Gold nanoparticles (AuNPs) have been used as scaffolds to create a colorimetric sensing system for various substances as they have high extinction coefficients and unique distance-dependent optical properties. The extinction coefficient of AuNPs is $10^5$ to $10^6$ higher than those of organic dyes, and AuNPs generally have an optical absorption peak around 520 nm caused by surface plasmon resonance and exhibit a red color. Aggregation of AuNPs shifts the surface plasmon resonance toward longer wavelengths and changes the color of AuNP solutions to purple.

The distance-dependent optical properties can be chemically controlled using specific host compounds such as DNA, antibodies, and various chemicals. DNA in these host compounds has been used intensively for functionalizing AuNPs in order to create colorimetric sensing systems since the changing sequence of oligonucleotides can create various host compounds and AuNPs are functionalized easily with oligonucleotides. DNA-functionalized AuNPs have been developed as colorimetric sensing systems for metal ions, oligonucleotides, and proteins. These methods are almost all based on the cross-linking mechanism by which analytes interconnect with the DNA on the AuNPs. Recently, Maeda et al. found that DNA functionalized AuNPs aggregate together in the presence of DNA that is perfectly complementary to the DNA on the AuNPs in sequence as well as in chain length. They developed a colorimetric sensing system to detect a single-base mismatch in target oligonucleotides and mercury ions, respectively, based on the non-cross-linking mechanism.

Although the systems used to detect target oligonucleotides and mercury ions are less sensitive than the cross-linking systems, these systems are simple because they require only AuNPs functionalized with one type of single-stranded DNA and yield a rapid response. However, few sensing systems based on the non-cross-linking mechanism have been reported.

DNA binding molecules have been developed as potential anticancer drugs. Many of these molecules, such as doxorubicin and daunorubicin, are known to be reversible binders to DNA and were approved as anticancer drugs by the FDA. As a result, large libraries of DNA-binding molecules have been synthesized for the development of anticancer drugs through combinatorial chemistry. Screening methods for the activities of DNA-binding molecules have been developed, including mass spectroscopy, nuclear magnetic resonance, light scattering, and electrochemistry. However, these methods are not applicable to high-throughput screening. Only fluorescent intercalator displacement assays and cross-linking mechanism-based AuNP systems have been developed as high throughput screening methods for DNA-binding molecules. Therefore, another approach for screening DNA-binding molecules is needed.

As mentioned above, single-stranded DNA functionalized AuNPs aggregate rapidly in the presence of DNA that is perfectly complementary to the single-stranded DNA at high salt concentrations. However, the addition of complementary DNA with a single-base mismatch at the end to single-strand-DNA functionalized AuNPs does not cause aggregate formation, even at high salt concentrations. Theoretical simulations indicate that double strand DNAs on gold nanoparticles dissociate with higher probability at the end furthest from the surface than any site on the double strand DNAs.

Based on this information, we designed a colorimetric assay system for determining the relative binding affinities between DNA binding molecules and duplexed DNA. When single strand-DNA functionalized AuNPs (probe-AuNPs) and a copy of perfectly complementary (cDNA) to the single strand-DNA are combined, the probe-AuNPs aggregate rapidly by forming duplex DNA. The aggregates are dispersed when the end of the duplexed DNA dissociates at a specific temperature. However, DNA-binding molecules stabilize the duplexed DNA, which inhibits the dispersion of the AuNPs at the target temperature. This increased stability is reflected by an increase in the melting temperature. Therefore, by monitoring the blue-to-red color change of cDNA-probe-AuNPs conjugates in the presence of different compounds with increasing temperature, the relative strength of DNA-binding was determined according to Scheme 1.

This assay was prepared by combining probe-AuNPs (3 nM) and cDNA (300 nM) in 10 mM PBS buffer (pH = 7, 0.5 M NaCl). When the probe-AuNPs and cDNA were combined, they aggregated within 3 min by forming duplexes on the nanoparticle surface. The melting temperatures of the nanoparticle-DNA conjugates (probe-AuNPs and cDNA) were then determined in the presence of 4',6-diamidino-2-phenylindole (DAPI), ellipticine (EIPT), amsacrine (AMS), anthraquinone-2-carboxylic acid (AQ2A), ethidium bromide (EB), and 9-aminoacridine (9- AA) (5µM), respectively. Control experi-

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**Key Words** : Gold nanoparticle, Colorimetric assay, DNA-binding molecules, High-throughput screen
ments were evaluated by determining the melting temperatures of unmodified duplex DNA (DNA-1: 5'- CGA GTT AAG AAG AAA AAA GA-3', DNA-2: 3'- GCT CAA TTC TTC TTT TTT CT -5') in the presence of DNA binding molecules (5 µM). As shown in Table 1 and Figure 1, a comparison of the nanoparticle melting data and the unmodified duplex control melting data reveals that the trends in the melting temperatures are almost identical. Moreover, because the melting transition occurs over a very narrow temperature range, the AuNP-based assay system allows for a more precise analysis of temperature change than does the unmodified duplex DNA system. In addition, the absorbance changed by 0.05 in the melting transition of unmodified DNA and interfered with that of the DNA binding molecules as AuNPs have an extremely large extinction coefficient. These properties yield an enhanced signal:noise ratio when a large library of DNA-binding molecules is screened.  

