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Developing Novel On-Site Handheld Biosensors for PFAS Constituents

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Sixth International Symposium on Bioremediation and Sustainable Environmental Technologies
May 8-11, 2023| Austin, Texas

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Transformative biology for PFAS Biosensor

DISCOVER

BIOINFORMATICS

DEVELOP

TRANSFORMATIVE BIOLOGY

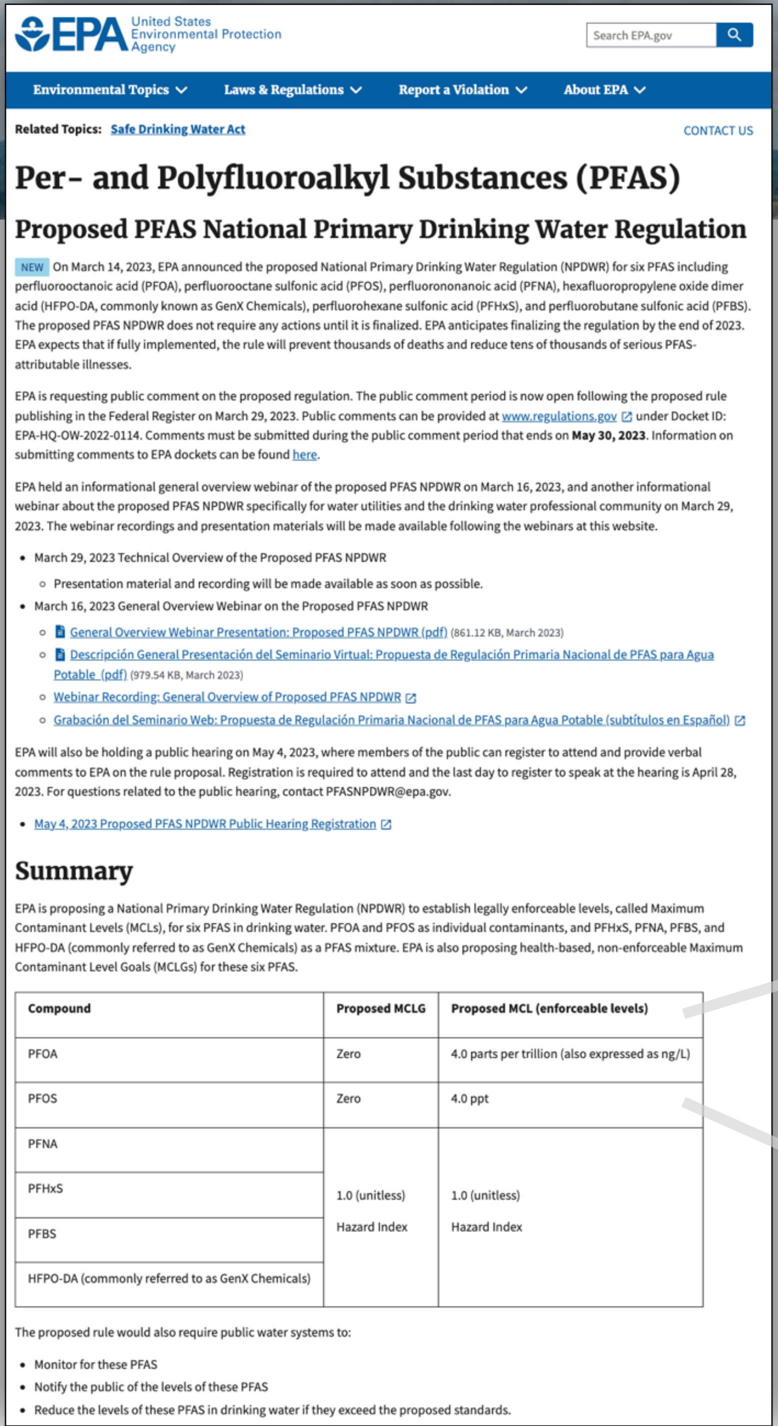
DEPLOY

INTEGRATED SYSTEMS

What is PFAS?

- Concerns around PFAS:
  - Hard to degrade (C-F bond is hard to break)
  - Moves though soil and contaminate water sources
  - Bioaccumulation
- Found in serum of nearly all people tested (https://www.cdc.gov/exposurereport/)

EPA proposal on PFAS levels



EPA United States Environmental Protection Agency

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Related Topics: Safe Drinking Water Act

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Per- and Polyfluoroalkyl Substances (PFAS)

Proposed PFAS National Primary Drinking Water Regulation

NEW On March 14, 2023, EPA announced the proposed National Primary Drinking Water Regulation (NPDWR) for six PFAS including perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), hexafluoropropylene oxide dimer acid (HFPO-DA, commonly known as GenX Chemicals), perfluorohexane sulfonic acid (PFHxS), and perfluorobutane sulfonic acid (PFBS). The proposed PFAS NPDWR does not require any actions until it is finalized. EPA anticipates finalizing the regulation by the end of 2023. EPA expects that if fully implemented, the rule will prevent thousands of deaths and reduce tens of thousands of serious PFAS-attributable illnesses.

