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1. Introduction

The need for rapid reliable detection of intoxicating controlled or other substances in their parent and/or metabolized forms is a critical issue in the realm of public health.[1] Advances in onsite drug testing can provide immediate information for officers investigating cases of driving under the influence of drugs or drug-facilitated sexual assault, as well as drug overdose situations being evaluated in hospital Emergency Departments or forensic laboratories.[2] Antibody-based immunoassays are currently used to screen for the presence of drugs in biological samples.[3,4] Although they enable fast and sensitive screening,[5] the specificity and accuracy of immunoassays varies depending on the quality of the antibodies being used to target a given drug or metabolite, and false-positives often result from poor antibody specificity and cross-reactivity to structurally similar or dissimilar interferents commonly found in typical samples.[1] There is therefore an urgent need for alternative strategies for rapid, non-invasive, non-instrument-based screening that address these issues. Nucleic acid-based aptamers isolated via *in vitro* systematic evolution of ligands by exponential enrichment (SELEX)[6] offer a promising alternative to antibodies, with better specificity and longer shelf-lives.[7] Aptamer-based sensors have gained popularity due to their simplicity, sensitivity and specificity,[7] and may even be superior to antibodies for onsite detection of drugs of abuse.

We propose a colorimetric detection platform based on a low-cost, portable, paper-based microfluidic device to simultaneously detect trace amounts of drug molecules in oral fluid with high specificity. We chose cocaine and methamphetamine as targets because they are of particular interest for those working in the health and criminal justice fields. In the proposed platform, new aptamers will be generated that specifically bind to the targeted drugs, achieving better specificity than immunoassays. Exonuclease III (Exo III) will be used to digest the target/aptamer complex and recycle the target, resulting in high sensitivity and a reduction in false negatives. Detection will be achieved with a color change resulting from gold nanoparticle (AuNP) aggregation. By transferring this assay into a portable, postage stamp-sized, paper-based device, we expect to achieve instrument-free, simultaneous detection of cocaine and methamphetamine at low ng/mL concentrations in μL volumes of oral fluid with a visible red-to-blue color change. The successful outcome of this work will provide **1**) an aptamer-based, Exo III-

amplified and AuNP-reported colorimetric method for drug molecule screening with high sensitivity and specificity; **2**) a set of new aptamers that specifically bind methamphetamine; and **3**) a paper-based, portable device for sensitive on-site drug screening in oral fluid within minutes. Upon the success of this pilot study, this platform/device can easily be generalized for rapid and simultaneous screening of different drugs of abuse or families of designer drugs in oral fluid.

2. Project Development: Results

2.1 Engineer cocaine-binding aptamer with high affinity.

To develop the robust colorimetric platform proposed above, we first split a well-characterized cocaine-binding aptamer (MNS-4.1)[8] and tested target-induced aptamer assembly. We observed that the percent of cocaine-induced assembly was 10–20% under optimized conditions, with the low efficiency most likely attributable to low aptamer target binding affinity. To engineer a cocaine-binding aptamer with a high target-binding affinity, we developed a new strategy based on an MNS-4.1-derived aptamer sensor that reports the presence of cocaine via the displacement and unquenching of a bound fluorophore dye molecule (2-amino-5,6,7-trimethyl-1,8-naphthyridine; ATMND). In the absence of cocaine, the aptamer forms three helical stems around a three-way junction. ATMND binds the aptamer at this junction, which quenches its fluorescence. This aptamer also binds cocaine, and the competitive binding of cocaine results in rapid displacement of ATMND. The released ATMND generates a high-intensity fluorescent signal, reporting the cocaine-binding event.[9] To enhance aptamer-binding affinity, we further engineered MNS-4.1 to generate a new cocaine-binding aptamer (38-GC), in which we have converted putative non-canonical base-pairs in stem 1 and stem 3 into matched Watson-Crick base-pairs. This increased structural stability led to an improved signal gain, with a 2.6-fold enhanced binding to cocaine (K_{dc} = 2.6 ± 1.0 μM) relative to MNS-4.1 that produced a two-fold stronger signal in response to 50 μM cocaine. This could 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.[9] Importantly, our assay achieves successful cocaine detection in body fluids (Fig. 1), with a limit of detection (LOD) of 10.4 μM, 18.4 μM

and 36 μM in undiluted saliva, urine and serum, respectively. These findings were recently published in Analytical Chemistry (Roncancio D. et al *Anal. Chem.*, 2014, 86, 11100 – 11106.).

