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A Label-Free Aptamer-Fluorophore Assembly for Rapid and Specific Detection of Cocaine in Biofluids

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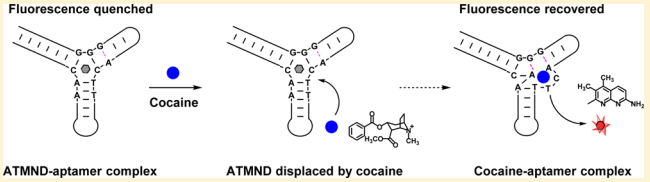
Supporting Information

ABSTRACT: We report a rapid and specific aptamer-based method for one-step cocaine detection with minimal reagent requirements. The feasibility of aptamer-based detection has been demonstrated with sensors that operate via target-induced conformational change mechanisms, but these have generally exhibited limited target sensitivity. We have discovered that the cocaine-binding aptamer MNS-4.1 can also bind the fluorescent molecule 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) and thereby quench its fluorescence. We subsequently introduced sequence changes into MNS-4.1 to engineer a new cocaine-binding aptamer (38-GC) that exhibits higher affinity to both ligands, with reduced background signal and increased signal gain. Using this aptamer, we have developed a new sensor platform that relies on the cocaine-mediated displacement of ATMND from 38-GC as a result of competitive binding. We demonstrate that our sensor can detect cocaine within seconds at concentrations as low as 200 nM, which is 50-fold lower than existing assays based on target-induced conformational change. More importantly, our assay achieves successful cocaine detection in body fluids, with a limit of detection of 10.4, 18.4, and 36 μM in undiluted saliva, urine, and serum samples, respectively.



Cocaine is a central nervous system stimulant that increases levels of dopamine[1,2] and potently inhibits neurotransmitter reuptake at the synapse.[3−7] Abuse of cocaine has been shown to cause anxiety, paranoia, mood disturbances, organ damage, and violent behavior.[8−10] Therefore, rapid detection of cocaine is needed to confirm suspicion of recent use in impaired driver investigations or to assist in overdose treatment in medical emergency settings. Various immunoassays have been developed for the detection of cocaine and/or its major metabolite benzoylecgonine in biofluids, including the enzyme-linked immunosorbent assay (ELISA)[11] and the EMIT II Plus Cocaine Metabolite Assay.[12] Unfortunately, the use of these assays is often limited because of the high cost of generating antibodies and issues with poor specificity. These antibody-based tests often cannot distinguish between the targeted drug and structurally similar substances, resulting in cross-reactivity-related false positives.[13,14]

Aptamers are single-stranded RNA or DNA molecules selected *in vitro* via Systematic Evolution of Ligands by Exponential Enrichment (SELEX)[15] to specifically bind to targets with high affinity, and they offer a practical alternative to antibodies for the detection of nucleic acids, proteins, and small molecules. Compared to antibodies, aptamers are relatively fast and cheap to produce and can be chemically synthesized with extreme accuracy and reproducibility.[16,17] Aptamer-based sensors have been gaining popularity because of their simplicity and specificity. For example, derivatives of the MNS-4.1 cocaine-binding aptamer[18] have been labeled with sensing elements such as fluorophore/quencher pairs,[19] magnetic or metallic nanoparticles,[20−22] quantum dots,[23,24] and methylene blue[25,26] to achieve specific detection of cocaine. In the absence of cocaine, the aptamer population exists in an equilibrium state consisting of both folded and unfolded structures,[27] where the folded structures generate a background signal. When challenged with cocaine, the unfolded aptamers undergo a target-induced conformational change and form a noncanonical three-way junction that binds cocaine,[27] producing a signal change. This limited target-induced fluorescence change resulted in a high detection limit (10 μM) even under optimal conditions, and the reason may be due to inefficient proximity quenching, low aptamer-target binding affinity, or both.[19]

Different strategies such as target displacement have been used to increase the sensitivity of aptamer-based detection. For example, Stojanovic's group used unmodified MNS-4.1 (Figure 1A, MNS-4.1) to construct a colorimetric cocaine sensor based on cocaine-mediated displacement of a cyanine dye (diethylthiotricarbocyanine iodide; Cy7) from the dye-aptamer com-

