Federal Agency: National Institute of Justice
Title: Electrochemical Aptamer-Based Sensor for Rapid Opioid Detection in Seized Substances

Grant Number: 2019-DU-BX-0024
Grant Period: 01/01/2020-12/31/2022

Principal Investigator:

DeEtta (Dee) Mills (PI)
Associate Professor
Department of Biological Sciences
Florida International University
11200 SW 8th Street
Miami, FL 33199
Tel: 305 348 7410
E-mail: millsd@fiu.edu

Yi Xiao (PI)
Associate Professor
Department of Chemistry
North Carolina State University
2620 Yarbrough Drive,
Raleigh, NC 27607
Tel: 919-515-5452
E-mail: yxiao34@ncsu.edu

Recipient Organization:

Florida International University
11200 SW 8th Street, Miami, FL 33199

Submitting Official:

Roberto Gutierrez
Assistant Vice President for Research
Office of Research and Economic Development
Florida International University
11200 SW 8th Street, MARC 430
Miami, FL 33199
Tel: 305-348-2494

Reporting Period: January 2020 – December 2022
Report Draft: Final Summary Overview
Submission Date: February 10, 2023
DUNS:
EIN:
Signature of Submitting Official:

Table of Contents

1. Introduction

The term 'opioids' refers to a class of psychoactive narcotic compounds that act on the central nervous system as agonists of opioid receptors. These drugs are commonly used for their analgesic and sedative properties in the medical field, but the misuse of such drugs can cause a variety of serious health consequences such as physical dependence, respiratory failure, and even death. Morphine-related natural and semi-synthetic opioids sharing the N-methyl-4,5-epoxymorphinan structure (*i.e.* morphine, codeine, heroin, oxycodone, hydrocodone, oxymorphone, and hydromorphone) are disproportionately responsible for the current opioid epidemic. These opioids accounted for 67% of all opioid-related seizures in 2017. On the other hand, fentanyl and its analogs, which share the 4-anilidopiperidine core structure, have also become increasingly common in street samples, often mixed with heroin as an adulterant. In 2017, fentanyl became the 5th most seized drug overall, and the most seized narcotic after heroin. Currently, screening of opioids in seized substances generally involves the use of chemical spot tests, Raman spectroscopy, and lateral-flow immunoassays. Chemical tests often suffer from interferent-related inconclusive results, false positives, and negatives. Surface-enhanced Raman spectroscopy has challenges for opioid-containing impure samples and relies on specialized reference libraries which are difficult to keep up with emerging fentanyl analogs and new drug formulations. Lateral flow immunoassays lack the necessary cross-reactivity and specificity for accurate and reliable opioid detection. So far, no lateral-flow immunoassay is available for field testing of fentanyl analogs. Thus, there is an urgent need for a rapid and accurate presumptive test that can detect opioids of interest in seized samples for law enforcement/forensic personnel.

We have developed an electrochemical aptamer-based (E-AB) sensor as a presumptive testing tool that can be easily employed by law enforcement agents, first responders, or forensic personnel to identify opioid families in seized substances. This sensor is capable of providing a "yes-or-no" readout in the presence of an opioid from either the morphine or fentanyl family within seconds for samples such as powders, tars, pastes, counterfeit pills/tablets, or other formulations, even trace residues on surfaces such as packages and bags. The sensor employs three aptamers. Two aptamers detect seven morphine-related opioids, while the other detects fentanyl and its analogs. This project entailed three major aims. In Aim 1, we performed library-immobilized SELEX with a 'parallel-and-serial' selection strategy to isolate two cross-reactive aptamers binding to all proposed morphine-related opioids. In Aim 2, we employed a similar selection protocol to isolate another aptamer that is cross-reactive to all proposed fentanyl-related opioids. During both selections, we performed counter-SELEX to ensure that the aptamers do not bind common cutting agents, adulterants, or other illicit drugs found in seized opioid samples, as well as to prevent the three aptamers from cross-reacting to each other's targets. In Aim 3, we fabricated an electrochemical aptamer-based (E-AB) sensor for sensitive detection of opioids in seized substances. Specifically, we first used an exonuclease III (Exo III)-directed truncation strategy to introduce structure-switching functionality into the isolated aptamers. We then modified these functionalized aptamers with an electrochemical signaling tag, methylene blue (MB, E = -0.2 V vs. Ag/AgCl). These modified aptamers were immobilized onto gold electrode surfaces. The binding of target to its respective aptamer induced a conformational change that generated a target-related current. Our E-AB sensor is cross-reactive to the proposed opioids at low ppb (ng/g or ng/mL) levels and is highly specific against interferents found in seized substances.

