A MULTI-APTASENSORS SYSTEM FOR THE DETECTION OF MARINE ALGAL TOXINS

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Co-financed by the EU under "The Ocean of Tomorrow" programme, the SMS project promotes the development of a novel automated networked system that will enable real-time in-situ monitoring of marine water chemical and ecological status in coastal areas. In this context, we are developing a multi-optical aptasensors system to detect several marine algal toxins that accumulate in vectors and impact human health through the consumption of contaminated shellfish and finfish or through water or aerosol exposure

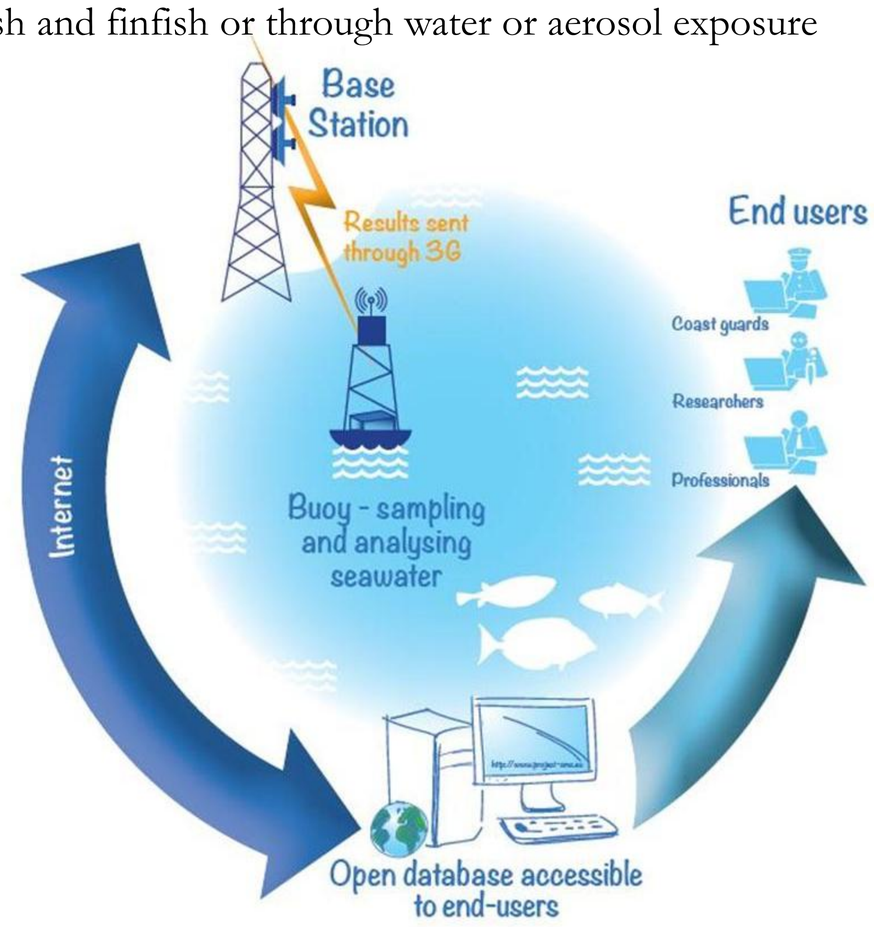

Figure 1. The goal of SMS project

Figure 2. Red tides and shellfish health warning signs at affected beaches

PROPOSED SENSING PRINCIPLES

1) Aptamers are ligand-binding nucleic acids whose affinities and selectivities can rival those of antibodies. By labelling aptamer, through the introduction of a fluorophore/quencher couple, aptamer signals the target presence through a binding-induced conformational change which brings the fluorophore close to the quencher thus decreasing the fluorescence signal (Fig. 3). We have used this strategy to detect Saxitoxin (STX), a toxin responsible for causing paralytic shellfish poisoning. (Fig. 5)