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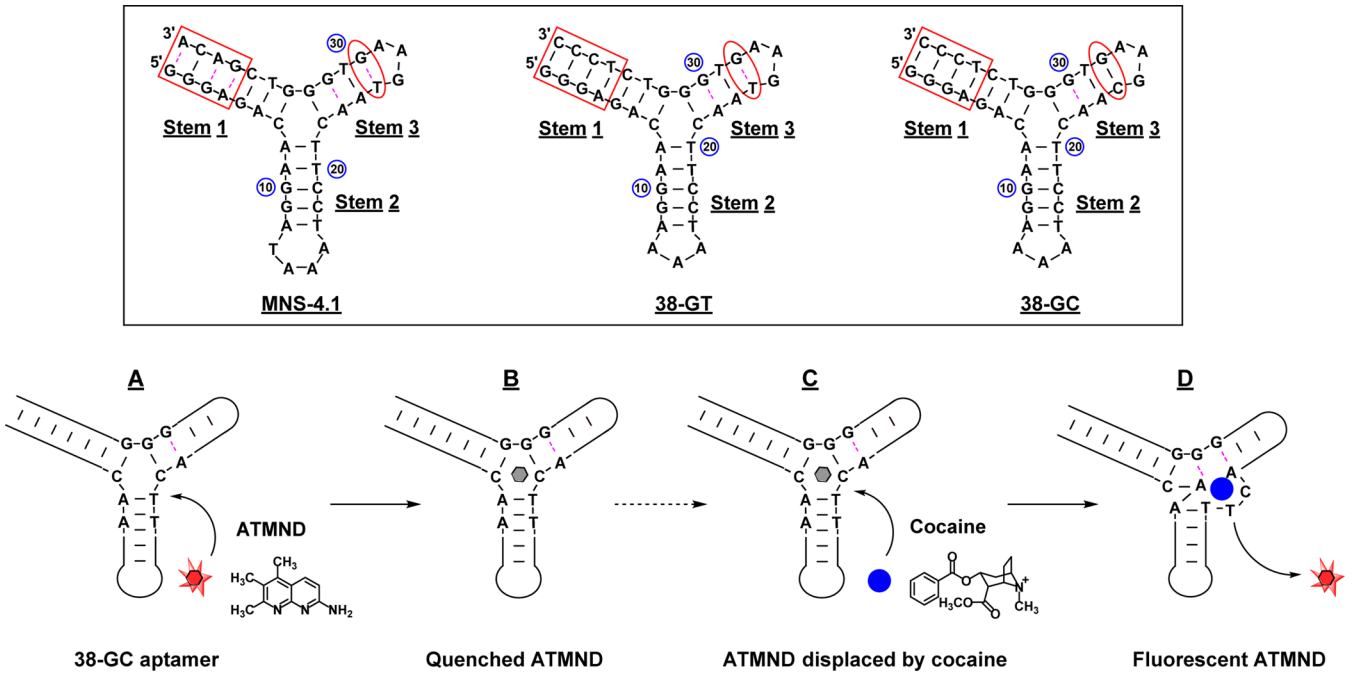

Figure 1. (Top) Structures of the MNS-4.1, 38-GT, and 38-GC aptamers. These aptamers are predicted to fold into three helical stems built around a three-way junction. Stem 1 of MNS-4.1 contains three putative noncanonical base pairs, which have been substituted for Watson−Crick base pairs in 38-GT. 38-GC was further stabilized by converting the G−T wobble pair in stem 3 to a matched G−C base pair. (Bottom) Scheme of cocaine detection with the 38-GC aptamer sensor. 38-GC stably folds in the absence of ligand at room temperature. ATMND binding to the aptamer quenches the molecule's fluorescence, but after challenging this complex with cocaine, the competitive binding displaces ATMND from the binding site. The released ATMND generates a readily detectable fluorescent signal, indicating the presence of the cocaine target.

plex.[28] They observed that the absorbance of Cy7 at 760 nm decreased with increasing cocaine concentrations in the range of 2 to 600 μM. Compared to the corresponding fluorescence sensor that exhibited less sensitivity due to intrusive covalent modifications on the aptamer,[19] the sensitivity of the Cy7-based displacement assay was improved due to the high binding affinity of the unmodified MNS-4.1 aptamer for cocaine.[19,28,29] However, the limit of detection of this sensor was still limited to 2 μM as a result of high background signal from the weak interaction between Cy7 and MNS-4.1.[28]

In this work, we found that the MNS-4.1 cocaine binding aptamer can also bind the fluorescent dye 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND), quenching its fluorescence. In the presence of cocaine, however, the bound ATMND is competitively displaced, generating a rapid fluorescent readout as the released molecule is highly fluorescent in solution. Based on our characterization of this competitive binding of the cocaine target and ATMND signal reporter to the same aptamer, we further improved the performance of our aptamer sensor by deriving a new variant (Figure 1A, 38-GC) of MNS-4.1 that stably assumes a predominantly folded conformation at equilibrium. Using this optimized aptamer, we developed a rapid and selective assay for the detection of cocaine at concentrations as low as 200 nM within 20 s, ∼50-fold lower than assays based on target-induced conformational change,[20] but in less time and with fewer reagent requirements.[30−32] Importantly, our sensor can also rapidly achieve cocaine detection at micromolar concentrations in body fluids such as urine, saliva, and serum.

