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Visual Cocaine Detection with Gold Nanoparticles and Rationally Engineered Aptamer Structures

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***A** novel bioassay strategy is designed to detect small-molecule targets such as cocaine, potassium, and adenosine, based on gold nanoparticles (AuNPs) and engineered DNA aptamers. In this design, an aptamer is engineered to be two pieces of random, coil-like single-stranded DNA, which reassembles into the intact aptamer tertiary structure in the presence of the specific target. AuNPs can effectively differentiate between these two states via their characteristic surface-plasmon resonance-based color change. Using this method, cocaine in the low-micromolar range is selectively detected within minutes. This strategy is also shown to be generic and applicable to the detection of several other small-molecule targets.*

Keywords:
- aptamers
- cocaine
- DNA
- nanoparticles

1. Introduction

Aptamers are in vitro selected artificial nucleic acid receptors that possess high affinity and specificity toward their ligands.[1,2] These DNA or RNA molecules, with unprecedented advantages such as synthetic convenience, chemical stability, and so on, have become increasingly important molecular tools for diagnostics and therapeutics.[3–5] In particular, aptamers have been popularly employed in the design of novel assay methods for small molecules, metal ions, and proteins, involving various signal-transduction approaches such as fluorescence, electrochemistry, and quartz crystal microbalance (QCM).[6–12] More recently, aptamer-based colorimetric assays have been developed as well, providing a way to detect molecular targets simply using the naked eye.[13–17] In this work, we report a potentially generalizable strategy for aptamer-based assays for cocaine and other small molecules. This strategy relies on the size-dependent surface plasmon resonance (SPR) properties of gold nanoparticle (AuNP) probes and rationally engineered aptamer structures.

AuNPs have been successfully employed for chemical and biological detection based on their unique optical properties. They possess very high extinction coefficients and show brilliant, size-dependent colors. The Mirkin group pioneered the use of AuNP-DNA conjugates, that is, AuNPs modified with thiolated oligonucleotide probes,[18,19] which led to a series of novel assay methods for the ultrasensitive detection of DNA and proteins.[20–23] Unmodified AuNPs are highly useful colorimetric probes as well. Li and Rothberg reported that unmodified AuNPs could effectively discriminate single-stranded (ss) DNA against hybridized, double-stranded (ds) DNA, which was ascribed to the significantly different binding affinities of ssDNA/dsDNA toward AuNPs.[24,25] Inspired by this observation, we developed an approach to colorimetrically detect potassium ions by exploiting the ligand-induced conformational variation of an anti-K^+ aptamer.[13] While this approach is straightforward and simple in design, the generality remains a problem since not all aptamers undergo sufficiently large ligand-induced conformation changes that can be sensed by AuNPs. In order to overcome this limitation, we recently developed a new displacement-based strategy.[16] Rather than using a single-stranded aptamer, we initially hybridized the aptamer with its complementary sequence. Then, the aptamer–target binding event led to the dehybridization of the duplex, releasing the complementary sequence in its single-stranded form that could stabilize AuNPs against color changes.[16] This strategy is potentially generic. However it usually suffers from slow kinetics due to the involved

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displacement process. Herein, we propose an alternative strategy that is not only generic, but fairly rapid, and sensitive.

2. Results and Discussion

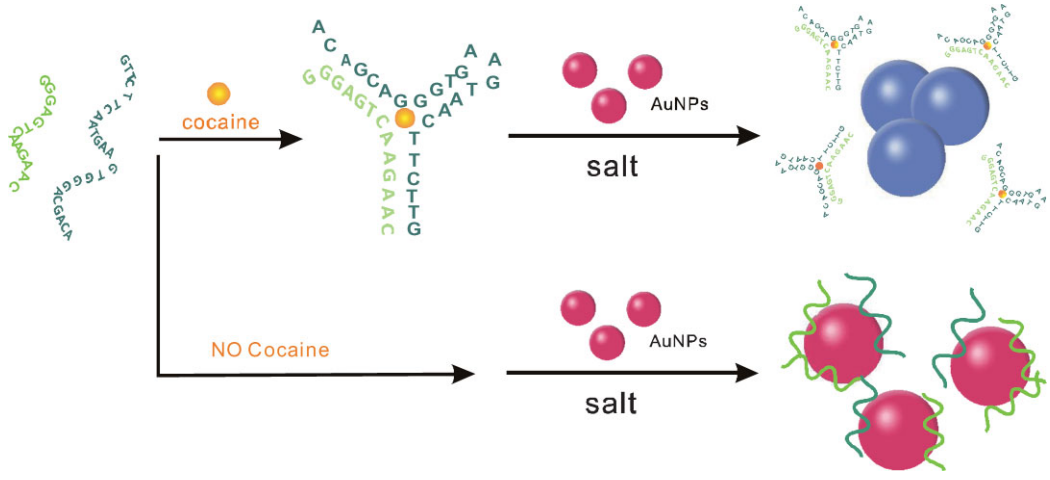
It has been demonstrated that aptamer structures can be rationally engineered, that is, modification or edition (insertion/deletion) of nonconserved regions, without significant perturbation of their ligand-binding abilities.[1,8,26–28] In particular, Stojanovic's and Kumar's groups developed sensors for proteins and small-molecule ligands by using engineered aptamers.[8,29] Inspired by their findings, we engineered aptamers as demonstrated in Scheme 1 and challenged them with AuNP-based assays. An aptamer is cut into two pieces, which are separated in the absence of the ligand. A specific ligand glues the two pieces, resulting in the equilibrium being moved to the folded state. We believe that AuNPs can differentiate between these two states via the characteristic SPR colors.

