Detecting Microplastics with Aptamer-Initiated HCR and AuNPs

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Background

Microplastics:

- Microplastics (MPs) are plastic fragments <5 mm[1], and are pervasive in oceans, fresh water, soil, and the human body,[2-4] where they accumulate in organs, leading to health complications including infertility and hormonal imbalance.[5-7]
- Current MP detection methods: slow, expensive, not sensitive.[6-7]

Aptamer-initiated Hybridization Chain Reaction (HCR):

- **Aptamers** are short single-stranded DNA sequences (ssDNA) that bind to a target; in our case, to microplastics.
- Hybridization Chain Reaction (HCR) amplifies binding signals.[8]
- Enzyme free, isothermal and cheaper than other amplification methods[8]

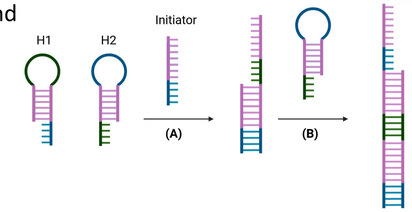

Figure 1. Schematic on how HCR works. **(A)** Initiator binds to the H1 probe, causing the H1 probe to unbind from its hairpin loop conformation. **(B)** Unwound H1 binds to the H2 probe and opens it, which then binds to a new hairpin H1 probe, causing a chain reaction.

Intent & Motivation

Designing and optimizing a **colorimetric**, **aptamer-based assay** to detect microplastics within drinking water, providing **accessible water quality testing** for the greater community. Tracking microplastic contamination in water samples is essential due to the toxic effects of microplastic ingestion.

Objective:
To develop an accessible, highly sensitive, and specific aptamer-based assay using **Gold Nanoparticles (AuNPs)** for a colorimetric output and **Hybridization Chain Reaction (HCR)** as an isothermal, enzyme-free method to improve assay sensitivity.

Acknowledgements and References

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References and Extra Information

Methods

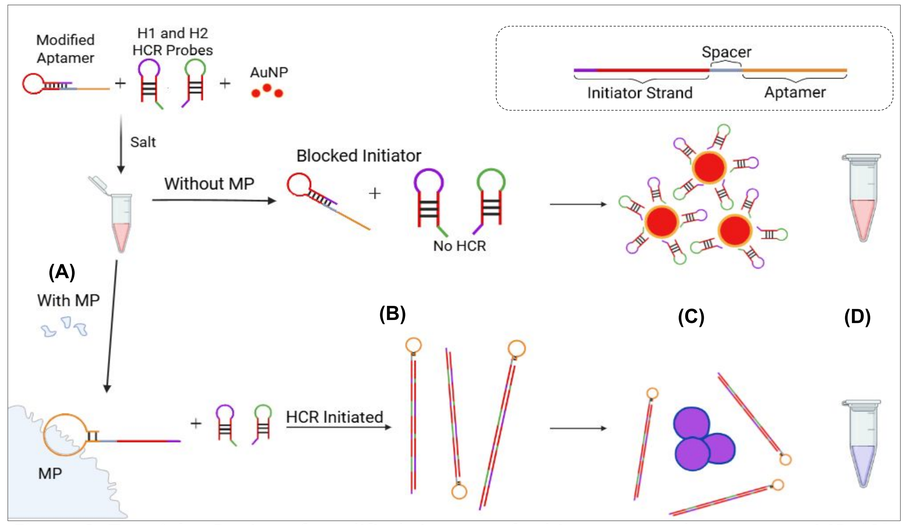

Figure 2. Top right: Design of modified Aptamer for biosensor to detect target MP **(A)** MP added, MP binding exposes initiator; no MP keeps it blocked **(B)** Exposed initiator triggers HCR, creating long dsDNA as products. Blocked initiator cannot start HCR. **(C)** HCR products allow aggregation of AuNP; whereas HCR probes do not, due to ssDNA adsorption. **(D)** AuNP changes color to violet in the presence of MP and stays the same(red) in its absence.

