Supporting Information

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SI Text

Mechanism Discussion. The mechanism underlying this detection method appears to be linked to the different affinities with which double- and single-stranded DNA bind to the conjugated polymer (1–3). Because of this difference, the polymer does not bind all double-stranded DNA to prevent aggregation but efficiently removes single-stranded DNA from the gold nanoparticles, which, in turn, allows the nanoparticles to aggregate and change color. In support of this argument, dye-modified single-stranded DNA is quenched far more effectively by conjugated polyelectrolytes than is the equivalent dye-modified double-stranded DNA (Fig. S1 *A* and *B*), indicating that the polymer binds to single-stranded DNA binds more tightly than it binds double-stranded DNA. Although the single-stranded DNA has less charge density than double-stranded DNA, the additional hydrophobic interactions in ssDNA may compensate for the weaker electrostatic interactions due to its flexible structure and more exposed hydrophobic region (1–6).

Test of Bio-Specificity. 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 (Fig. S2) produce the same blue color as control samples lacking any target.

Optimizing the Conditions. The color change, induced by bio-specific binding to the unmodified gold nanoparticles, occurs under predetermined and separately optimized conditions. For example, our approach relies on the ability to detect double-stranded probe-target duplexes. A potential disturbance is thus that any double-stranded DNA contaminating the sample will stabilize the nanoparticles. However we could optimize test conditions, the pretreatment of the sample with Exonuclease III provides a ready and convenient solution to this problem. Specifically, this enzyme catalyzes the removal of mononucleotides from the 3′-end of double-stranded DNA but does not degrade single-stranded DNA. For example, an actual sample could contain some kind of double-stranded DNA (here, 27 bases double-stranded DNA). If so, when the sample contains no perfect DNA (here, 7-mismatch-DNA) to the probe, the double-stranded DNA could stabilize AuNPs using the above described assay (Fig. S4*A*). If, however, we first treat the sample with Exonuclease III followed by heat deactivation of the enzyme (via immersion for 5 min in a 65 °C water bath), the problem is solved without disrupting the method's ability to detect the authentic target (Fig. S3 *B*, *C*, and *D*).

Recognition of dsDNA in Serum. To demonstrate that the new assay is able to recognize dsDNA in biological media, we prepared the ssDNA and dsDNA ample in 56 mM NaCl solution containing 30 vol% serum. Serum is plasma without fibrinogen or other clotting factors; serum contains proteins, glucose, mineral ions, hormones, and other biological substances. We added the above solution into the AuNPs and followed added CP. The final concentration for the serum is 3 vol%. And both of the solutions are red at this stage. Then we centrifuged the two samples for 2 min at 14,100 rcf. Finally the sample contains dsDNA retains red, while the sample with ssDNA turns to very light blue (Fig. S4).

Details of the Assay. Tables S1, S2, S3, and S4 show the details of the assay.

Materials. Gold nanoparticles (20 nm) were obtained from Sigma-Aldrich. The AuNP solution was centrifuged to a concentrated solution; the optimum conditions were centrifuging for 15 min at 4 °C, with a RCF of 16100 g. Exonuclease III was obtained from Sigma-Aldrich. DNase I (RNase-free) was obtained from New England Biolabs. Human R-thrombin was obtained from Haematologic Technologies Inc.; Essex Junction, VT. Cocaine, mercury ion and serum were used as received. The sequences of the involved oligonucleotides, which were all purchased from Biosearch Technologies, Inc., are listed in following. UV/Vis absorption spectroscopy was performed with a Beckman Coulter DU 800 spectrophotometer and photographs were taken with a Nikon digital camera. The Polymer, poly[(9,9-bis(6′-N,N,N-trimethylammonium)hexyl)fluorene-alt-1,4-phenylene] bromide (PFP-Br) was prepared according to the published references (7, 8).

