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Table of Contents

1. Introduction

Synthetic cathinones (SCs) are designer drugs sharing a similar core structure with amphetamines and 3,4-methylenedioxy-methamphetamine.[1] They are highly addictive central nervous system stimulants, and are associated with many negative health consequences, including death.[1] Although these drugs have emerged recently, abuse of SCs has become a threat to public health and safety due to their severe toxicity, increasingly broad availability, and difficulty of regulation. Although presumptive drug testing needs to be first conducted in the field to narrow down the identity of the substance, SCs currently cannot be effectively screened using traditional field test kits. Current field drug screening generally entails the use of either chemical spot tests or lateral-flow immunoassays. Spot tests, which are based on color-producing chemical reactions between testing reagents and certain functional groups of SCs, are simple, rapid, and require only sample/reagent(s) mixing.[2] However, such tests have low specificity: the employed reagents often react with non-target compounds, including common cutting agents, adulterants, and other irrelevant drugs present within samples, which can result in false positives or inconclusive results.[2] On the other hand, many SCs do not react with one or more of the testing reagents due to the introduction of new side-chain substituents, resulting in false negatives.[2] Immunoassays are another alternative for SC screening.[3] These tests are based on the specific binding between antibodies and the analyte, allowing accurate identification of selected targets.[4] However, these assays suffer from false negatives due to the fact that SCs, as designer drugs, are rapidly and routinely modified based on their core structure, and even such minor structural modifications can greatly impair binding with existing antibodies.[3] The process of generating and validating new antibodies is too slow and costly to keep pace with designer drug evolution, and thus the utility of these assays is constrained by the range of available, target-specific antibodies. So far, no lateral-flow immunoassay is available for field testing of SCs. Thus, a highly effective presumptive screening assay that can perform rapid, sensitive and accurate on-site detection of the entire SC family is urgently needed.

We have devised a strategy for the development of such a screening assay, which uses a DNA-based affinity element – aptamer – to cross-reactively detect a broad range of SC drugs in a colorimetric and electrochemical format. There are two specific aims in this project. In **Aim 1**, we use systematic

evolution of ligands by exponential enrichment (SELEX),[5] a 'parallel-and-serial' selection strategy, and a carefully-designed counter-SELEX regimen to isolate a single class-specific aptamer that binds to the SC family. This process is expected to yield the first cross-reactive aptamer capable of recognizing virtually any SC based on their related core structure such that small chemical modifications should not significantly affect the aptamer's binding affinity. Moreover, by employing counter-SELEX, the isolated aptamer is not responsive to common cutting agents, adulterants, and other illicit drugs found in street samples. In **Aim 2**, we integrate the resulting cross-reactive aptamer into both a colorimetric assay[6] and an electrochemical aptamer-based (E-AB) sensor[7] for naked-eye and digital detection of SCs, respectively. We confirm the interference-free performance of our E-AB sensor in various sample mixtures.

2. Project Development: Results

2.1 *In Vitro* Isolation of Class-Specific aptamers for SCs

Parallel selection. Our parallel-and-serial selection strategy involves two steps: 1) challenging individual DNA libraries with structurally-similar targets in parallel to pre-enrich cross-reactive sequences and 2) combining these obtained parallel pools and challenging with all targets serially to isolate aptamers that specifically recognize the targeted family. SCs share the same β-keto phenethylamine chemical core structure with four substituent sites (**Fig. 1A, core structure**).[1] To isolate a class-specific SC-binding aptamer, we first performed parallel selection using three targets: α-pyrrolidinovalerophenone (α-PVP), ethylone, and butylone (**Fig. 1A**). These targets share the same core structure but have variations at all of the substitution sites that are typically modified in the SC family. Parallel selection was performed using three different initial library pools, with one pool being challenged with α-PVP, one with ethylone, and one with butylone (**Fig. 1B, top**). During the first round, each initial library pool was challenged with the respective target, and eluted strands were collected and amplified for the next round of selection. To further establish class-specificity, from the second round onward we performed counter-SELEX prior to the positive selection step to remove aptamers binding to structurally-similar interferents (e.g. cocaine, procaine, and lidocaine) that have the same functional groups or partial structural features as our targets. After the fifth round, a gel-elution assay[6] was performed to determine the target-binding affinity of each pool for its

respective target. We determined that aptamers in the ethylone and butylone pools had been enriched through parallel selection. Both pools cross-reacted to ethylone, butylone, and α-PVP. In contrast, enrichment remained low for the α-PVP pool. Given this, we performed four additional rounds of selection for the α-PVP pool. We performed the gel-elution assay for the round nine pool and observed a clear target concentration-dependent elution profile for α-PVP, indicating sufficient enrichment. We also determined that this pool displayed affinity to ethylone and butylone but was less responsive towards the various interferents, which can be attributed to the fact that more rounds of counter-SELEX were performed. Given that the pools enriched with individual targets also cross-reacted to other targets, we believe that those pools contained cross-reactive aptamers.