2. Project Development: Results

In total, the funding from this grant supported the completion of four publications and two filed patents, three manuscripts currently under review, and two manuscripts in preparation.

2.1 Immobilization Strategies for Enhancing Sensitivity of Electrochemical Aptamer-Based Sensors. Electrochemical aptamer-based (E-AB) sensors are a versatile sensing platform that can achieve rapid and robust target detection in complex matrices. However, the limited sensitivity of these sensors has impeded their translation from proof-of-concept to commercial products. Surface-bound aptamers must be sufficiently spaced to bind targets and subsequently fold for signal transduction. We hypothesized that electrodes fabricated using conventional methods result in sensing surfaces where only a fraction of aptamers are appropriately spaced to actively respond to the target. As an alternative, we presented a novel aptamer immobilization approach that favors sufficient spacing between aptamers at the microscale to achieve optimal target binding, folding, and signal transduction. We first demonstrated that immobilizing aptamers in their target-bound, folded state on gold electrode surfaces yields an aptamer monolayer that supports greater sensitivity and higher signal-to-noise ratio than traditionally prepared E-AB sensors. We also showed that performing aptamer immobilization under low ionic strength conditions rather than conventional high ionic strength buffer greatly improves E-AB sensor performance. We successfully tested our approach with three different small-molecule-binding aptamers, demonstrating its generalizability. On the basis of these results, we believe our electrode fabrication approach will accelerate development of high-performance sensors with the sensitivity required for real-world analytical applications. This work was published in *ACS Applied Materials and Interfaces* (**2021**, 13, 9491–9499).

2.2 DNA Aptamer–Cyanine Complexes as Generic Colorimetric Small-Molecule Sensors. Aptamers are promising biorecognition elements for sensors. However, aptamer-based assays often lack the requisite levels of sensitivity and/or selectivity because they typically employ structure-switching aptamers with attenuated affinity and/or utilize reporters that require aptamer labeling or which are susceptible to false positives. Dye-displacement assays offer a label-free, sensitive means for overcoming these issues, wherein target binding liberates a dye that is complexed with the aptamer, producing an optical readout. However, broad utilization of these assays has been limited. Here, we demonstrate a rational approach to develop colorimetric cyanine dye-displacement assays that can be broadly applied to DNA aptamers regardless of their structure, sequence, affinity, or the physicochemical properties of their targets. Our approach should accelerate the development of mix-and-measure assays that could be applied for diverse analytical applications. This work was published in *Angewandte Chemie International Edition* (**2022**, 61, e202112305).

2.3 Platinum-Nanoparticle-Modified Single-Walled Carbon Nanotube-Laden Paper Electrodes. Platinum nanostructures have been used as electrocatalysts on various electrode substrates for applications such as molecular sensing and fuel storage. Lightweight and flexible paper-based devices embedded with micro- or nanoscale metallic electrodes could prove highly useful for wearable devices and other portable applications. We here demonstrate that nanometer-thickness single-walled carbon nanotube (SWCNT)-laden paper prepared via ambient vacuum filtration offers an excellent conducting electrode substrate for the deposition of platinum through either electrodeposition or vacuum filtration. Characterization of the resulting paper electrodes reveals the formation of crystalline, interspersed, discrete spiky platinum nanoclusters (PtNCs) on the SWCNT film fabricated through electrodeposition, while those made via vacuum filtration feature interconnected spherical platinum nanoparticles (PtNPs). Using methanol as a benchmark molecule, we demonstrate that PtNP-SWCNT paper electrodes prepared via vacuum filtration have higher electrocatalytic efficiency compared to electrodes made via electrodeposition, and this is most likely due to the greater electroactive surface area of the PtNP-SWCNT paper electrode. This combination

of the superior catalytic properties of platinum nanostructures with the flexible, thin, and conductive SWCNT paper substrate could prove highly valuable for the low-cost manufacturing of high-quality, disposable electrodes for a variety of applications. This work was published in *ACS Applied Nano Materials* (**2021**, 4, 13798-13806).