One additional advantage of this system is that it enables detection of the relative binding affinities of DNA-binding molecules with the naked eye; instrumentation is not necessary.

**Table 1.** Melting temperatures (Tm) of nanoassemblies and control duplexes in the presence of intercalators

<table>
<thead>
<tr>
<th>Intercalator</th>
<th>Tm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tm&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.1</td>
<td>61.8</td>
</tr>
<tr>
<td>DAPI</td>
<td>36.3</td>
<td>67.3</td>
</tr>
<tr>
<td>EB</td>
<td>34.0</td>
<td>62.7</td>
</tr>
<tr>
<td>9AA</td>
<td>31.6</td>
<td>62.2</td>
</tr>
<tr>
<td>AQ2A</td>
<td>30.5</td>
<td>62.2</td>
</tr>
<tr>
<td>EIPT</td>
<td>30.4</td>
<td>62.0</td>
</tr>
<tr>
<td>AMSA</td>
<td>30.2</td>
<td>62.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Conditions: Probe-AuNP (3.0 nM) and cDNA (300 nM) in 10 mM sodium phosphate buffer (pH 7.0) containing 0.5 M sodium chloride.

<sup>b</sup>Conditions: DNA-1 and DNA-2 (each 2.0 µM) in 10 mM sodium phosphate buffer (pH 7.0) containing 0.5 M sodium chloride.

**Figure 1.** Melting curves of (a) DNA-1 and DNA-2 (no nanoparticles) and (b) nano-conjugates in the presence of DNA binding molecules.
required. As the temperature increases, a color change from blue to red occurs at specific temperatures (Figure 2). At 25 °C all seven cells (one control and six DNA-binding molecules) appear light blue/purple. At 30 °C, the nano-conjugates containing the control and weak DNA-binding molecules turn red, leaving only the conjugates containing DAPI and EB as blue/purple. Increasing the temperature to 35 °C causes all of the samples to turn red except the nano-conjugates containing DAPI, a strong DNA-binding molecule. This allows for discrimination between weak, intermediate, and strong DNA-binding molecules by an easily identifiable color change. The trend in DNA-binding molecule binding affinities for DNA was determined to be DAPI > EB > other molecules, which is consistent with the control experiments. Although the reported AuNP-based method using a cross-linking mechanism can more precisely discriminate the relative binding affinities of DNA binding molecules than can the current method, the prior method requires a long incubation time to form aggregates to determine the relative binding affinities. In contrast, the current method requires an incubation time of only three minutes before the nano-conjugates form and requires only one species of AuNP probe.

In summary, we have developed an AuNP-based colorimetric assay for determining the binding strength of DNA binding molecules to duplex DNA using a non-cross-linking mechanism. This method allows for discrimination between weak, intermediate, and strong intercalators using spectrophotometric instruments and the naked eye. Although this screening method requires a more precise temperature control to discriminate the relative binding affinities of the DNA binding molecules, compared to AuNP based assay system using cross-linking mechanism, this assay requires only one species of AuNP probe and prepared nano-conjugate and requires only a three minute incubation time.

**Experimental Section**

**Preparation of Probe-AuNPs.** Citric acid stabilized Au particles with a diameter of 13 nm were prepared by adding 50 mL of citrate solution (38.8 mM) to 500 mL of boiling 1.0 mM HAuCl₄·3H₂O with vigorous stirring. After the appearance of a deep red color, boiling and stirring were continued for 15 minutes. The solution was then allowed to cool to room temperature with continued stirring. The disulfide group of the probe DNA purchased from Integrated DNA Technologies (IDT) was reduced by soaking it in a 0.1 M dithiothreitol phosphate buffer solution (0.17 M phosphate, pH 8.0) for 30 minutes. The cleaved DNA strands were purified using a NAP-5 column (GE Healthcare) and added to the AuNP solution (the final probe DNA concentration was approximately 3 µM). The solution was buffered to 0.15 M NaCl and 10 mM phosphate by simultaneously adding 2 M NaCl solution and 0.1 M phosphate buffer solution (pH 7.4). After incubation overnight at room temperature with gentle shaking, the AuNP solution was centrifuged and redispersed in distilled water after the supernatant was removed. The particles were washed two times more, and finally redispersed in distilled water.

**Determination of Melting Temperature of the Nano-conjugates.** The assay was initiated by mixing probe AuNPs (3 nM) and cDNA (300 nM) in 10 mM PBS buffer (pH = 7.0, NaCl 0.5 M). The melting temperatures of the nano-assemblies were then determined in the presence of 4',6-diamidino-2-phenylindole (DAPI), ellipticine (EIPT), amsacrine (AMSA), anthraquinone-2-carboxylic acid (AQ2A), ethidium bromide (EB), and 9-aminocaridine (9-AA) (5 µM), respectively. UV melting profiles were measured by monitoring the absorbance at 520 nm at a scan rate of 0.5 °C min⁻¹.

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**References**