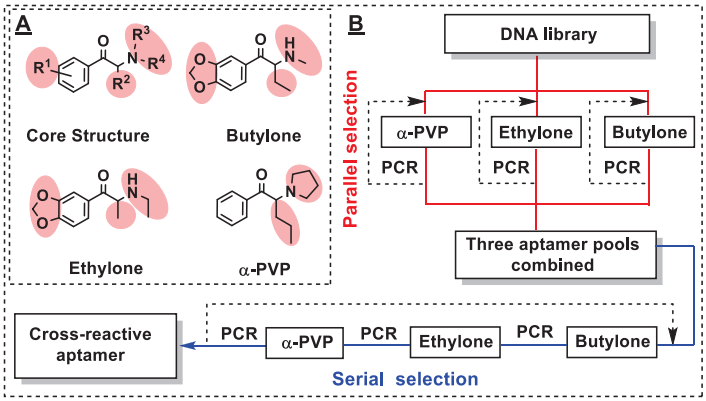

Figure 1. Isolation of a class-specific aptamer via SELEX. (**A**) The core structure of SCs and the three targets chosen for SELEX, with substituent moieties shaded in red. (**B**) Schematic diagram of parallel-and-serial SELEX.

Serial selection. We then performed serial selection to enrich cross-reactive aptamers and exclude aptamers specific to individual targets (**Fig. 1B, bottom**). We combined three enriched pools from parallel selection as a starting library. For each cycle of serial selection, we challenged the combined pool with each target sequentially for a total of three rounds of selection using butylone (first round), ethylone (second round), and α-PVP (third round). In each round, we performed a series of counter-SELEX screens with cocaine, procaine, and lidocaine. After the first cycle of serial selection, we performed the gel-elution assay to determine the cross-reactivity and specificity of the resulting pool. We observed that the cross-reactivity towards ethylone and butylone had substantially increased (dissociation constant (K_D) = 82 μM and 77 μM, respectively) relative to the individual pools obtained for these targets at the end of parallel selection, while affinity towards α-PVP was essentially unchanged (K_D = 34 μM). Importantly, this pool exhibited greatly improved specificity, with minimal affinity for cocaine and lidocaine and only a moderate response to procaine. We then performed a second cycle of serial selection with an identical selection procedure but with a counter-SELEX process against

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ephedrine, pseudoephedrine, acetaminophen, methamphetamine, amphetamine, cocaine, procaine, lidocaine, and promazine. We believe that the inclusion of these additional counter-targets, which are similar in structure to SCs and commonly encountered in seized substances, further enhance the specificity of the enriched pool. After this cycle, we evaluated the pool binding affinity (**Fig. 2A**). We found that the pool affinity towards ethylone and butylone had increased by ~10-fold (K_D = 6.9 μM and 9.5 μM, respectively), whereas the affinity towards α-PVP only marginally increased (K_D = 21 μM).

Characterization and sequencing of the enriched pool. We concluded that at this stage, the enriched pool