2.4 High-throughput quantitative binding analysis of DNA aptamers using exonucleases. Aptamers are nucleic acid bioreceptors that have been used in various applications including medical diagnostics and as therapeutic agents. Identifying the most optimal aptamer for a particular application is very challenging. Here, we for the first time have developed a high-throughput method for accurately quantifying aptamer binding affinity, specificity, and cross-reactivity via the kinetics of aptamer digestion by exonucleases. We demonstrate the utility of this approach by isolating a set of new aptamers for fentanyl and its analogs, and then characterizing the binding properties of 655 aptamer–ligand pairs using our exonuclease digestion assay and validating the results with gold-standard methodologies. These data were used to select optimal aptamers for the development of new sensors that detect fentanyl and its analogs in different analytical contexts. Our approach dramatically accelerates the aptamer characterization process and streamlines sensor development, and if coupled with robotics, could enable high-throughput quantitative analysis of thousands of aptamer–ligand pairs. This work was published in *Nucleic Acids Research* (**2023**, 51, e19. https://doi.org/10.1093/nar/gkac1210).

2.5 A Suite of Aptamer-Based Sensors for the Detection of Fentanyl and its Analogs. Fentanyl and its analogs are potent synthetic opioids that are commonly abused and are currently the number one cause of drug overdose death in the United States. The ability to detect fentanyl with simple, rapid, and low-cost tools is crucial for forensics, medical care, and public safety. Conventional on-site testing options for fentanyl detection—including chemical spot tests, lateral flow immunoassays, and portable Raman spectrometers—each have their own unique flaws that limit their analytical utility. Here, we have developed a series of new aptamer-based assays and sensors that can detect fentanyl as well as several of its analogs in a reliable, accurate, rapid, and economic manner. These include colorimetric, fluorescent, and electrochemical sensors, which can collectively detect and quantify minute quantities of fentanyl and many of its analogs with no response to other illicit drugs, cutting agents, or adulterants—even in interferent-ridden binary mixtures containing as little as 1% fentanyl. Given the high performance of these novel analytical tools, we foresee the potential for routine use by medical and law enforcement personnel as well as the general public to aid in rapid and accurate fentanyl identification. This work was published in *ACS Sensors* (https://doi.org/10.1021/acssensors.2c02463).

2.6 Ultrasensitive Fentanyl Detection in Biomatrices with High Affinity Aptamers Isolated via Kinetic-SELEX. Pharmaceutical drugs are often dosed based on a 'one size fits all' approach, even though factors such as health status necessitate alternative dosing regimens. Methods that can quantify the concentration of drugs at the bedside will facilitate therapeutic drug monitoring and personalized drug dosing. A commonly used drug with an established need for monitoring is fentanyl, an opioid used to relieve pain and induce anesthesia with a very narrow therapeutic index. Existing methods for fentanyl detection in biological samples, such as immunoassays and mass spectrometry, are highly sensitive, but they are unable to deliver information in a timely manner that can initiate and expedite rescue efforts (in the case of overdose) or aid healthcare teams in making critical decisions (i.e., during surgery). To address these problems, we here develop a series of aptamer-based sensors that can detect fentanyl rapidly, sensitively,

and specifically. First, we isolate new high-affinity DNA aptamers that specifically bind to fentanyl under physiologically relevant conditions using the systematic evolution of ligands by exponential enrichment (SELEX) technique. Then, we characterize the binding properties of the resulting aptamers using isothermal titration calorimetry and an exonuclease digestion assay. We finally use these aptamers to develop colorimetric, fluorescence, and electrochemical sensors that can detect medically relevant concentrations (i.e., low nanomolar) of fentanyl in biological matrices such as human urine, saliva, and serum. Given the exceptional performance of these sensors, we anticipate them maturing into point-of-care devices that can enable rapid fentanyl detection in real-world environments for diagnostics and therapeutic drug monitoring. This work was submitted to *Angewandte Chemie International Edition* and is currently under revision.

