



Development and Characterization of a DNA-Based Biosensor for Nanomolar Fluoride Detection in Environmental Waters

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Research Problem

Significance of fluoride

WHO guidelines recommend:

0.5 mg/L⁻¹ -1.5 mg/L⁻¹

If >1.5 mg/L⁻¹ and <0.5 mg/L⁻¹.



Current detection methods

- Fluoride Ion Selective Electrode : μM detection
- Biosensors: nM detection possible.

Limitations

On site detection
Sensitivity
Specificity

Applications of the Biosensor

- Environmental monitoring
- In research of engineering of defluorinating enzymes: a remedy for 'forever chemicals'.



Main Objective

To develop and characterize a biosensor capable of rapid and on-site detection of nanomolar concentrations of fluoride levels in environmental waters.

Aptasensor Mechanism: FRET

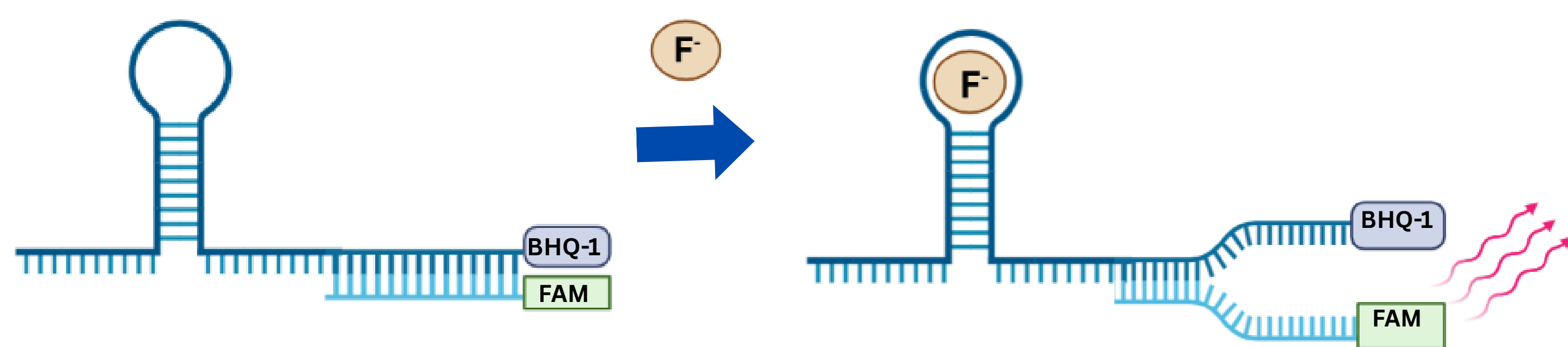


Figure 01: Binding of fluoride to the aptasensor derived from *crcB* motif of *B. cereus* produces a fluorescent signal.

Characterization of the biosensor

Table 01: Optimized parameters for the FRET assay.

Parameters tested	Optimal Condition selected
Sample volume	50 μL
Aptamer/ACE concentration	1 μM
Optical/Gain settings (instrument)	Bottom/auto

