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Room-Temperature Colorimetric Detection of Coralyne Using DNA-Functionalized Nanoparticle Probes

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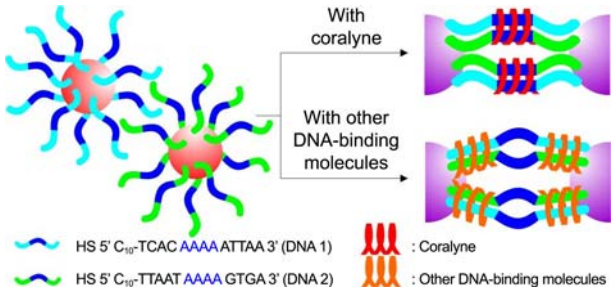
Coralyne is a crescent-shaped planar heterocyclic molecule that is capable of binding to duplex and triplex DNA. As a synthetic derivative of protoberberine alkaloids, coralyne exhibits powerful anticancer activity against P388 and L1210 leukemias in animal models such as mice.[1] The potential medicinal impact of coralyne in cancer therapeutics is particularly attractive because of its relatively low toxicity, which has led to the deep investigation of its molecular interactions with nucleic acids and the synthetic development of various coralyne derivatives.[2] One of the most important chemical and biological properties of coralyne is its molecular recognition of specific nucleic acids, or polyadenine (poly-A), with a strong binding affinity (binding constant = 1.8×10^6 M^{-1}) and a stoichiometry of one coralyne to four adenine bases.[3-5] In fact, most mRNA sequences contain a number of poly-A residues at their 3' end, which is essential for determining the mRNA stability and maturation, and for initiation of translation.[6] Considering that the drug design associated with gene regulation typically requires specific binding to unique structural regions in mRNA such as poly-A, the chemical, biological, and physiological applications of coralyne still essentially require further vast investigation.

Based upon such importance, there have been a few reports for the detection of coralyne in aqueous media using unmodified DNA sequences,[7] fluorophores,[8] unmodified silver nanoparticles,[9] and unmodified or DNA-modified gold nanoparticles.[10,11] These methods are in common based upon the non Watson-Crick base pair interactions of adenine-adenine (A-A), where coralyne (CR) strongly intercalates the poly-A/poly-A duplex *via* A-CR-A chemistry.[3-5] While sensitive, however, they often suffer from costly instrumentation for photoluminescence, employ a temperature controller, and most of all, exhibit limited or unconfirmed selectivity. Therefore, the development of a selective and sensitive assay to detect coralyne is highly demanded.

In this *Note*, we present a new colorimetric assay to detect coralyne based upon the kinetic observation of the DNA-functionalized gold nanoparticles (DNA-AuNPs) and their hybridization in the presence of coralyne. We have taken advantage of (1) the difference in kinetics of DNA-AuNPs' hybridization determined by their DNA loading, (2) the distance-dependent optical properties of DNA-AuNPs based upon surface plasmon resonance (SPR), and (3) the specific A-CR-A intercalation chemistry.[12-14] This assay is conducted at room temperature and does not require any expensive instrumentation. Importantly, we have hypothesized that the DNA-AuNP hybridization kinetics is dramatically enhanced in the presence of coralyne than the other DNA-binding molecules, which would lead to a target-specific visible response of the system to coralyne.

The assay began by combining the DNA-AuNP probes (**DNA 1** and **DNA 2**)[15] with various DNA-binding molecules (Scheme 1) at room temperature, and monitoring their extinction at 525 nm as a function of time ([**DNA 1**] = [**DNA 2**] = 0.5 nM, [DNA-binding molecule] = 5 μM). In the absence of a DNA-binding molecule (Blank), the DNA-AuNPs assembled to form aggregates slowly *via* Watson-Crick base pairing with a decrease in extinction at 525 nm. On the other hand, however, coralyne dramatically accelerated the macroscopic aggregate formation of the DNA-AuNPs with a concomitant color change from red to purple, indicating that the DNA-DNA hybridization took place *via* A-CR-A intercalation chemistry in a kinetically favorable manner (Fig. 1(a)). To systematically evaluate the selective binding of coralyne for A-A mismatches, we prepared eight identical batches of the DNA-AuNP probe solutions and combined each of them with pure water (Blank), coralyne (CR), and structurally compatible and physiologically relevant other intercalators such as ethidium bromide (EB), neomycin (NM), 4,6-diamidino-2-phenylindole (DAPI), 9-aminoacridine (AA), palmatine (PT), and berberine (BR), respectively. After 15 minutes, only the mixture containing



