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PFASter Final Report MUDP-project

MUDP

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Environmental Technology Development and Demonstration Programme

The project described in this report is supported by the Environmental Technology Development and Demonstration Program (MUDP) under the Ministry of the Environment, which supports the development, testing and demonstration of environmental technology.

MUDP invests in the development of the environmental technology of the future for the benefit of the climate and environment in Denmark and globally, while strengthening Danish growth and employment. The program supports the broader environmental agenda, including clean water, clean air and safe chemicals, but also supports the government's objectives within climate, biodiversity and circular economy.

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Content

1. Summary

PFASter is a technology development project funded by the Environmental Technology Development and Demonstration Program (MUDP) under the Ministry of the Environment. The project addresses the pressing environmental and public health challenge posed by per- and polyfluoroalkyl substances (PFAS) in Denmark's water systems.

PFAS are a family of synthetic fluorinated compounds that have been widely used in industrial and consumer products for decades due to their chemical stability and water- and grease-resistant properties. However, their persistence in the environment, combined with mounting evidence of their toxicity, has elevated PFAS to a high-priority concern for regulators, water authorities, and the general public. In Denmark and globally, PFAS are now associated with contamination of groundwater, surface water, soils, and even food chains.

Recent studies have documented PFAS exposure in humans through drinking water and food consumption, and have linked this exposure to adverse health effects, including reduced immune response, endocrine disruption, and possible carcinogenicity. In response to these risks, the Danish government has tightened the acceptable threshold for PFAS, such as in drinking water with a combined limit of 2 ng/L. This regulation represents one of the most stringent in Europe and imposes new requirements on detection capabilities, frequency of sampling, and responsiveness of testing systems.

1.1 Problem and Rationale

The detection and monitoring of PFAS are currently hampered by technological and practical limitations. Standard testing relies on highly sensitive laboratory techniques, typically involving liquid chromatography and tandem mass spectrometry (LC-MS/MS). While accurate, these methods are expensive, time-consuming, and only available in a limited number of specialized laboratories. The process of collecting, shipping, and analyzing samples can delay decision-making by days or even weeks, which is particularly problematic when time-sensitive interventions are needed.

Moreover, many municipalities and landowners lack the resources or capacity to conduct large-scale PFAS monitoring, making it difficult to map contamination across broad areas. This creates uncertainty around where PFAS exposure is occurring and how best to prioritize remediation. The absence of a practical, field-level screening tool creates a bottleneck in the national strategy for managing PFAS pollution.

PFASter was conceived as a direct response to this gap. Its aim is to develop a portable, low-cost, and rapid testing solution for detecting elevated PFAS concentrations in water. The vision is to enable non-specialists—such as municipal staff, environmental consultants, or even concerned citizens—to conduct on-site testing using a disposable device that provides an immediate visual result. The tool would serve as a first-line screening instrument, complementing (but not replacing) laboratory-based methods by identifying sites that warrant further investigation.

1.2 Project Objectives

PFASter focuses on the creation of a lateral flow assay (LFA) designed to detect the presence of key PFAS substances at levels relevant to Danish regulatory thresholds. The LFA format, already well-established in medical diagnostics (e.g., pregnancy tests and COVID-19 antigen tests), offers a number of advantages: ease of use, rapid results, affordability, and minimal equipment requirements.

To achieve this, the project is structured around two core objectives:

Objective 1 - Development of Aptamer-Based Detection Chemistry:
Aptamers are short, single-stranded DNA or RNA molecules that can be engineered to bind selectively to target molecules. In PFASter, aptamers are developed to bind with high specificity to the four regulated PFAS compounds. Aptamers are chosen over antibodies due to several key benefits: they are chemically synthesized (eliminating batch variation), stable at room temperature, and can be rapidly tailored to new targets. In environmental testing, these properties are crucial, as field conditions can be variable and demanding.

Objective 2 - Integration into a Functional LFA Device:
The second objective is to incorporate the selected aptamers into a lateral flow strip that enables a visual indication (typically a line or color change) when PFAS are present in a sample above a certain concentration. The assay is designed to be qualitative, serving as a rapid pre-screening tool to determine whether further laboratory testing is warranted.

1.3 Partnership and Roles

The PFASter consortium brings together complementary expertise from both the private sector and academic research institutions:

Pisco ApS (Project lead):
Participants: Jacob Højgaard, Thomas Rayner, Jonas Højgaard, Bent Højgaard.
A Danish SME specialized in sustainable land-based aquaculture, offering consultancy, system design, and innovation services. In this project, Pisco leads coordination while supporting LFA hardware development and testing.

DTU Health Technology – Center for Diagnostics:
Participants: Jesper Sørensen, Ilaria Rocca, Nigel Caterer, Helene Larsen, Ying Jie Ma, Kasama Larsen, Charikleia-Despoina Vagianou, Sanne Berger.
Responsible for the development, selection, and validation of aptamers, including in vitro testing, sequence optimization, and characterization of molecular interactions with PFAS compounds. The center also leads the development and optimization of the LFA format based on selected aptamers, translating molecular findings into a functional prototype for field use.

DTU Sustain:
Participants: Kamilla Kaarsholm, Henrik Andersen.
Provides expertise in environmental chemistry and PFAS analysis. Responsible for validating the test system using real and simulated water samples with known PFAS contamination via LC-MS. This ensures that the aptamer-based assay is benchmarked against established analytical standards.

The collaboration was structured around two primary work packages: WP1 (Aptamer Development) and WP2 (LFA Development). Both tracks progress in parallel, with points of convergence around aptamer integration and early prototyping.

1.4 Relevance of Targeted PFAS Compounds

The project specifically targets the four compounds—PFOA, PFOS, PFNA, and PFHxS—that are regulated under the Danish drinking water threshold. These compounds were chosen because:

- They are among the most commonly detected PFAS in groundwater and surface water.

- They are well-characterized toxicologically and environmentally.
- They have been used historically in fire-fighting foams, textiles, and industrial applications, leading to legacy pollution at many known and unknown sites.
- They form part of a harmonized risk assessment framework within both Danish and European regulatory systems.

Focusing on these four PFAS allows PFASter to directly address an urgent national monitoring challenge while designing a flexible test platform that could later be expanded to include additional PFAS compounds.

1.5 Anticipated Use Cases

PFASter was envisioned as a tool with multiple field applications. These include:

1. Triage testing by municipal utilities prior to initiating full laboratory testing campaigns.
2. Pre-screening at known or suspected contamination sites, such as areas affected by historical use of firefighting foams.
3. Rapid testing in field studies, including hydrological or ecological assessments of PFAS mobility.
4. Public communication, where private well users or citizen science groups may participate in decentralized monitoring.

Each of these use cases reflects the project's intention to democratize access to PFAS screening tools by lowering the technical and financial barriers associated with traditional analytical methods.

1.6 Alignment with MUDP Goals

The PFASter project contributes to MUDP’s goals in multiple ways:

1. Environmental protection: Supports the national objective of reducing PFAS exposure by enabling more responsive monitoring and mitigation.
2. Technological innovation: Advances biosensing and diagnostic platforms into the environmental sector, promoting novel uses of aptamer and LFA technologies.
3. Circular economy and safe chemistry: Assists in tracing contamination sources and preventing environmental dispersal of harmful substances.
4. Growth and exports: Lays the foundation for a scalable product that can be further developed and potentially commercialized for export, especially to countries facing similar PFAS challenges.

PFASter demonstrates how targeted technology development can contribute both to Danish environmental policy and to broader global efforts to manage persistent pollutants.

1.7 Summary of the project

The PFASter project aimed to develop a field-deployable, cost-effective, and selective detection method for per- and polyfluoroalkyl substances (PFAS) in water using aptamer-based lateral flow assays (LFA). The target compounds — PFOA, PFNA, PFHxA, and PFHxS — were selected based on environmental prevalence, regulatory focus, and structural diversity across chain length and functional groups.

The project was structured across two main phases:
- Aptamer development (WP1)
- LFA development (WP2)

Regarding the aptamer development (WP1), this work package focused on developing aptamers capable of specifically and sensitively binding PFAS molecules, which would serve as the core recognition element in the PFASter test. The project team at DTU Sundhedsteknologi carried out SELEX-based selection procedures against the key PFAS molecules (PFOA, PFOS, PFNA, and PFHxS), with an aim to identify broadly binding aptamers due to their structural similarities.

Despite known challenges with small-molecule aptamer selection, high-affinity candidates were identified through iterative rounds of selection and NGS-based sequencing. LNA-modified aptamers were also explored to improve stability and binding. Binding affinities were analyzed using SPR and displacement assays, although SPR results revealed both unexpected non-specific DNA-PFAS interactions and complex kinetic behaviors. These findings were critical in guiding assay format decisions and were corroborated by results from LFA experiments later in the project.