EXPERIMENTAL SECTION

Materials. ATMND was purchased from Ryan Scientific, and 3,3′-diethylthiatricarbocyanine iodide (Cy7) was purchased from Sigma-Aldrich; any dilution or stock solution was prepared with dimethyl sulfoxide (DMSO). Cocaine hydrochloride was purchased from Sigma-Aldrich, and benzoylecgonine tetrahydrate was purchased from Cerilliant Corporation. Both 50 mM stock solutions were prepared in HCl solution (pH 4.5) and stored at 4 °C. All DNA aptamers were ordered from Integrated DNA Technologies with HPLC purification and dissolved to a concentration of 500 μM in 1 × filtered TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). DNA concentrations were measured on a NanoDrop 2000 (Thermo Scientific). DNA sequences are listed below:

38-GT: 5′ GGG AGA CAA GGA AAA TCC TTC AAT GAA GTG GGT CTC CC 3′;
38-GC: 5′ GGG AGA CAA GGA AAA TCC TTC AAC GAA GTG GGT CTC CC 3′;
38-GC M1: 5′ GGG AGA CAA GGA AAA TCC TCT AAC GAA GTG GGT CTC CC 3′;
38-GC M2: 5′ GGG AGA CAA GGA AAA TCC TAC AAC GAA GTG GGT CTC CC 3′;
MNS-4.1: 5′ GGG AGA CAA GGA TAA ATC CTT CAA TGA AGT GGG TCG ATA 3′

ATMND Binding and Cocaine Displacement Experiments. For cocaine detection, we prepared 96 μL of reaction buffer (10 mM Tris, 0.01 mM $MgCl_2$, pH 7.4), 1 μL of aptamer (final concentration 2 μM), and 1 μL of ATMND (final concentration 0.25 μM) solution. Each 98 μL reaction was loaded into one well of a 96-well plate. Fluorescence readings were taken 3 min apart to determine the stability of the signal, with excitation at 358 nm and emission at 405 nm. After the signal stabilized for 15 min, a 2 μL cocaine solution (0−2.5 mM) was added to each well using a multichannel pipet while

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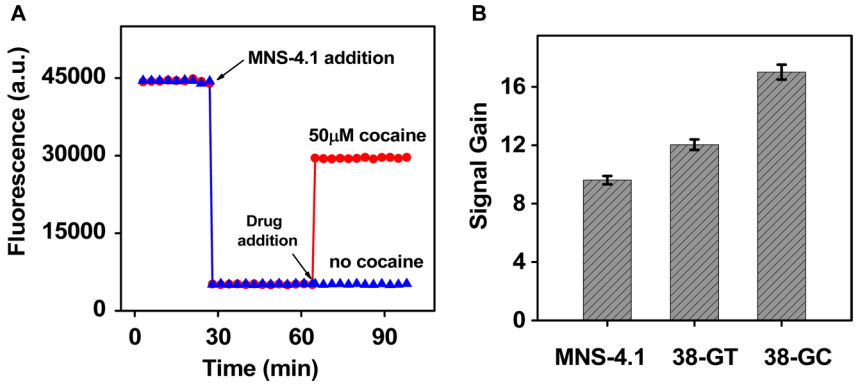

Figure 2. Our aptamer-based sensor rapidly responds in the presence of cocaine. (A) Time course of ATMND release. ATMND is rapidly bound and quenched upon addition of the MNS-4.1 aptamer. In the presence of cocaine, the release of ATMND results in a strong fluorescent signal. (B) The impact of aptamer stability on signaling performance. The signal gain observed in the presence of cocaine was dependent on the composition of stem 1 and stem 3, which contributes to aptamer stability and ATMND/cocaine binding. Experimental conditions: [DNA] = 2 μM, [ATMND] = 250 nM, [cocaine] = 50 μM. Error bars represent the standard deviation of three measurements.

monitoring the fluorescence signal. Samples were prepared in triplicate with average values used to plot the figures.

