

# Colorimetric detection of DNA, small molecules, proteins, and ions using unmodified gold nanoparticles and conjugated polyelectrolytes

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We have demonstrated a novel sensing strategy employing single-stranded probe DNA, unmodified gold nanoparticles, and a positively charged, water-soluble conjugated polyelectrolyte to detect a broad range of targets including nucleic acid (DNA) sequences, proteins, small molecules, and inorganic ions. This nearly “universal” biosensor approach is based on the observation that, while the conjugated polyelectrolyte specifically inhibits the ability of single-stranded DNA to prevent the aggregation of gold nanoparticles, no such inhibition is observed with double-stranded or otherwise “folded” DNA structures. Colorimetric assays employing this mechanism for the detection of hybridization are sensitive and convenient—picomolar concentrations of target DNA are readily detected with the naked eye, and the sensor works even when challenged with complex sample matrices such as blood serum. Likewise, by employing the binding-induced folding or association of aptamers we have generalized the approach to the specific and convenient detection of proteins, small molecules, and inorganic ions. Finally, this new biosensor approach is quite straightforward and can be completed in minutes without significant equipment or training overhead.

biosensor | aptamer | visual detection | thrombin detection | cocaine detection

Gold nanoparticle colorimetric biosensors have seen significant applications in diagnostics, environmental monitoring, and antibiotechnology supporting unaided, visual readout (1–12). Commonly, the relevant nanoparticles are covalently modified with either a probe DNA or an aptamer such that hybridization (13–16) or aptamer-target interactions (17–27), for example the scanometric method developed by Mirkin (25), which is a very sensitive and specific tool, crosslink them, inducing aggregation. The second broad approach utilizes unmodified nanoparticles. (28–30) These two approaches, however, suffer from time-consuming (20–40 h of assembly) and relatively poor (low nanomolar) detection limits, respectively. Here, a unique, colorimetric sensing strategy employing a simple but selective combination of a single-stranded DNA probe, a positively charged, water-soluble conjugated polyelectrolyte, and unmodified gold nanoparticles is demonstrated. The universality of this method allows detection of a broad range of targets, including nucleic acid (DNA) sequences, proteins, small molecules, and inorganic ions. Our approach is rapid (turnaround time is 5–10 min) and sensitive (picomolar concentrations of target DNA are readily detected with the naked eye, even in complex sample matrices like blood serum). Hence, an operator with minimum scientific overhead can easily employ this technique.

Generally, the gold nanoparticle applications typically rely on a quantitative coupling between target recognition and the aggregation of the nanoparticles, which, in turn, leads to a dramatic change in the photonic properties—and thus the color—of the nanoparticle solution. This colorimetric “readout” avoids the relative complexity inherent in optical imaging/detection meth-

odologies and thus may prove suitable for point-of-care and developing world applications (9–12). Examples for the covalently modified nanoparticles include the work of Mirkin and collaborators, who pioneered the use of gold nanoparticle-DNA conjugates (22, 23) for the sensitive detection of DNA and proteins (24–27). For the unmodified nanoparticles, Li and Rothberg have shown that, at high ionic strength, single-stranded—but not double-stranded—DNA protects unmodified gold nanoparticles from aggregating, thus modifying their color (28–30). Employing this mechanism, these authors have demonstrated a colorimetric assay for specific DNA sequences.

Our approach to the application of gold nanoparticles in optical biosensing relies on the recent observations that (i) both single-stranded DNA and double-stranded DNA prevent aggregation of gold nanoparticles at low salt concentrations (28–30); (ii) conjugated polyelectrolytes lead to the ready aggregation of such nanoparticles (31), and (iii) previous work has demonstrated that, at certain conditions, the cationic conjugated polyelectrolyte poly[(9,9-bis(6'-N,N,N-trimethylammonium) hexyl) fluorene-alt-1,4-phenylene] bromide (PFP-Br) binds single-stranded DNA preferentially to double-stranded or otherwise “folded” DNA, (32–34). This appears to arise due to the greater hydrophobicity of single-stranded DNA. Upon reducing the strength of the hydrophobic interactions, the electrostatic attraction becomes the important interaction that regulates the binding between the water-soluble conjugated polymer and DNA (33). The different affinities between the cationic conjugated polymer and various forms of DNA (single-stranded DNA and double-stranded DNA and single-stranded DNA and complex DNA folds) can be used to design a variety of biosensors (32–34).