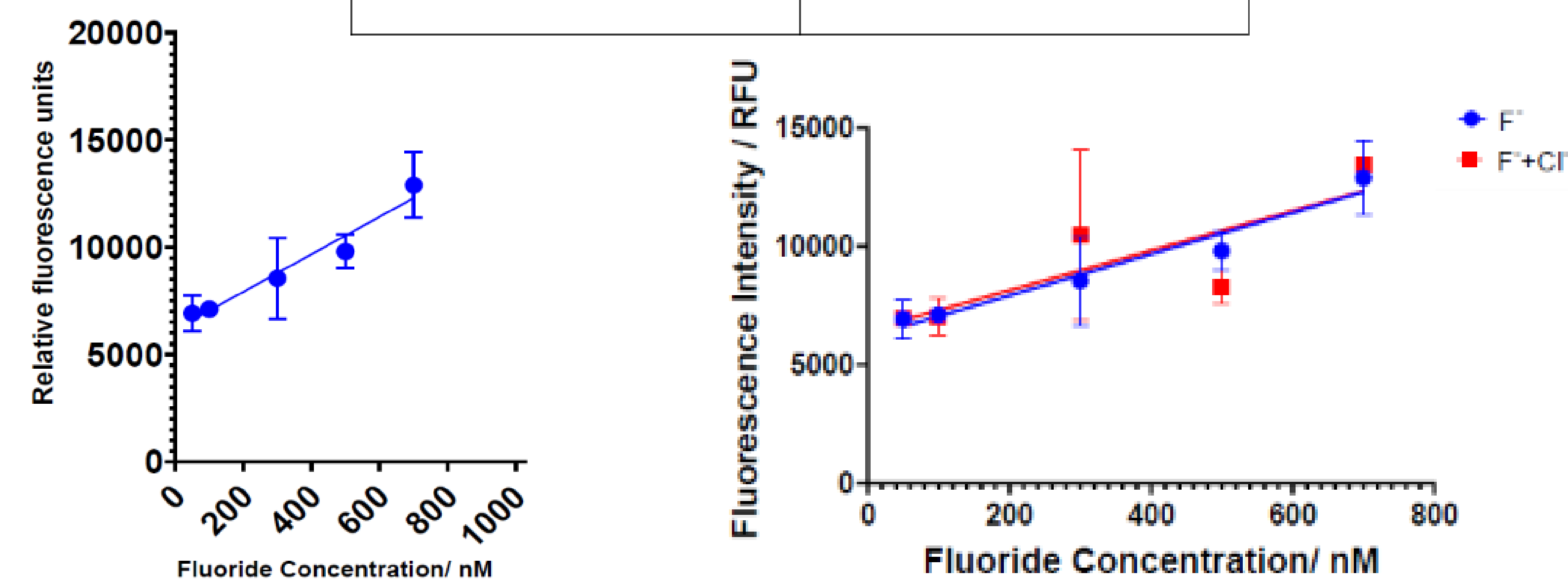
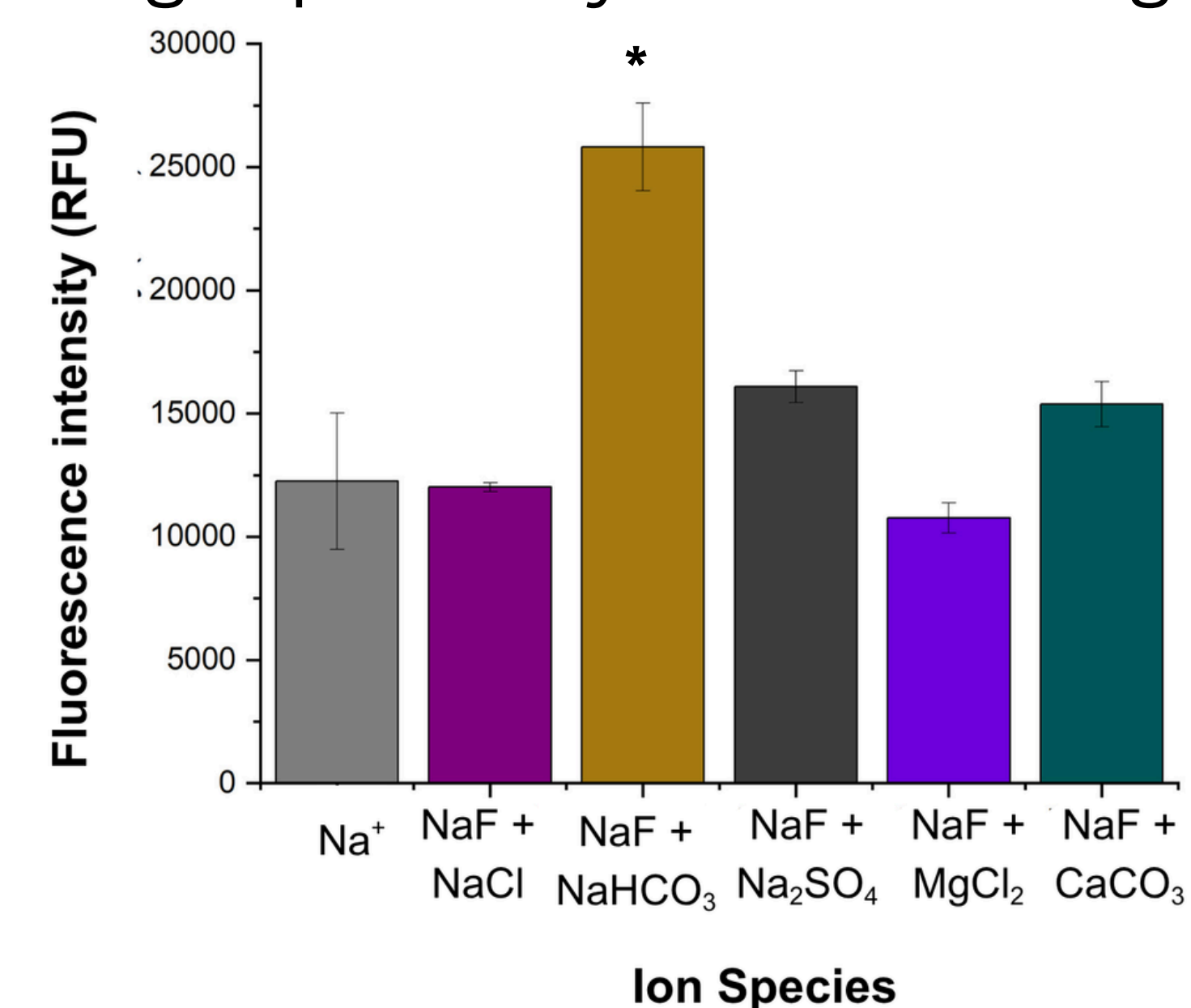


Figure 02: The standard curves of the fluorescence signal (RFU) vs fluoride concentrations (nM) generated for the FRET assay, with sensitivity at concentrations as low as 100 nM, producing a linear response in the nM range and high specificity to fluorides against interfering ions such as chlorides.