In a typical experiment, an anticocaine aptamer (ACA) was used to test the applicability of this novel strategy. Cocaine-bound ACA forms a specific stem–bulge–stem structure; however, ACA itself contains several secondary structures even in the absence of cocaine. Consequently, cocaine cannot be directly sensed by using the strategy employed in our previous work.[13] In this work, cocaine was cut into two flexible ssDNA pieces, ACA-1 and ACA-2, with small sequence modifications in order to avoid interstrand binding.

The red-colored AuNPs (≈13 nm in diameter) have an intense SPR absorption located at 520 nm. The as-prepared AuNPs, stabilized by the adsorbed citrate anion at the surface, are readily aggregated in the presence of ≈20 mM NaCl, which screens the electrostatic repulsion between particles. Such aggregation shifts the SPR absorption to the longer wavelength, resulting in the characteristic red–blue color change.[13] Interestingly, in the presence of cocaine-free ACA-1 and ACA-2 (10 μM each), AuNPs retained the red color upon the addition of 50 mM NaCl (Figure 1A). This observation coincides well with previously reported results,[25] suggesting that highly negatively charged ssDNA spontaneously binds to AuNPs through interactions between gold and nitrogen-containing bases, and effectively stabilizes AuNPs against salt-induced aggregations. However, in the presence of 200 μM cocaine, we found that the AuNPs solution readily turned blue (Figure 1B). This color change suggests that ACA-1 and ACA-2 form a tertiary structure along with cocaine, which does not possess an affinity to AuNPs strong enough to resist salt-induced aggregation. It is also important to note that the assay is fairly rapid and can be finished within 10 min, including a 5 min reaction time.

A series of control experiments were carried out to confirm that such a color change was only specific to the binding of cocaine with the engineered aptamer. Firstly, cocaine itself did not show any visible effect on AuNPs. Even at a cocaine concentration as high as 500 μM, the color of AuNPs remains the same as as-prepared AuNPs (Figure 1C). Secondly, when ACA-1 was replaced with a random sequence, no color change was observed over a wide range of cocaine concentrations (≈20 nM–200 μM; Figure 1D). The result clearly indicated that the sequence-specific structural formation was essential for the cocaine binding. Thirdly, two cocaine metabolites were employed as cocaine analogues. When challenged with the same assay protocol, neither could induce the red–blue color change (Figure 1B), which implied that the engineered anticocaine aptamer retained its high selectivity toward cocaine molecules. Absorption spectroscopy further confirmed that only cocaine, not its analogues, induced a distinct increase at ≈650 nm.

Figure 2 demonstrates the concentration profile of the cocaine-induced color change. With the increase of cocaine concentration, the solution of AuNPs gradually turned blue, implying an increase in the aggregation of AuNPs. We could easily observe the color change for a cocaine concentration as low as 20 μM. Figure 2B displays the concentration curve for A520/A650, the ratio between the absorption at 520 nm and 650 nm. The detection limit of the described method is comparable to the previously reported fluorescent cocaine assays,[8] which clearly shows that AuNPs are a sensitive colorimetric probe for cocaine detection. In principle, the smallest amount



Scheme 1. Scheme for the engineered cocaine aptamer and the visual detection of cocaine based on the red-to-blue color change of gold nanoparticles.