Although some candidates showed promising binding profiles, issues like broad binding and low signal-to-noise in functional formats led to continued optimization and post-modification efforts. Final aptamer candidates were selected for downstream testing in LFA configurations.

Regarding the LFA development (WP2), this work package aimed to translate the aptamer chemistry into a functional lateral flow assay for field-ready PFAS detection. Initially, a standard LFA setup was tested where PFOA was immobilized on Bovine Serum Albumin (BSA) and spotted on membranes. However, this assay design produced poor signals and high background, prompting a major design shift.
Inspired by the literature, a displacement-based fluorescence assay was implemented. This involved aptamers labeled with fluorescein hybridized to complementary quencher strands. Upon PFAS addition, the aptamer was displaced, releasing the signal. This design showed promise and was validated in ELISA plate format before transitioning into LFA.
Two LFA prototypes were developed: one using blue latex beads and another using gold nanoparticles (AuNPs). The latex bead version underperformed due to poor signal resolution and non-specific interactions. AuNP-based tests showed more robust visual signals and better reproducibility.
However, a major challenge identified during this stage was the tendency of PFAS to bind non-specifically to DNA, as seen in both SPR and LFA assays. This behavior impacted assay specificity and led to challenges in interpreting weak signals, especially in environmental samples. Additional optimization efforts included improving blocking buffers, membrane materials, and sample clean-up methods (e.g., syringe filtration) to mitigate matrix effects.
Ultimately, a working prototype using AuNPs was established. While not yet at commercial readiness, the project succeeded in identifying aptamer-LFA formats with potential for further optimization and field testing.

1.7.1 Realization and conclusion

Over the course of the PFASter project, significant progress was made in developing aptamer-based detection strategies for PFAS, despite the challenges posed by this class of small-molecule targets. The team successfully implemented and optimized a solution-phase SELEX protocol, leading to the selection of enriched aptamer candidates through NGS and bioinformatic analysis.
A comprehensive evaluation pipeline was established—including colorimetric gold nanoparticle assays, fluorescence displacement formats, SPR, and lateral flow devices—to characterize aptamer performance across multiple platforms. This multi-modal strategy revealed consistent and unexpected behavior: unmodified ssDNA aptamers displayed interactions with PFAS compounds that were often non-sequence-specific, suggesting that electrostatic or hydrophobic forces may drive binding rather than canonical aptamer-target affinity.

Attempts to translate these aptamers into a lateral flow assay, while conceptually appealing, faced multiple technical obstacles such as nanoparticle aggregation, test line clogging, and irreproducible signal generation. These issues, combined with weak target-induced responses and the absence of consistent dose-responsiveness, ultimately limited the success of the platform in its current form.
Nonetheless, the project provided critical lessons about the biophysical behavior of PFAS, the constraints of aptamer selection, and the challenges of assay integration, particularly for displacement-based formats. These insights now point to promising next steps: such as using chemically modified aptamers, incorporating structure-switching designs, or exploring orthogonal detection mechanisms that bypass the limitations of non-specific PFAS–DNA interactions.
In summary, while the final detection system did not achieve point-of-use validation, the PFASter project established a robust scientific foundation for future work and generated broadly applicable knowledge for aptamer development, biosensor design, and small-molecule diagnostics.

1.8 Main conclusion(s)

The PFASter project resulted in several important scientific and methodological outcomes:

- A robust and flexible SELEX pipeline was successfully implemented and optimized for the selection of DNA aptamers capable of recognizing PFAS compounds in solution. The protocol included both positive and negative selection rounds and was tailored to preserve the native conformation of small-molecule targets.

- Next-generation sequencing (NGS) and bioinformatic analysis enabled the identification of enriched aptamer sequences, laying the foundation for a unique panel of candidate binders specific to PFOA, PFOS, PFNA, and PFHxS.

- A suite of complementary validation assays was developed and optimized, including a gold nanoparticle colorimetric assay, fluorescence displacement assays, and surface plasmon resonance (SPR). These assays enabled a multifaceted evaluation of aptamer–PFAS binding behavior and facilitated rapid down-selection of candidate sequences.

- While a fully functional LFA sensor was not achieved, several lateral flow prototypes were developed and tested, using both latex and gold nanoparticle platforms. These experiments provided critical insight into fluidics, hybridization strategies, and aptamer–target interaction dynamics under flow conditions.

- Across platforms, a consistent challenge was identified: non-specific binding of PFAS compounds to DNA, regardless of aptamer sequence. This finding, confirmed across SPR, colorimetric, and LFA formats, suggests intrinsic interaction modes between PFAS molecules and nucleic acids that limit selectivity under unmodified conditions.

- Although the detection system remains at a pre-commercial development stage, the project delivered a fully characterized aptamer panel, established analytical workflows, and generated detailed technical knowledge of aptamer–PFAS interactions. These assets form a strong basis for future iterations of the detection platform, potentially including chemically modified aptamers or hybrid sensor systems.

1.9 Derivative effects

While the PFASter project did not yield a commercially deployable detection platform, it resulted in substantial knowledge generation, methodological advancements, and strengthened collaborations between academic researchers and the private enterprise. These outcomes provide a valuable foundation for future development and broader application of aptamer-based technologies in environmental monitoring.

For the academic research team, the project delivered:
- A fully developed, adaptable SELEX pipeline for aptamer selection against small molecules in solution, including PFAS — a class traditionally considered highly challenging for biosensor recognition.
- Extensive experience with assay development tools such as gold nanoparticle colorimetric systems, fluorescence displacement formats, SPR platforms, and lateral flow assay (LFA) construction.
- Detailed insight into non-specific interactions between nucleic acids and PFAS molecules, informing future aptamer design strategies and guiding new lines of investigation into aptamer–analyte binding mechanisms.
- A strong basis for scientific dissemination, student training, and follow-up grant applications targeting sensor optimization or the detection of other environmental micropollutants.

For Pisco, the project generated:
- A comprehensive technical overview of the possibilities and current limitations of rapid PFAS detection technologies, with relevance to monitoring water quality.
- A better understanding of how aptamer-based methods might integrate into real-world screening workflows for environmental contaminants — including the challenges of developing user-friendly formats like LFAs for on-site use.
- Early exposure to biosensing platforms and prototype technologies that, with further development, may offer cost-effective, in-house solutions for maintaining water quality standards.

At the partnership level, PFASter catalyzed:
- The development of shared experimental protocols, technical know-how, and data, transferable to other environmental sensing challenges.
- A concrete platform for applying to additional funding programs, focused on translating the aptamer panel and assay frameworks into more robust and selective sensor technologies, possibly incorporating chemical modifications or hybrid recognition strategies.

Altogether, PFASter has laid critical groundwork for both scientific and industrial advancement. It has equipped Pisco and its research collaborators with the insight needed to reframe the next steps in PFAS detection and future product development.

1.10 Perspectives

The PFASter project contributes meaningfully to a growing societal and environmental need: the detection and mitigation of persistent, bioaccumulative substances like PFAS in water systems. Although the final sensor system is still under refinement, the project has generated foundational tools, aptamer candidates, and technical knowledge that could, in future iterations, lead to low-cost, field-deployable detection solutions accessible to a wide range of stakeholders.
For society, improved PFAS detection tools would support more effective environmental monitoring, empowering municipalities, utilities, and industrial actors to detect contamination early and respond proactively. This is especially important in light of growing public awareness,

health concerns, and international regulatory efforts to limit PFAS exposure. A successful aptamer-based sensor could offer an affordable and portable alternative for environmental screening of which currently rely on laboratory-based techniques such as LC-MS.
From a technological development perspective, PFASter has advanced the frontier of aptamer-based biosensors for small, non-immunogenic molecules — a class of targets traditionally considered difficult to detect selectively without high-end equipment. The project's iterative work across SELEX, NGS, and multiple assay platforms has laid the groundwork for future innovation in biosensing, including the use of chemically modified aptamers, nanomaterial integration, and displacement-based formats that bypass antibody limitations.
Furthermore, the project highlights critical insights into PFAS–DNA interactions, which may influence not only sensor design but also scientific understanding of PFAS behavior in biological systems and engineered environments. This could open new lines of inquiry across toxicology, remediation, and molecular recognition fields.
Lastly, PFASter contributes to the policy and regulatory landscape, by demonstrating both the challenges and the promise of next-generation detection technologies. As regulatory limits tighten and demand for routine PFAS monitoring grows, the outcomes of this project help define what is technically feasible, where gaps remain, and how public–private collaboration can accelerate tool development in the environmental diagnostics sector.