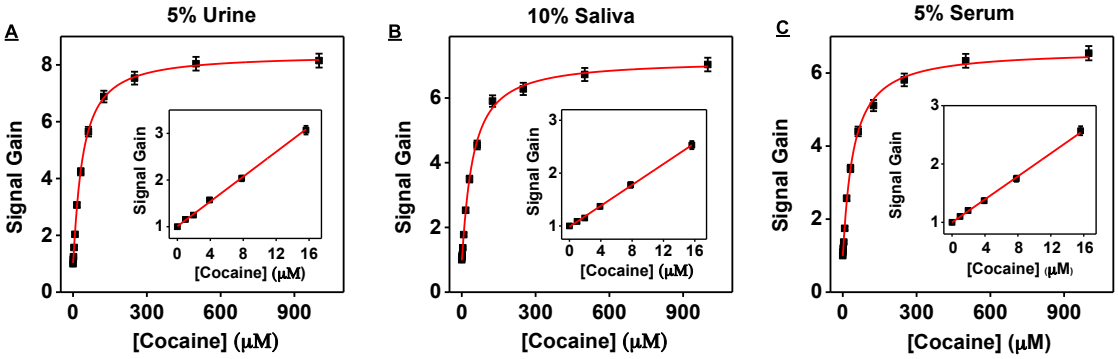

Figure 1. Calibration curves for 38-GC-based cocaine detection in biological samples. We observed a strong concentration-dependent response to cocaine in (**A**) 2.5% urine (**B**) 5% saliva and (C) 5 % serum. Experimental conditions: [38-GC] = 2 μM, [ATMND] = 250 nM.

2.2 Engineer cocaine-binding aptamer with high specificity.

Our 38-GC-displacement-based assay is remarkably simple, fast, and specific. Detection can be performed in a single tube containing the 38-GC-ATMND complex and the sample of interest. However, we observed false positives when major metabolites of cocaine and structurally-dissimilar interferents were tested. To increase the specificity of our assay, we utilized Exo III to preferentially digest the unbound folded aptamer. In the absence of cocaine, Exo III catalyzes 3'-to-5' digestion of 38-GC by the stepwise removal of mononucleotides, forming short, single-stranded products due to its preferential recognition of blunt termini.[10,11] In the presence of cocaine, however, 38-GC undergoes a subtle shift of a few nucleotides within the three-way junction involved with cocaine binding.[12,13] We have observed that Exo III is highly sensitive to this minor reorganization within the binding domain, and is no longer capable of digesting the cocaine-bound aptamer. This is the first such report of this nuclease's sensitivity to such subtle structural alterations in the target-aptamer complex. The extent of aptamer digestion is cocaine concentration-dependent, and can be readily quantified by SYBR Green I (SGI) fluorescent dye, which labels the remaining aptamers without the need for prior aptamer modification. Since the folded 38-GC is completely digested in the absence of cocaine, the background remains very low, achieving an

improved LOD of 1 μM in buffer. Importantly, Exo III's structure selectivity is sufficiently strong to prevent recognition of aptamer complexes formed via weaker interactions with other, non-target interferents. This results in excellent target specificity against common cocaine interferents (**Fig. 2A**) in comparison with our previously reported 38-GC-based cocaine assay (**Fig. 2B**).[9] We found that the sensitivity of Exo III to subtle structural alterations in the target-aptamer complex is generalizable for other small-molecule targets. We are currently preparing a manuscript (Wang Z.W. et al) that reports these findings with an anticipated submission of November, 2016.