## Results

Inspired by the above, we have developed a unique colorimetric assay for the detection of nucleic acids, small-molecules, proteins, and inorganic ions that relies on unmodified gold nanoparticles but that employs the conjugated, positively charged, water-soluble polymer poly[(9,9-bis(6'-N,N,N-trimethylammonium) hexyl) fluorene-alt-1,4-phenylene] bromide. We have employed cationic conjugated polyelectrolytes to efficiently sequester single-stranded—but not double-stranded or otherwise “folded”—DNA from nanoparticles, leading to their aggregation and color change (Fig. S1) (32–34). We have then used this effect as the

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The color change associated with the lack of double-stranded DNA allows us to easily differentiate the single-stranded DNA probe and the double-stranded probe-target duplex via visual inspection at concentrations as low as 1.25 pM (Fig. 1 *E* and *F*), a detection limit comparable to those of many previously reported fluorescent DNA assays and enzyme-linked amplified assays (35). A more quantitative analysis can be made using UV-Vis spectroscopy. Specifically, the absorbance of the solution at 520 nm increases and the absorbance at 700 nm decreases when the concentration of double-stranded DNA is increased from 1.25 pM to 125 nM (Fig. 1 *C* and *D*). A series of control experiments confirm the specificity of the assay: As expected, duplexes between the 34-base pair probe and targets containing 3-, 5- or 7 base-pair mismatches do not stabilize the nanoparticles against aggregation, leading to the characteristic blue color (Fig. S2).

A key feature of our method is that, unlike approaches that utilize functionalized nanoparticles, hybridization and binding occur under conditions that can be independently regulated and optimized. For example, our approach relies on an ability to detect double-stranded probe-target duplexes. Thus, a potential disturbance is that any double-stranded DNA contaminating the sample will also stabilize the nanoparticles. Because we can optimize test conditions, the pretreatment of the sample with Exonuclease III, which provides a ready and convenient solution degrading any double-stranded DNA initially present in the sample (Fig. S3). Thus our assay can be made quite specific. Likewise the assay is selective and performs well even when challenged directly in complex biological media, such as target-doped blood serum (Fig. S4).

## Discussion

While the detection of DNA is of significant clinical utility in its own right, by employing aptamers, nucleic acid molecules that have been selected *in vitro* to bind to specific molecular targets (36–39), our colorimetric assay can also be expanded to the detection of nonnucleic acid targets. Specifically, because it is relatively easy to reengineer aptamers such that they undergo a transition from largely single-stranded to a folded, compact structure upon target binding (40–44), and because the conjugated polyelectrolyte only weakly binds with these compact, folded structures (31–34), we can adapt our assay to the detection of the aptamer's binding partner (Fig. 2).

As a first example of the broader applicability of our approach, we developed an assay for the small molecule cocaine using the anticocaine aptamer of Stojanovic (39–42). To do so we modified the aptamer sequence slightly (see *Methods*) to prevent inter-strand binding and then cut the sequence into two short, single-stranded pieces (40, 41). We then prepared two samples: a control sample containing only these fragments and a test sample containing the two aptamer fragments and cocaine. Upon adding a solution of ~20 nm gold nanoparticles, both samples turn red

due to the intense surface plasmon resonance absorption of the nanoparticles. Upon the addition of the conjugated polyelectrolyte, the nanoparticles in the control solution aggregate and the solution turns blue. The test sample, in contrast, retains its red color, presumably because cocaine binding drives the two pieces to associate (40–42) to form a well-folded, largely double-stranded aptamer that only weakly binds with the conjugated polyelectrolytes and thus remains free to inhibit nanoparticle aggregation (Fig. 2). This color change supports the ready detection of cocaine via visual inspection. As expected, this sensor does not respond when challenged with other small molecules, such as ATP (Fig. 2).