2. Reporting on activities

2.1 Work package 1: Aptamer Development

2.1.1 Selection via SELEX and NGS

The foundation of the project relied on the systematic selection of DNA aptamers capable of binding per- and polyfluoroalkyl substances (PFAS), specifically PFOA, PFOS, PFNA, and PFHxS. To achieve this, a robust and iterative Capture SELEX (Systematic Evolution of Ligands by Exponential Enrichment) protocol, based on a displacement technique and adapted from Park et al. (2022), was established and continuously optimized throughout the project period. The approach involved in-solution selection, enabling aptamers to recognize PFAS molecules in their native, free form—an essential design decision to maintain the molecular presentation encountered in real samples and during assay development.

Eight candidate DNA libraries with a 40-nucleotide random region were initially screened for stability and amplification quality. These libraries were selected based on primer compatibility, structural properties, and prior use in aptamer discovery for small molecules. Each library underwent ten consecutive PCR rounds to assess amplification fidelity. Libraries 1, 7, and 8 were disregarded during the first cycles of experiments due to their insufficient amplification (data not shown). Despite libraries 2,3,4,5,6 could amplify nicely, only libraries 3 and 4 did not generate any by-products after all the rounds of amplification (FIGURE 1). Library 4 was chosen as the ideal template library for aptamer SELEX targeting PFAS. One of the key advantages of library 4 is its unique structural feature, which comprises a long stem composed of complementary nucleotides. This structural characteristic holds promise for the immobilization of the aptamer on a solid support, such as a sensor surface, without compromising the integrity of its binding site. Finally, Library 4 has been successfully employed in the previous studies for the identification of aptamers targeting PFAS by the Park et al. (2022).

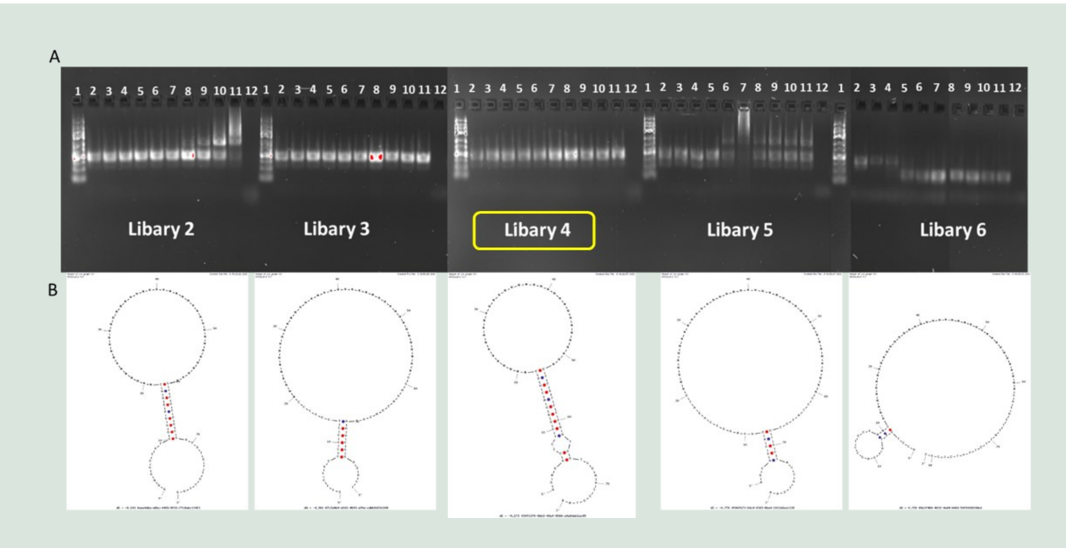

FIGURE 1. PCR validation of ssDNA libraries led to the selection of Library 4 for the Capture SELEX.

The SELEX process began with immobilizing the ssDNA library on streptavidin-coated beads via a biotinylated complementary strand. Upon incubation with the PFAS target, aptamers with affinity for the analyte dissociated into solution, while non-binding sequences remained bead-bound. These eluted sequences were collected, amplified via PCR (using qPCR-determined thresholds), enzymatically converted to ssDNA, and purified for reuse in subsequent rounds. Across the selection process, buffer composition, incubation time, and target concentration were carefully adjusted to improve specificity and binding strength. A simplified Capture SELEX workflow is shown in FIGURE 2.

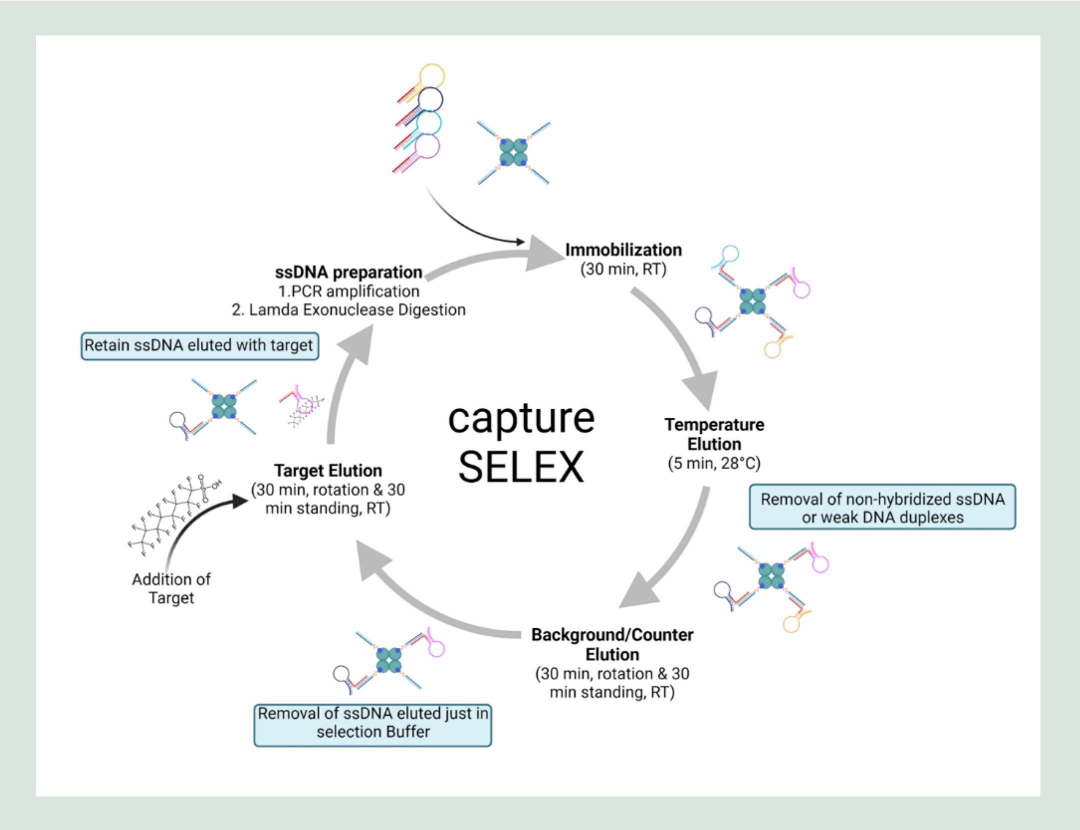

FIGURE 2. Capture SELEX workflow showing ssDNA library immobilization on a streptavidin-coated beads, removal of weak or nonspecific binders through temperature and background elution steps, and selective elution of target-binding ssDNA for enrichment and further rounds of selection.

15 SELEX rounds were carried out, where both positive and negative selection pressures were used to fine-tune specificity. Positive rounds consisted of PFAS targets, while negative rounds consisted of incubating the aptamer-immobilized beads with octanoic acid as a structurally similar non-target, before incubation with PFAS. This allowed the isolation of sequences that preferentially bind PFAS with minimal cross-reactivity. Next-generation sequencing (NGS) was performed on selected rounds (RA4, RA10, RA12, RA15) to identify enriched aptamer candidates. Bioinformatic analysis using the AptaSUITE software platform enabled classification of sequences as singletons, unique fractions, or enriched species based on frequency and round-by-round enrichment trends. Enrichment patterns confirmed the progressive selection of sequences with specificity to PFAS, with limited carryover into negative rounds, suggesting good target selectivity (FIGURE 3).

FIGURE 3. Classification of sequences from SELEX rounds using AptaSUITE. Bar plots show the proportions of singletons, enriched species, and unique fractions across positive, negative, and control selection cycles, illustrating progressive enrichment and target-specific selection.

These enriched sequences were subsequently carried forward into binding validation assays (e.g., fluorescence displacement and lateral flow formats), supporting the development of novel aptamers optimized for PFAS recognition under solution-phase and assay-relevant conditions.