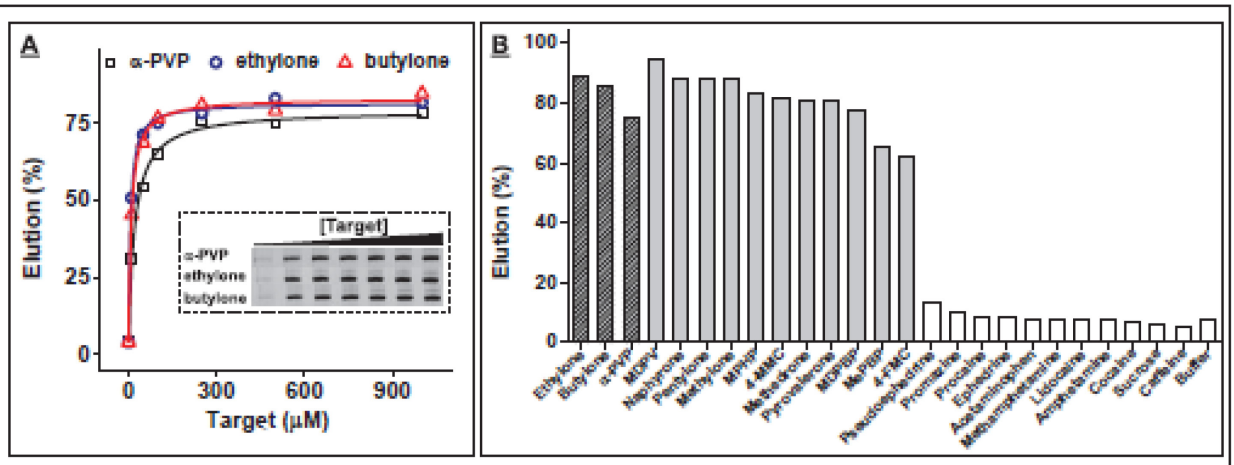

Figure 2. Characterization of the affinity and specificity of the final enriched pool via a gel-elution assay. (**A**) PAGE results depict the target elution profile, with lanes representing samples of the pool eluted with 0, 10, 50, 100, 250, 500, or 1,000 μM (from left to right) α-PVP, ethylone, or butylone. The percent of target-eluted pool was plotted against the concentration of target to determine the binding affinity of the enriched pool. (**B**) Percent elution values for 14 SCs and 11 interferents at a concentration of 50 μM and buffer alone.

was largely comprised of cross-reactive SC-binding aptamers. To confirm this, we used the gel-elution assay to test the target cross-reactivity of this pool by challenging it with the three targets as well as 11 other SCs. All of them demonstrated >60% target elution at a concentration of 50 μM (**Fig. 2B**). This shows that the aptamer could recognize the core structure of SCs, while being tolerant even to side-chain substituents that were not encountered during SELEX. To evaluate the target specificity of the enriched pool, we challenged the pool with 50 μM of the counter targets and other potential interferents and found that none of them showed increased elution compared with buffer alone (**Fig. 2B**). We therefore cloned and sequenced this final enriched pool. Sequencing results revealed three highly-prevalent sequences, termed SCA1.1 (7 of 50 clones), SCA1.2 (4 of 50), and SCA2.1 (30 of 50 clones).

Characterization of the aptamer's affinity. We characterized the affinity of SCA2.1 for the selection targets using isothermal titration calorimetry (ITC).[8] We titrated a 300–400 μM solution of target into a 20 μM solution of the aptamer. We found that binding of target to aptamer does not occur in a 1:1 ratio. Given that SCs are chiral and a racemic mixture of the targets was employed for SELEX, we hypothesized that the aptamer may have differential binding affinity for each enantiomer. The pure enantiomers of the three selection targets were not commercially available, but the high cross-reactivity of the aptamer allowed us to use enantiomers for another SC, (-)- and (+)-methylenedioxypyrovalerone (MDPV), to confirm our hypothesis. ITC data indicated that the aptamer binds to either one (-)-MDPV molecule with K_D = 46.5 nM or (+)-MDPV molecule with K_D = 3.61 μM. We also an enantiomer binding model to determine the binding parameters for α-PVP, butylone, and ethylone to SCA2.1, and observed similar nanomolar K_D for one of the two enantiomers.

2.2 Development of aptamer-based Cy7-displacement assay based on the isolated aptamer. We then demonstrated the analytical utility of SCA2.1 in a colorimetric 'dye-displacement' assay. Diethylthiotricarbocyanine (Cy7) is a small-molecule dye that exists in equilibrium between monomer and dimer forms, which have absorbance peaks at 760 and 670 nm, respectively.[9] Previous studies have shown that Cy7 monomers can bind to hydrophobic target-binding domains of aptamers, which results in strong enhancement of absorbance at 760 nm.[6,10] However, the binding of target to the aptamer can displace Cy7 monomer from the binding domain within seconds, which causes the dye to dimerize in aqueous solution, resulting in the reduction of absorbance at 760 nm and enhancement of absorbance at 670 nm. This approach can thus be used as a colorimetric indicator for small molecule detection.[6] We first titrated different concentrations of butylone into a mixture of Cy7 and SCA2.1, and found that increasing concentrations of butylone progressively reduced the absorbance of Cy7 at 775 nm while enhancing absorbance at 670 nm. This change can be attributed to dimerization of the Cy7 monomer when displaced from the aptamer into solution.[6,10] We used the absorbance ratio between 670 nm and 775 nm to calculate the signal gain and generate a calibration curve, which displayed a linear range of 0–10 μM and a measurable detection limit of 250 nM. We obtained equivalent results with both ethylone and α-PVP.