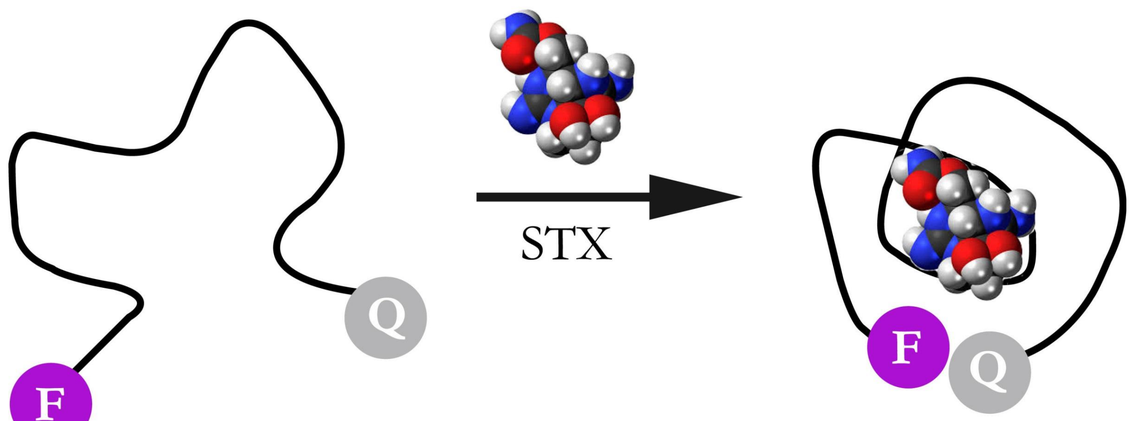

Figure 3. Principle of optical aptasensor. F and Q stand for fluorophore and quencher.

2) Aptamers can also be joined to nucleic acid enzymes to create allosteric RNAzymes. By introducing an aptamer domain in an hammerhead ribozyme, RNA self-cleavage is dependent on the presence of effector. By modifying the structure of hammerhead with a fluorofore/quencer couple, upon binding of effector, the ribozyme undergoes self-cleavage and releases a short florescein-labeled product oligonucleotide (Fig.4). We are using this strategy to detect Palytoxin, an intense vasoconstrictor, and Domoic Acid, responsible for causing amnesic shellfish poisoning (Fig. 6).

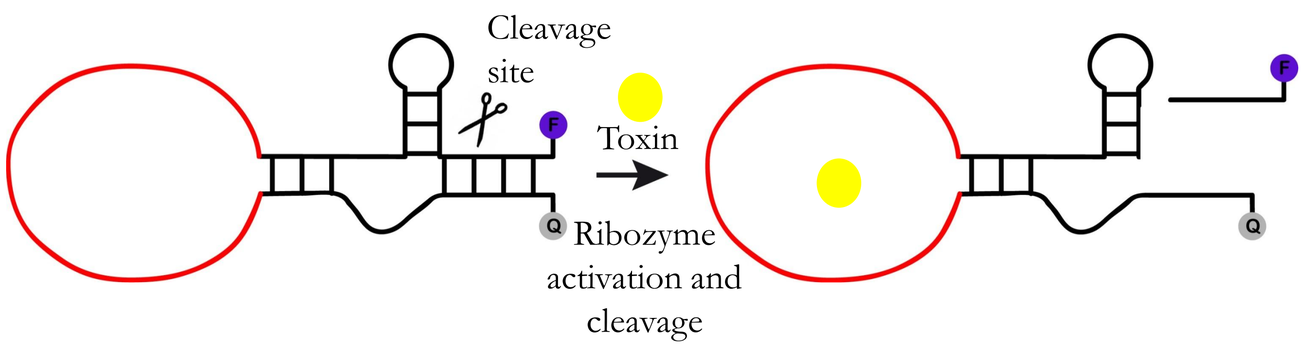

Figure 4. Principle of optical RNAzyme. In red the aptamer domain. F and Q stand for fluorophore and quencher.