The sequences of the DNA probes and targets used in this work. *34-mer-Linear-Probe*: 5′-TGGATCGGCGTTTTATTCTTGTTCAGATATTCAA-3′

34-mer-Linear-Target 0 mismatch: 5′-TTGAATATCTGAACAAGAATAAAACGCCGATCCA-3′

34-mer-Linear-Target 3 mismatch: 5′-TTGAATATCTGAACAA**CTT**TAAAACGCCGATCCA-3′

34-mer-Linear-Target 5 mismatch: 5′-TTGAATATCTGAACA-**TCTTA**AAAACGCCGATCCA-3′

34-mer–Linear-Target 7 mismatch: 5′-TTGAATATCTGAAC-**TTCTTAT**AAACGCCGATCCA-3′

27-mer–Linear-Probe: 5′-GACACTGGATCGGCGTTTTATTCACAG-3′

27-mer–Linear-Target 0 mismatch: 5′-CTGTGAATAAAACGCCGATCCAGTGTC-3′

27-mer–Linear-Target 7 mismatch: 5′-CTGTGAATAA**TTGCGGC**ATCCAGTGTC-3′

3 fragment for anticocaine aptamer: 5′-AGACAAGGAAAA-3′

5 fragment for anticocaine aptamer: 5′-TCCTTCAATGAAGTGGGTCG-3′

Thrombin Aptamer: 5′-TAAGTTCATCTCCCCGGTTGGTGTGGTTGGT-3′

Spiegelmer: 5′-TAAGTTCATCTCCCCGGTTGGTGTGGTTGGT-3′

Mercury-Specific Oligonucleotide (MSO) probe: 5′-TGTTTCTTTCTTCCCCTTGTTTGTTTCA-3′

Mercury-Control sequence: 5′-TAGCTATGGAATTCCTCGTAGGCA-3′

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4. Liu B, Gaylord BS, Wang S, & Bazan GC (2003) Effect of chromophore-charge distance on the energy transfer properties of water-soluble conjugated oligomers. *J Am Chem Soc* 125(22):6705–6714.
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7. Yang RQ, Wu HB, Cao Y, Bazan GC (2006) Control of cationic conjugated polymer performance in light emitting diodes by choice of counterion. *J Am Chem* 128(45): 1422–14423.

8. Hoven C et al. (2007) Ion motion in conjugated polyelectrolyte electron transporting layers. *J Am Chem Soc* 129(36):10976–10977.

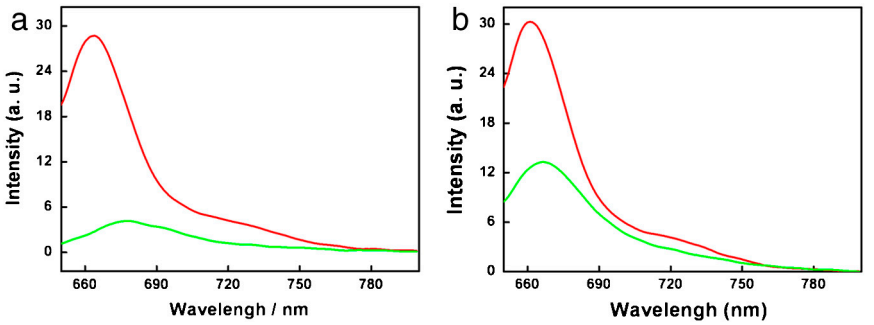

Fig. S1. Experiments for preferential affinity between ssDNA with PFP-Br. (*A*) Fluorescence emitted from Cy5 attached to 34-mer-DNA probe (red curve) and after adding PFP-Br (green curve). (*B*) Fluorescence emitted from Cy5 attached to 34-mer-DNA probe with target DNA (red curve) and after adding PFP-Br (green curve).