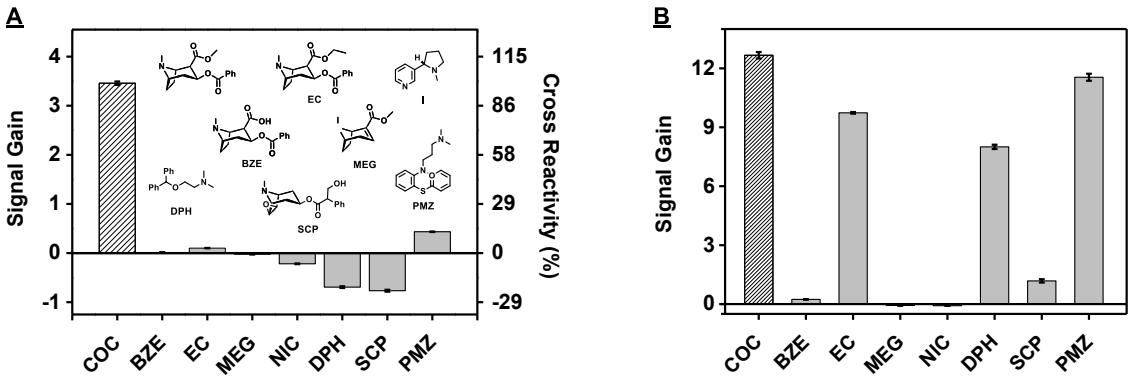

Figure 2. High specificity for cocaine achieved by Exo III fluorescence assay. (**A**) Specificity of Exo III digestion for cocaine (COC), benzoylecgonine (BZE), cocaethylene (EC), methylecgonidine (MEG), nicotine (NIC), diphenhydramine hydrochloride (DPH), (–)-scopolamine hydrochloride (SCP), and promazine hydrochloride (PMZ). (**B**) Specificity of detection with 38-GC in an ATMND-displacement assay using the same set of drugs. [Drug targets]: 250 μM, [38-GC]: 2 μM, [ATMND]: 0.25 μM.

2.3 Engineer cocaine-binding aptamer with sensitive target response.

We then split 38-GC at the non-binding region (*i.e.*, the loop of stem 2), yielding a short fragment (16 nt) and a long fragment (31 nt). This split aptamer was engineered to incorporate two additional base pairs in stem 2 and a $(T)_4$ sequence at the 3' end of the long fragment, which respectively allow for favorable cocaine-induced aptamer assembly and prevent enzymatic digestion of the long fragment. Cocaine-induced aptamer assembly was tested using SGI, a fluorescent dye that binds to double-stranded (ds) DNA with up to ten-fold greater efficiency relative to single-stranded (ss) DNA. Our results showed that the percent of cocaine-induced assembly under optimized conditions was 40–50%. However, the signal-to-noise ratio was <2 due to non-specific assembly, and we calculated that this accounted for roughly

50% of the total signal. To achieve a more effective target response, we have developed a novel split aptamer that achieves enhanced target-binding affinity through cooperative binding.[14] This split cocaine-binding aptamer incorporates two binding domains, such that target binding at one domain greatly increases the affinity of the second domain. We demonstrated that the resulting cooperative-binding split aptamer (CBSA) exhibits higher target-binding affinity and is far more responsive in terms of target-induced aptamer assembly compared to the single-domain parent split aptamer (PSA) from which it was derived. To the best of our knowledge, CBSA-5335 has the highest cocaine affinity of any split aptamer described to date and demonstrates excellent target detection in complex samples. By engineering the short fragment of our CBSA to incorporate a fluorophore-quencher pair, we achieved specific, ultra-sensitive, one-step fluorescent detection of cocaine within fifteen minutes at concentrations as low as 50 nM in 10% saliva (**Fig 3A**). This LOD meets the standards recommended by the European Union's Driving under the Influence of Drugs, Alcohol and Medicines program. Our assay also demonstrates excellent cocaine specificity: our results demonstrated no measurable signal from 50 μM benzoylecgonine, methylecgonidine, or nicotine and only 19% and 3% cross-reactivity to 50 μM and 5 μM cocaethylene, respectively, in 10% saliva (**Fig. 3B**). These findings were recently published in Chemical Science (Yu H.X. et al *Chem. Sci.*, (2016) DOI: 10.1039/C6SC01833E.).

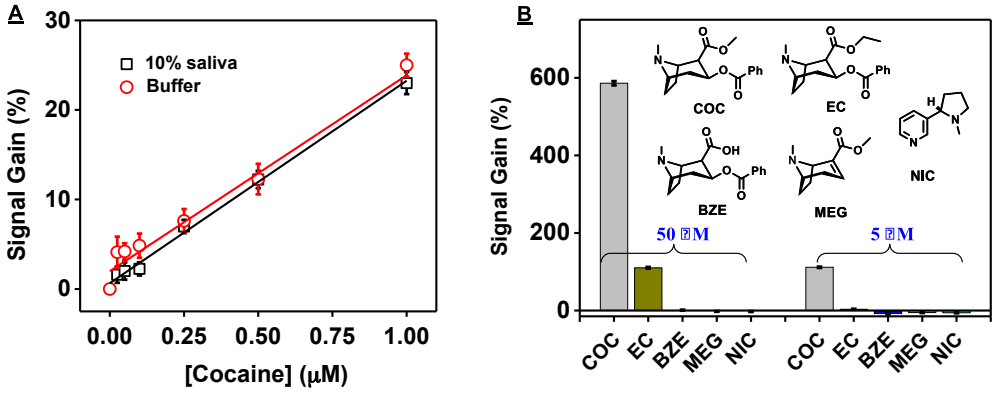

Figure 3. High sensitivity and specificity of our CBSA-5335-based fluorophore/quencher assay for cocaine detection in saliva. (**A**) Calibration curve for the assay in buffer and 10% saliva. (**B**) Signal gain in the presence of 50 μM (left) and 5 μM (right) COC or potential interferents including EC, BZE, MEG and NIC in 10% saliva. Inset shows the interferent structures.

