laser scanner (CRBio II, Hitachi Software Engineering). Fluorescence intensity from the probe oligonucleotides was obtained both in the presence and absence of the target oligonucleotides. The intensities were quantified by measuring emission of Cy3 or Cy5 with excitation light at 532 nm for Cy3 and 635 nm for Cy5, using a fluorescence image analysis software package (DNASIS; Hitachi Software Engineering). The fluorescence intensity per a single fluorophore was calculated from the fluorescence intensity per unit area divided by the amount of immobilized probe oligonucleotide per unit area as measured by the SPR.

3 RESULT AND DISCUSSIONS

3.1 Fabricated Sensors

Figure 2 shows gold nanoparticles immobilized on the amine-terminated surface. The gold nanoparticles were spatially isolated with submonolayer coverage. At pH levels less than 7.0, the gold nanoparticles are negatively charged due to adsorption of anions, whereas the amine groups are positively charged. Thus, the electrostatically attractive interactions between the nanoparticles and the amine-terminated surface allow immobilization of the nanoparticles on the surface. In addition, a repulsive interaction among the nanoparticles prevents the particles from aggregating on the surface [13].

After the amine-terminated surface was blocked with PEG-NHS and subsequently the thiolated probe oligonucleotide with Cy3 or Cy5 (Table 1) was immobilized, SEM images confirmed that the immobilized gold nanoparticles were not detached or aggregated. The amount of non-specifically adsorbed probe oligonucleotides on the surface was below the detectable limit using fluorescence measurements.

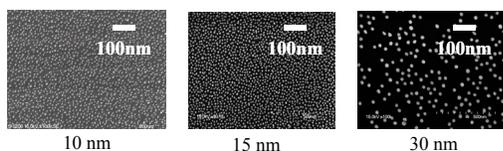


Figure 2. Scanning electron microscopy (SEM) images of immobilized 10 nm, 15 nm, and 30-nm gold nanoparticles.

3.2 Fluorescence Quenching and Enhancement

Fluorescence intensities per a single fluorophore Cy5 attached to the 50 mer probe oligonucleotide on the surface were obtained with and without 10 nm and 15 nm gold nanoparticles (Figure 3). With gold nanoparticles, the fluorescence intensities of Cy5 before hybridization were lower than the intensities without gold nanoparticles. This result reveals that the fluorescence is quenched by the gold nanoparticles when the fluorophore is in contact with the nanoparticle surface due to a conformationally flexible single-stranded oligonucleotide [4-5].

Then, fluorescence intensities were obtained after hybridization with 1 μM complementary target oligonucleotide. Without gold nanoparticles, fluorescence intensities before and after hybridization were not drastically different. With gold nanoparticles, in contrast, the fluorescence intensities increased after hybridization. After hybridization of the target oligonucleotides, the rigidity of the double-stranded oligonucleotides allows the fluorophore to separate from the surface of the gold nanoparticles and leave the quenching region. Since the energy transfer rate is inversely proportional to the sixth power of the distance between the fluorophore and the center of the nanoparticle [8], the transfer rate is decreased dramatically after hybridization. Alternatively, enhancement of fluorescence intensity occurred on the gold nanoparticles. In the vicinity of the gold nanoparticles, the local electromagnetic field is enhanced by the high polarizability of the small particles [14-15]. This enhancement of the local field increases the near field scattering cross section of the particle [16-17]. Large scattering intensity can increase the intensity of excitation light, which enhances fluorescence intensity. In addition, the local field around the gold nanoparticles could increase the quantum yield of fluorophores due to an increase in the radiative decay rate [8]. From these results, we conclude that a double layer is formed in the vicinity of gold nanoparticles; the inner layer acts as a fluorescence quenching region and the outer layer acts as a fluorescence enhancement region as shown in Figure 4. By using both regions effectively, the ensemble of gold nanoparticles and oligonucleotides with a fluorophore can function as a DNA sensing transducer.

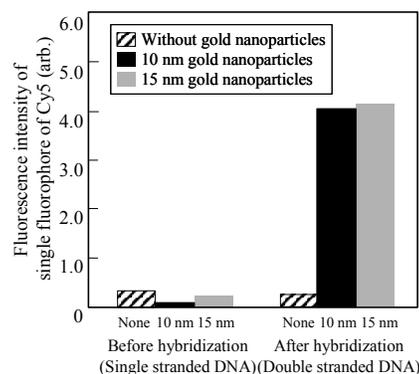


Figure 3. Fluorescence intensities of a single fluorophore of Cy5 in the vicinity of the surface in the presence of gold nanoparticles and in the absence of gold nanoparticles.