Detection with Cy7. We performed the same set of experiments with the same solutions as described above for ATMND, but with 1 μL (final concentration 7 μM) of Cy7 instead of ATMND. The absorbance was recorded at 760 nm.

ITC Experiments. ITC experiments were performed with a MicroCal iTC200 instrument (GE Healthcare). All measurements were performed in 10 mM Tris buffer (pH 7.4) with 0.01 mM $MgCl_2$ and 5% DMSO. The sample cell contained the aptamer solution, while the titrant was loaded in the syringe. In order to detect tight binding, the aptamer concentration was kept at 20 μM while the titrant (cocaine or ATMND) concentration was 500 μM. We introduced 19 total injections of 2 μL each and a purge injection of 0.4 μL. The purge injection was not included in the calculations. Two sets of each experiment with cocaine or ATMND were performed at 25 °C. The raw data were averaged and fitted to the two sets of sites and single-site binding models and adjusted for the heat of the titrant.

Detection of Cocaine in Urine, Serum, and Saliva. Different concentrations of biofluids were obtained by dilution with deionized water. To test the quenching effect of biofluids, we mixed ATMND (500 nM) in 2× reaction buffer (20 mM Tris, 0.02 mM $MgCl_2$, pH 7.4) with an equal volume of biofluids, followed by fluorescence measurements. To test the signal gain of cocaine in different biofluid dilutions, we spiked cocaine (500 μM) into 50%, 25%, 10%, 5%, and 0% urine, serum, and saliva. These were then mixed with an equal volume of 2× reaction buffer containing ATMND (500 nM) and 38-GC (4 μM), followed by subsequent fluorescence recording. To perform cocaine detection in biofluids, different cocaine concentrations were spiked into 5% urine, 5% serum, or 10% saliva. Equal volumes of cocaine-spiked biofluids and 2× reaction buffer containing ATMND (500 nM) and 38-GC (4 μM) were mixed to read the fluorescence. Unless otherwise indicated, fluorescence measurements were obtained with excitation at 358 nm and emission at 405 nm.

RESULTS AND DISCUSSION

We devised our platform based on cocaine-mediated displacement, employing an aptamer sensor that reports the presence of cocaine via the displacement and unquenching of a bound fluorophore molecule. In the absence of ligand, the cocaine binding aptamer forms three helical stems around a three-way junction. ATMND binds the aptamer at this junction, which results in the quenching of its fluorescence (Figure 1B). Since this aptamer also binds cocaine, the competitive binding of cocaine results in a rapid displacement of the ATMND from the aptamer. The released ATMND generates a high-intensity fluorescent signal, reporting the cocaine-binding event.

To develop a cocaine displacement based sensor platform, we need a signal reporter which binds to the cocaine-binding aptamer but can also be displaced by cocaine, reporting the presence of the target. We recently discovered that the MNS-4.1 binds to ATMND and the cocaine binding can competitively displace ATMND from the aptamer−dye complex. Free ATMND is highly fluorescent in the buffer, but these dye molecules are rapidly bound by MNS-4.1 upon addition of the aptamer, and we observed that ATMND fluorescence was greatly quenched within seconds. When we incubated 250 nM ATMND with 2 μM MNS-4.1, roughly 93% of the fluorophore was quenched, with the remaining 7% contributing to the low level of fluorescence background (Figure 2A). The aptamer−ATMND complex is very stable, and we did not observe any detectable fluorescence change over the course of 1 h. We characterized sensor performance in terms of signal gain, which is the ratio of the background-subtracted fluorescence obtained with cocaine relative to that obtained in the absence of cocaine, such that a larger signal gain is indicative of better sensitivity. The addition of cocaine is predicted to trigger a conformational rearrangement in the aptamer (Figure 1B), with two adjacent GA base pairs and a dinucleotide bulge (T20 and C21) within the aptamer binding pocket.[27] Upon 50 μM cocaine addition, we observed a competitive target binding to MNS-4.1, resulting in successful displacement of ATMND from the aptamer with a signal gain of 9.6 (Figure 2A). This fluorescence increase was stable for at least several hours (data not shown). We assume that this competitive binding results from a pure site-to-site displacement, although it could also be explained by a heterotropic allosteric effect. In contrast, we observed no measurable signal change in the absence of cocaine (Figure 2A).