2.2 Work package 2: LFA Development

2.2.1 Gold Nanoparticle Colorimetric Assay

As a first step toward evaluating aptamer functionality, a gold nanoparticle (AuNP)-based colorimetric assay was implemented as a qualitative validation method. This assay is widely described in the literature for rapid screening of aptamer–target interactions due to its simplicity, speed, and ability to visually detect binding events without the need for complex instrumentation. In our laboratory, the assay was adapted from published protocols and optimized for use with PFAS compounds as target analytes.

The assay operates on the principle that single-stranded DNA aptamers adsorb onto the surface of citrate-stabilized AuNPs, forming a negatively charged coating that stabilizes the particles in suspension. In this dispersed state, the colloidal solution appears red. Upon addition of the target molecule—such as a PFAS compound—the aptamers may bind the analyte and undergo a conformational change or detach from the nanoparticle surface. This disrupts the electrostatic repulsion between nanoparticles, promoting aggregation when salt is added, and causing a visible color shift from red to blue or purple. This color change can be detected by eye or quantified using UV–Vis spectrophotometry, typically by monitoring absorbance shifts from ~520 nm (dispersed) to ~650 nm (aggregated). FIGURE 4 depicts the AuNPs assay concept.

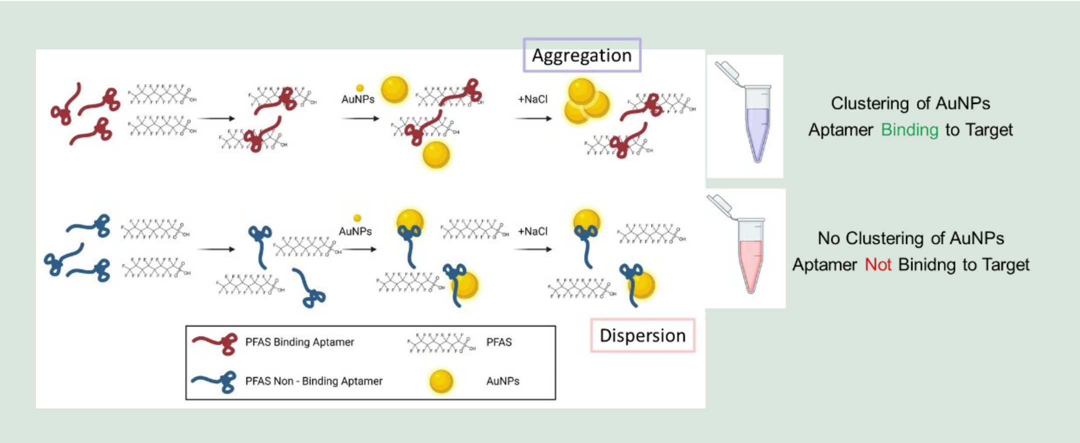

FIGURE 4. Schematic of the AuNP-based colorimetric assay for aptamer–PFAS binding. In the presence of target-bound aptamers, nanoparticle aggregation leads to a color shift (purple). Without binding, aptamers stabilize dispersed nanoparticles, maintaining the red color.

This assay was employed as an initial screening tool during the in-house aptamer selection process via SELEX and NGS. While awaiting the arrival of our own aptamer candidates, we validated the method by testing ten published aptamers from Park et al. (2022), which were originally developed for PFOA detection using a fluorescence-based system. Notably, the sensor from Park et al. (2022) was reported to detect PFOA down to 0.17 µM in water, making it a relevant and promising benchmark.

Each of the Park aptamers was tested in two configurations—with and without their primer binding regions (PBRs)—against three PFAS targets: PFOA, PFOS, and PFNA. The screening revealed that aptamer JYP8 with and without PBR provided the most reproducible and pronounced aggregation-based signal shift in the AuNP colorimetric assay. Based on this result, JYP8 with PBR was selected for further analysis using surface plasmon resonance (SPR), with the dual purpose of confirming its binding properties and establishing a robust workflow for future SPR studies with in-house aptamers.

However, as additional aptamers and experimental repetitions were tested, a key realization emerged during this phase: the AuNP colorimetric assay, while fast and simple, showed significant limitations in reproducibility and stability. The assay's performance was highly sensitive to experimental conditions such as salt concentration, aptamer sequence, and nanoparticle surface chemistry. In particular, inconsistent aggregation responses were observed for several aptamer–PFAS combinations, making it difficult to reliably compare binding across sequences.

Due to these challenges, and to obtain more robust and quantifiable results for our in-house aptamers, we decided to shift toward a fluorescence displacement assay in a microplate (ELISA-like) format, adapted from the method developed by Park et al. (2022). The assay principle is displayed in FIGURE 5 and relies on the displacement of a fluorophore-labeled aptamer (FAM) from a complementary quencher-labeled strand (D). In the absence of the target, the FAM-labeled aptamer is hybridized with the quencher strand, suppressing fluorescence through Förster resonance energy transfer (FRET) or contact quenching. Upon addition of the target molecule—such as PFOA—the aptamer preferentially binds the analyte, leading to its release from the quencher strand. This conformational switch restores fluorescence, which can be detected and quantified using a microplate reader.

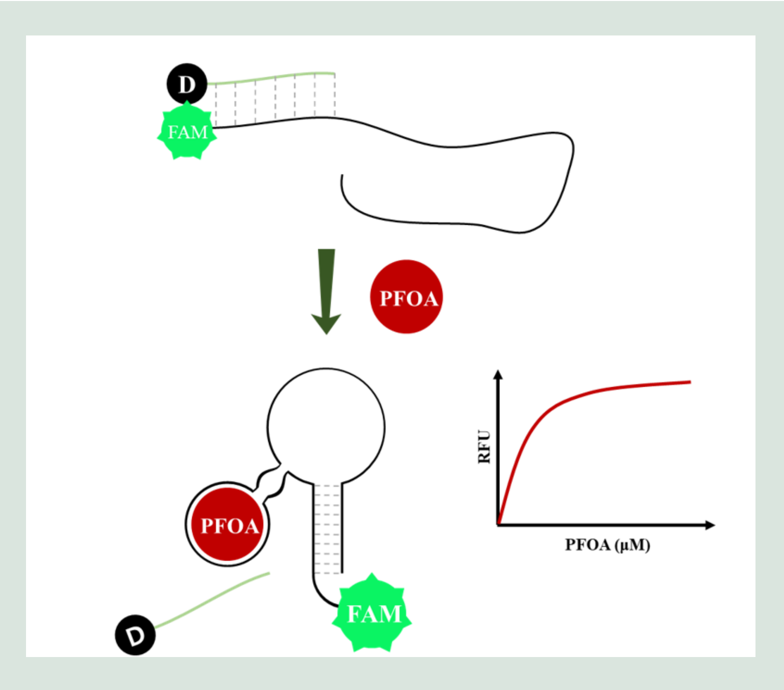

FIGURE 5. Schematic of the fluorescence displacement assay. A FAM-labeled aptamer is quenched by a complementary strand (D). Upon binding to PFOA, the aptamer is displaced, restoring fluorescence in a concentration-dependent manner. Tailored from Park et al., 2022.

This approach offered improved control over reaction conditions and enhanced reproducibility, while maintaining the advantages of aptamer-based small-molecule detection.

An aptamer named 162458, originally selected against PFNA, was evaluated using a fluorescence displacement assay to investigate its binding properties and the influence of secondary structures. Results showed that fluorescence intensity was notably higher in the presence of PFNA compared to no-target conditions, particularly at quencher concentrations between 125–250 nM for the 10- and 7-nucleotide quenchers. This suggests successful target-induced displacement. However, for the 13-nucleotide quencher, displacement occurred only at 62.5 nM, indicating stronger hybridization between the longer quencher and the aptamer. A consistent drop in fluorescence was observed between 62.5 and 15 nM PFNA across all quenchers, which likely reflects the formation of stable self-quenching secondary structures that can only be disrupted at higher target concentrations (FIGURE 6A, B, C).

