Engineering optical aptasensors for the detection of fungicides

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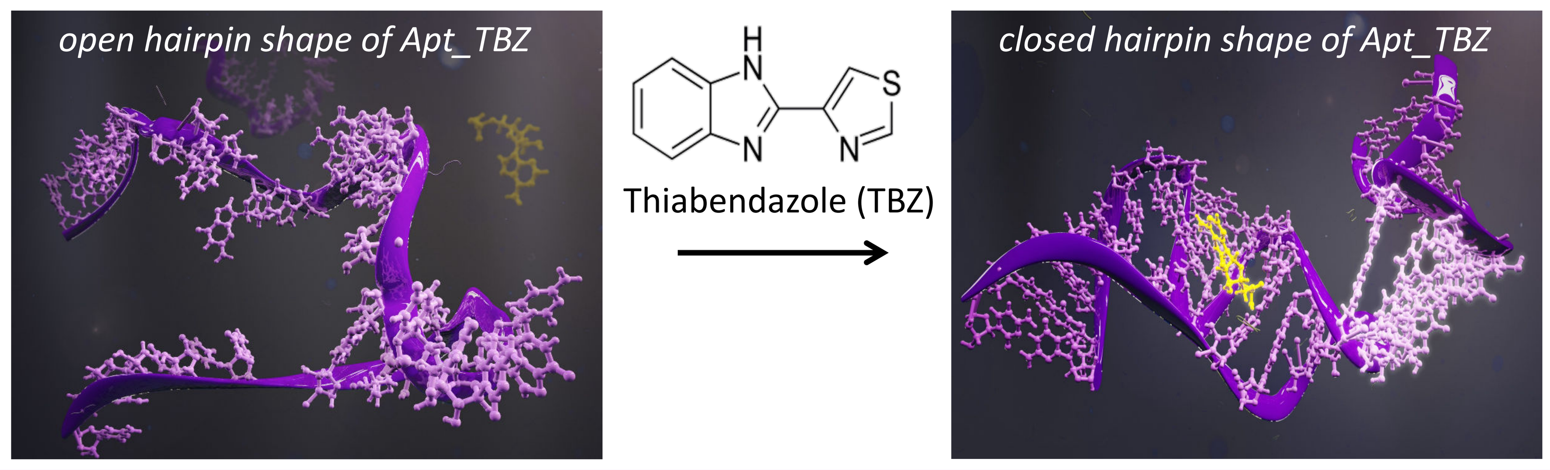
Environmental applications of aptasensors

Fungicides are commonly used in agriculture for the plant protection. Their presence in the environment and food chain has to be actively monitored as they can be carcinogenic and endocrine-disrupting at high doses. Traditional analytical techniques are time-consuming and use large, expensive equipment, which makes them unsuitable for on-site measurements and untrained users. Development of simple, portable devices for the selective detection of fungicide residues is an imperative and our efforts are devoted to the engineering of such biosensors.

Aptamers, single-stranded oligonucleotides which fold into 3D structures, bind to a wide range of targets with high affinity and specificity. They are synthetically evolved to recognize their targets, and therefore represent a cost-effective, easily modifiable alternative to antibodies.

Novaptech's innovative technology, **NOVAswitch,** allows the selection of sensing aptamers, which undergo conformational change upon small-molecule recognition.

Novaptech has selected, sequenced and optimized aptamers against different, but chemically related fungicides. Here, we present the optimized, 38 nucleotide-long, anti-thiabendazole aptamer (Apt_TBZ) and its integration in real-time, user-friendly, multiplexed biosensors for the quantitative detection of this fungicide.