EPA is requesting public comment on the proposed regulation. The public comment period is now open following the proposed rule publishing in the Federal Register on March 29, 2023. Public comments can be provided at www.regulations.gov under Docket ID: EPA-HQ-OW-2022-0114. Comments must be submitted during the public comment period that ends on **May 30, 2023**. Information on submitting comments to EPA dockets can be found here.

EPA held an informational general overview webinar of the proposed PFAS NPDWR on March 16, 2023, and another informational webinar about the proposed PFAS NPDWR specifically for water utilities and the drinking water professional community on March 29, 2023. The webinar recordings and presentation materials will be made available following the webinars at this website.

- March 29, 2023 Technical Overview of the Proposed PFAS NPDWR
  - Presentation material and recording will be made available as soon as possible.
- March 16, 2023 General Overview Webinar on the Proposed PFAS NPDWR
  - General Overview Webinar Presentation: Proposed PFAS NPDWR (pdf) (861.12 KB, March 2023)
  - Descripción General Presentación del Seminario Virtual: Propuesta de Regulación Primaria Nacional de PFAS para Agua Potable (pdf) (979.54 KB, March 2023)
  - Webinar Recording: General Overview of Proposed PFAS NPDWR
  - Grabación del Seminario Web: Propuesta de Regulación Primaria Nacional de PFAS para Agua Potable (subtítulos en Español)

EPA will also be holding a public hearing on May 4, 2023, where members of the public can register to attend and provide verbal comments to EPA on the rule proposal. Registration is required to attend and the last day to register to speak at the hearing is April 28, 2023. For questions related to the public hearing, contact PFASNPDWR@epa.gov.

- May 4, 2023 Proposed PFAS NPDWR Public Hearing Registration

Summary

EPA is proposing a National Primary Drinking Water Regulation (NPDWR) to establish legally enforceable levels, called Maximum Contaminant Levels (MCLs), for six PFAS in drinking water. PFOA and PFOS as individual contaminants, and PFHxS, PFNA, PFBS, and HFPO-DA (commonly referred to as GenX Chemicals) as a PFAS mixture. EPA is also proposing health-based, non-enforceable Maximum Contaminant Level Goals (MCLGs) for these six PFAS.

Compound	Proposed MCLG	Proposed MCL (enforceable levels)
PFOA	Zero	4.0 parts per trillion (also expressed as ng/L)
PFOS	Zero	4.0 ppt
PFNA	1.0 (unitless) Hazard Index	1.0 (unitless) Hazard Index
PFHxS		
PFBS		
HFPO-DA (commonly referred to as GenX Chemicals)		

The proposed rule would also require public water systems to:

- Monitor for these PFAS
- Notify the public of the levels of these PFAS
- Reduce the levels of these PFAS in drinking water if they exceed the proposed standards.

Compound	Proposed MCLG	Proposed MCL (enforceable levels)
PFOA	Zero	4.0 parts per trillion (also expressed as ng/L)

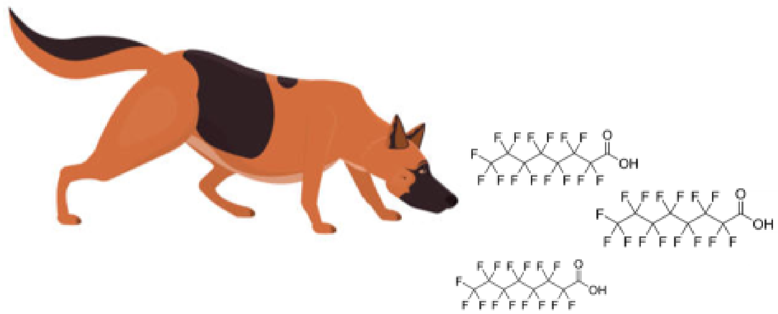
The Challenge:

PFAS can only be detected by use of off-site labs, which are expensive and time-intensive

The Unmet Market Need:

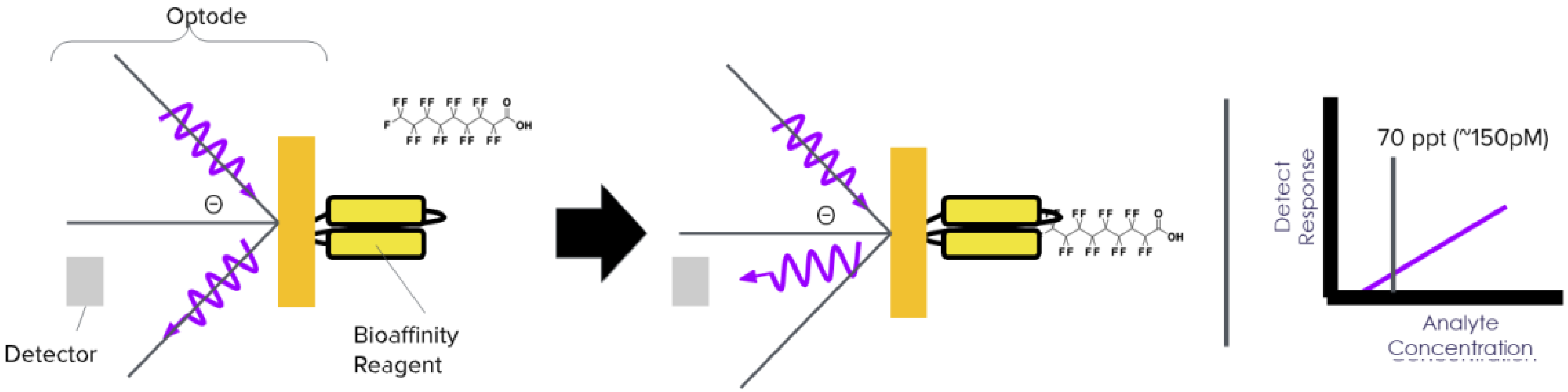
Portable tools for real-time PFAS detection results

Biosensor



- Biosensors are analytical devices that measure biological or chemical reactions proportional to an analyte
- Widely used in human health for quick diagnostics (COVID test)
- A fast, reliable, cost-effective way to detect pollutants

Bioaffinity Reagent-Based Biosensor



Developing a PFAS Biosensor

DETECT DOWN TO PARTS PER TRILLION

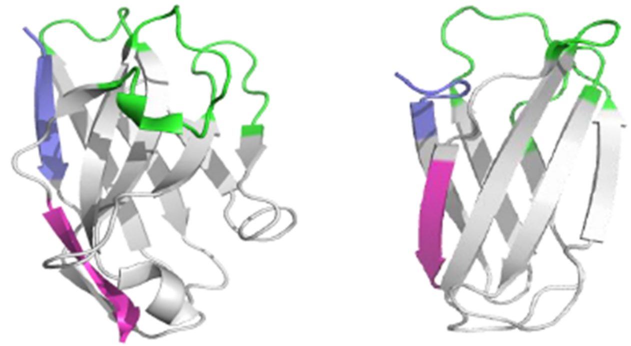
Allonnia's patent pending protein can detect and quantify PFAS to parts per trillion levels

REDUCE LAB COSTS <50%

With quantifiable detection results in the field, samples sent to labs for costly analysis can be greatly reduced

REAL-TIME RESULTS

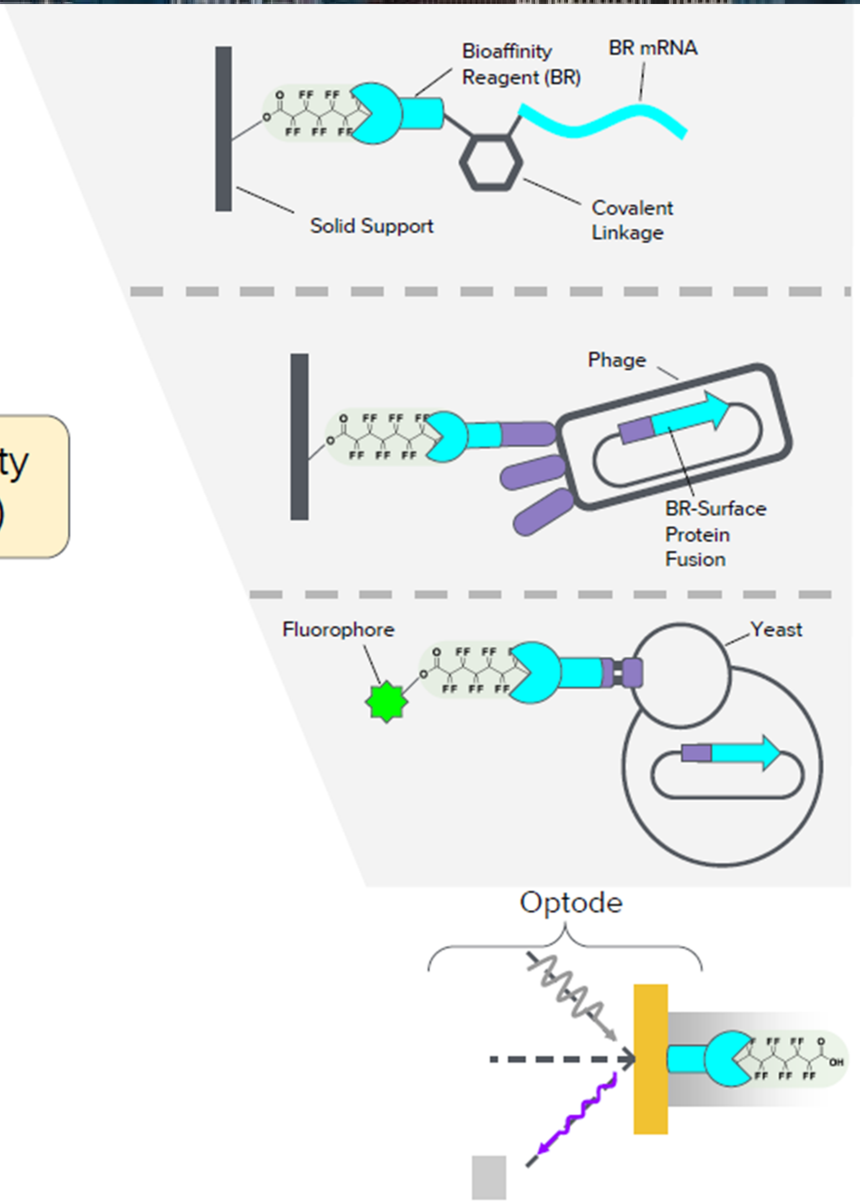
Real-time results facilitate accurate site delineation in fewer mobilizations and more comprehensive monitoring of treatment system performance allonnia