We then tested the cross-reactivity of this assay for nine other SCs, including naphyrone, MDPV, pentylone, methylone, 4-MMC, 4-FMC, 3-FMC, methcathinone and cathinone at a concentration of 50 μM. As expected, despite the diversity of the side chains substituents, all SCs produced a signal gain ranging from 45% to 130% relative to ethylone (**Fig. 3A**). This implies that SCA2.1 mainly recognizes the β-keto phenethylamine core structure, and variations in the side chains do not significantly affect target-binding affinity. Notably, the aptamer is more cross-reactive to a broad range of SCs than antibodies used in current existing immunoassays, which achieve >20% cross-reactivity for only five SCs[3]. Importantly, our assay has excellent specificity, as the aptamer does not cross-react to non-SC interferents. We tested our assay with 12 different interferent compounds, including common illicit drugs (amphetamine, methamphetamine and cocaine) and cutting agents found in street samples (pseudoephedrine, ephedrine, procaine, lidocaine, benzocaine, caffeine, acetaminophen and sucrose) at a concentration of 50 μM. The assay yielded no response to any of these interferents (**Fig. 3A**), even though many contained a partial β-keto phenethylamine structure, demonstrating that aptamer specificity can be precisely controlled through a well-designed counter SELEX approach. We further fine-tuned our Cy7-displacement assay by using a higher concentration of the dye and aptamer in order to intensify the target-induced color change and thereby enable naked-eye detection. In the absence of target, the solution of aptamer-Cy7 complex is practically colorless (**Fig. 3B**, -). However, when Cy7 is displaced by the target, Cy7 forms dimers which produce a

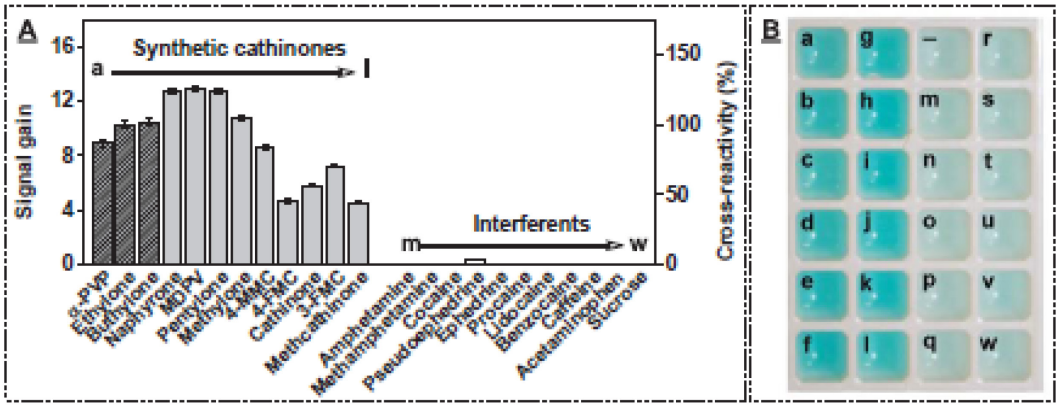

Figure 3. Colorimetric detection of SCs using a Cy7-displacement assay. (**A**) Signal gain measured via a plate reader from the Cy7-displacement assay with 12 SCs (gray, with the three selection targets shaded) and 11 interferents (white) at a concentration of 50 μM. Error bars show standard deviations from three measurements. (**B**) Naked-eye detection of SCs using the Cy7-displacement assay. The color appears faint blue in the absence of target (-) or 50 μM of interferents (m-w). However, the color of the solution changes to bright blue upon addition of 50 μM SCs (a-l).

bright, clearly visible blue color. We observed that all 12 SCs immediately induced a clear-to-blue color change in the solution, while no color change was identified upon addition of any of the interferents (**Fig. 3B**). As a demonstration, we observed that as low as 2 ng/mL of ethylone can be visually detected with the naked eye. These results demonstrate the feasibility of this assay for on-site drug screening applications.

3. Development of Electrochemical Aptamer-Based (E-AB) Sensors for SCs Detection.

Generating structure-switching aptamers using exonuclease-based method. We also used the isolated aptamers to develop an E-AB sensor for rapid, robust, and digital SC detection. E-AB sensors are fabricated by immobilizing aptamers covalently labeled with an electroactive tag (*e.g.* methylene blue) on a gold electrode.[11] The sensing mechanism is based on target-induced 'structure switching' of the aptamer.[12] In the absence of target, the aptamer is flexible and disorganized, which positions the tag far away from the electrode surface, resulting in low current. In the presence of target, the aptamer undergoes a large target-induced conformational change, which relocates the tag close to the electrode surface, yielding a large increase in current. The isolated three aptamers are pre-folded and do not have structure-switching functionality. We used our previously reported enzyme-based to introduce this functionality into the aptamers.[13] Specifically, SCA2.1 was digested by a mixture of exonuclease III (Exo III) and exonuclease I (Exo I), resulting in a 40-nt major and 39-nt minor products in the presence MDPV. In the absence of MDPV, the aptamer was completely digested by the exonucleases. On the other hand, Exo III digestion of