- $p > 0.05$ for all competing ions except NaHCO_3 – Tukey post-hoc.
- Attributed to the pH mediated disruption of the biosensor.

Figure 03: The bar graph represents the response generated in the FRET assay depicting specificity to fluoride (300 nM) against common environmental ions (5 μM) with significant interference detected by HCO_3^- ions.

In silico pipeline : work in progress

- 3D structure prediction by AlphaFold3
- Building the system using CHARMM-GUI
- Performing MD simulations using GROMACS (2021.4)
- Docking studies of fluoride ion with GNINA

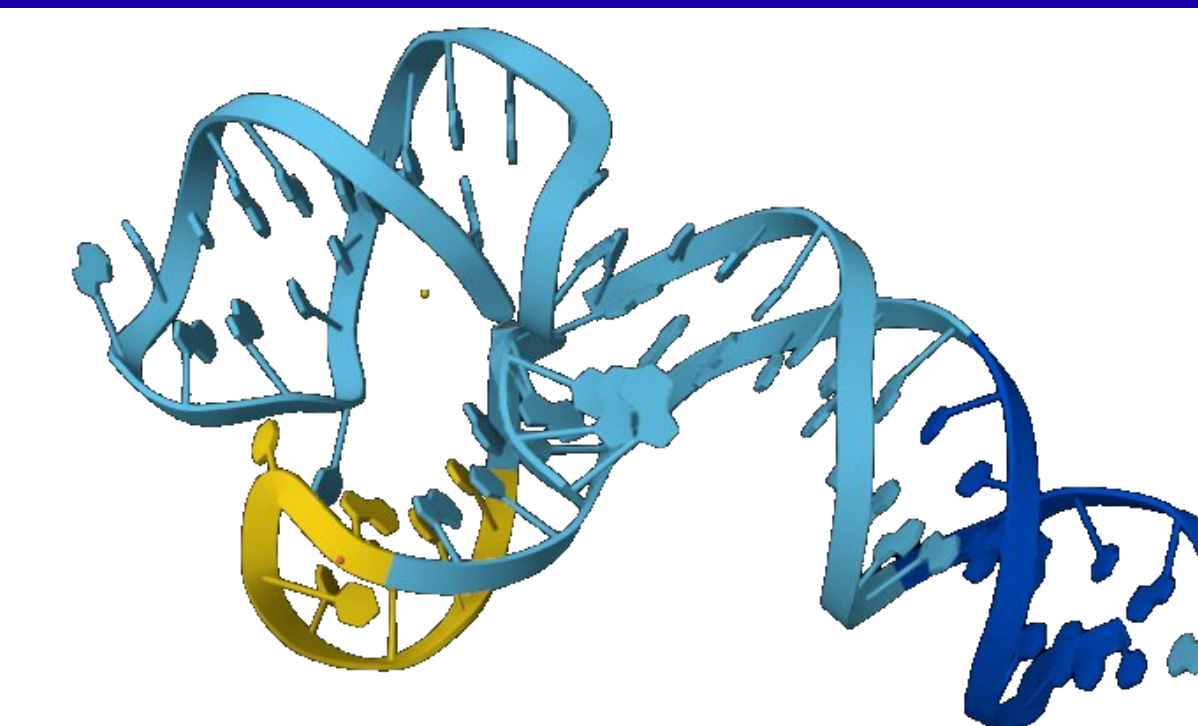


Figure 04: The predicted 3D structure for aptamer/ACE complex with 3 Mg^{2+} ions by AlphaFold3 (yellow coloured dots represent Mg^{2+} ions).

Conclusion

This aptasensor selectively detects fluoride at nanomolar concentrations in the presence of environmentally common ions, surpassing the sensitivity of conventional methods such as the fluoride ion-selective electrode – enabling rapid, on-site environmental monitoring.

Future work

- To conduct selectivity assay to detect the FRET response from environmentally common ions.
- To utilise the aptasensor as a nanomolar fluoride reporter for high-throughput screening of defluorinating enzymes.

Acknowledgement

Undergraduate research funds, Department of Chemistry, University of Colombo

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