Nanobody   Monobody

Start with a very large library of Bioaffinity Reagents (nanobodies or monobodies)

Objective



Select for binders in a very large (1e12) library using *in vitro* display

↓

Select for binders in a large (1e9) library using phage display

↓

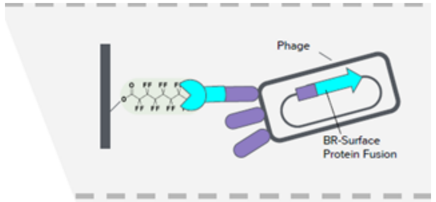
Select for binders in a medium (1e5) library using yeast surface display

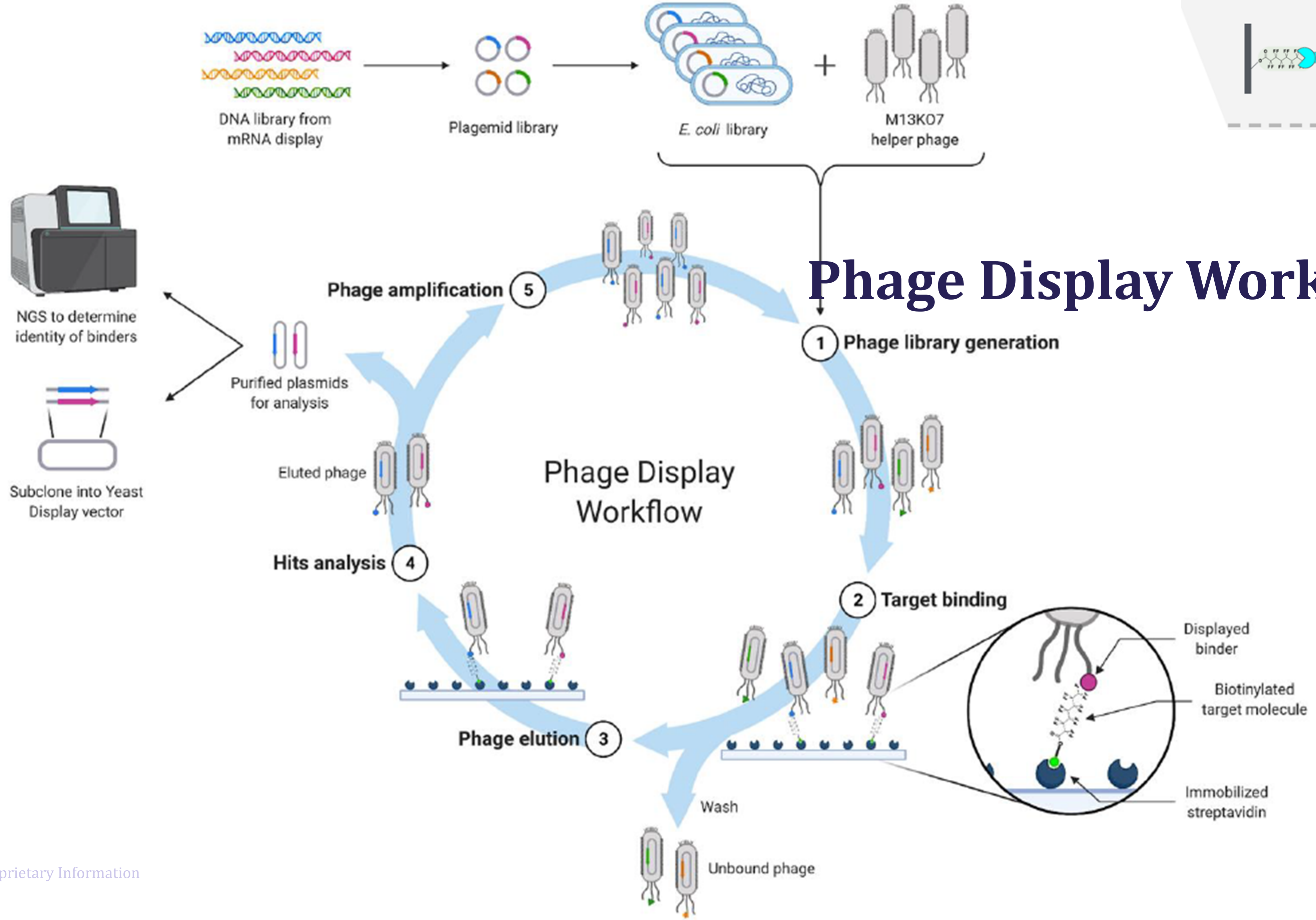
↓

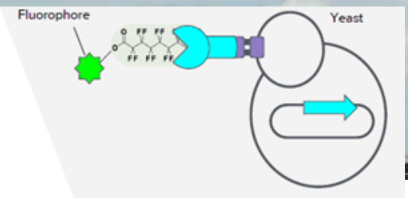
Characterize hits on the Octet or using SPR