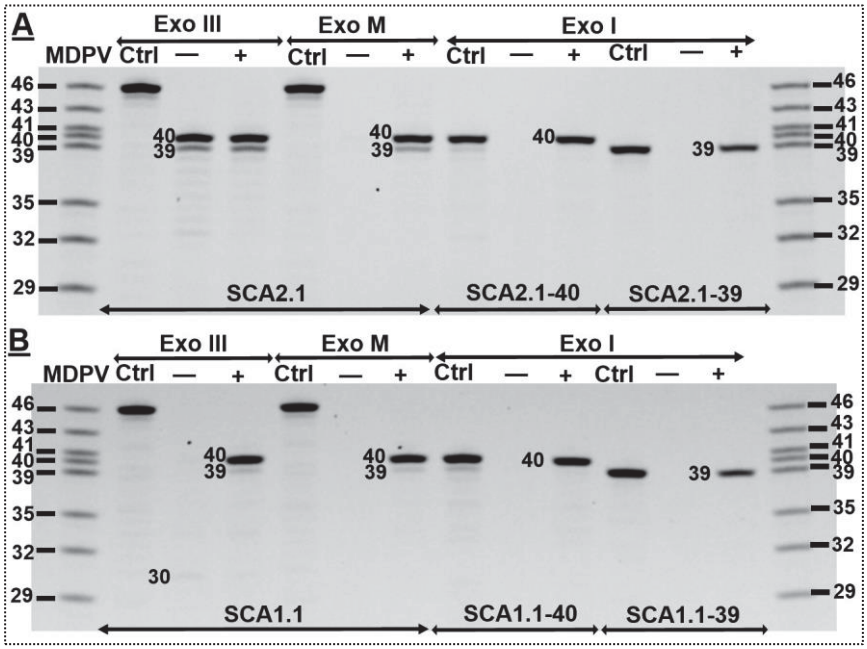

Figure 4. PAGE analysis of exonuclease digestion in absence (-) and presence (+) of MDPV. (A) Digestion products of SCA2.1 by Exo III or Exo mixture and SCA2.1-40 and SCA2.1-39 by Exo I. (B) Digestion products of SCA1.1 by Exo III or Exo mixture and SCA1.1-40 and SCA1.1-39 by Exo I.

SCA 2.1 alone yielded 40-nt and 39-nt products regardless of the presence or absence of MDPV (**Fig. 4A**). We believed that the 40-nt product had structure-switching functionality. We confirmed this with circular dichroism.[14] The same digestion experiments were performed on SCA1.1. We found that a 40-nt major product was generated by the exonuclease mixture in the presence of target, and the aptamer was completely digested in the absence of target. Digestion with Exo III alone yielded the same results (**Fig. 4B**). Circular dichroism confirmed that the 40-nt product had structure-switching functionality.

Fabrication of E-AB sensors for SCs detection. The structure-switching aptamers (SCA2.1-40 and SCA1.1-40) were then utilized to fabricate E-AB sensors for SC detection. Both sensors displayed a linear range of 0 to 1 μM for MDPV, with a detection limit of 100 nM. We tested the cross-reactivity of both sensors for eleven other SCs including α-PVP, ethylone, butylone, naphyrone, pentylone, methylone, 4-MMC, 4-FMC, cathinone, 3-FMC, and methcathinone at a concentration of 10 μM. For SCA-2.1 E-AB sensor, these SCs demonstrated cross-reactivity ranging from 30% to 100% relative to MDPV. The SCA-1.1 E-AB sensor showed higher cross-reactivity; all 11 SCs had cross-reactivities between 50% to 110% relative to MDPV. To investigate the specificity of both E-AB sensors, we tested fifteen structurally-similar and -dissimilar interferents including illicit drugs (amphetamine, ketamine, cocaine, MDMA, and heroin) and common cutting agents and adulterants (caffeine, benzocaine, lidocaine, levamisole, phenacetine, paracetamol, quinine, sucrose, lactose, and mannitol). We found that the SCA-2.1 E-AB sensor demonstrated less than 20% cross-reactivity relative to MDPV towards all 15 interferents at 10-fold higher interferent concentrations. The SCA-1.1 E-AB sensor showed less specificity towards certain interferents, yielding a cross-reactivity of 62%, 35%, and 60% for MDMA, levamisole, and quinine, respectively. Clearly, the SCA-2.1 sensor exhibits high specificity towards interferents but low cross-reactivity towards SCs, while the SCA-1.1 sensor demonstrated high cross-reactivity towards SCs and poor specificity against interferents.