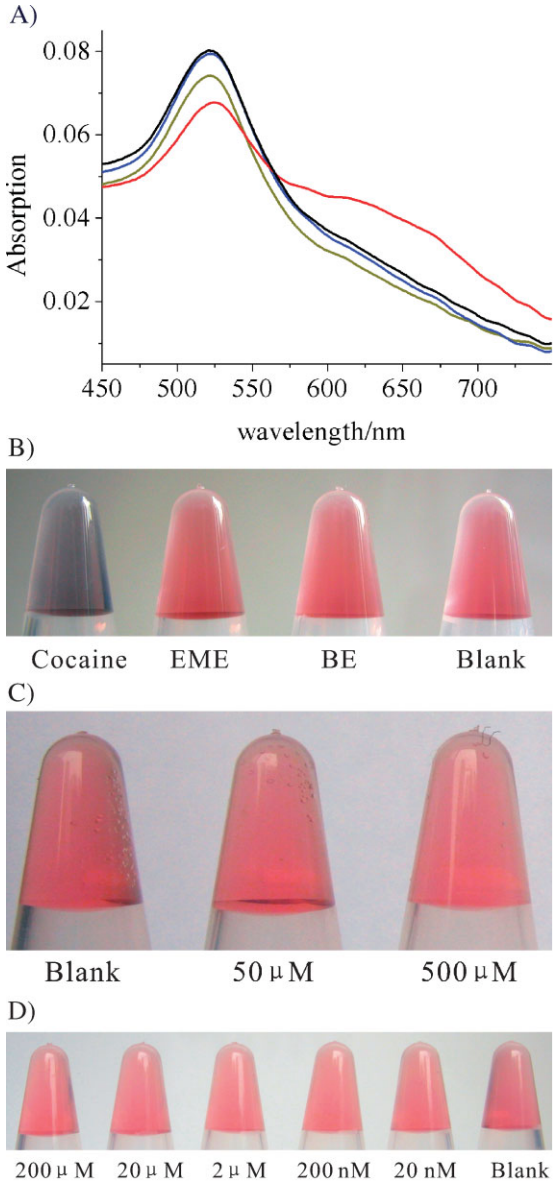


Figure 1. UV/Vis spectra (A) and visual detection (B–D) for AuNP-based cocaine assays. A) Selectivity for cocaine over two analogues, ecgonine methyl ester (EME) and benzoyl ecgonine (BE); the curves have different colors for blank (black), EME (blue), BE (brown) and cocaine (red). B) Visual detection of cocaine over two analogues, EME and BE. C) The effect of cocaine on AuNPs in the absence of aptamers. D) Assays with the control sequence in the presence of cocaine at different concentrations.

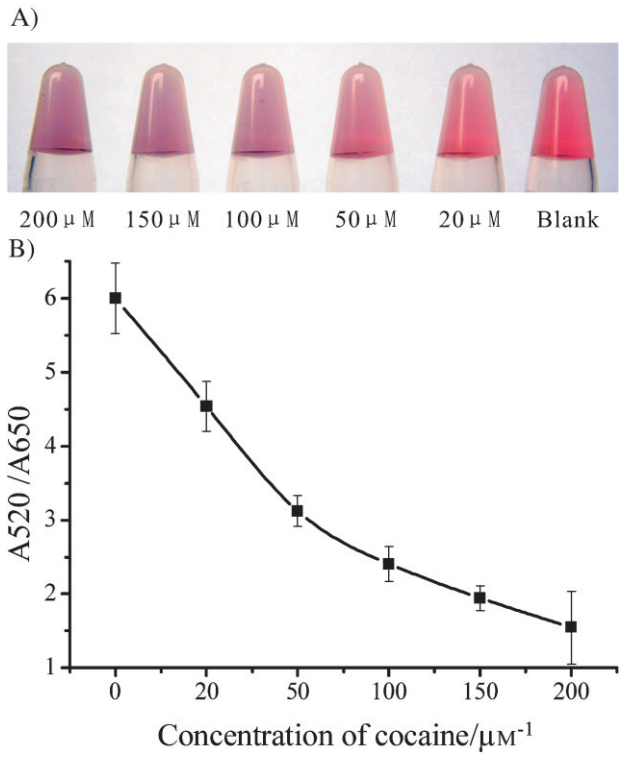


Figure 2. Visual detection (A) and the titration curve (B) for cocaine assays with a 10 μM aptamer probe. The *y* axis of the titration curve stands for the ratio of UV/Vis absorption at 520 nm and 650 nm.

of probe that could stabilize AuNPs should lead to the highest sensitivity. When the concentration of aptamer probes was reduced from 10 to 1 μM, AuNPs were still well stabilized and we could detect as little as 2 μM cocaine concentration with the naked eye (Figure S1 of the Supporting Information). We expect that sophisticated optimization of the ratio between AuNPs and aptamer probes might lead to even higher detection sensitivity.