PRELIMINARY RESULTS

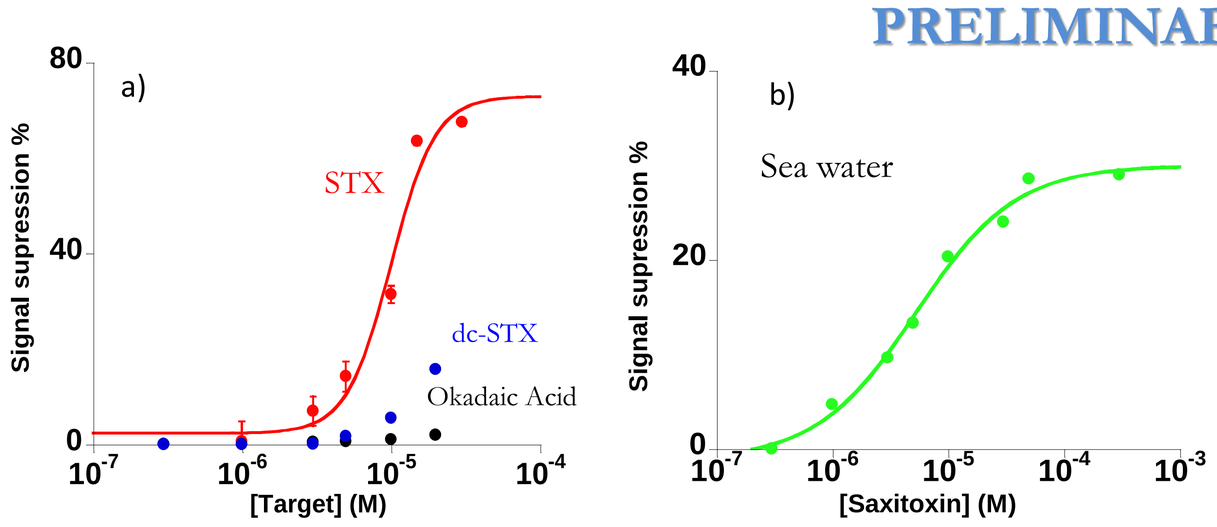

Figure 5. a) Fluorescence binding curve of aptamer with the target (STX) (red curve), the decarbamoyl-STX (dc-STX) (blue curve) and the OA (black curve) in HEPES 10 mM + 10 mM NaCl pH 7.4. b) Binding curve of aptamer (50 nM) with STX in sea water.

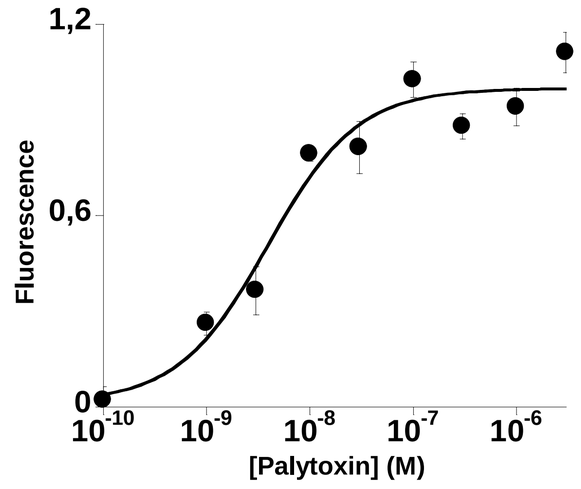

Figure 6. Binding curve of aptamer (10 nM) with Palytoxin in TRIS-Hcl 50 mM $MgCl_2$ 10 mM pH 7.4

CONCLUSIONS

We propose different aptamer based approaches to detect marine algal toxins. The developed method for the detection of STX is simple, high specific and selective even if a preconcentration step is needed (K_D = 8.0 ± 0.8 μM; LOD = 3.0 ± 0.5 μM). A more sensitive method, based on the use of hammerhead ribozyme is in developing to detect Palytoxin and Domoic Acid.

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