A

	PFNA	1mM	250uM	62.5	15	3.9	0.97	0.244	61nM	15	3.8	0.95	0
Quencher 13 (nM)	162458	1	2	3	4	5	6	7	8	9	10	11	12
250	A	2806	2778	2094	2586	2856	2598	2903	2655	3022	2502	2832	2162
125	B	5338	4552	3830	4464	3880	3766	4158	3846	3765	3804	3868	3297
62,5	C	10048	9242	7262	5724	5566	5282	5496	5996	5386	5499	5101	5074
31,25	D	16119	12202	14567	6989	8210	7598	7947	8066	8448	6378	8247	7664
15,625	E	16427	17456	16037	8203	8536	9159	9199	9007	7548	9183	8776	7955
7,8125	F	18429	18364	19158	10006	9458	9486	10048	10555	8435	8317	7681	9379
3,90625	G	19079	20152	17386	10432	9891	9080	10471	9695	9561	8421	8418	8193
0	H	17826	19387	18457	9399	8654	7742	8154	7956	8818	8316	8835	9068

B

	PFNA	1mM	250uM	62.5	15	3.9	0.97	0.244	61nM	15	3.8	0.95	0
Quencher 10 (nM)	162458	1	2	3	4	5	6	7	8	9	10	11	12
250	A	15136	16665	17214	8672	8647	8833	7895	8373	7953	8219	7572	8244
125	B	17652	19373	18660	8647	8449	10764	8837	8886	8065	7277	8756	10949
62,5	C	18618	20769	20166	9589	9420	9472	9569	9116	10352	9226	8280	8544
31,25	D	19820	21314	21325	10182	9949	9765	10765	10459	9705	9592	8774	10496
15,625	E	19144	21866	19749	10392	9940	10789	11212	10161	10428	10520	9213	10129
7,8125	F	19763	21789	20583	10683	10280	10189	11852	10508	11557	10792	10072	9191
3,90625	G	20212	21548	19511	10867	10483	10857	11625	10391	10265	9466	9279	9272
0	H	21091	22222	20891	11168	9475	9792	10495	10136	10095	10015	10318	9744

C

	PFNA	1mM	250uM	62.5	15	3.9	0.97	0.244	61nM	15	3.8	0.95	0
Quencher 7 (nM)	162458	1	2	3	4	5	6	7	8	9	10	11	12
250	A	16787	18088	17548	6977	7359	6513	8238	6793	6383	6132	6555	6384
125	B	18060	19735	18688	8220	8981	8664	8346	8088	7750	8079	7940	7040
62,5	C	18900	20679	19829	8698	8414	8887	8558	8483	8640	8137	7987	8150
31,25	D	18086	21225	19970	9678	9032	9548	9522	9315	9139	9391	8288	8042
15,625	E	16901	20008	18362	8456	9227	9943	9231	8662	8770	8656	8251	9065
7,8125	F	16564	20377	18774	9213	9507	9261	8818	9886	9422	8667	8168	8706
3,90625	G	16990	19786	19328	8714	9039	9475	9234	9679	8752	8205	7087	7364
0	H	15941	19791	19098	8243	8526	8282	8449	9278	9432	8565	7936	7802

FIGURE 6. Raw fluorescence displacement assay data for aptamer 162458 across three quencher lengths (13, 10, and 7 nt), shown for easy visualization of signal trends in response to PFNA and to identify displacement and self-quenching effects across target concentrations.

Importantly, similar signal patterns were observed in a scrambled DNA control, raising concerns about non-specific interactions or artifacts (FIGURE 6). To further probe specificity, the assay was repeated by probing octanoic acid—a structurally similar but non-fluorinated compound— to both aptamer 162458 and scrambled, and it failed to induce comparable fluorescence changes, even at high concentrations (FIGURE 7A, B, C). This suggests that while PFAS compounds (like PFNA) can partially disrupt aptamer structures and possibly bind with low specificity, octanoic acid cannot. Collectively, these results indicate that aptamer 162458 may possess some degree of selective interaction with PFAS, though structural artifacts and self-quenching effects complicate interpretation at lower concentrations.

A

	PFNA	1mM	500uM	250uM	125uM	62,5	31,25	15,625	7,8125	3,90625	1,953125	0,976563	0
Quencher 13 (nM)	scrambled	1	2	3	4	5	6	7	8	9	10	11	12
250	A	507	540	605	617	610	640	569	579	640	676	685	675
125	B	1380	1102	1413	1106	955	1084	1013	1055	1207	1325	1255	1282
62.5	C	5958	6144	4904	3201	2258	2285	2048	1961	2164	2701	2478	2347
31,25	D	8419	7144	7751	4126	3035	2827	2998	2670	3287	3132	3404	3508
15,625	E	9074	9146	8100	4932	3915	3559	3189	3447	3458	4036	3909	3857
7,8125	F	10196	8468	9414	5238	3888	3699	3497	3261	3872	4439	4052	3439
3,90625	G	10122	10422	9474	5096	3912	4024	3682	3729	3825	4096	3979	3732
0	H	8045	7941	8136	4407	3365	3102	3162	3371	3552	4015	4009	3232

B

	octanoic acid	1mM	500uM	250uM	125uM	62,5	31,25	15,625	7,8125	3,90625	1,953125	0,976563	0
Quencher 13 (nM)	162458 Apt	1	2	3	4	5	6	7	8	9	10	11	12
250	A	2876	3412	2422	3428	3007	2298	2976	2621	2595	2627	2543	2696
125	B	3147	4029	4006	3850	3332	3719	3077	3313	3754	3388	3302	3319
62.5	C	6460	7117	7233	7319	6063	6564	5564	5027	4992	5236	5163	5036
31,25	D	9329	9726	10128	9245	8031	8972	7217	7989	8297	7447	7634	7688
15,625	E	9293	9689	10193	10064	8666	8184	8567	8532	12906	8625	8770	7954
7,8125	F	10505	11874	10548	12235	10460	10089	9777	9616	9588	8393	7731	8961
3,90625	G	10128	12287	10437	12468	9679	9437	9448	9032	9286	10158	8342	7293
0	H	8366	9286	9545	9035	8497	7192	7938	7454	6972	7289	6009	7459

C

	octanoic acid	1mM	500uM	250uM	125uM	62,5	31,25	15,625	7,8125	3,90625	1,953125	0,976563	0
Quencher 13 (nM)	scrambled	1	2	3	4	5	6	7	8	9	10	11	12
250	A	733	828	729	755	809	850	749	817	825	780	787	755
125	B	1049	1076	1075	970	950	1037	994	927	1196	1087	1000	1131
62.5	C	1931	1873	2022	2011	1760	1661	1823	1552	1967	2127	2015	2134
31,25	D	3832	3725	3774	3566	3427	3372	3322	3638	3190	3785	3344	3395
15,625	E	4397	3812	4091	3055	3002	2855	3009	3085	3778	3636	3764	3621
7,8125	F	4392	4399	4377	4402	3346	3319	3147	3358	4023	4058	4067	3570
3,90625	G	4636	4820	4360	4399	3476	3326	3572	3547	3945	4109	3849	3611
0	H	4309	4133	3918	3494	3970	2668	3466	3453	3523	3619	3849	3856

FIGURE 7. Raw fluorescence data showing responses scrambled DNA to PFNA (A) and responses of aptamer 162458 and scrambled DNA to octanoic acid across a range of concentrations (B, C). Data are shown for easy visualization of signal trends and to assess target specificity, confirming that octanoic acid does not induce significant displacement compared to PFNA.

2.2.2 Biding Affinity Characterization via Surface Plasmon Resonance (SPR)

To complement the solution-based assays and gain quantitative insights into aptamer–PFAS interactions, Surface Plasmon Resonance (SPR) was used as a label-free, real-time method for affinity characterization. The Biacore X-100 platform was employed to monitor binding events and evaluate dissociation constants (K_D) for selected aptamer candidates. Initial SPR experiments focused on validating aptamer JYP8 from Park et al., which had previously shown favorable performance in the colorimetric assay.

Two immobilization strategies were explored: (1) direct coupling of biotinylated aptamers onto streptavidin (SA) sensor chips, and (2) an alternative hybridization-based method involving a PolyA capture probe immobilized on the chip surface and subsequent annealing of PolyT-tailed aptamers. These dual approaches were tested to improve throughput and assay reproducibility, and to facilitate screening of a broader aptamer panel under consistent conditions.

Biotinylated JYP8 aptamers were tested against PFOA, PFOS, and PFNA, with octanoic acid included as a negative control. Preliminary responses suggested very weak or noisy interactions, and comparable signal patterns were observed when using scrambled control sequences, raising questions about non-specific effects and the reliability of the detected binding.

The SPR method presented multiple challenges. Signal inconsistencies across replicates raised concerns about non-specific effects such as bulk refractive index shifts or unspecific matrix interactions. For example, similar response patterns were observed when comparing active and scrambled aptamers, indicating that part of the signal may not be attributed to true binding. Additionally, efforts to immobilize aptamers via amine coupling were unsuccessful or produced high background noise.

These issues limited the robustness of SPR as a standalone validation tool in this context. However, the platform proved useful for protocol development and served as a bridge between qualitative screening (e.g., AuNP assay) and more scalable assays (e.g., fluorescence displacement). Further development would be required to improve signal specificity and reproducibility before SPR could be reliably used for aptamer validation in this application.