To achieve both high cross-reactivity to SCs and high specificity against interferents, we fabricated an E-AB sensor by co-immobilizing both aptamers (SCA2.1-40 and SCA1.1-40) on the same gold electrode. We determined that a 1:1 mole ratio of both aptamers yielded the most optimal target-cross-reactivity and

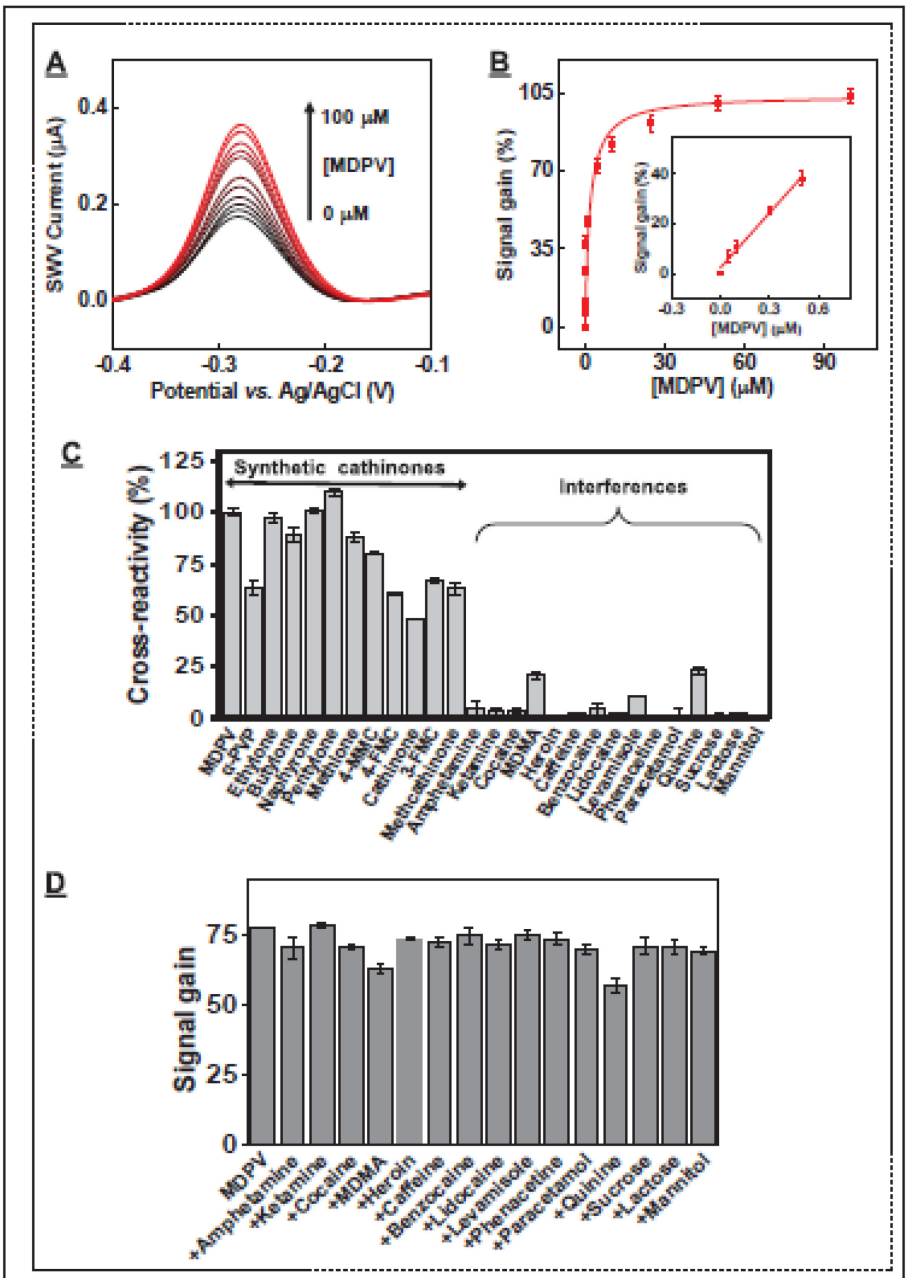

Figure 5. Sensitive and specific SC detection using dual-aptamer-modified E-AB sensor. (A) SWV spectra at various concentrations of MDPV. (B) Calibration curve derived from SWV spectra. (C) Cross reactivity of 12 10 μM SCs and 15 100 μM interferents. (D) Signal gain for a binary mixture containing 10 μM MDPV and one of each 15 interferents with concentration of 100 μM.