2.7 Exonuclease Digestion for Aptamer Characterization, Engineering, and Sensing. Aptamers are short single-stranded nucleic acids typically 10 – 100 nucleotides in length that bind to specific molecules with high affinity. They are isolated from random libraries through an in vitro method termed systematic evolution of ligands by exponential enrichment to bind a variety of targets such as nutrients, metabolites, drugs, toxins, metal ions, proteins, and cells. Aptamers have several appealing qualities over antibodies that have made them widely used as biorecognition elements in sensors for applications including medical diagnostics, environmental monitoring, food safety, and forensic analysis. However, while aptamer sensors have made several great strides in terms of sensitivity, specificity, turnaround time, and ease of use, there remain several gaps that hinder the broad adoption of these sensors for practical use. These challenges include the inadequate sensitivity of aptamer sensors, limitations in aptamer binding characterization to identify the most optimal candidates, and the costly and labor-intensive aptamer engineering for use in sensors. In this account, we describe our successes in using nucleases, enzymes that process nucleic acids like DNA, to address these problems. Nearly five years ago, our laboratory began our initial work with nucleases to enhance the sensitivity of split aptamer sensors via enzyme assisted target recycling. While performing these studies, we discovered for the first time that the digestion of DNA aptamers by exonucleases, which occurs unimpeded in the absence of target, is inhibited when a ligand is bound to an aptamer. This finding served as the foundation for the development of three novel aptamer-related methodologies in our laboratory. First, we used exonucleases as a means of intelligently truncating non-essential nucleotides from aptamers to generate structure-switching aptamers in a single step, which greatly simplified the aptamer engineering process. Second, we used exonucleases to develop a novel label-free aptamer-based detection platform that can utilize aptamers straight from in vitro selection without any prior sequence engineering to detect arbitrary analytes with ultra-low background levels and high sensitivity. Through this approach, we were able to detect analytes at nanomolar levels in biological samples with the added capability of performing simultaneous multitarget detection. Third, we used exonucleases to develop a high throughput means of characterizing the binding profile of aptamers (e.g. affinity and specificity) to a variety of ligands, which has allowed for more comprehensive analysis of aptamers in terms of greatly increasing number of candidates identified from in vitro selection that can be characterized as well as the number of ligands that can be tested for an aptamer in a single experiment. We have demonstrated the success of this approach for identifying new mutant aptamers with augmented binding properties (e.g., a highly specific adenosine aptamer engineered from the well-known cross-reactive ATP aptamer) and to even quantify aptamer-target affinity. We believe our enzymatic technologies significantly streamline the aptamer characterization and sensor development process and, with the adoption of robotics or liquid handling systems, should enable rapid identification of the most suitable aptamers from hundreds of

candidates found through SELEX or mutagenesis screens for a particular application with just a single experiment. This work was submitted to *Accounts of Chemical Research* and is currently under revision.

2.8 Developing aptamer-based colorimetric opioid tests. Opioids are psychoactive drugs that have analgesic and sedative effects and are prone to misuse and abuse. The detection of opioids in seized drug samples is currently achieved using chemical tests such as the Marquis test which, although it is rapid and simple to perform, is highly prone to false results. Aptamers are short oligonucleotides that bind to targets with high affinity and specificity. They are isolated from random libraries through an in vitro technique termed systematic evolution of ligands by exponential enrichment. Here, we used SELEX to isolate DNA aptamers for natural and semi-synthetic opioids of the morphinan family. First, we utilized a parallel-and-serial selection strategy to isolate aptamers that bind the opioids heroin and morphine. Then, we utilized a toggle-selection approach to isolate aptamers for the targets, oxycodone and oxymorphone. After sequencing the enriched pools from these selections, we identified several candidate aptamers, which we screened using our recently reported exonuclease digestion fluorescence assay. We discovered seven aptamers that bind to heroin and morphine strongly with high affinity and with the capability to recognize codeine as well as three aptamers that bind to oxycodone and oxymorphone with cross-reactivity to hydrocodone and hydromorphone. Notably, these aptamers are highly specific and do not bind to other common drugs of abuse, cutting agents, adulterants, and, importantly, even opioid antagonists such as naloxone. Using isothermal titration calorimetry, we found the heroin/morphine aptamers have target-binding affinities of 2 – 6 μM and the oxycodone/oxymorphone aptamers have target-binding affinities of 0.5 – 3 μM. Finally, we utilized these aptamers to develop electrochemical aptamer-based sensors that can detect these opioids at nanomolar levels with high specificity. These sensors should prove useful for expediting the screening of opioids in seized substances, thereby increasing the efficacy of drug interdiction. This work was submitted to *Angewandte Chemie International Edition* and is currently under review.