2.4 Utilize CBSA to develop ultra-sensitive Exo III-assisted target recycling (EATR) assays.

We have since utilized our fluorophore-quencher-modified CBSA assay in combination with Exo III-assisted target-recycling (EATR) strategy to amplify the signal generated by cocaine binding events (**Fig. 4A**). We inserted an apurinic (AP) site (marked as X) into the short CBSA fragment, which had also been modified to include an Iowa Black RQ black quencher at the 5' terminus and a Cy5 fluorophore at the 3' terminus, protected by a 3' inverted dT. In the absence of cocaine, the short and long CBSA fragments remain unassembled and Exo III cannot cleave the single-stranded, short fragment due to its strong preference for duplex DNA. The fluorophore remains in close proximity to the quencher linked by the short fragment, and is therefore significantly quenched. Upon addition of cocaine, the two CBSA fragments assemble to form a duplexed AP site that can be cleaved by Exo III's AP endonuclease activity. Once the short fragment is cleaved, the CBSA disassembles and the fluorophore is released from the quencher, resulting in a fluorescence increase. The cocaine and long CBSA fragment disassemble from the cleaved CBSA, and can be recycled anew to generate an amplified signal via further rounds of binding and enzymatic digestion. Under optimized conditions, we obtained a calibration curve in the range of 10 nM to 1 mM with a LOD of 100 nM (**Fig. 4B**). We are currently preparing a manuscript (Yu H.X. et al) that reports these findings with an anticipated submission of November, 2016.

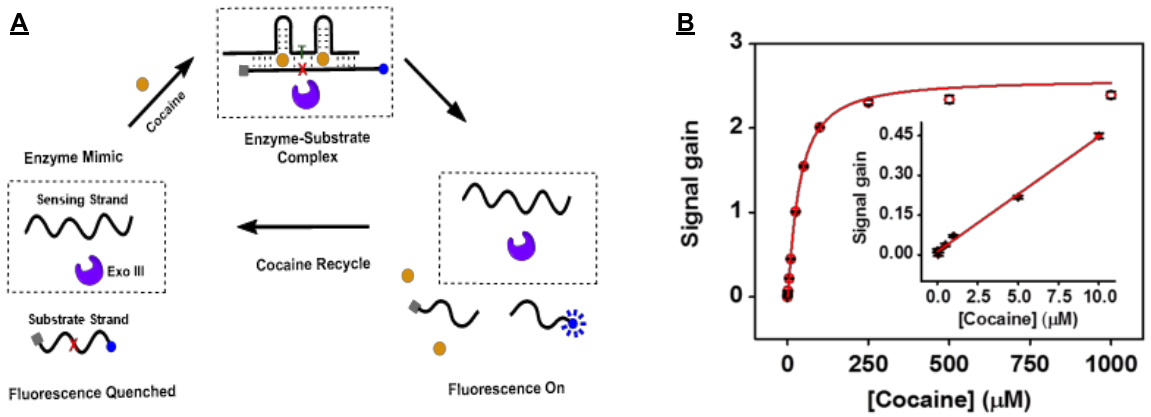

Figure 4. A CBSA-based, EATR-amplified assay for ultra-sensitive cocaine detection. (**A**) The working principle of the CBSA-based EATR sensor. (**B**) Calibration curve for cocaine detection in buffer. The assay was performed at 30°C with 1 μM of long and short fragments, 0.05 U/μL Exo III.

We then immobilized the short CBSA fragments on 13-nm AuNPs to achieve EATR-amplified sensitive CBSA-based colorimetric detection of cocaine. In the presence of cocaine, the free long CBSA

fragment assembles with the immobilized short CBSA fragment, forming aptamer/target complexes on the AuNP surface. Exo III then recognizes the complex and selectively digests the immobilized CBSA fragment, releasing the free long CBSA fragment and cocaine for another round of target binding and Exo III digestion. Once the exonuclease has digested all the short CBSA fragments on the AuNP surface, the sheared AuNPs aggregate and produce a visible red-to-blue color change. In the absence of target, the split short CBSA fragments cannot assemble, and no Exo III-assisted digestion occurs; thus, the solution remains red (**Fig. 5A)**. Using our CBSA-based EATR assay, we have achieved naked-eye detection of cocaine within fifteen minutes at concentrations as low as 1 μM in buffer (**Fig. 5B**). Currently, we are utilizing this CBSA-based platform to achieve naked-eye detection of low nM concentrations of cocaine in saliva samples within fifteen minutes. We are currently preparing a manuscript (Yu H.X. et al) that reports these findings with an anticipated submission of December, 2016.