Previous studies have suggested that the composition of stem 1 of the cocaine-binding aptamer has the greatest impact on the secondary structure of the aptamer as well as the target binding affinity.[27] MNS-4.1 is only partially folded at equilibrium,[28] and

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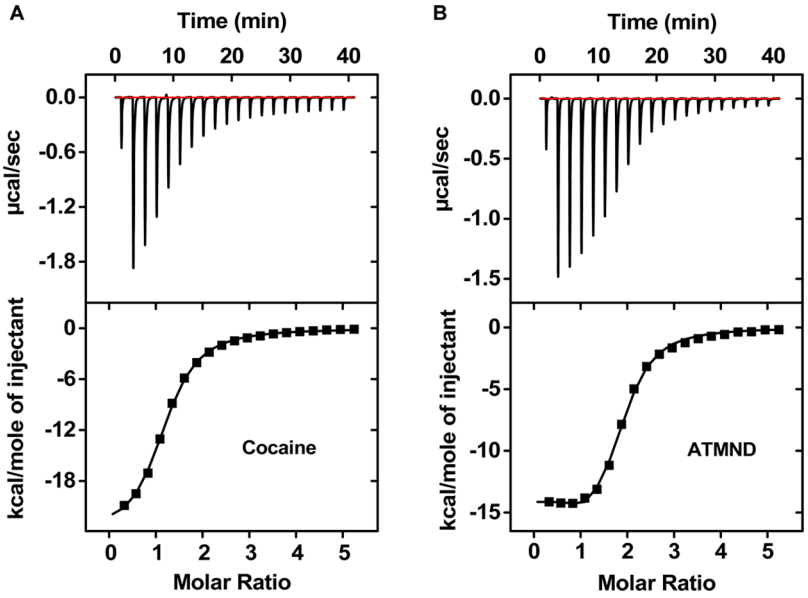

Figure 3. 38-GC binds both cocaine and ATMND. (Top) ITC data showing heat generated from each injection of (A) cocaine or (B) ATMND into the 38-GC solution. (Bottom) The integrated heat plot after correcting for the heat of dilution. Experimental conditions: [38-GC] = 20 μM, [ATMND] = 500 μM, and [cocaine] = 500 μM.

this is most likely due to the presence of three noncanonical base pairs in stem 1, which would be expected to reduce the efficiency of ATMND binding and quenching and thereby result in high background fluorescence. Neves's group[27] used 2D NMR spectral changes to confirm that the cocaine-binding aptamer with six Watson−Crick base pairs in stem 1 is fully folded in the free form. To test whether such a completely folded aptamer could reduce background fluorescence and boost signal gain, we engineered the 38-GT aptamer (Figure 1A, 38-GT) by converting the three putative noncanonical base pairs in stem 1 to Watson−Crick base pairs, forming a seven-base-pair stem. The 38-GT resulted in reduced background fluorescence, with 95% ATMND quenched (data not shown), indicating that ATMND binds tightly to 38-GT. We did not observe any loss of cocaine-displaced signal and obtained an increased signal gain of 12 in the presence of 50 μM cocaine (Figure 2B). To further reduce the background signal, we subsequently converted the G−T wobble pair in stem 3 of 38-GT to a matched G−C base pair (Figure 1A, 38-GC). This increased structural stability led to a further increase of ATMND quenching efficiency (97%) and an improved signal gain of 17 (Figure 2B) with 50 μM cocaine.

We presume that the greatly enhanced stability of 38-GC contributes to its high affinity toward both ligands, favoring formation of stable aptamer−ligand complexes and resulting in low background and high target-displaced signal gain. We used isothermal titration calorimetry (ITC) to investigate the binding affinity and thermodynamics of MNS-4.1, 38-GT, and 38-GC with regard to both ATMND and cocaine. The results confirmed that the aptamers bind both molecules, and that binding in both cases is enthalpically driven and entropically unfavorable (Supporting Information (SI), Table S1). ITC stoichiometry data indicated that each aptamer binds to one cocaine molecule or two ATMND molecules. After correction of the dilution heat, we used the single-site binding model to obtain equilibrium dissociation constants (K_{dc}) of 6.7 ± 1.3 μM (SI, Figure S1A), 10 ± 1 μM (SI, Figure S1C) for cocaine binding to MNS-4.1 and 38-GT, respectively. We used the two sets of sites binding model to obtain affinity measurements for ATMND binding sites on MNS-4.1 (K_{d1}, 6.6 ± 0.8 μM; K_{d2}, 25 ± 1 μM) (SI, Figure S1B) and 38-GT (K_{d1}, 0.16 ± 0.08 μM; K_{d2}, 6.3 ± 1.2 μM) (SI, Figure S1D). ITC data also showed that the G−C change in 38-GC further increases the stability of the aptamer, leading to increased binding affinity for both ligands. Compared with 38-GT, we observed 4-fold enhanced binding to cocaine (K_{dc} = 2.6 ± 1.0 μM) and 10-fold tighter binding to ATMND (K_{d1} = 0.016 ± 0.001 μM; K_{d2} = 2.6 ± 1.0 μM) (Figure 3).