Portable and connected detection

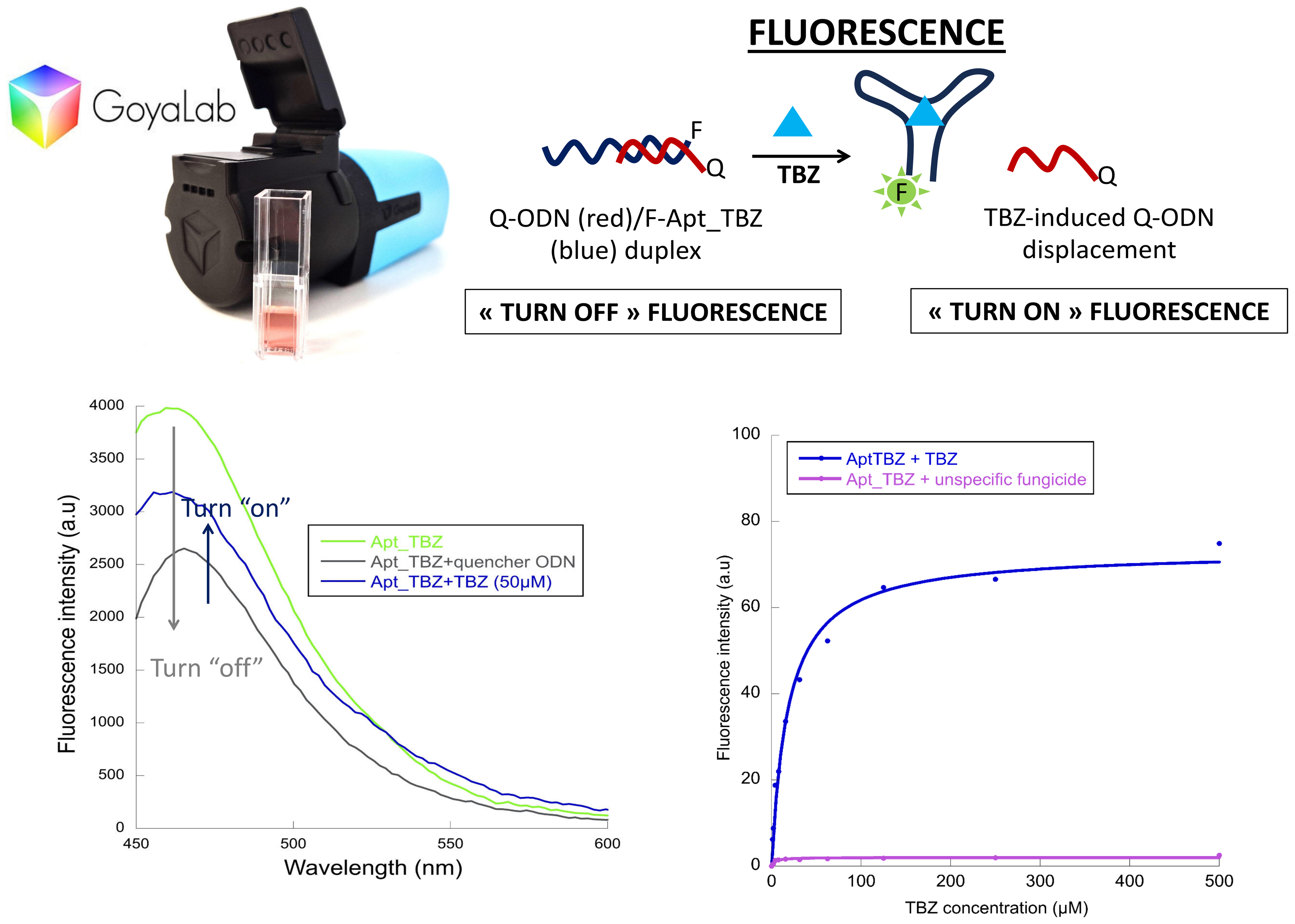


*Figure 1. Design and **fluorescence** responses of the aptasensor allowing the quantitative detection of TBZ.*

Fluorophore-labelled F-Apt_TBZ was hybridized to the quencher-labelled Q-ODN, resulting in the fluorescence quenching. TBZ addition induced the conformational change of F-Apt_TBZ, displacing the quencher ODN and generating a fluorescent signal.
Quantitative TBZ detection with the LOD of **0.7 ppm** was achieved. In addition, fluorescence signal wasn't observed in the presence of metazachlor, a control fungicide, indicating the specificity of the assay.
Measurements can be carried out using Indigo, a simple, portable spectrophotometer, allowing the detection of fungicides in the field with transmission of the results to a smartphone.