2.2.3 Lateral Flow Assay (LFA) Development

Developing a functional lateral flow assay (LFA) for PFAS detection proved to be one of the most experimentally challenging components of the project, requiring repeated redesigns, troubleshooting, and assay adaptation over the course of the years. The initial LFA concept was based on a competitive binding format, in which PFOA was conjugated to BSA and immobilized onto the test line. This setup aimed to detect free PFAS through competitive binding with aptamers labeled on colored particles. However, this classical small-molecule assay design failed to yield reliable or specific signals. A likely contributing factor was the fundamental mismatch between the selection and detection formats: since the SELEX protocol was performed entirely in solution, the aptamers were optimized to recognize free, unbound PFAS molecules in their native conformation. When PFOA was immobilized onto a surface via BSA conjugation, this may have masked key recognition epitopes or altered the spatial presentation of the target, thereby disrupting aptamer binding. Additional challenges such as steric hindrance at the test line and matrix interferences may have further compounded the assay's poor performance under flow conditions.

As a result, the assay architecture was completely re-evaluated. Drawing inspiration from Park et al. (2022), the team shifted toward the previously described displacement-based assay design, where a FAM-fluorescently labeled aptamer is hybridized to a quencher-labeled complementary strand. In the absence of PFAS, the fluorophore and quencher remain in close proximity, quenching the signal. Upon PFAS binding, the aptamer dissociates from the quencher strand, resulting in a measurable signal change. This method was first tested in an ELISA-like microplate format, enabling controlled incubation and precise monitoring of fluorescence intensity before and after PFAS addition. The assay demonstrated measurable displacement behavior with some aptamers at high concentration but not with control sequences (scrambled) or in the presence of octanoic acid, indicating its potential as a detection strategy (see FIGURE 6 and FIGURE 7).

After this proof-of-concept, the team translated the displacement system to an LFA format. Several approaches were tested using different nanoparticle platforms and hybridization configurations. In one version, latex beads were conjugated with DNA capture strands that hybridized with biotinylated aptamers. The beads were applied to the LFA strip, which featured a streptavidin test line to capture the full duplex in the absence of PFAS. Displacement of the aptamer by PFAS was expected to reduce test line signal (color). However, this format presented persistent technical issues: latex beads often clogged the sample pad, exhibited poor flow dynamics, and led to faint or non-reproducible test line signals—even under optimal buffer conditions.

To address this, the team switched to a more stable and commercially validated platform using gold nanoparticles (AuNPs) pre-conjugated with anti-digoxin antibodies. The complementary strand to the aptamer (capture strand) was modified with a digoxigenin label, and the aptamer carried a biotin tag. In this setup, the aptamer–capture strand duplex was prehybridized before loading onto the test line where streptavidin was immobilized and ready to bind the biotinylated complex. If PFAS was present in the sample, it would displace the biotinylated aptamer, preventing capture at the streptavidin test line. In the absence of PFAS, the complex would flow and accumulate at the test line, producing a visible red band.

When 5nM biotinylated aptamer 162458 was tested against dilution buffer spiked with 5mM PFNA, the test line disappeared compared to not-spiked dilution buffer, proving the occurrence of the displacement, despite the fact no titration for lower concentrations could be appreciated (FIGURE 8).

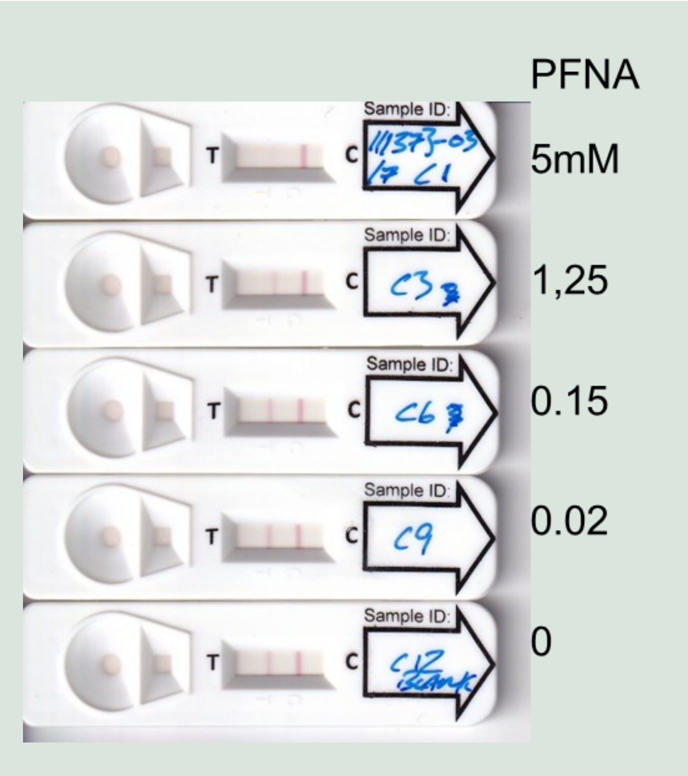

FIGURE 8. LFA results using biotinylated aptamer 162458 in the displacement-based format. Test strips were run with dilution buffer spiked with 5 mM PFNA (top) or unspiked buffer (bottom).

When the same LFA setup was applied to a real water sample and its serial dilutions, the undiluted sample showed a visibly weaker test line compared to the other diluted sample and undiluted one, suggesting the presence of potential interfering substances in the original matrix (FIGURE 9A). To determine whether this signal reduction was due to PFAS, the same water sample was spiked with 5 mM PFNA and tested in parallel dilutions (FIGURE 9B). The spiked sample exhibited a similar trend in test line intensity, with no further reduction compared to the not-spiked condition. These results suggest that the initial signal suppression observed in the undiluted water sample may not be caused directly by PFAS, but rather by matrix effects such as ionic strength, particulates, or other interfering compounds that impact aptamer performance or nanoparticle flow under LFA conditions.

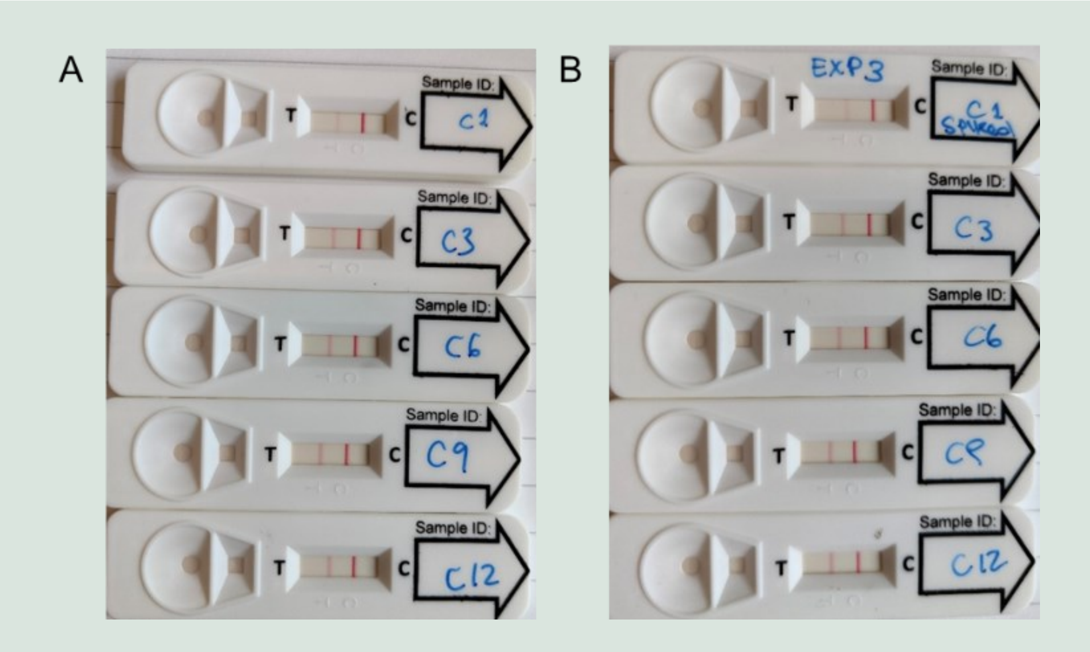

FIGURE 9. LFA results for a real water sample serially diluted (from C1 to C12) and tested using the displacement-based assay. (A) Undiluted real-life water sample (C1) shows signal interference comparing to the diluted ones. (B) The same water sample spiked with 5 mM PFNA (C1) shows a similar trend, suggesting that the signal reduction in the undiluted sample could be primarily due to matrix effects rather than PFAS-specific binding.