These results demonstrate the potential of ATMND as an excellent transduction element; its tight binding to the aptamer results in a very low background signal until it becomes displaced via competitive binding of cocaine and recovers its fluorescence. To minimize the background signal and obtain a high signal-to-noise ratio, we used a fixed concentration of 38-GC (2 μM) to optimize the concentration of ATMND and thus obtained an optimized 38-GC:ATMND ratio of 8:1 (SI, Figure S2); under these conditions, we calculated that 99.3% dye−aptamer complexes contained only one ATMND molecule bound at the strong binding site, while the concentration of complexes containing two ATMND molecules was sufficiently low as to be negligible (detailed calculations provided in SI).

Our sensor is modeled on the understanding that both ATMND and cocaine compete for the same aptamer, and we assume that the binding site of both ligands is located within the hydrophobic three-way junction pocket. To confirm this assumption, we examined the extent to which targeted nucleotide changes affects ligand binding and target competition. We designed mutants of 38-GC in which we introduced a nucleotide switch between T20 and C21 (38-GC-M1; SI, Figure S3) or a replacement of thymine at position 20 with an adenine (38-GC-M2; SI, Figure S3). We tested these mutant aptamers in our competitive cocaine-binding fluorescence assay and observed significantly reduced binding to both ATMND and cocaine, with a high fluorescence background and a low cocaine-displaced fluorescence recovery (SI, Figure S3). This

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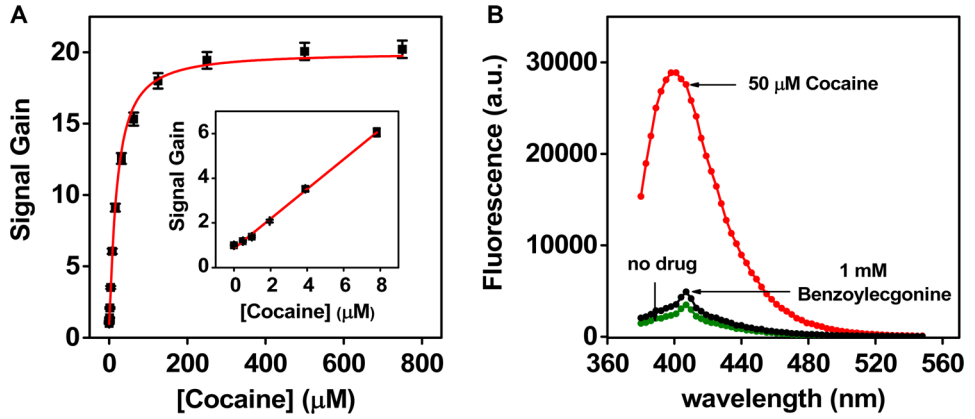

Figure 4. Sensitivity and specificity of the ATMND-based sensor in the reaction buffer. (A) Calibration curve shows a strong concentration-dependent response to cocaine by 38-GC-ATMND. (B) Our sensor specifically responds to cocaine but exhibits almost no response to its major metabolite benzoylecgonine. Experimental conditions: [DNA] = 2 μM, [ATMND] = 250 nM. Error bars in plot A represent the standard deviation of three measurements conducted at each cocaine concentration.

poor ATMND binding and weak cocaine displacement resulted in a signal gain of just 1.3 and 1.2 with 50 μM cocaine for 38-GC-M1 and 38-GC-M2, respectively. This very weak binding affinity of mutant aptamers to both ATMND and cocaine was also confirmed by ITC (SI, Figure S4). This is consistent with previous studies showing that mutations to nucleotides that contribute to formation of this hydrophobic pocket, including the unpaired T20 and C21, can profoundly alter the aptamer's binding affinity for cocaine.[33]