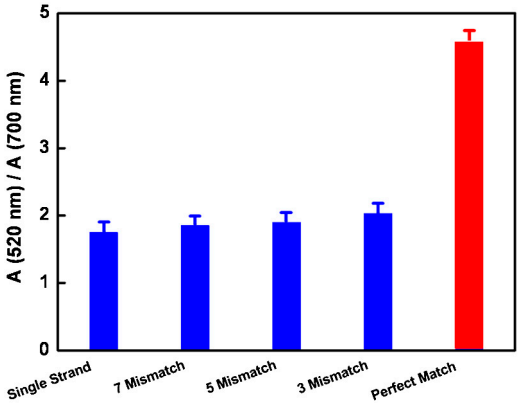

Fig. S2. The absorbance ratio (A_{520}/A_{700}) versus each corresponding DNA/AuNPs/CPs solution.

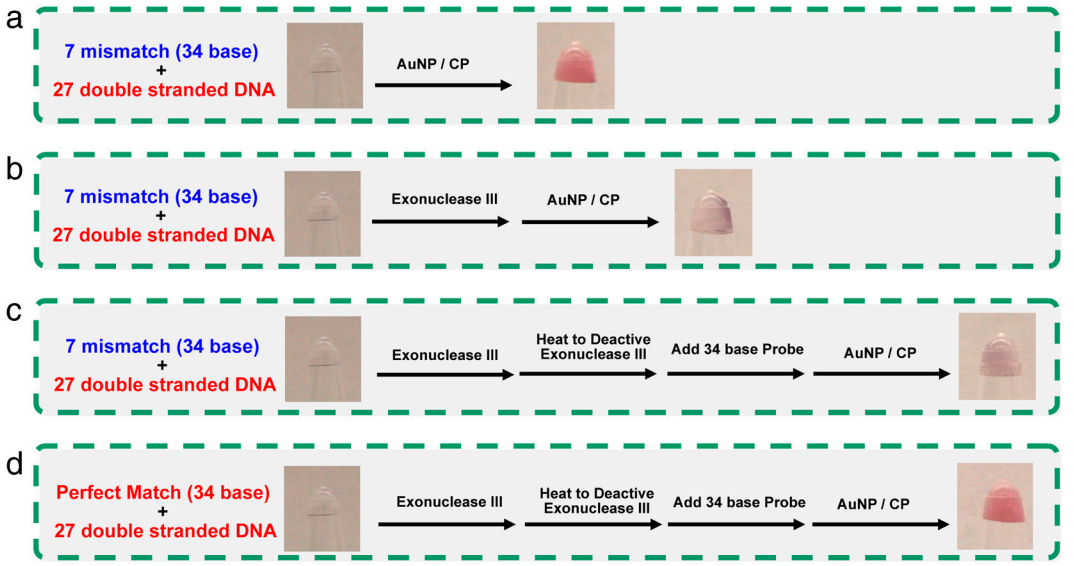

Fig. S3. Optimizing the conditions in the DNA Test via pretreatment with Exonuclease III. (*A*) A test sample containing a contaminating double-stranded DNA adopts the characteristic red color associated with the presence of no-authentic sample. (*B*) If the sample is pretreated with Exonuclease III this sample could not stabilize AuNPs (see *C*). (*D*) Finally, a sample containing both contaminating double-stranded DNA and authentic, single-stranded target provides a correct, positive response after this treatment protocol.

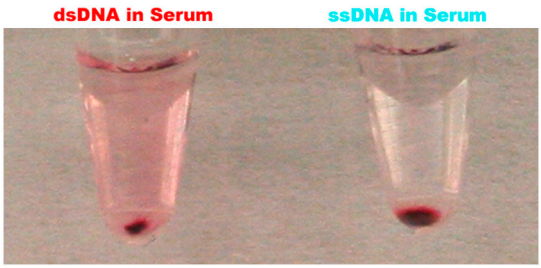

Fig. S4. Colorimetric detection of oligonucleotide hybridization in serum. 34-mer-linear-probes detect the target (dsDNA) and control (ssDNA).