2.2.4 LFA Testing on Environmental Water Samples

Real-world LFA testing on surface waters from the environment was done to test the potential of this assay as a screening tool for PFAS detection with regards to effectiveness, ease-of-use and reliability. A total of 4 sampling sites were visited where 2L of water samples were taken. Three of the sites are located in the northern part of the Copenhagen area east of the Furesø lake whereas the 4th site is located on South Zealand east of Næstved. The aforementioned lake is interconnected with other lakes via channels and streams; and it was at these channels and smaller lakes that the samples were taken, being at:

- Holte Harbor – At Vejlesø, characterized as a calm and eutrophic lake.
- Frederiksdal – Mølleå river where it is narrow with a steady stream of water.
- Brede – Mølleå river where it widens and water is calm.

The sample from South Zealand was taken from a small creek named Rønnebækken just north-west of the village of Rønnebæk.

FIGURE 10. Sampling locations.

Subsamples for each sampling site were prepared and spiked with PFAS to match the concentrations used in the previously describe laboratory tests. Four 100 mL glass bottles for each site were filled with 93, 75, 87.5 and 87.5 mL of sample water and the bottle with 93 mL sample water was added 7 mL of a highly concentrated mixture of PFAS (PFOS, PFOA, PFNA and PFHnX). The solution was mixed, and 25 mL was thereafter transferred to the bottle containing 75 mL for a 4-times-dilution. Next, 12.5 mL of the x4 diluted solution was transferred to one of the 87.5 mL bottles to achieve a x32 dilution. This same step was repeated to the last bottle containing 87.5 mL to achieve a x256 dilution from the highest concentration. These dilution steps were performed for each sampling site, resulting in 4 spiked subsamples of differing PFAS concentrations for each sampling site.

To determine the final concentration of PFAS in the spike samples as well as un-spiked samples for background values, LC-MS analysis was performed. Sample aliquots from each bottle were spiked with a mixture of C13 labelled PFAS corresponding to the analysed compounds as internal standards. The analysis was performed directly on the filtered water samples for the spiked samples by LC-MS-MS. Background samples without spike were additionally preconcentrated by SPE extraction to increase the quantification limit.

One LFA for each spiked sample was used as well as one LFA for the un-spiked sample. One LFA was also added tap water, hereby acting as a blank. The six LFAs were lined up and glued onto a plastic sheet in order to better transfer them into a scanner. Two to three mL of each sample were filtered through a 0.2 micron syringe-filter, whereafter 10 µL of each filtered solution were added to the LFAs. This was replicated 3 times for each sampling site.

FIGURE 11. Scan of LFA tests for Brede samples for illustrative purposes. C1, C3, C6 and C9 represent ×1, ×4, ×32 and ×256 dilution, respectively. 0a represents un-spiked sample. 0b represents tap water.

After 20 minutes of running time, the LFAs were scanned in an OKI MC573 smart printer and the resulting images were uploaded into a proprietary image software where the test line, control line and empty surface between the lines (blank) were read. The program gave the read surfaces as a RGB intensity value, and the green intensity (GI) values were hereby used. The GI from the blank readings were subtracted from the GI values from the test and control lines, whereafter the test line value was divided by the value for the control line to give a relative value of intensity (RI).

$$RI = \frac{GI_T - GI_B}{GI_C - GI_B}$$

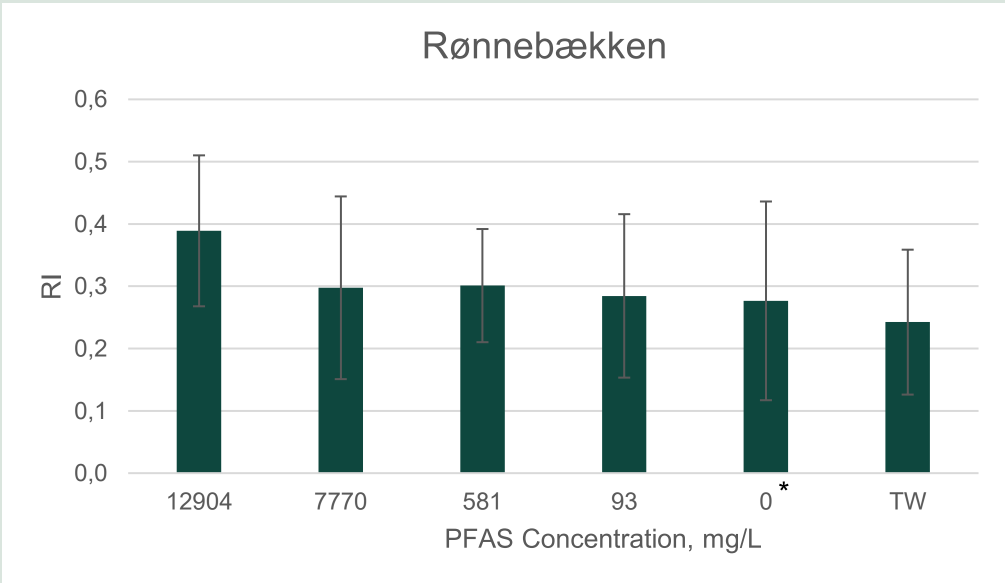

FIGURE 12. Signal intensity at different PFAS concentrations. RI: Relative Intensity. TW: Tap Water. *8.8 ng/L PFAS (un-spiked). Error-bars indicate 95% confidence intervals.

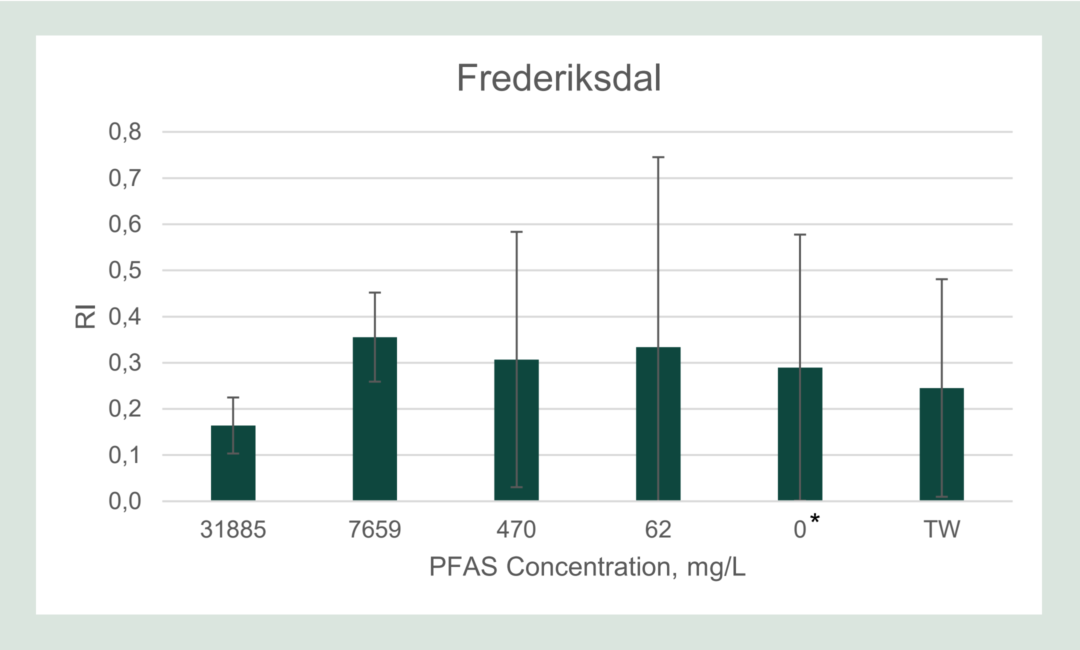

FIGURE 13. Signal intensity at different PFAS concentrations. RI: Relative Intensity. TW: Tap Water. *40.2 ng/L PFAS (un-spiked). Error-bars indicate 95% confidence intervals.