Our mutation experiments indicate that ATMND binding is also heavily dependent on C21 and T20. Nakatani et al.[34,35] used ^{15}N NMR experiments to reveal that 1,8-naphthridine selectively binds to cytosine or thymine via a three-point hydrogen bond[36] and that the binding affinity of ATMND to various target nucleotides is as follows: cytosine > thymine > adenine > guanine.[37] Thus, it is likely that ATMND binds either C21 or T20 within the three-way junction of the aptamer through a three-point hydrogen bond.[38,39] Additionally, we observed a large negative enthalpy change in ITC experiments, which also indicates the formation of hydrogen bonds between ATMND and the aptamer. We believe that ATMND forms these hydrogen bonds and possibly cooperates with adjacent nucleotides via π-stacking interaction to significantly quench its fluorescence.[38−41]

Our sensor is based on a simple, one-pot, one-step reaction that entails the simple mixing of 38-GC, ATMND, and cocaine at room temperature and then exploiting the rapid competition between cocaine and ATMND for limited aptamer binding sites to quantitatively detect cocaine in samples. We observed that the signal gain increased with cocaine concentration, reaching a saturated signal gain of 19 with 250 μM cocaine (Figure 4A), with a linear range of 0−8 μM (Figure 4A, inset). The low background resulting from the high affinity interaction of 38-GC with ATMND yields an excellent limit of detection (LOD). In a reaction with 2 μM 38-GC and 250 nM ATMND, we achieved an LOD of 200 nM within 20 s (calculated LOD as $S/N > 3$), more than 50-fold better than most existing aptamer-based systems[19,23,26,32,42,43] and comparable to sensitive assays that require enzymatic amplification (SI, Table S2).

The 38-GC-ATMND complex offers a superior signal transduction mechanism for immediate and specific cocaine detection with a robust signal gain. Since Cy7 has been used as a signal reporter in a previously reported cocaine-mediated aptamer-based assay,[28] we compared the sensor performance of 38-GC with either Cy7 or ATMND under optimized conditions and confirmed that 38-GC exhibits a much better signal gain and sensitivity with ATMND. For example, our ATMND-based "signal-on" sensor produces an approximate signal gain of 17 with only 1.5% of relative standard deviation (RSD) in the presence of 50 μM cocaine (Figure 4A), while the same cocaine concentration produces only a 35% signal decrease with 5% of RSD for the Cy7-based "signal-off" sensor (SI, Figure S5).

Cocaine-binding aptamers generally exhibit good specificity in terms of distinguishing cocaine from structurally similar major metabolites.[18] We tested the specificity of our assay with benzoylecgonine, which is the corresponding carboxylic acid derivative of cocaine with an otherwise almost identical structure.[44,45] Relative to the signal gain of 17 obtained with 50 μM cocaine, we did not detect a measurable signal change upon addition of 50 μM benzoylecgonine and observed a signal gain of just 1.7 when we increased the benzoylecgonine concentration to 1 mM (Figure 4B), which is consistent with the good specificity observed with previous aptamer-based sensors (SI, Table S2).

The average concentration of cocaine in different body fluids within 24 h after ingestion is typically greatest in the urine (milligrams per liter), with lower concentrations found in saliva and serum, respectively.[46] We explored the practicality of our assay for performing cocaine detection in different body fluids collected from healthy donors. We first tested our assay with 250 μM cocaine spiked into various dilutions of urine (SI, Figure S6), saliva (SI, Figure S7), or serum (SI, Figure S8) samples. We observed that the signal gain increased in inverse proportion to the biofluid concentration, with a maximum signal gain of 8.2, 9.6, and 5.6 for 2.5% urine, saliva, and serum, respectively. Human urine normally contains very small amounts of protein (<0.14 mg/mL)[47] and a large number of fluorescent metabolites.[48] We observed that urine samples indeed generated a strong background fluorescence at 400−550 nm (SI, Figures S9 and S10) and metabolites such as pterins, flavins, porphyrins, and 4-pyridoxic acid may be contributing to this fluorescence.[49] Although the urine matrix exhibited a high background fluorescence, we still achieved successful detection of cocaine. We observed that the signal gain increased with

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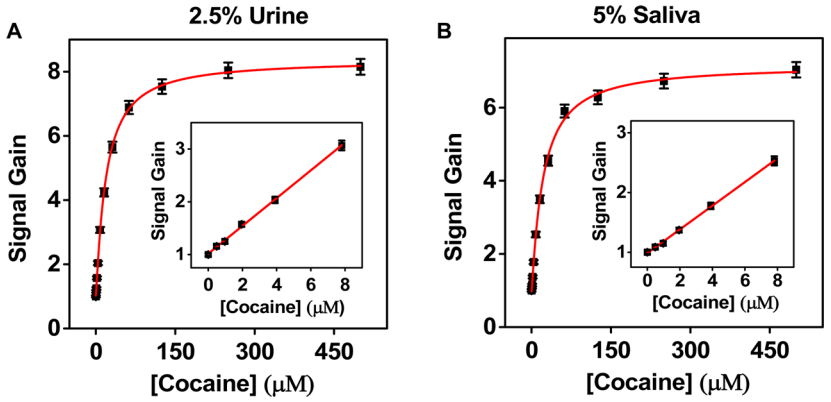