interferent-specificity. This E-AB sensor was determined to have a surface coverage of 3.20 pmole/cm^2. The sensor had a linear range of 0 to 0.5 μM for MDPV, with an improved detection limit of 50 nM (**Fig. 5A and 5B**). As expected, the sensor exhibited high cross-reactivity to all tested SCs at a concentration of 10 μM, ranging from 50% to 110% relative to MDPV (**Fig. 5C**). Meanwhile, the sensor showed excellent specificity against interferents at a 10-fold higher concentration than target, yielding < 10% to all interferents except MDMA (22%) and quinine (17%) relative to MDPV (**Fig. 5C**). These results demonstrated this E-AB sensor has both high cross-reactivity to SCs and high specificity against interferents. Finally, we tested sample matrix effects by challenging the sensor with binary mixtures of MDPV and an interferent (amphetamine, ketamine, cocaine, MDMA, heroin, caffeine, benzocaine, lidocaine, levamisole,

phenacetine, paracetamol, quinine, sucrose, lactose, or mannitol) at a molar ratio of 1:10. We observed that the presence of 10-fold higher interferent resulted in similar signal gains relative to that achieved with MDPV alone (**Fig. 5D**), indicating the great potential for accurate SC detection in seized substances.

3. Conclusion

The overall goal of the proposed project is to develop aptamer-based assays that can perform rapid, on-site detection of SCs in seized substances with high sensitivity and specificity. We have successfully produced **1**) a new DNA aptamer that cross-reactively binds to 15 SCs with high affinity; **2**) a single-step, aptamer-based Cy7-displacement colorimetric assay that instantaneously detects SCs in seized substances with superior specificity against interferents and greater target-cross-reactivity than all existing antibodies; and **3**) an E-AB sensor that screens for SCs with high sensitivity without being influenced by 15 interferents found in street samples. Both the colorimetric assay and E-AB sensor are rapid (seconds-scale), sensitive, specific, inexpensive, and user-friendly, which makes them useful for on-site presumptive testing of SCs.

4. Impact on the Criminal Justice System

Our methods are anticipated to solve the low sensitivity, poor selectivity, and limited cross-reactivity issues inherent of the commonly-used chemical spot test and immunoassays that typically yield false negative and false positive results for drug screening. The development of our aptamer-based colorimetric and electrochemical sensors will permit on-site screening of SCs in seized substances within seconds. Our sensors are designed to be generically sensitive to all members of the synthetic cathinone family, and would thus be valuable in providing a simple 'yes/no' response for the presence of such drugs that would be immediately valuable for law enforcement. In addition, the project will establish the protocol for future detection many of new psychoactive substances such as synthetic cannabinoids and opioids. Because of the anticipated reliability, low cost, and portability of the sensor, this technology has the potential to revolutionize on-site drug testing and can be particularly valuable for law enforcement officials investigating seized substances, driving under the influence of drugs, and drug overdose situations.

5. Impact on Technology Transfer:

A provisional patent has been filed in December 2018.

Appendices

1. References

(1) Prosser, J. M.; Nelson, L. S. The Toxicology of Bath Salts: A Review of Synthetic Cathinones. *J. Med. Toxicol.* **2012**, *8*, 33–42.

(2) O'Neal, C. L.; Crouch, D. J.; Fatah, A. A. Validation of Twelve Chemical Spot Tests for the Detection of Drugs of Abuse. *Forensic Sci. Int.* **2000**, *109*, 189–201.

(3) Ellefsen, K. N.; Anizan, S.; Castaneto, M. S.; Desrosiers, N. A.; Martin, T. M.; Klette, K. L.; Huestis, M. A. Validation of the Only Commercially Available Immunoassay for Synthetic Cathinones in Urine: Randox Drugs of Abuse V Biochip Array Technology. *Drug Test. Anal.* **2014**, *6*, 728–738.

(4) Tsai, J. S.-C.; Lin, G. L. *Drugs of Abuse Body Fluid Testing*; Wong, R. C.; Tse, H. Y., Eds.; Humana Press: Totowa, NJ, 2005.

(5) Tuerk, C.; Gold, L. Systematic Evolution of Ligands by Exponential Enrichment: RNA Ligands to Bacteriophage T4 DNA Polymerase. *Science* **1990**, *249*, 505–510.

(6) Yu, H.; Yang, W.; Alkhamis, O.; Canoura, J.; Yang, K.-A.; Xiao, Y. In Vitro Isolation of Small-Molecule-Binding Aptamers with Intrinsic Dye-Displacement Functionality. *Nucleic Acids Res.* **2018**, *46*, e43.

(7) Xiao, Y.; Lubin, A. A.; Heeger, A. J.; Plaxco, K. W. Label-Free Electronic Detection of Thrombin in Blood Serum by Using an Aptamer-Based Sensor. *Angew. Chem., Int. Ed.* **2005**, *44*, 5456–5459.