2.9 Aptamer isolation and sensor development for remifentanil, and 3-cis methyl fentanyl. Fentanyl is a synthetic opioid that has analgesic and sedative properties and is approximately 100-fold more potent than morphine. In 2022, nearly 100,000 people died from fentanyl overdose in the United States, far outnumbering deaths relative to other drugs such as heroin, cocaine, and methamphetamine. Fentanyl is part of a drug family of which analogs share the 4-anilinopiperidine core structure. There have been almost one hundred unique fentanyl analogs identified thus far; several of them are 10-100-fold more potent than fentanyl. The detection of fentanyl analogs is important for diagnosing opioid overdose and identifying such drugs in seized substances for forensic purposes. However, existing screening methods for fentanyl, such as lateral flow immunoassays, can detect only a handful of analogs. Aptamers are nucleic acid affinity reagents that have the capability to address this issue. They are isolated from random oligonucleotide libraries to bind specific molecules with high affinity. We have recently isolated DNA aptamers that can specifically bind fentanyl and 17 of its analogs with high affinity, such as acetyl fentanyl, furanyl fentanyl, and *p*-fluoroisobutyryl fentanyl, which are analogs that primarily vary at the anilino and amide moiety. However, these aptamers are unable to recognize analogs with modifications at other sites, such as the piperidine ring and the phenethyl moiety. To address this issue, in this work we performed SELEX with a parallel-and-serial selection strategy to isolate aptamers cross-reactive to fentanyl analogs modified at these moieties using the targets 3-methylfentanyl, remifentanil, and alfentanil. After several rounds of selection against these targets individually in parallel, we combined the enriched pools and performed selection with

each target in series. After selection, we performed high-throughput sequencing to identify aptamer candidates and discovered that the resulting aptamers have high specificity for 3-methylfentanyl but do not bind the other two targets. In light of this, we continued selection with the remifentanil and alfentanil pools to isolate aptamers specific for these targets. After high-throughput sequencing of these enriched pools, we identified several aptamer candidates and screened their binding affinity and specificity using an exonuclease digestion fluorescence assay. We identified aptamers that have high affinity and specificity respectively for 3-methylfentanyl, remifentanil, and alfentanil. Isothermal titration calorimetry results for the 3-methylfentanyl aptamers indicate they bind with nanomolar affinity. We will continue to characterize other aptamers using isothermal titration calorimetry and then utilize top-performing aptamers to develop sensors for the detection of these fentanyl analogs. This work is being prepared for submission.

3. Conclusion

The overall goal of the proposed project is to develop aptamer-based assays that can perform rapid, on-site detection of opioid families in seized substances with high sensitivity and specificity. We have successfully produced 1) a new DNA aptamer that cross-reactively binds to fentanyl and at least 15 analogs sharing the 4-anilidopiperidine core structure with high affinity and excellent specificity; 2) two new aptamers that bind to seven morphine-related opiods sharing the N-methyl-4,5-epoxymorphinan core structure with nanomolar affinity; 3) electrochemical aptamer-based sensors for sensitive and specific analyte detection of opioids in seized substances with superior specificity against interferents and greater target-cross-reactivity than all existing antibodies; and 4) a new aptamer-based dye-displacement assay that sensitively screens for fentanyl and its analogs with naked eye. 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 opioid families.

4. Impact on the criminal justice system

The E-AB sensor represents an ideal solution for combatting the current opioid epidemic. Our E-AB sensor is designed to be generically sensitive to both morphine-related opioids as well as fentanyl and its analogs, producing a qualitative “yes-or-no” readout for the presence of such opioids in seized substances. Testing is simple and can be performed by dissolving a small quantity of sample into a few drops of buffer and dipping the electrode into the solution; a readout can be obtained within seconds. Such a tool would be highly valuable for law enforcement and forensic personnel. This sensor will be superior to current methods, as it is more specific than chemical spot tests, less prone to inconclusive results than handheld Raman spectrometers, and more cross-reactive to opioids than lateral-flow immunoassays. Additionally, our assay is label-free and the cost ($1 per test) is much lower than handheld Raman spectrometers and antibody-based immunoassays due to the inexpensive process of aptamer synthesis and electrode fabrication. We therefore envision that our E-AB sensor will most likely replace existing presumptive tests for natural, semi-synthetic, and synthetic opioids for forensic purposes. Upon the success of this proposal, our plan is to expand our SELEX strategy and sensing platform for the simultaneous screening of other families of illicit drugs, such as synthetic cannabinoids, tryptamines, substituted amphetamines, and benzodiazepines, greatly improving the efficiency of drug interdiction and collection of drug intelligence.

5. Impact on Technology Transfer

One patent has been issued in 2022 and one patent provision was filed in 2023 .

1. Xiao Y. & Canoura J. “Aptamer-based sensors for detection of fentanyl opioids”. 2022, US11408850B2.
2. Xiao Y. & Canoura J. “Development of aptamer-based sensors for detection of fentanyl in biomatrices”. 2023, Patent provision.