As a further step, we attempted to prove the general applicability of our strategy by using two other aptamers for potassium and adenosine, respectively. As shown in Figure 3, both the antiadenosine aptamer (AAA) and G-quartet (or antipotassium aptamer, APA) were cut into two pieces, that is, AAA-1 and AAA-2, APA-1 and APA-2, respectively. Three bases were appended to APA-1 (TTA) at the 3′-end and to APA-2 (TAA) at the 5′-end to increase the binding affinity of the two pieces.

As demonstrated in Figure 3, our novel AuNP-based strategy can selectively detect adenosine and potassium ion. We found that only adenosine led to a characteristic red–blue color change, suggesting that it glued AAA-1 and AAA-2 to form the tertiary aptamer structure, while all adenosine analogues, guanine, thymine, cytosine, and uracil did not induce color change of AuNPs, consistent with the fact that they could not bind to AAA-1 and AAA-2. Similarly, the APA-1/APA-2 pair showed high selectivity toward K^+ over other cations such as Na^+ and Li^+.

The described strategy has several unprecedented advantages. Firstly, we have shown that aptamers can, in principle, be cut into several pieces through the elaborate design, which can be readily assembled in the presence of specific ligands. This is a widely applicable strategy and may significantly broaden the application of AuNP-based colorimetric assays. Secondly, relatively long aptamer sequences are cut into smaller pieces, which brings about faster binding kinetics for DNA-AuNP adsorption.[30] For example, it is relatively difficult for the original 39-mer ACA aptamer to bind to AuNPs; however, the engineered 24-mer ACA-1 and 13-mer ACA-2 readily interact with AuNPs within only 5 min, which significantly reduces the assay time. Thirdly, engineered aptamers bring about higher sensitivity than the native ones

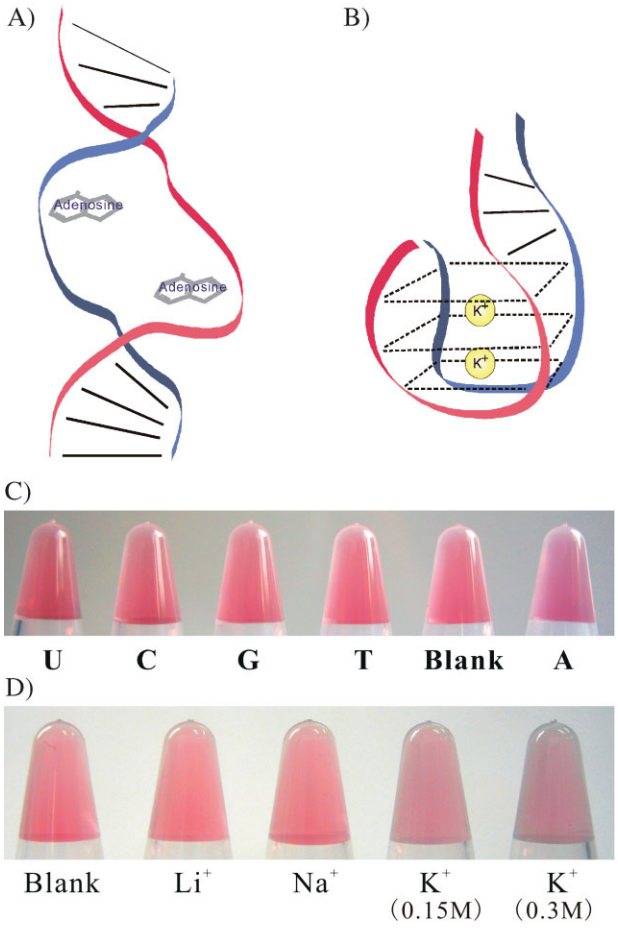


Figure 3. Engineered A) antiadenosine aptamer (AAA) and B) antipotassium aptamer (APA). The intact aptamers are divided into two pieces shown in different colors. Visual detection of C) adenosine and D) K^+ based on the color change of gold nanoparticles. In adenosine detection (C), all controls for urodine, cytidine, guanosine, and thymine do not show red-to-blue color change. In potassium detection (D), both LiCl and NaCl at 0.3 M do not induce the color change, while KCl of 0.15 M and 0.3 M induced the red-to-blue color change of gold nanoparticles.

