

Multiplexed Aptamer-based Fluorescent Sensor Array for the Detection of Neurotoxins

Keywords

Biosensor, Toxin, Multiplex, Aptamer-based fluorescent sensor array, Zone-specific sensor array

Background

Toxins represents one of the major sources of human illnesses and deaths but also have a significant ability to disrupt the international economy. A biosensor for toxins is relevant and important for many reasons: antidote procurement for treatment against venomous animals, preliminary information for biological warfare attacks, and for checking if foods & beverages are safe to consume.

While there are a great spectrum of toxins that exist - there are several available methods that can be used to detect toxins. To name a few: Hybrid Sandwich-ALISA can be used to detect Staphylococcal enterotoxin B, Modified ELISA for Alpha-toxin, Spectrofluorometric for Botulinum neurotoxin, and Gold Nanoparticles can be used to detect CDT [1].

For this proposal, I will be using the sensor array to detect for three neurotoxins: Saxitoxin(STX), Anatoxin-A (ATX), and Tetrodotoxin (TTX). Saxitoxin is a relatively low molecular weight toxin; however, it is one of the most dangerous marine toxins - primarily produced by certain cyanobacteriums as well as several dinoflagellates. Anatoxin-A, also low molecular weight and produced by several freshwater cyanobacteriums is linked in the lethal poisons of domesticated animals and wildlife. Tetrodotoxin is a strong neurotoxin that is carried by many animals: blue-ringed octopus, pufferfish, and frogs [2].

Objectives

The aim of this study is to develop an aptamer-based fluorescent sensor array that can detect a multiplex of neurotoxins in the field or in hospitals without the need of external lab equipment. In specific, the biosensor would detect various types of neurotoxins in solution- Saxitoxin(STX), Anatoxin-A (ATX), and Tetrodotoxin (TTX). I will be using the DNA aptamers shown in Table 1 as the target sensing platform while I will use Quant-iT OliGreen™ as a fluorescent dye in expression platform.

Rationale

There are many vectors by which a neurotoxin can affect a person and often times a person that has been bit by a venomous animal does not know which so getting the correct antidote to the victim is difficult. Neurotoxins are also known to be potential agents of biological weapons, thus the relevance for a biosensor for the detection of neurotoxins is huge. A biosensor for neurotoxins may not only be used for detection and identification but for treatment as well. This may further research in developing antidotes for toxins.

Approach

As mentioned in Figure 1, I will be using DNA aptamers APT^{STX}, ATX8, and A3 to bind Saxitoxin, Anatoxin-A, and Tetrodotoxin, respectively [3]. Quant-iT OliGreen™ will be used as the fluorescent dye which will then be conjugated to the aptamers. Afterwards, we will apply it to the zones of the sensor array which will fluoresce in the presence of the toxin. Shown below, Figure 2 describes the fluorescent sensor array setup.

Figures

Table 1

Target	Aptamer Name	Sequence (5' - 3')
Saxitoxin (STX)	ATP ^{STX}	GGTATTGAGGGTCGCATCCCGTGGAAACATGTTTCAT TGGGCGCACTCCGCTTTCTGTAGATGGCTCTAACTC TCCTCT
Anatoxin-A (ATX)	ATX8	TGGCGACAAGAAGACGTACAAACACGCACCAGGC CGGAGTGGAGTATTCTGAGGTCGG
Tetrodotoxin (TTX)	A3	GGGAGCTCAGAATAAACGCTCAACCCTGCCGGGGGCTT CTCCTTGCTGCTCTGCTCTGTTCGACATGAGGCCCGGATC

Figure 1

Biosensor Design

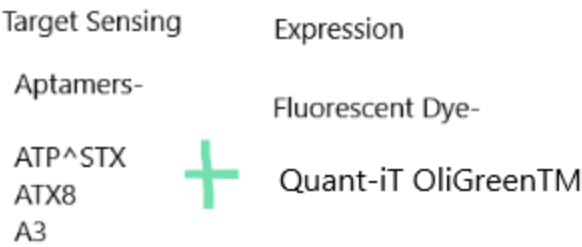


Figure 1: The figure above describes the design of the biosensor that will be used in each zone of the fluorescent sensor array.

Figure 2

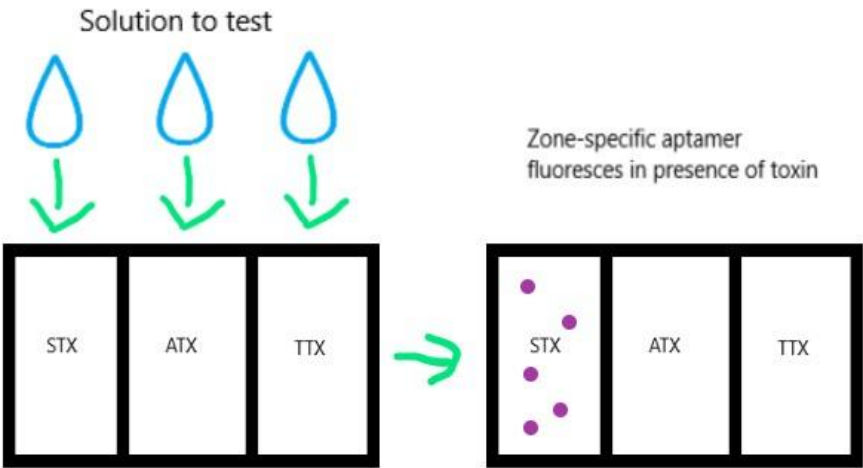


Figure 2: The solution with an unknown toxin is applied to the sensor array. After an amount of time for the aptamers on the array to work, it will fluoresce in the neurotoxin-specific zones.

Works Cited

- 1) Alizadeh, N., Memar, M., Mehramuz, B., Hemmati, F., & Kafil, H.A (2018). Current advances in aptamer-assisted technologies for detecting bacterial and fungal toxins.
- 2) Chau, R., Kalaitzis, J. A., & Neilan, B. A. (2011). On the origins and biosynthesis of tetrodotoxin. *Aquatic Toxicology*, 104(1-2), 61–72.
- 3) Zhao, L., Huang, Y., Dong, Y., Han, X., Wang, S., & Liang, X. (2018). Aptamers and Aptasensors for Highly Specific Recognition and Sensitive Detection of Marine Biotoxins: Recent Advances and Perspectives. *Toxins*, 10(11), 427.