Scheme 1. A scheme illustrating the coralyne detection using DNA-AuNP probes. The two types of probes are combined at 1.0 M NaCl.

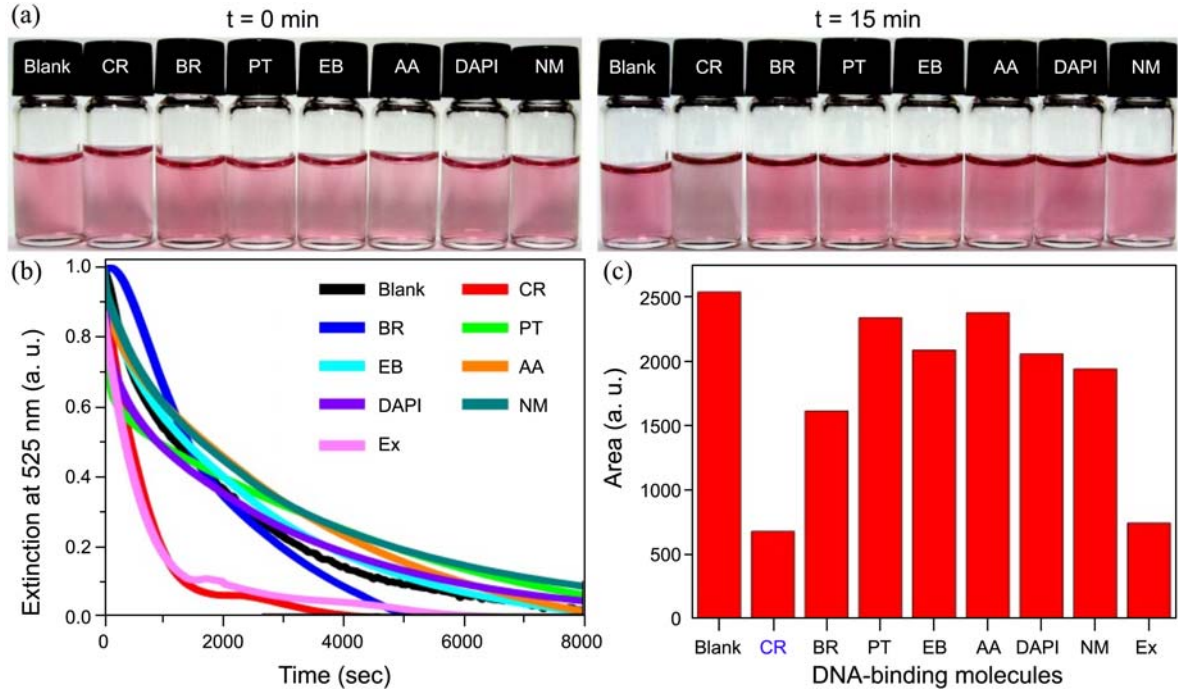


Figure 1. (a) Color change of the DNA-AuNP probes with low DNA loading (DNA-AuNPs whose DNA sequences were conjugated at 0.2 M NaCl, each DNA-AuNP at 0.5 nM) in the presence of various representative DNA-binding molecules (each at 5 μM) observed at t = 0 and t = ~15 min, respectively. (b) The hybridization transitions of each mixture. (c) The area below the hybridization transition of each mixture.