Table S1. The assay of 34 base probe

Step 1. Solution 1					
	34 base DNA Probe	34 base DNA Probe+34 base DNA target (perfect match to probe)	34 base DNA Probe+34 base DNA control (3 mismatch to probe)	34 base DNA Probe+34 base DNA control (5 mismatch to probe)	34 base DNA Probe+34 base DNA control (7 mismatch to probe)
Color	Colorless	Colorless	Colorless	Colorless	Colorless
Step 2. Adding solution 1 to AuNPs solutions to make solution 2					
	34 base DNA Probe+AuNPs	34 base DNA Probe+34 base DNA target (perfect match to probe)+AuNPs	34 base DNA Probe+34 base DNA control (3 mismatch to probe)+AuNPs	34 base DNA Probe+34 base DNA control (5 mismatch to probe)+AuNPs	34 base DNA Probe+34 base DNA control (7 mismatch to probe)+AuNPs
Color	Red	Red	Red	Red	Red
Step 3. Adding conjugated polyelectrolyte (CP) into solution 2					
	34 base DNA Probe+AuNPs +CP	34 base DNA Probe+34 base DNA target (perfect match to probe)+AuNPs+CP	34 base DNA Probe+34 base DNA control (3 mismatch to probe)+AuNPs+CP	34 base DNA Probe+34 base DNA control (5 mismatch to probe)+AuNPs+CP	34 base DNA Probe+34 base DNA control (7 mismatch to probe)+AuNPs+CP
Color	Blue Probe	Red Probe+Target	Blue Probe+Control	Blue Probe+Control	Blue Probe+Control

Table S2. The assay for cocaine aptamer

Step 1. Solution 1			
	Cocaine aptamer 3′ fragment and 5′ fragment	Cocaine aptamer 3′ fragment and 5′ fragment+Cocaine	Cocaine aptamer 3′ fragment and 5′ fragment+ATP
Color	Colorless	Colorless	Colorless
Step 2. Adding solution 1 to AuNPs solutions to make solution 2			
	Cocaine aptamer 3′ fragment and 5′ fragment+AuNPs	Cocaine aptamer 3′ fragment and 5′ fragment+Cocaine+AuNPs	Cocaine aptamer 3′ fragment and 5′ fragment+ATP +AuNPs
Color	Red	Red	Red
Step 3. Adding conjugated polyelectrolyte (CP) into solution 2			
	Cocaine aptamer 3′ fragment and 5′ fragment+AuNPs+CP	Cocaine aptamer 3′ fragment and 5′ fragment+Cocaine+AuNPs+CP	Cocaine aptamer 3′ fragment and 5′ fragment+ATP+AuNPs+CP
Color	Blue Probe	Red Probe+Target	Blue Probe+Control

Table S3. The assay of Thrombin aptamer

Step 1. Solution 1			
	Thrombin aptamer	Thrombin aptamer+Thrombin	Spiegelmer+Thrombin
Color	Colorless	Colorless	Colorless
Step 2. Adding solution 1 to AuNPs solutions to make solution 2			
	Thrombin aptamer+AuNPs	Thrombin aptamer+Thrombin+AuNPs	Spiegelmer+Thrombin+AuNPs
Color	Red	Red	Red
Step 3. Adding conjugated polyelectrolyte (CP) into solution 2			
	Thrombin aptamer+AuNPs+CP	Thrombin aptamer+Thrombin+AuNPs+CP	Spiegelmer+Thrombin+AuNPs+CP
Color	Blue Probe	Red Probe+Target	Blue Control+Target

Table S4. The assay for mercury ions

Step 1. Solution 1			
Color	MSO probe Colorless	MSO probe+Mercury ion Colorless	Control sequence+Mercury ion Colorless
Step 2. Adding solution 1 to AuNPs solutions to make solution 2			
Color	MSO probe+AuNPs Red	MSO probe+Mercury ion+AuNPs Red	Contro sequence+Mercury ion+AuNPs Red
Step 3. Adding conjugated polyelectrolyte (CP) into solution 2			
Color	MSO probe+AuNPs+CP Blue Probe	MSO probe+Mercury ion+AuNPs+CP Red Probe+Target	Contro sequence+Mercury ion+AuNPs+CP Blue Control+Target