Figure 4. A double layer consisting of an inner fluorescence quenching region and outer fluorescence enhancement region in the vicinity of gold nanoparticles.

3.3 Fluorescence Detection of DNA on Gold Nanoparticles

Figure 5 shows the relative increase in fluorescence intensity after hybridization of the complementary target on various sizes of gold nanoparticles. The relative increases were calculated simply as the ratio of fluorescence intensity after hybridization to that before hybridization. The value was maximized on 10 nm gold nanoparticles for those probe oligonucleotides. We discuss the mechanism of the appearance of the maximal value. The polarizability of the gold nanoparticle of radius r is given by [10, 17]

$$\alpha = 4\pi r^3 \frac{\epsilon_1(\omega) - \epsilon_2}{\epsilon_1(\omega) + 2\epsilon_2}$$

where the $\epsilon_1(\omega)$ is the dielectric constant of the nanoparticle and ϵ_2 is the dielectric constant of the ambient. Since the polarizability is proportional to the third power of the radius, the smaller nanoparticles hold smaller polarizability, followed by weaker local electromagnetic field around the nanoparticles. Thus, we assume that weaker field around gold nanoparticles smaller than 5 nm causes smaller fluorescence enhancement, resulting in lower fluorescence intensity after hybridization. Conversely, larger gold nanoparticles with a diameter larger than 15 nm hold higher polarizability, and produce a larger local electromagnetic field. Consequently, it produces large fluorescence enhancement, which apparently dilutes the quenching effect; accordingly, fluorescence intensity before hybridization increases. Hence, relative increase values in fluorescence intensity after hybridization decrease on gold nanoparticles smaller than 5 nm as well as larger than 15-

nm. We conclude that the optimal gold nanoparticle diameter of approximately 10 nm utilizes both quenching and enhancement factors effectively, allowing the highest relative increase of about 50-fold for detecting DNA. The relative increases of Cy5 were higher than those of Cy3 for all gold nanoparticles. Since the excitation wavelength of Cy3 is similar to the localized surface plasmon resonance wavelength of the gold nanoparticles (approximately 520 nm), the resonance can produce a stronger electromagnetic field near the nanoparticles and reduce the quenching effect. It follows increase in fluorescence intensity before hybridization resulting in the smaller relative increase value for Cy3.

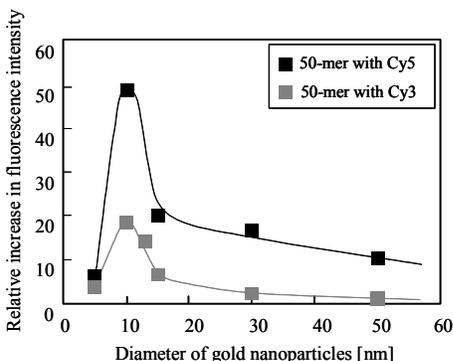


Figure 5. Relative increase in fluorescence intensity after hybridization of 50 mer target oligonucleotides on various sizes of gold nanoparticles.

Figure 6 shows the relative increase when target oligonucleotides with complementary sequences and random sequences were hybridized with the 50 mer probes on 15 nm gold nanoparticles. The relative values increase with the concentration of the complementary target oligonucleotides; the correlation was approximated by the Langmuir model. For random target sequences, however, the values were close to unity for all the concentrations. These results indicated that DNA could be detected selectively and quantitatively.

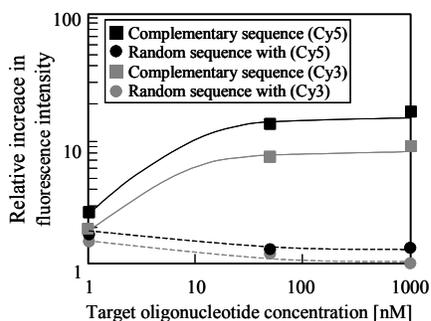


Figure 6. Relative increase in fluorescence intensity after hybridization with a 50 mer complementary sequence and a 50 mer random sequence on 15 nm gold nanoparticles. The solid lines represent calculated values with the Langmuir model.

4 CONCLUSION

We have developed a novel DNA detection system in which a single-stranded oligonucleotide with a fluorophore for probing DNAs was immobilized on two-dimensionally assembled gold nanoparticles. We established processes for fabricating the surface of the detection system by selectively tethering probe oligonucleotides with fluorophores on gold nanoparticles. This system allows quantitative detection of a non-labeled complementary target DNA using fluorescence quenching and enhancement mechanism. The relative increase in fluorescence intensity after hybridization was maximized to 50-fold on 10 nm gold nanoparticles, since the diameter size enabled the use of both quenching and enhancement factors effectively. DNA recognition properties of this system will be explored for highly parallel gene expression analysis.

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