Novel aptamer development for tetrodoxin detection in puffer fish

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Tetrodotoxin (TTX) is a paralytic marine neurotoxin [1] causing seafood poisoning after the consumption of contaminated marine species such as puffer fish and shellfish [2]. Liquid chromatography-mass spectroscopy is routinely used for laboratory-based analysis of field samples [3]. Competitive immunoassays have also been developed and are available in the market for TTX detection. Aptamers are attractive alternatives to antibodies and have great potential in analytical applications [4]. They are artificial synthetic nucleic acids (RNA/DNA) that bind specifically to their target and they are selected through an in vitro iterative process called Systematic Evolution of Ligands by Exponential enrichment (SELEX) [5]. In this work we sought to develop novel aptamers binding to TTX and exploit them for TTX detection in puffer fish. Using a variation of SELEX suitable for small molecules (Capture-SELEX) in combination with high-throughput Next Generation Sequencing, TTX aptamers were identified, and their binding properties were characterized. Finally, a highly sensitive and user-friendly hybrid antibody-aptamer sandwich assay was developed with superior performance compared to several assays reported in the literature and commercial immunoassay kits. The assay exhibited excellent recoveries for the detection of TTX spiked in fish extracts and it was also successfully applied for the quantification of TTX in puffer fish extracts. Ongoing work is focused on the development of a lateral flow assay which would allow the rapid and facile analysis of field samples.

References

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Figures

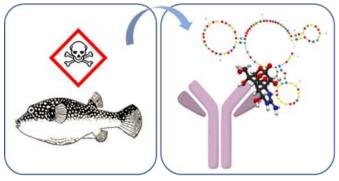


Figure 1. Hybrid antibody-aptamer assay for TTX detection

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