in AuNP-based assays. For example, one cocaine molecule binds to two pieces of DNA (ACA-1 and ACA-2), forming one structured DNA. Since the color change of AuNPs is directly associated with the amount of bound DNA strands, the detection sensitivity of AuNPs toward cocaine is doubled compared to the one-to-one reaction in our original design.[16] As a result, the cocaine assay achieves low micromolar sensitivity, which compares favorably to previously reported instrument-based methods (Table 1). For example, the detection limit of the present method is even comparable to cocaine sensors with either fluorescent or electrochemical transducers.[10,31] However, we also note that this method is not applicable for colored samples and, as other colorimetric assays, is most useful in rapid screening with the qualitative requirement. Also of note, this strategy has its own limitations, for example, it is difficult to do the sequence design for aptamers possessing complicated interstrand secondary structures, or containing only a single loop.

3. Conclusions

In summary, we have developed a novel bioassay strategy based on AuNPs and engineered aptamers. In this strategy, an aptamer is cut into two pieces of random coil-like ssDNA through elaborate design. The color of AuNPs is employed to identify ligand-free ssDNA and ligand-induced aptamer structures. By using this strategy, we are able to selectively detect micromolar quantities of cocaine within minutes. This strategy is also shown to be generic and applicable to the assay of both adenosine and potassium.

4. Experimental Section

Materials: Hydrogen tetrachloroaurate(III) ($HAuCl_4.4H_2O$, 99.99%) was purchased from China National Pharmaceutical Group Corporation. Adenine, thymine, guanine, cytosine, and uracil were obtained from Sigma–Aldrich. All other chemicals were of analytical grade. All chemicals were used without further purification. Water was purified using a Millipore filtration system. AuNPs of 13 nm (≈3.5 nM) were synthesized by the citrate reduction of $HAuCl_4$ as previously reported.[31] DNA oligonucleotides were synthesized and high-performance liquid chromatography (HPLC) purified in Sangon Biotechnology Inc. (Shanghai, China). The sequences of the involved oligonucleotides are listed in Table S1 of the Supporting Information. UV/Vis absorption spectroscopy was performed with a Hitachi U-3010 spectrophotometer and photographs were taken with an Olympus C-5060 digital camera.

Table 1. Comparison of sensors for cocaine detection.

Detection method	Strategy	Detection limit	Existing problems
Visual (this work)	Engineered apatmer and AuNPs	2 μM	Not applicable for colored samples
Colorimetric[32]	Organic dye replacement	500 μM[a]; 2 ≈ 600 μM[b]	Time consuming (≈12 h)
Colorimetric[15]	Aggregation of AuNPs assembled with thiolated DNA aptamers	1 mM[a]; 50 ≈ 500 μM[b]	Cumbersome preparation of coated AuNPs
Fluorescence[7]	FRET[c]	10 μM	Dual-labeled oligos
Fluorescence[33]	Aptamer-based Machine	5 μM	Complicated amplification steps, time consuming (70 min)
Electrochemistry[10]	Electron transfer	10 μM	Dual-labeled oligos

[a]Visual detection; [b]optical detection; [c]fluorescence resonance energy transfer.

Assay protocol: In a typical cocaine assay, the ACA-1 and ACA-2 of equal molar ratio were mixed in a Tris buffer solution (25 mM Tris-HCl, 0.15 M NaCl) at pH 8.2. Such solution (1 μL) was added to cocaine sample (99 μL) in the Tris buffer and the obtained solution was incubated for 5 min at room temperature. Of note, the concentrations of aptamer probes are 10 μM in this solution unless specially indicated. All analyte concentrations mentioned in this work refer to the original concentration of cocaine samples. In adenosine and potassium assays, buffer solutions of 25 mM Tris-HCl and 0.3 M NaCl with pH 8.2 and 25 mM Tris-HCl at pH 8.0 were used, respectively, while all other conditions were the same.

2 μL of the test solution were added to an AuNP solution of 100 μL. After incubation for 2–3 min, phosphate buffered saline (PBS) solution (100 mM PB, 1 M NaCl, pH 8.0, 5 μL) was introduced to the mixed solution, followed by either visual observation or UV/Vis characterization.

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