(8) Cowan, J. A.; Ohyama, T.; Wang, D.; Natarajan, K. Recognition of a Cognate RNA Aptamer by Neomycin B: Quantitative Evaluation of Hydrogen Bonding and Electrostatic Interactions. *Nucleic Acids Res.* **2000**, *28*, 2935–2942.

(9) Garoff, R. A.; Litzinger, E. A.; Connor, R. E.; Fishman, I.; Armitage, B. A. Helical Aggregation of Cyanine Dyes on DNA Templates: Effect of Dye Structure on Formation of Homo- and Heteroaggregates. *Langmuir* **2002**, *18*, 6330–6337.

(10) Stojanovic, M. N.; Landry, D. W. Aptamer-Based Colorimetric Probe for Cocaine. *J. Am. Chem. Soc.* **2002**, *124*, 9678–9679.

(11) Xiao, Y.; Lai, R. Y.; Plaxco, K. W. Preparation of Electrode-Immobilized, Redox-Modified Oligonucleotides for Electrochemical DNA and Aptamer-Based Sensing. *Nat. Protoc.* **2007**, *2*, 2875–2880.

(12) Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. An Electronic, Aptamer-Based Small-Molecule Sensor for the Rapid, Label-Free Detection of Cocaine in Adulterated Samples and Biological Fluids. *J. Am. Chem. Soc.* **2006**, *128*, 3138–3139.

(13) Canoura, J.; Wang, Z.; Yu, H.; Alkhamis, O.; Fu, F.; Xiao, Y. No Structure-Switching Required: A Generalizable Exonuclease-Mediated Aptamer-Based Assay for Small-Molecule Detection. *J. Am. Chem. Soc.* **2018**, *140*, 9961–9971.

(14) Kypr, J.; Kejnovská, I.; Renčiuk, D.; Vorlíčková, M. Circular Dichroism and Conformational Polymorphism of DNA. *Nucleic Acids Res.* **2009**, *37*, 1713–1725.

2. Publications and Presentations

Patent:

1. Yang W.J., Yu H.X., Liu Y.Z. & Xiao Y. "Method for isolating cross-reactive aptamer and use thereof". Patent provision, 2018 (Application Number: 16/174,764)

Publications in progress:

1. Yang W.J., Yu H.X., Alkhamis O., Liu Y.Z., Canoura J., Fu F.F. & Xiao Y. *In Vitro* Isolation of a Class-Specific Oligonucleotide-Based Small-Molecule Receptors. *Submitted*.
2. Luo Y.P., Yu H.X., Alkhamis O., Liu Y.Z., Lou X.H., Yu B.Y. & Xiao Y. Label-Free, Visual Detection of Small Molecules Using Highly Target-Responsive Multi-Module Split Aptamer Constructs. *Submitted*.

3. Liu Y.Z., Yu H.X., Alkhamis O., Moliver J. & Xiao Y. For best of both worlds: dual-aptamer-based Electrochemical sensors for sensitive and specific detection of synthetic cathinones. *In preparation.*
4. Liu Y.Z., Yu H.X., Alkhamis O., Canoura J. & Xiao Y. Utilizing High Throughout Sequencing to Monitor the Evolution of Small-Molecule-Binding Aptamers During Parallel-and-Serial SELEX. *In preparation.*

Presentations:

1. Xiao Y. (2019) Beauty Through Simplicity – Functionalized Aptamers for On-Site Small-Molecule Detection. Symposium (Strategies for Uncovering and Tracing Biomarkers in Complex Biomedical Systems), Pittcon 2019, Philadelphia, Pennsylvania, USA, March 17 – 21.
2. Yang W.J., Yu H.X., Alkhamis O., Fu F.F. & Xiao Y. (2019) Rapid, Naked-Eye Screening for Synthetic Cathinones Based on Cross-Reactive Aptamers. NIJ Poster Session, Pittcon 2019, Philadelphia, Pennsylvania, USA, March 17 – 21.
3. Liu Y.Z., Yu H.X., Alkhamis O., Moliver J. & Xiao Y. (2019) Development of Electrochemical Aptamer-Based Sensors for On-Site Synthetic Cathinone Detection. Oral Sessions (Homeland Security/Forensics - Illicit Drugs), Pittcon 2019, Philadelphia, Pennsylvania, USA, March 17 – 21.
4. Xiao Y. (2018) Beauty Through Simplicity – Functionalized Aptamers for On-Site Small-Molecule Detection. *Invited talk,* University of Notre Dame, USA, September 17.
5. Xiao Y. (2018) Aptamer-based Assays for On-site Drug Detection. *Oral presentation,* Pittcon 2018, Orlando, USA, February 26 – March 1.