Iterate as needed

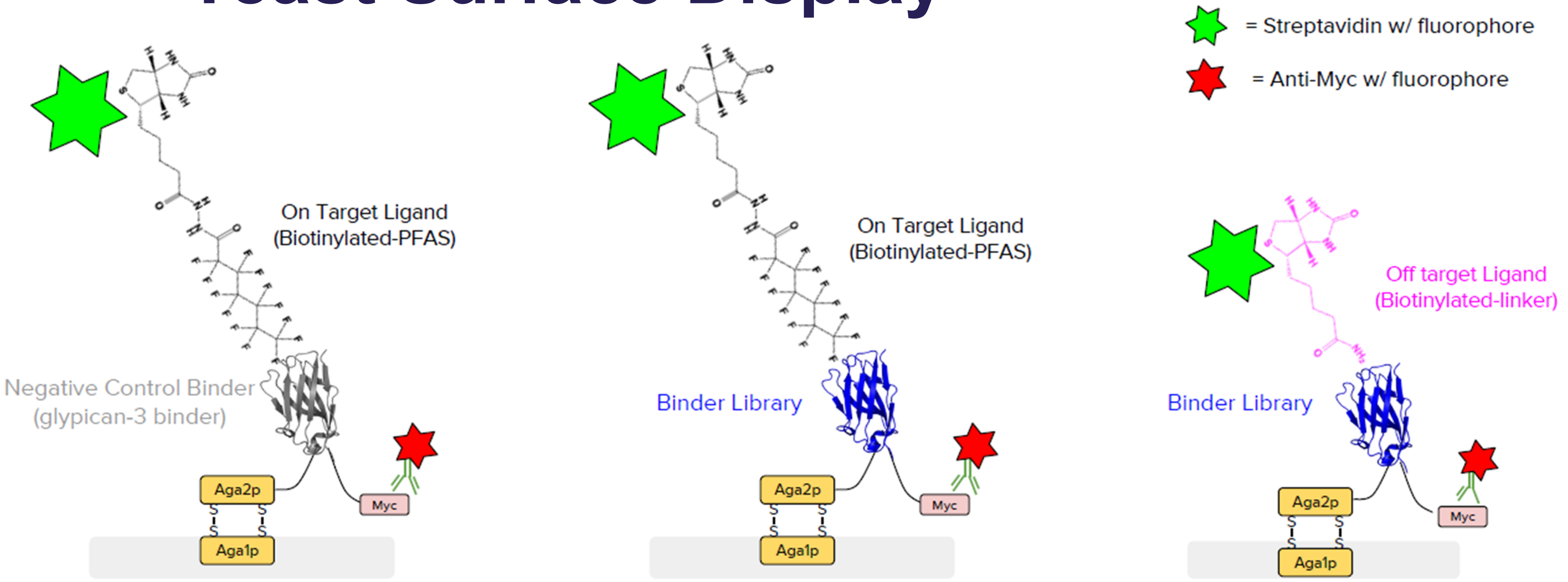
Phage Display Workflow







Yeast Surface Display

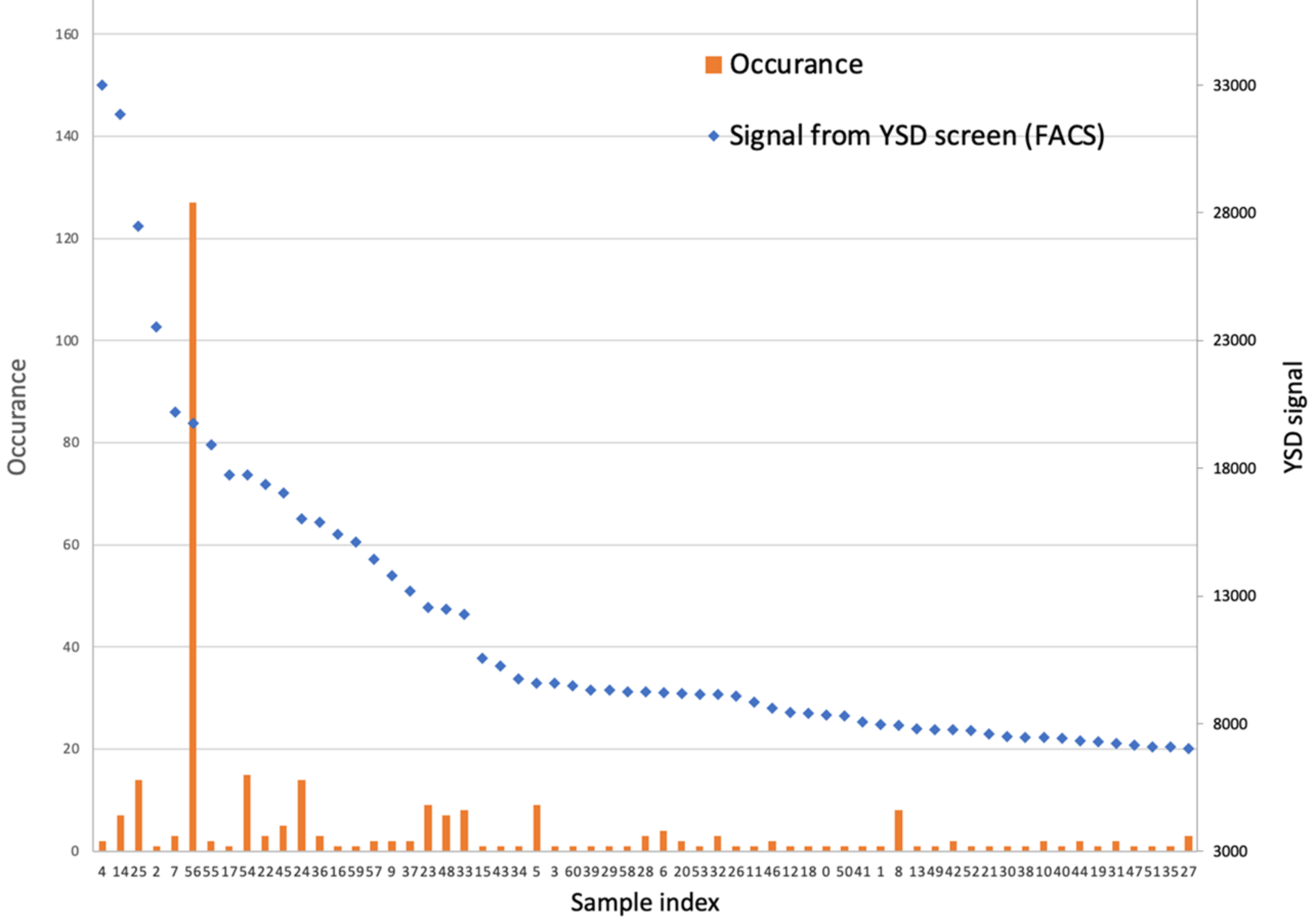


Negative Control Binder
+
On-Target Ligand

Binder Library
+
On-Target Ligand

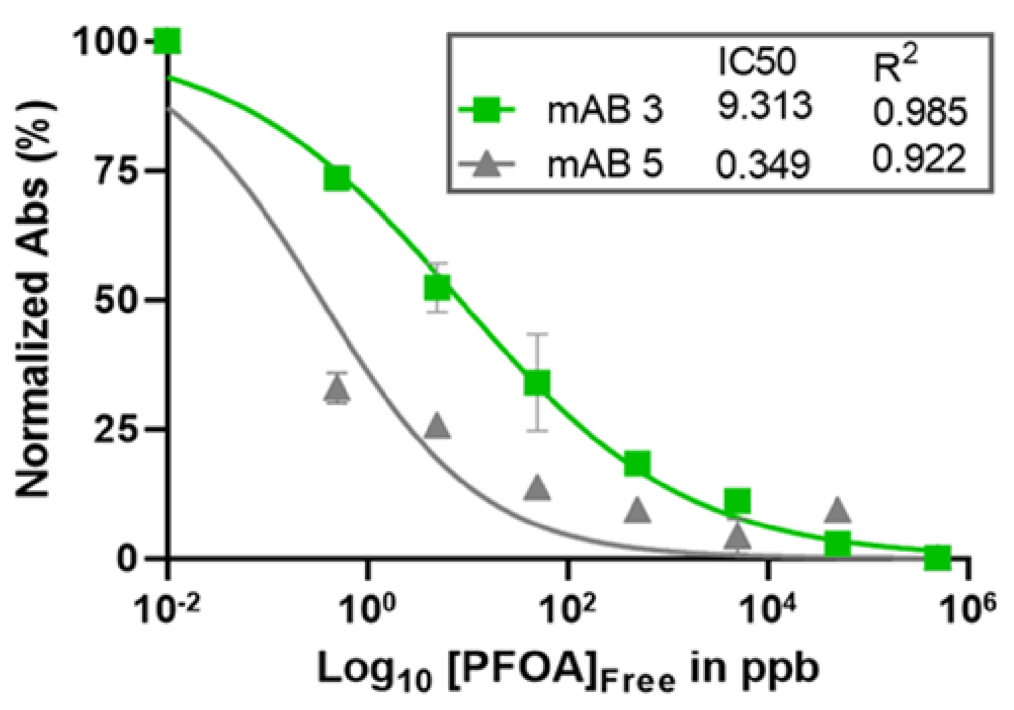
Binder Library
+
Off-Target Ligand