Abundant literature suggests that it is relatively easy to modify aptamers such that they only fold upon target binding (40–45), an effect that also supports our assay. As examples we have used Bock's thrombin-binding aptamer, which folds from a single-stranded state to a largely G-quadruplex structure upon target binding (43, 44) and a mercury-responsive sequence that folds in the presence of Hg (II) (45–49). Once again, we employ control (aptamer-only) and test (aptamer-plus-target) solutions, which, upon mixing with ~20 nm gold nanoparticles, produce the characteristic absorption at 520 nm. In the absence of target, both aptamers are single-stranded and thus do not stabilize the gold nanoparticles against aggregation in the presence of the polymer. Upon target binding, however, the aptamers fold, reducing their interactions with the conjugated polyelectrolytes and allowing them to prevent nanoparticle aggregation (Fig. 2). The resultant color change supports the easy differentiation between target-containing and target-free samples via visual inspection.

The strategy described in this report offers advantages over many existing sensing methodologies. First, our approach is likely universal; here, for example, we have shown that it is applicable to the detection of nucleic acids, small molecules, proteins, and inorganic ions. Second, our approach is convenient, requiring only the mixing of several solutions at room temperature to achieve rapid, semiquantitative detection via visual inspection or quantitative detection via visible light absorbance spectroscopy. Third, the detection of DNA using this approach is sensitive. We achieve, for example, low picomolar sensitivity for DNA detection, comparing very favorably with previously reported methods (Table 1) (28, 35, 50–52). Fourth, each step in the process (pretreatment with Exonuclease, the binding of the target to the DNA probe, the addition of conjugated polyelectrolytes) is performed separately, allowing each step to be optimized independently. Finally, the detection process is rapid and convenient.

## Methods

In a typical gold nanoparticles/CP assay, the DNA probes were dissolved in a salt solution (112.5 mM NaCl) to form 1.25 μM probe solution at room temperature. Such solution (2.5 μL) was added to gold nanoparticles solution (25 μL), and the obtained solution was incubated for 1 min at room temperature. The concentrations of probes are 125 nM in this solution unless specially indicated. After that CP (5 μL, 1 μM) was added to the above solution,

**Table 1. Comparison of sensors for DNA detection**

Detection method	Strategy	Detection limit	Existing problems
Colorimetric (this work)	AuNP & conjugated polyelectrolyte	1 pM (naked eyes)	Not applicable for colored samples
Colorimetric (28)	AuNP & salt	4.3 nM (naked eyes)	Detect limit not applicable for colored samples
Colorimetric (50)	Enzyme amplification magnetic separation	100 pM (naked eyes)	Multistep process, cumbersome preparation of functionalized gold nanoparticles and magnetic bead. Time-consuming (>1 day), not applicable for colored samples
Electrochemistry (51)	Electron transfer	10 pM	Dual-labeled oligos. Time-consuming (≈5 hours)
Electrochemistry (52)	Enzyme Amplification & PNA Probe & AgNP	10 fM	Multistep process, stability of enzyme, dual label of DNA, reagent intensive. Time-consuming (≈5 hours)
Fluorescence (35)	Conjugated Polyelectrolyte FRET	2.14 pM	Using expensive and complicated instruments for detection. Time-consuming (≈3 hours)

followed by either visual observation or UV/Vis characterization. The total volume of the final solutions in this work may differ, but the volume ratio of the above DNA, gold nanoparticles solution, and CPs is 1:10:2. In cocaine assays, the 3' fragment and 5' fragment were dissolved in salt solution (112.5 mM NaCl) to form 0.625  $\mu$ M 3' fragment and 0.625  $\mu$ M 5' fragment probe solution at room temperature. Then cocaine was added to the above solution. This solution (2.5  $\mu$ L) was added to gold nanoparticles solution (25  $\mu$ L), and the obtained solution was incubated for 2 min at room temperature. After that CP (5  $\mu$ L, 1  $\mu$ M) was added, followed by either visual observation or UV/Vis characterization. For the thrombin assay, the thrombin aptamer (or spiegelmer) was dissolved in a salt solution (112.5 mM NaCl)

to form 1.25  $\mu$ M probe solution at room temperature. Then thrombin was added followed by incubation for 20 min. After that CP (5  $\mu$ L, 1  $\mu$ M) was added, followed by either visual observation or UV/Vis characterization.

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