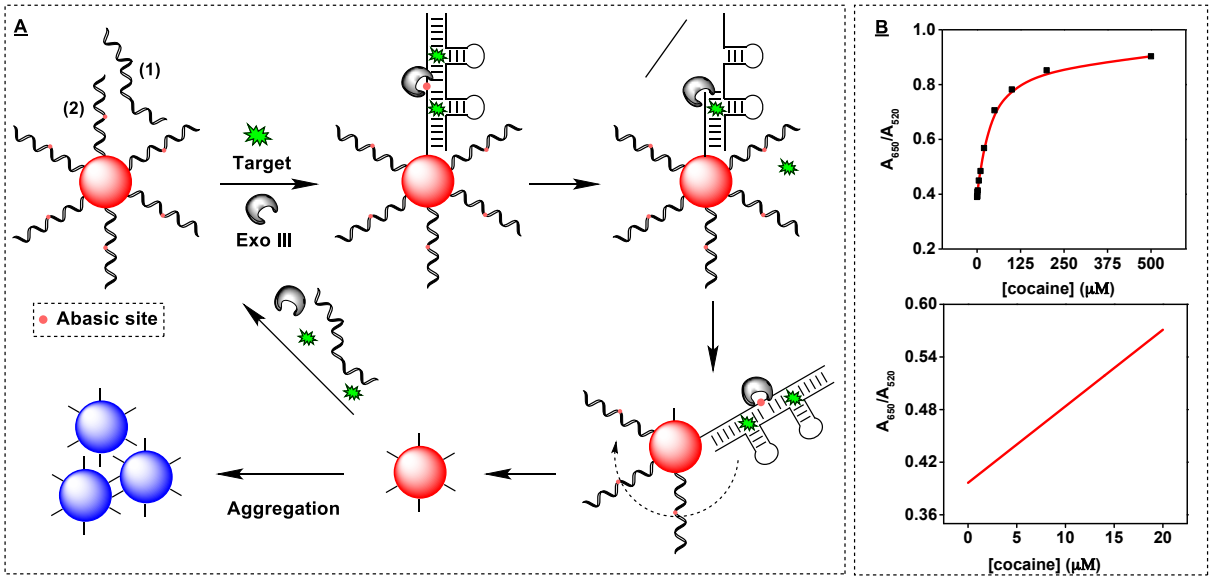

Figure 5. A CBSA-based, EATR-amplified AuNP-reported assay for ultra-sensitive cocaine detection. (**A**) Scheme of the colorimetric sensor. (**B**) Calibration curve for cocaine detection in buffer.

2.5 Develop paper microfluidic device for drug detection based on chemical reaction.

We have developed a paper microfluidic device for the presumptive testing of seized drugs in forensic casework.[15] We created hydrophilic channels on chromatographic paper using wax printing and thermal lamination. These are connected to a single central channel that draws an unknown analyte solution up

into six different lanes (**Fig. 6**). A different colorimetric reaction occurs within each lane, permitting multiplexed detection of a variety of different compounds, including cocaine, opiates, ketamine, and various phenethyl amines. The device, not much larger than a postage stamp, produces color changes from a few micrograms of compound that are detectable by the naked-eye in under 5 minutes. The procedure requires only small quantities of reagents in an easily stored format, and demonstrates improved sensitivity compared to solution-based colorimetric testing. In addition, since the reagents are adsorbed onto the paper, the procedure is much easier to perform and less hazardous to use. The multichannel system permits the simultaneous detection of numerous compounds using a variety of different reagents. This process greatly increases the specificity of the method. The system also permits the user to distinguish between illicit drugs, drug diluents, and common powders. Overall we expect this procedure to greatly benefit forensic testing, customs, and other applications where quick, portable testing of unknown powders is necessary. These findings were recently published in Analytical Methods (Musile, G. et al *Anal. Methods* 2015, 7, 8025–8033.).

Figure 6. A demonstration of our microfluidic paper-based device for the detection of seized drugs in a blank sample (**A**) and a sample adulterated with morphine (Morp) (**B**). Each lane is labeled with the name of analytes and the color they should produce. Lane 1, ephedrine (Eph), metamphetamine (MA) and MDMA; Lane 2, cocaine (Coc), codeine (Cod), ketamine (Ket) and thebaine (The); Lane 3, Cod, MA, MDMA and Morp; Lane 4, Ket and Morp; Lane 5, amphetamine (Amp); Lane 6, Morp and MDMA.