1

In silico design of **HCR probes** and **modified aptamer** sequences, aptamer sequence from Zandieh et al. (2024)

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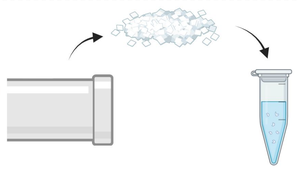

Preparing **microplastics** by shaving **PVC**, dissolving DI water at 85°C and filtering via **vacuum filtration (5 µm)** used in **(B)**

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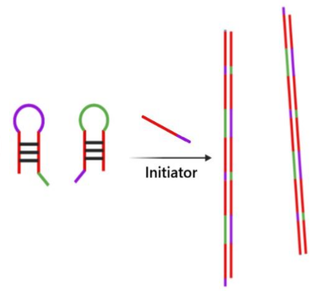

- **HCR reaction buffer** was **optimized** and tested with varying **NaCl, $MgCl_2$**
- HCR **ssDNA concentration(H1,H2 and Initiator)** were varied and optimized for ideal results.
- **Time and cooling method**(fridge vs room temp) for **probe folding** time into proper **hairpins** were tested and **optimized**.
- **HCR reaction time** was tested for overnight, 1 hr, 2 hr, and **45 mins.**

Results

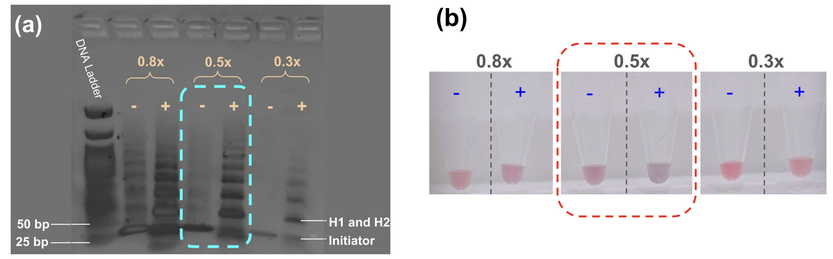

Figure 3. Optimization of ssDNA concentration for HCR and AuNP aggregation. **(a)** 2% agarose gel of HCR products at varying concentrations (1x = 1 µM H1, 1 µM H2, 3 µM Initiator) in 15 mM $MgCl_2$, 300 mM NaCl, 150 mM Tris-HCl. "+" lanes contain initiator; "−" are negative controls. **(b)** AuNP colorimetric shifts at 1:30 (v/v) HCR product:AuNP ratio. The 0.5x condition (dashed box) showed the greatest HCR amplification and colorimetric shift from red to purple.

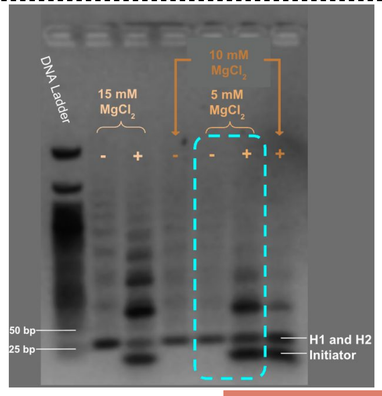

Figure 4. Optimization of $MgCl_2$ concentration for HCR. 2% agarose gel of HCR products using the 0.5x ssDNA concentration found in Figure 3. "+" lanes contain initiator; "−" are negative controls.

Discussion

- **Figure 3 (a)** demonstrates that concentrations of 0.5 µM of H1 and H2 probes and 1.5 µM for the initiator strand were optimal, due to clear smearing in positive and the least smearing in the negative because HCR triggers only in the presence of the initiator. **(b)** demonstrates that the same optimal concentrations in **(a)** produced the clearest red/blue change in AuNPs.
- **Figure 4 (a)** demonstrates that a concentration of 5 mM $MgCl_2$ was optimal for HCR, due to clear smearing in the positive and the least smearing in the negative, showing that HCR triggers only in the presence of the initiator.

Future Work

- Use Nile Red to quantify microplastics in spiked samples.
  - Once quantified, retest aptamer binding with UV-Vis or look into another method (flourescence measurements were not yielding conclusive results).
- Test aptamer and probe sequences *in silico* and modify them if necessary.
- Keep optimizing HCR to get more consistent and expected gel and AuNP results.