Results of YSD Screen

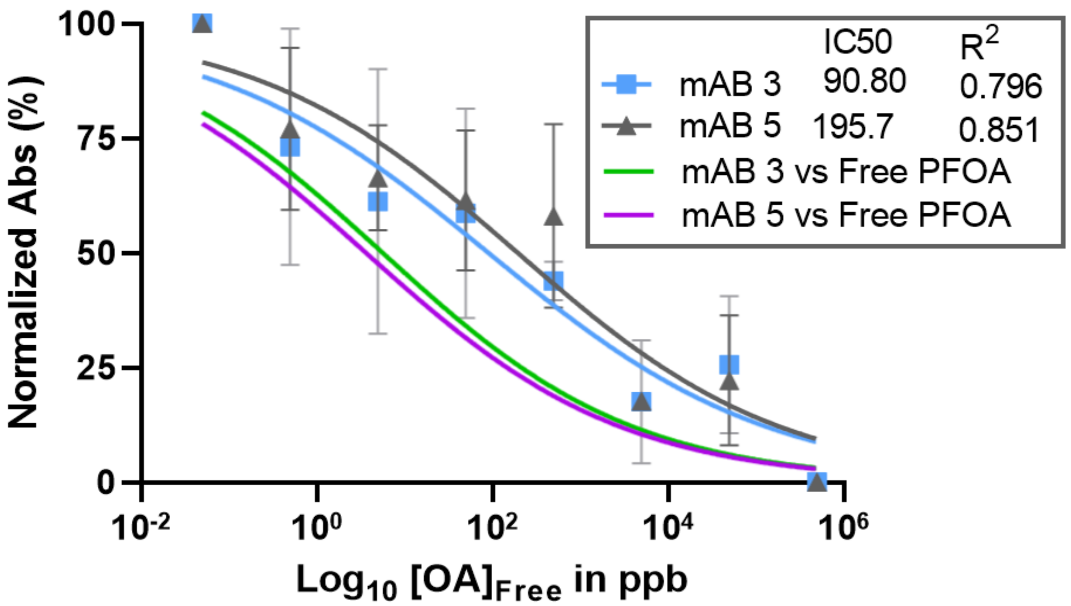


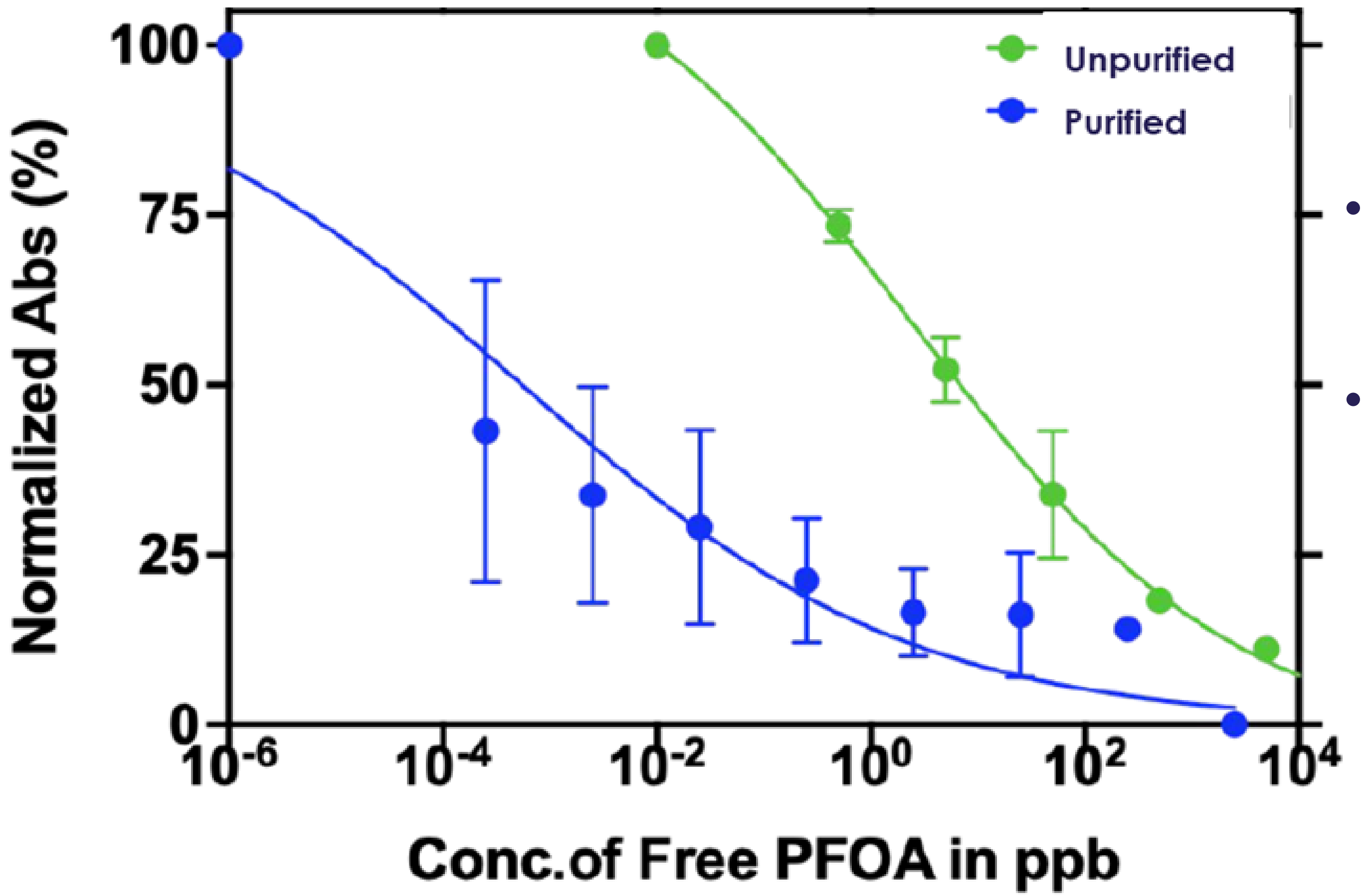
The Results

1. Biotin-PFOA vs Free PFOA



- Discovered 2 proteins that can be used as a PFAS biosensor
- Proteins have higher binding specificity to PFOA vs. OA

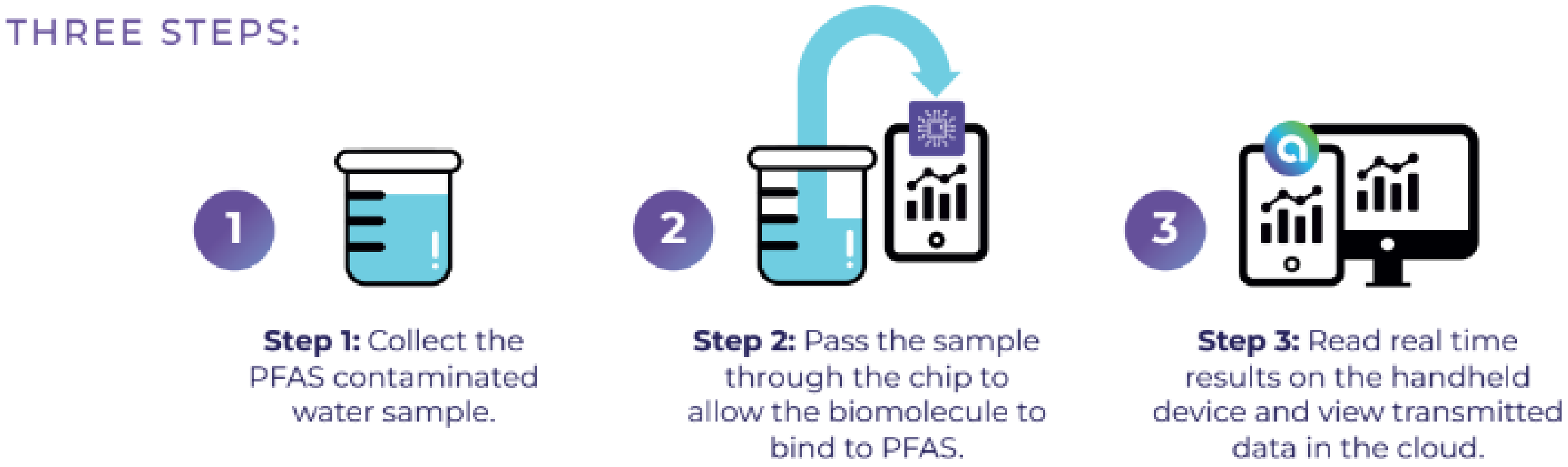




- Purification of the proteins produces better sensitivity
- More testing required in relevant matrices to identify interference due to other groundwater components

The Roadmap

- Develop this transformative bio-solution into a field test kit
- Integrate the sensor with our PFAS removal system (SAFF) and other PFAS remediation systems
- Continue work to increase the sensitivity of the protein and its specificity to PFAS in real world groundwater



Acknowledgement

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Thank you

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