Figure 5. Calibration curves for ATMND-38-GC-displaced cocaine detection in biological samples. We observed a strong concentration-dependent response to cocaine in (A) 2.5% urine and in (B) 5% saliva. Experimental conditions: [DNA] = 2 μM, [ATMND] = 250 nM. Error bars represent the standard deviation of three measurements conducted at each cocaine concentration.

increasing cocaine concentrations, with an LOD of 460 nM cocaine in 2.5% urine (Figure 5A).

Serum samples generate weak background fluorescence (SI, Figures S9 and S10). However, since serum contains ~67 mg/mL protein,[50] which might bind ATMND molecules within hydrophobic patches and thereby quench its fluorescence (SI, Figure S11), we expected these samples to be significantly challenging to work with. Even though serum samples demonstrated considerable ATMND quenching at 405 nm (SI, Figure S11), we were still able to detect cocaine in 2.5% serum with a detection limit of 900 nM (SI, Figure S12). Saliva is a cleaner matrix with no background fluorescence (SI, Figure S10), containing only ~1.6 mg/mL proteins,[51] and we were thus able to use 5% saliva to achieve cocaine detection and establish a calibration curve with an LOD of 520 nM (Figure 5B). Based on these findings, we calculated detection limits of 10.4, 18.4, and 36 μM in undiluted saliva, urine, and serum, respectively. These experiments successfully demonstrate the feasibility of using our sensor to detect cocaine in body fluids.

CONCLUSIONS

We have demonstrated the rapid, sensitive, and specific detection of cocaine based on competitive aptamer binding between the cocaine target and the ATMND fluorescent signal reporter. Our 38-GC aptamer is derived from the previously reported MNS-4.1 aptamer and incorporates additional complementary base pairs at multiple sites that stabilize aptamer folding and thereby increase binding affinity to both ligands and reduce background fluorescence. ATMND fluorescence is significantly quenched upon binding to 38-GC. In the presence of cocaine, however, ATMND is displaced from the dye−aptamer complex, generating an intense fluorescence signal. We confirmed the competitive binding of the two ligands to the 38-GC and demonstrated that targeted mutagenesis clearly affects sensor performance.

Our assay is remarkably simple, fast, and specific. Detection is performed in a single tube containing the 38-GC-ATMND complex and the sample of interest. The assay is label-free, and detection only requires 20 s at room temperature to achieve a linear range of 0−8 μM with an LOD of 200 nM in buffer, about 50-fold lower than assays based on target-induced conformational change.[18−26] More importantly, our assay can achieve successful cocaine detection in body fluids. We observed that the signal gain increased with increasing cocaine concentrations, obtaining LODs of 10.4, 18.4, and 36 μM in undiluted saliva, urine, and serum samples, respectively. We anticipate that the well-established SELEX technique should likewise make it possible to derive similar target-ligand displacement sensors that exhibit equally high specificity and affinity for other small molecules. This approach therefore potentially offers a general framework for performing rapid and specific high-throughput on-site drug testing.

ASSOCIATED CONTENT

Supporting Information

Modeling data for binding of ATMND to 38-GC; Binding and thermodynamic parameters of 38-GT and 38-GC aptamers for ATMND and cocaine; Comparison of aptamer-based sensors for cocaine detection; ITC data demonstrate that 38-GT binds both cocaine and ATMND; Effect of fluorescence signal gain on different concentrations of ATMND; Modifications of 38-GT to convert a GT base pairing to a GC base pairing in stem 3 further increase its ligand binding affinity, whereas mutations in the binding pocket reduce ligand affinity; ITC data for 38-GC-M1 and 38-GC-M2; Calibration curves for a 38-GC-Cy7-based sensor; Successful detection of cocaine spiked into different dilutions of urine, saliva, and serum; Fluorescence spectra of 5% urine and 5% serum; Fluorescence intensities for different concentrations of biofluids; Fluorescence intensities of ATMND in different concentrations of saliva and serum; Calibration curves for cocaine detection with an ATMND-38-GC-based sensor in 2.5% serum. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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