coralyne eventually turned purple owing to the hybridization of the DNA-AuNPs associated the A-CR-A intercalation, which was soon (t = ~30 minutes) followed by the hybridization of DNA-AuNP probes in mixtures containing other DNA-binding molecules (Fig. 1(a)). Importantly, this result indicates that all the binding molecules examined in this assay are thermodynamically capable of forming the nanoparticle assemblies under the conditions studies, which could

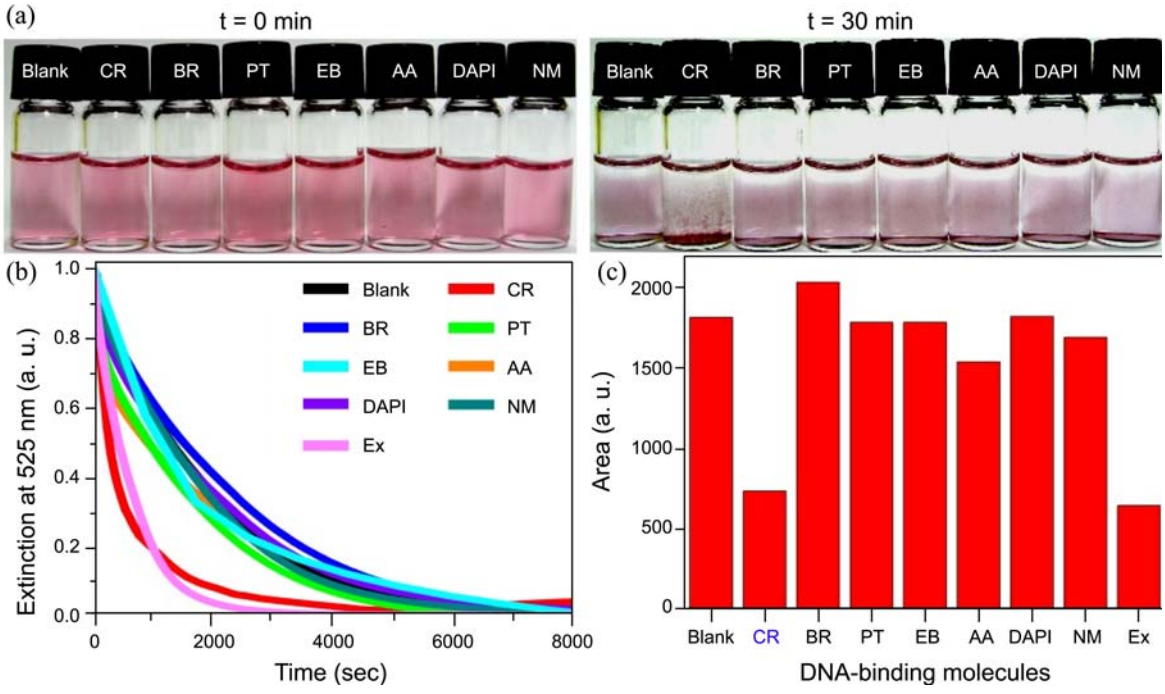


Figure 2. (a) Color change of the DNA-AuNP probes with high DNA loading (DNA-AuNPs whose DNA sequences were conjugated at 1.2 M NaCl, each DNA-AuNP at 0.5 nM) in the presence of various representative DNA-binding molecules (each at 5 μM) observed at t = 0 and t = ~30 min, respectively. (b) The hybridization transitions of each mixture. (c) The area below the hybridization transition of each mixture.