6. Publications and Presentations

6.1 Publications and manuscripts in progress:

1. Wang L.L., Canoura J. & Xiao Y. Aptamer isolation and sensor development for alfentanil, remifentanil, and 3-cis methyl fentanyl. *In preparation.*
2. Canoura J., Alkhamis O., Venzke M., Ly P.T. & Xiao Y. Developing aptamer-based colorimetric opioid tests. *Under review for Angew. Chem. Int. Ed.*, (IF: 16.82).
3. Alkhamis O., Canoura J., Ly P.T. & Xiao Y. (2023) Exonuclease digestion assays for aptamer characterization, engineering, and sensing. *Under revision for Acc. Chem. Res.* (IF: 24.47)
4. Canoura J., Liu Y.Z., Alkhamis O., Willis C., Perry, J. & Xiao Y. (2023) Ultrasensitive Fentanyl Detection in Biomatrices with High Affinity Aptamers Isolated via Kinetic-SELEX. *Under revision for Angew. Chem. Int. Ed.*, (IF: 16.82)
5. Canoura J., Liu Y.Z., Willis, C., Perry, J. & Xiao Y.* (2023) A suite of aptamer-based sensors for fentanyl and analog detection. *ACS Sensors Accepted* (IF: 9.618)
6. Canoura J., Alkhamis O., Liu Y.Z., Willis C. & Xiao Y. High-throughput quantitative binding analysis of DNA aptamers using exonucleases. *Nucleic Acids Res.*, 2022, *https://doi.org/10.1093/nar/gkac1210* (IF: 19.16)
7. Paudyal J., Wang P., Zhou F., Liu Y.Z., Cai Y. & Xiao Y. Platinum-nanoparticle-modified single-walled carbon nanotube-laden paper electrodes for electrocatalytic oxidation of methanol. *ACS Appl. Nano Mater*. 2021, 4, 13798 – 13806 (IF: 5.64)
8. Alkhamis O., Canoura J., Bukhryakov K.V., Tarifa A., DeCaprio A.P. & Xiao Y. DNA aptamer-cyanine complexes as generic colorimetric small-molecule sensors. *Angew. Chem. Int. Ed.*, 2022, 61, e202112305 (IF: 16.82)
9. Liu Y.Z., Canoura J, Alkhamis O. & Xiao Y. Immobilization Strategies for Enhancing Sensitivity of Electrochemical Aptamer-Based Sensors. *ACS Appl. Mater. Interfaces.* 2021, 13, 9491 – 9499 (IF: 10.38)

6.2 Presentations:

1. Canoura, J. & Xiao, Y. (2023) Isolation of fentanyl-binding aptamers and construction of aptamer-based sensors for the detection of fentanyl and its analogs. Poster presentation, Pittcon Conference & Expo, Philadelphia, PA, March 18–22.
2. Xiao Y. (2023) Development of aptamer-based sensors for rapid opioid detection in seized substances. Invited talk, Pittcon Conference & Expo, Philadelphia, PA, March 18–22.
3. Xiao Y. (2023) Exonuclease digestion assay for quantifying binding affinity of aptamer to ligands. Invited talk, Pittcon Conference & Expo, Philadelphia, PA, March 18–22.
4. Canoura, J. & Xiao, Y. (2021) Accelerating aptamer characterization, engineering and sensor development using exonuclease digestion. Poster presentation, NCSU Virbela recruitment weekend, March 12.
5. Canoura, J. & Xiao, Y. (2021) Accelerating aptamer characterization, engineering and sensor development using exonuclease digestion. Poster presentation, Triangle Student Research

Competition, Durham, NC, October 7.

6. Canoura, J., Liu, Y. & Xiao, Y. (2021) Electrochemical aptamer-based sensor platform for detection of fentanyl and its analogs. Oral presentation, Crossing Forensic Borders Global Webinar Series, April 14.
7. Xiao Y., Canoura J. & Liu Y.Z. (2021) Development of Aptamer-Based Sensors for Sensitive and Specific Detection of Fentanyl Opioids. Invited talk, Pittcon Conference & Expo, Zoom, March 11.
8. Xiao Y. & Alkhamis O. (2021) Bring biosensors onsite – aptamer isolation, engineering, and aptamer-based assays. Poster presentation, NC State Virbela Recruitment Weekend, March 12.