

## Developing Novel On-Site Handheld Biosensors for PFAS Constituents

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**Background/Objectives.** Samples from sites contaminated with emerging contaminants like per- and polyfluoroalkyl substances (PFAS) must be sent for off-site analysis in order to measure contamination levels. Current analytical methods mostly employ lab-based liquid chromatography mass spectrometry (LCMS) which have the advantage of high sensitivity and low detection limits but suffer from being expensive, time-intensive (usually 2- to 3-week turnaround time), and require specialized personnel for operation. While this is inconvenient for site characterization and limits the ability to perform a “triad”-style expedited approach, it is even more troublesome when it comes to measuring treatment system effluent because exceedances will not be known for weeks after the sample is collected. Portable tools that can allow for real-time, inexpensive measuring of contaminants in the field are therefore highly desirable.

**Approach/Activities.** A program is underway to develop biosensors that enable in-field, or non-laboratory based, detection of PFAS. The objective is biosensor-based detection that is sensitive and specific without interference from non-target PFAS compounds or common PFAS co-contaminants. Literature precedent suggests Bioaffinity based biosensors (BRBs) can achieve PFAS LODs in the ppt range when used in the context of surface plasmon resonance-based optodes. Accordingly, Allonnia’s BRB work aims to deliver sensors that bind target PFAS with sufficient affinity and specificity to enable PFAS detection in the ppt range. A highly diverse library of about  $10^{12}$  protein variants with the potential for PFAS binding was constructed and screened for binding of PFOA. Three selection methods, Ribozyme display, Phage display and Yeast display, were used to narrow down the library size and to select for PFOA binders. Binding affinity of the top hits were measured using Octet and Surface Plasmon Resonance (SPR).

**Results/Lessons Learned.** In this presentation we will describe Allonnia’s approach to development of versatile biosensor tool that fulfils an unmet market need. Biosensors can convert a bio-signal from a highly specific interaction between the target contaminant and into a measurable electrochemical response to detect contaminants with high sensitivity. Using the above-described selection methods, we were able to identify around 70 proteins, out of the library of  $10^{12}$  proteins, that showed binding affinity for PFOA. Dynamic range was determined using competitive ELISA during which we ascertained that the top two hits were able to detect PFOA at concentrations of <500ppt (0.5ppb). Specificity binding to PFOA as compared to octanoic acid and found that the top hits have around 78-fold higher binding specificity to PFOA as compared to octanoic acid (a common non PFAS co-contaminant). Currently, we are in the process of developing a biosensor that is easy to operate, robust, rapid (short response time), and portable detection tools with high selectivity and specificity. Biosensors are more efficient and economical as they allow for direct detection of the analyte without pretreatment or with minimal sample pretreatment and eliminate the costs associated with sample shipment.