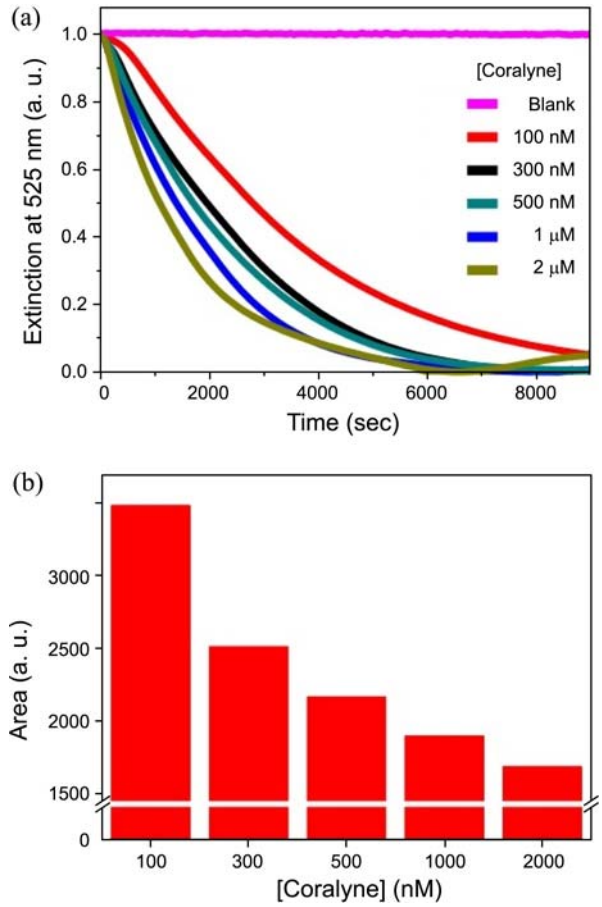


Figure 3. (a) Hybridization transitions of DNA-AuNP probes with coralyne at various concentrations (0 to 2 μM). (b) The area below the hybridization transitions as a function of the coralyne concentration. Note that the limit of detection is determined to be 100 nM.

hamper the selective detection of coralyne if the color change is not observed in a critical timely manner. The time-dependent hybridization progress of each mixture was further monitored by observing its extinction at 525 nm using UV-vis spectroscopy (Fig. 1(b)) and quantitatively analyzed by plotting the area under the transition curves obtained from each mixture (Fig. 1(c)). Additionally, we evaluated the assay for coralyne in the presence of a background DNA sequence (**Ex**: 5' CCA CAT GGA GAT ACA GCA GT 3', 1 μM) and obtained almost the same result (Fig. 1(b) and (c)).

To fundamentally enhance the selectivity of the assay based upon our recent study on controlling the hybridization kinetics, we employed a new type of AuNP probes that are much more heavily loaded with DNA. Because the elevated DNA loading would lead to the stronger repulsive force between the negatively charged DNA-AuNPs and thus decrease the hybridization rate, eventually an enhanced stringency condition is provided to the assay system to improve the selectivity of the assay.[16] We conducted the assay with eight aliquots of the new densely DNA-loaded AuNP probe solutions by combining each of them with the seven binding molecules (CR, EB, NM, DAPI, AA, PT, BR) or water (Blank), respectively. Interestingly, only the one with coralyne selectively turned purple and precipitated almost completely after ~30 minutes, while the others still stayed reddish purple (Fig. 2(a)). The other mixtures began to precipitate after 2 hours, which is much slower than the hybridization rate of less DNA-loaded DNA-AuNPs with non-coralyne binding molecules (~30 minutes). Figure 2(b) demonstrates the hybridization transitions of the mixtures containing 5 μM of each DNA-binding molecule, which exhibit larger difference between coralyne and the others compared to the hybridization transitions previously obtained with less DNA-loaded AuNP probes (Fig. 1(b)). We also evaluated the assay for coralyne in the presence of a background DNA sequence (**Ex**: 5' CCA CAT GGA GAT ACA GCA GT 3', 1 μM) and obtained almost the same result (Fig. 2(b) and (c)). Significantly, the assay selectivity has been substantially improved by a factor of two, assuming that the selectivity of the assay is quantitatively determined by the ratio of the time when the color change of the DNA-AuNPs containing coralyne (target) took place to the time when the color change of other mixtures took place.