2.6 Develop paper microfluidic device for drug detection based on aptamer assembly.

An alternative platform for cocaine detection was prepared using a different AuNP/aptamer design[16] obtained from the literature using paper microfluidic devices. This alternative set of aptamers was implemented into a paper microfluidic chip design that utilizes the binding of gold nanoparticles and aptamers to produce a color change (**Fig. 7**). Basically, the aptamers cannot bind AuNPs when cocaine is

present. The chip is designed such that powdered and liquid samples can be dissolved in a carrier solvent in vials and then applied to the paper-based device just prior to analysis. The paper chips are easy to prepare and inexpensive to operate. Furthermore, they can be conveniently stored for later use. The paper microfluidic devices were prepared using a wax-ink printer, thermal laminator, chromatography paper, aptamers, and AuNPs. The wax-ink printer and a thermal laminator produce hydrophilic channels defined by melted wax on the paper. Next, AuNPs and aptamers are prepared and placed in a hydrophilic channel. Cocaine samples in both acid and basic form are dissolved in solutions and then transferred to the chips. Cocaine travels down the channel via capillary action, interacting with the aptamers and causing a color change to occur due to the aggregation of the nanoparticles. When cocaine is not present the nanoparticles cannot aggregate and no color change occurs as aptamers are then free to bind the gold nanoparticles. Interference testing demonstrated that the paper devices produced a negative result with controlled drugs such as ketamine, ephedrine, amphetamine, JWH-073, morphine, methamphetamine, and codeine. Negative results were also obtained using common adulterants such as caffeine and procaine, as well as diluents such as sugar, inositol, and sucrose. Common powders such as Excedrine^{TM}, ibuprofen, foot powder, baking soda, sea salts, flour and baking powder also produced a negative result. The entire process takes ~5 minutes with detection limits as low as 3 nM. We are currently preparing a manuscript (Wang L. et al), which will be submitted once long-term stability testing is complete.

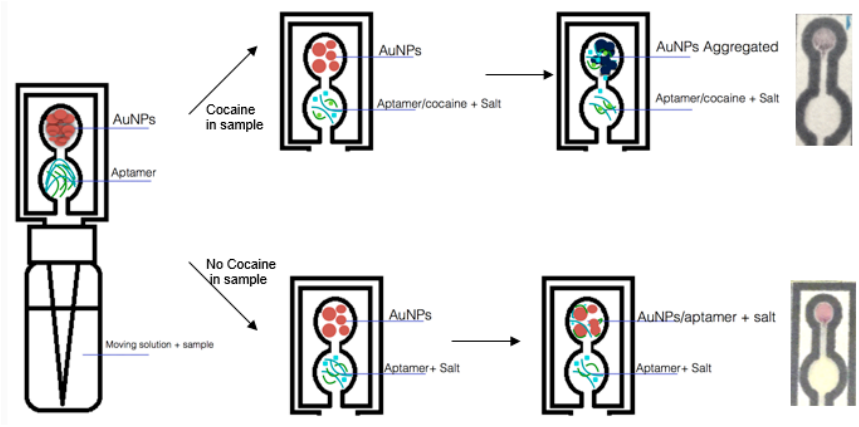

Figure 7. The AuNPs/aptamer modified paper chip. Two wells are present, one with aptamer and the second with gold nanoparticles. A sample is dissolved in a sucrose/$MgCl_2$ solution. In the presence of cocaine, aptamers bind to the cocaine, and gold nanoparticles aggregate. If the sample has no cocaine, aptamers bind to the surface of gold nanoparticles and prohibit aggregation.

3. Conclusion

The overall goal of the proposed project is to develop a portable, paper-based microfluidic device that can perform rapid and accurate colorimetric results from on-site screening for drug molecules in a μL-scale oral fluid sample with high sensitivity and specificity. We have successfully developed a specific, EATR-amplified and AuNP-reported screening platform for cocaine, using our newly-engineered cocaine-binding CBSA and have achieved colorimetric detection of 1 μM cocaine within 15 minutes. We have also modified different colorimetric assays for use in a six-channel paper-based microfluidic device. This paper microfluidic device can detect multiple drugs of abuse within 5 minutes, and the paper chips are stable for up to 3 months. In parallel, we have also developed a rapid assay based on split aptamers for the detection of cocaine and are in the process of adapting this assay for use in a paper-based microfluidic device. These paper microfluidic devices are inexpensive, portable, and user-friendly, making them useful for roadside testing, emergency room screening, and other on-site applications.