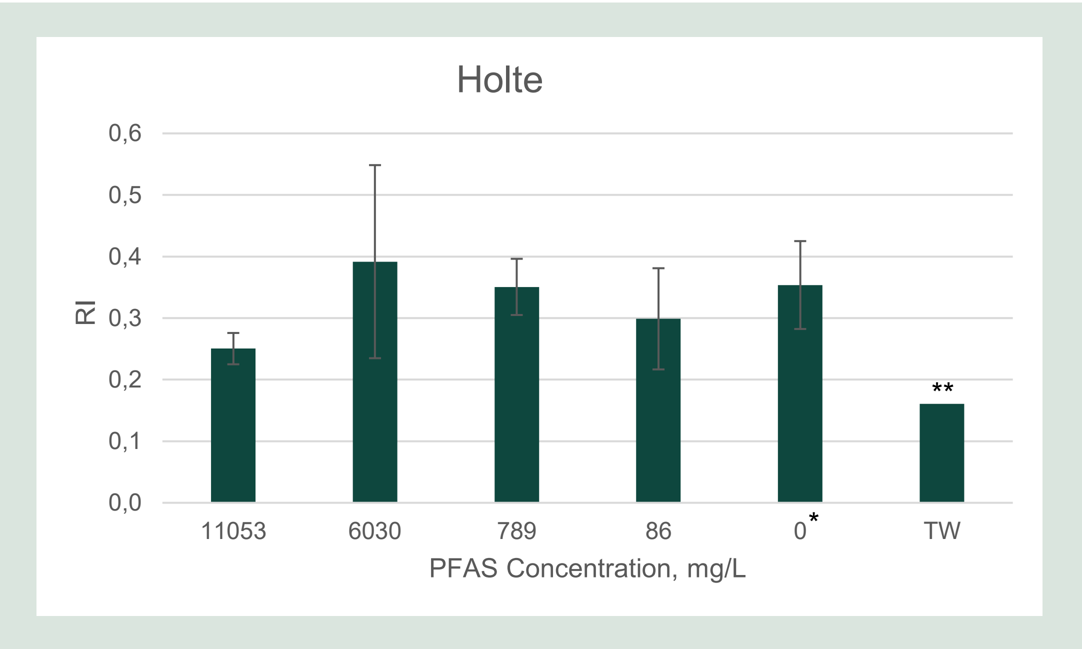

FIGURE 14. Signal intensity at different PFAS concentrations. RI: Relative Intensity. TW: Tap Water. *30.3 ng/L PFAS (un-spiked). **n = 1 due to faulty LFA tests. Error-bars indicate 95% confidence intervals.

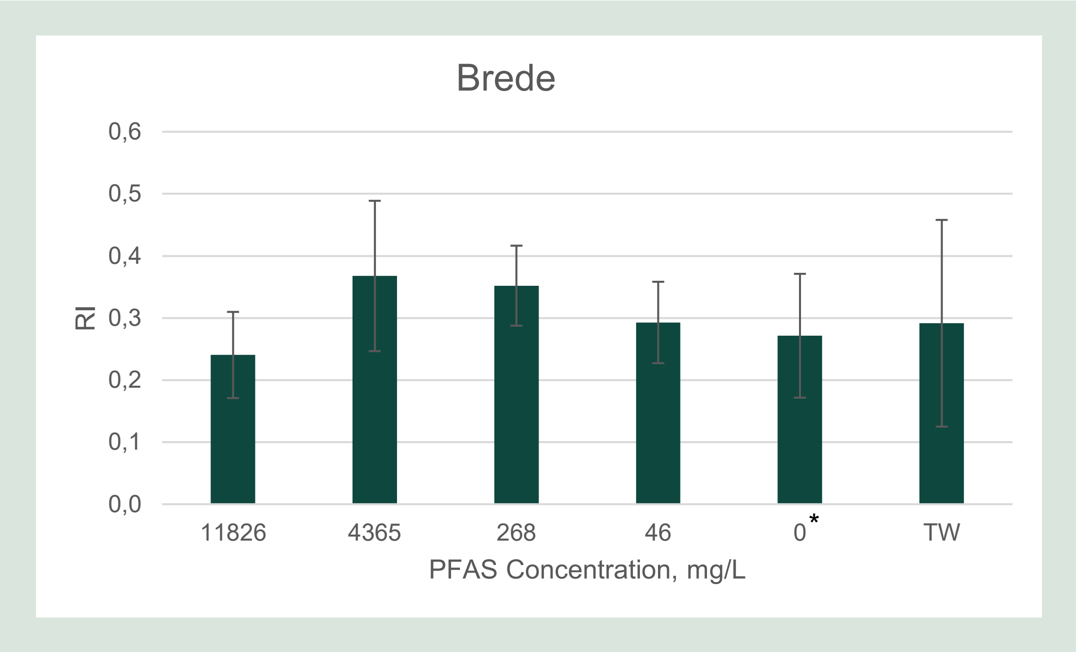

FIGURE 15. Signal intensity at different PFAS concentrations. RI: Relative Intensity. TW: Tap Water. *34.2 ng/L PFAS (un-spiked). Error-bars indicate 95% confidence intervals.

Out of all the LFA tests conducted (72 in total), only 2 were defective (weak control lines) (FIGURE 14), which was all due to faulty packaging (not airtight), indicating good reliability. The outcome of the tests was similar to what was observed in previously mentioned laboratory tests. A very high concentration of PFAS was required for the LFA test to show a signal (reduction in color) with the exception being for spiked water from Rønnebækken (FIGURE 12). It is most likely that the water matrix plays a large role in the LFAs behavior. The highest signal loss was observed for the Frederiksdal samples (FIGURE 13), which also had the highest concentrations of PFAS, although they also exhibited the highest variation between the concentrations, which might indicate that the LFAs performance is somewhat hindered by the high PFAS concentrations. Readings for samples taken from Holte (FIGURE 14) and Brede (FIGURE 15) showed a more muted response at the highest concentration with variability being lower compared to the Frederiksdal readings (likely due to the less intense concentration levels of PFAS). A commonality between the Holte and Brede sites is that the water was calm (higher water retention), whereas the other two sites had flowing water (river/creek), indicating that waters with little mechanical disturbances may need less pre-treatment. Although the LFAs were not going to show low level detection of PFAS to begin with, samples from 3 out of the 4 sampling sites revealed similar results to those tested in the laboratory (see previous section), which may suggest that an improvement in the capture probe (aptamers) could result in an effective assay as the tested LFA platform showed reliability and was somewhat consistent.

2.2.5 Closing Remarks

Given the uncertainties observed in signal behavior, the displacement-based LFA system—while easy to handle and visually interpretable—also produced inconsistent and difficult-to-interpret results. Notably, when the LFA was tested using a scrambled DNA sequence in the presence of PFNA, displacement was still observed (data not shown), mirroring the outcomes from the fluorescence displacement assays. These consistent responses across platforms suggest that PFAS–DNA interactions are not strictly sequence-specific. Instead, the results point toward non-specific binding mechanisms, likely driven by a combination of hydrophobic interactions and electrostatic attraction between the amphiphilic PFAS molecules and the negatively charged DNA backbone. This indicates some degree of chemical selectivity for fluorinated compounds, but not necessarily at the level of sequence-specific aptamer recognition. A critical limitation of the system was the failure to produce a reliable dose–response curve, even at high PFAS concentrations. This suggests that non-specific PFAS–DNA interactions may overshadow true aptamer-target binding, particularly in displacement-based detection formats. These findings are consistent with observations from SPR assays, where both specific and scrambled sequences exhibited similar weak responses, often lacking reproducibility. Despite extensive troubleshooting—including variations in buffer composition, hybridization conditions, and aptamer-to-bead ratios—the LFA system did not yield a reproducible or validated detection platform. However, the iterative development process revealed key insights into the limitations of current aptamer formats and nanoparticle-based assay designs and highlighted the need for future improvements.

2.2.6 References

Junyoung Park, Kyung-Ae Yang, Yongju Choi, Jong Kwon Choe, Novel ssDNA aptamer-based fluorescence sensor for perfluorooctanoic acid detection in water, Environment International, Volume 158, 2022, 107000, ISSN 0160-4120.

PFASter

PFASter focused on the creation of a lateral flow assay (LFA) designed to detect the presence of PFAS substances in surface waters. LFAs are already well-established in medical diagnostics (e.g., pregnancy tests and COVID-19 antigen tests) and offer advantages such as ease of use, rapid results, affordability, and minimal equipment requirements.

Over the course of the PFASter project, significant progress was made in developing aptamer-based detection strategies for PFAS, despite the challenges posed by this class of small-molecule targets. These strategies revealed consistent and unexpected behavior: unmodified ssDNA aptamers displayed interactions with PFAS compounds that were often non-sequence-specific, suggesting that electrostatic or hydrophobic forces may drive binding rather than canonical aptamer-target affinity. While development of an LFA prototype was achieved and multiple technical obstacles were overcome, such as nanoparticle aggregation, test line clogging, and irreproducible signal generation, the capture-probes' (aptamers) weak target-induced responses ultimately limited the success of the platform in its current form.

While the PFASter project did not yield a commercially deployable detection platform, it resulted in substantial knowledge generation and methodological advancements. These outcomes provide a valuable foundation for future development and broader application of aptamer-based technologies in environmental monitoring.

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