We finally evaluated the sensitivity of the assay by quantitatively analyzing the area below the hybridization transitions obtained with different concentrations of coralyne. Unlike previous experiments, Poly A sequences (HS-$\mathbf{A}_{10}$) were conjugated to AuNPs instead of **DNA 1** and **DNA 2**. **DNA 1** and **DNA 2** are not suitable for the detection scheme because they have complementary part that can bind together to hybridize even without coralyne. As the concentration of coralyne increased, the DNA-AuNPs hybridized more rapidly (Fig. 3(a)), which indicates that coralyne plays a significant role in the rate determining step of the A-A hybridization. Based on the areas below the hybridization transitions at each coralyne concentration (Fig. 3(b)), the limit of detection was determined to be 100 nM, which is comparable to those based on fluorophore or unmodified plasmonic nanoparticles, and is far more sensitive than other assays using DNA-AuNPs.[7-11]

In conclusion, we have developed a sensitive and selective colorimetric assay for detecting coralyne using DNA-AuNP probes based on the specific A-CR-A chemistry and controlled DNA loading on the probes. The kinetic observation of the nanoparticle hybridization allows one to understand the various chemical responses of kinetic properties to DNA-binding molecules with different molecular structures. Furthermore, the design of this assay clearly emphasizes the importance of DNA-AuNPs based on the chemically programmable assembly properties, which could be further extended to detect other DNA-binding molecules capable of recognizing specific bases.[17-19]

Experimental Section

Materials and Instruments. The HPLC-purified monothiol oligonucleotides (**DNA-1**: 5' HS-C_{10}-TCA C**AA AA**A TTA A 3'; **DNA-2**: 5' HS-C_{10}-TTA AT**A AAA** GTG A 3'; **Ex**: 5' CCA CAT GGA GAT ACA GCA GT 3', **Poly A**: 5' HS-A_{10} 3') were purchased from Genotech (Daejeon, Republic

of Korea). Berberine (Cat. # 14050), coralyne (Cat. # R278122), dithiothreitol (DTT, Cat. # 43815), ethidium bromide (Cat. # E8751), gold chloride trihydrate (Cat. # 520918), neomycin (Cat. # N6386), palmatine (Cat. # 361615), sodium dodecyl sulfate (SDS, Cat. # L4522), trisodium citrate dihydrate (Cat. # S4641), 4,6-diamindino-2-phenylindole (Cat. # D9542), 9-aminoacridine (Cat. # A38401) and other chemicals used for the buffer preparation were purchased from Sigma-Aldrich (Milwaukee, WI, USA). NAP-5 Sephadex columns were purchased from GE Healthcare (Piscataway, USA). Ultrapure water purified by a Direct-Q3 system (18.2 MΩ·cm, Millipore, Billerica, MA, USA) was used to prepare all solutions. Agilent 8453 UV-vis spectrophotometer equipped with a Peltier temperature controller was used to measure the extinction or absorbance in all experiments.

DNA-Gold Nanoparticle Conjugate (DNA-AuNP) Synthesis. Gold nanoparticles were synthesized following a previously reported literature method.[16] The monothiol DNA sequences (**DNA-1** and **DNA-2**) were deprotected with a 0.10 M DTT (0.17 M phosphate buffer, pH 8.0) solution to reduce the terminal disulfide group. Using a NAP-5 column, the deprotected DNA was completely purified. The purified DNA was combined with the 3 nM gold nanoparticle solution (the final DNA concentration is ~4.7 μM). The mixture was buffered with a phosphate buffer solution (0.01% SDS, pH 7.4, 10 mM phosphate) and salted up to 0.2 or 1.2 M NaCl. The final mixtures were incubated at room temperature for 24 h.

Hybridization Kinetics Observation. In a buffered solution (pH 7.4 10 mM phosphate, 0.01% TWEEN 20, [NaCl] = 1.0 M, [DNA-AuNP] = 0.5 nM each) two types of the DNA-gold nanoparticle conjugates (**DNA-1** and **DNA-2**) were conbined to prepare seven identical mixtures at 0.2 or 1.2 M NaCl concentrations, respectively, to one of which one of the binding molecules was spiked for the kinetic evaluation. The mixture was continously stirred with a magnetic stir bar at 850 RPM at 25 °C. The hybridization rate was measured by monitoring the change in extinction at 525 nm every one minute.

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