4. Impact on the Criminal Justice System:

Our method is anticipated to solve the low sensitivity, poor selectivity, and high cross-reactivity issues inherent in the commonly-used immunoassay that is apt to generate false negative and false positive results. The development of this amplified colorimetric method will permit an on-site/roadside drug screening of cocaine and/or methamphetamine in microliters of oral fluid on a portable, paper-based, microfluidic device in 10 minutes. In addition, the project will establish the protocol for future detection many of the new designer drugs such as synthetic cannabinoids and cathinones. Because of the anticipated reliability, low cost, and extreme portability of the device, this procedure has the potential to revolutionize on-site/roadside drug testing. It should be particularly valuable for officers investigating driving under the influence of drugs (DUID) and drug overdose situations as well as for a quick presumptive screen in additional settings.

5. Impact on Technology Transfer:

A patent has been issued and we are developing licensing arrangements with the potential companies.

Appendices

1. References

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2. Publications and Presentations

Patent:

1. Roncancio D., Yu H.X., Xu X.W. & Xiao Y. "Materials and Methods for Rapid and Specific Detection of Cocaine". Patent No. US 20160131668.

Publications:

1. Yu H.X., Canoura J., Guntupalli B., Lou X.H. & Xiao Y. A Cooperative-Binding Split Aptamer Assay for Rapid, Specific and Ultra-Sensitive Fluorescence Detection of Cocaine in Saliva. *Chem. Sci.* **2016,** DOI: 10.1039/C6SC01833E.
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3. Roncancio D., Yu H.X., Xu X.W., Wu S., Liu R., Debord J., Lou X.H. & Xiao Y. A Label-Free Aptamer-Fluorophore Assembly for Rapid and Specific Detection of Cocaine in Biofluids. *Anal. Chem.* **2014**, *86*, 11100–11106.

Work in progress:

4. Yu H.X., Xu X.W., Liang P.P., Loh K.Y., Guntupalli B. & Xiao Y. (2016) A Broadly-Applicable Assay for Accurately and Rapidly Quantifying DNA Surface Coverage on Diverse Particles. *Submitted*.
5. Wang Z.W., Yu H.X., Roncancio D., Fu F.F., Wu Z.J. & Xiao Y. (2016) Utilizing Nuclease Screening of Ligand-Aptamer Complexes to Enhance Specificity of Aptamers. *In preparation*.
6. Yu H.X., Guntupalli B., Canoura J. & Xiao Y. (2016) Cooperative Binding Split Aptamer Based, Exonuclease III-Assisted Target Recycling for Sensitive Detection of Small-Molecule Targets. *In preparation*.
7. Liang P.P., Canoura J., Yu H.X. & Xiao Y. (2016) DTT-Regulated, DNA-Modified AuNPs for Rapid Exo III-Engaged Colorimetric Detection of DNA and Small-Molecule Targets. *In preparation*.
8. Wang L. & McCord B. (2016) A paper-based device for rapid and sensitive detection of cocaine based on aptamer recognition. *In preparation*.

Presentations:

1. Canoura J., Wang Z.W., Yu H.X., Ng B., Roncancio D. & Xiao Y. (2016) Utilizing nuclease screening of ligand-aptamer complexes to enhance specificity of an aptamer-based cocaine assay. *Poster presentation,* 2016 American Chemical Society (ACS) Florida Annual Meeting and Exposition, Tampa, FL, May 5 – 7.
2. Yu H.X., Guntupalli B. & Xiao Y. (2016) A cooperative-binding split aptamer assay for rapid, specific and ultra-sensitive fluorescence detection of cocaine in saliva. *Oral presentation,* 2016 Conference of Undergraduate Research at FIU (CURFIU), Miami, FL, March 30 – 31.
3. Canoura J., Wang Z.W., Yu H.X., Ng B., Roncancio D. & Xiao Y. (2016) Utilizing nuclease screening of ligand-aptamer complexes to enhance specificity of an aptamer-based cocaine assay. *Oral presentation,* 2016 CURFIU, Miami, FL, March 30 – 31.
4. Roncancio D., Yu H.X., Xu X.W., Wu S., Liu R., Debord J., Lou X.H. & Xiao Y. (2016) A label-free aptamer-fluorophore assembly for highly sensitive and specific detection of cocaine. *Oral presentation,* 2016 CURFIU Miami, FL, March 30 – 31.
5. Roncancio D., Yu H.X., Xu X.W., Wu S., Liu R., Debord J., Lou X.H. & Xiao Y. (2016) A label-free aptamer-fluorophore assembly for highly sensitive and specific detection of cocaine. *Oral presentation,* FIU's annual Scholarly Forum during Graduate Student Appreciation Week (GSAW), Florida International University, Miami, FL, March 28 – 29.
6. Yu H.X., Guntupalli B. & Xiao Y. (2016) A cooperative-binding split aptamer assay for rapid, specific and ultra-sensitive fluorescence detection of cocaine in saliva. *Oral presentation,* FIU's annual Scholarly Forum during GSAW, Florida International University, Miami, FL, March 28 – 29.
7. Yu H.X., Guntupalli B. & Xiao Y. (2016) A cooperative-binding split aptamer assay for rapid, specific and ultra-sensitive fluorescence detection of cocaine in saliva. *Oral presentation,* Fifth Annual Forensic Science Symposium, Miami, FL, March 15 – 16.
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9. Yu H.X., Wang Z.W., Ng B., Canoura J. & Xiao Y. (2015) Simple, rapid and ultra-sensitive fluorescence detection of cocaine in oral fluid based on cooperative binding split aptamer. *Oral presentation,* Society of Forensic Toxicology (SOFT) 2015, Atlanta, Georgia, October 19 – 23.
10. Wang Z.W., Yu H.X., Ng B., Roncancio D., Canoura J. & Xiao Y. (2015) Amplified colorimetric detection of cocaine in oral fluid based on exonuclease-assisted aptamer strand recycling. *Poster presentation,* SOFT 2015, Atlanta, Georgia, October 19 – 23.
11. Xiao Y. (2015) Aptamer-based, exonuclease-amplified, paper device for point of collection

screening of cocaine and methamphetamine in oral fluid. *Invited talk,* 2015 Forensic Science R&D Grantees Meeting, Orlando, FL, February 17.

12. Xu X.W., Guntupalli B., Yu H.X. & Xiao Y. (2014) Target recycling-based assay for rapid and accurate determination of DNA surface coverage on gold nanoparticles. *Oral presentation,* 2014 Materials Research Society (MRS) Fall Meeting & Exhibit, Boston, Massachusetts, November 30 – December 5.
13. Roncancio D., Wu S., Yu H.X., Xu X.W., Liu R., Debord J., Lou X.H. & Xiao Y. (2014) A label-free aptamer-fluorophore assembly for highly sensitive and specific detection of cocaine. *Poster presentation,* 2014 MRS Fall Meeting & Exhibit, Boston, Massachusetts, November 30 – December 5.
14. Wang L., Musile G., Bottoms J., Tagliaro F. & McCord B., (2016) Colorimetric based paper microfluidic devices for the presumptive determiantion of seized drugs. *Oral presentation,* Florida International University GSAW, Miami, FL, March 28.
15. Wang L., Musile G., Bottoms J., Tagliaro F. & McCord B., (2016) Colorimetric based paper microfluidic devices for the presumptive determination of seized drugs. *Post presentation,* 5th Annual Forensic Science Symposium, Miami, FL, March 15 – 16.
16. Wang L., Musile G., Bottoms J., Tagliaro F. & McCord B., (2016) Colorimetric based paper microfluidic devices for the presumptive determination of seized drugs. *Post presentation,* AAFS 68th Annual Scientific Meeting, Las Vegas, NV, February 22 – 27.
17. Wang L., Musile G., Bottoms J., Tagliaro F. & McCord B., (2015) Presumptive determination of seized drugs using paper microfluidic devices with colorimetric reagents. *Poster presentation,* 91th FAME, Innisbnook, FL, May 6 – 9.
18. Wang L., Musile G., Bottoms J. & McCord B., (2015) The development of paper microfluidic devices for the presumptive determination of seized drugs. *Poster presentation,*4th Annual Forensic Science Symposium, Miami FL, May 5 – 6.
19. Wang L., Musile G., Bottoms J. & McCord B., (2015) The development of paper microfluidic devices for the presumptive determination of seized drugs. *Poster presentation,* AAFS 67th Annual Scientific Meeting, Hyatt Regency Orlando, Orlando, FL, February 16 – 21.
20. McCord B., (2015) Microfluidics and Nanotechnology in Forensic Science. *Invited talk,* George Washington University, Washington, DC, June 16.
21. Wang L. & McCord B., (2014) Detections for drugs of abuses with paper microfluidic devices assisted colorimetric tests. *Poster presentation,* 90th FAME, Innisbnook, FL, May 8 – 10.