Prescription drug overdose and abuse is a leading cause of death in the United States. It is a serious issue and has become increasingly problematic as opioids are being prescribed with a higher frequency. For this reason, fast, accurate detection of small drug molecules is crucial. The current standard for use in clinical drug detection is an enzyme-linked immunosorbent assay (ELISA) that uses a series of antibodies to bind to the target drug and enable quantification via a colorimetric output. However, the antibodies used in an ELISA often cannot distinguish between similar molecules. They are generated in vivo, causing them to have a limited potential target scope as well as being costly. Deoxyribonucleic acids (DNA) can have a wide variety of functions outside of simply encoding genetic information. Aptamers are short sequences of DNA that are capable of binding target small-molecules. They have emerged as a promising alternative to antibodies, as they are generated in vitro, where negative selections can be used to increase target selectivity. These aptamers can be cleaved to make split aptamers that only assemble in the presence of the target small molecule.

We developed a method of detection analogous to that of an ELISA where the cocaine split aptamer is used in conjunction with Split Aptamer Ligation (StAPL) technology. The detection is dependent upon a cycloaddition reaction that joins the two split aptamer fragments together and is carried out on a microplate. An attached biotin/streptavidin-horseradish peroxidase complex allows for a colorimetric output upon addition of TMB substrate. We successfully used this system to detect varying concentrations of cocaine in buffer and biological fluids.

We then investigated the problem of limited numbers of split aptamers for small-molecule targets. While there are many known aptamers, there are very few known split aptamers that bind small-molecules. This research generated four new steroid binding split aptamers from their three-way junction counterparts. Optimization, sequence changes, and selectivity of these new split aptamers were explored. We successfully demonstrated a reliable method of separating aptamers with a privileged structure to generate new split aptamers for more targets.

Finally, we worked towards making the split aptamer ligation system compatible with qPCR. This would allow for a semi automatable system of detection, which would be more useful for potential clinical applications. In order to accomplish this, we altered the covalent linkage from a cycloaddition adduct to a morpholino linkage. This, as well as a primer binding region on the 5’ end of one of the split aptamer fragments, allowed the ligated split aptamer to be PCR readable. This was exciting progress in the effort toward creating a semi automatable system of detection for small drug molecules using split aptamers. Further research will focus on developing this system and exploring its range of potential applications.

In conclusion, we have made strides toward the development of DNA based biosensors for use in clinical drug detection.