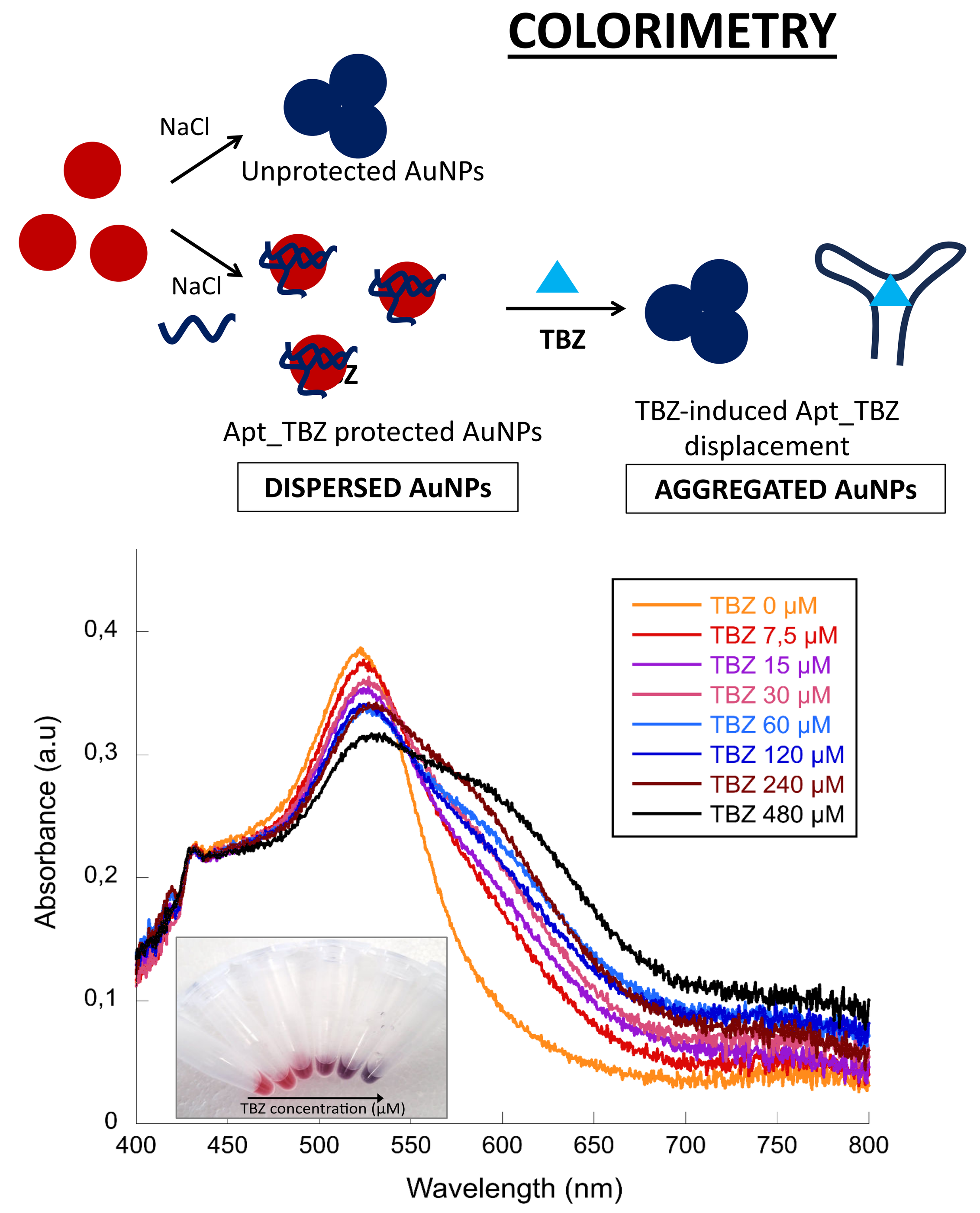
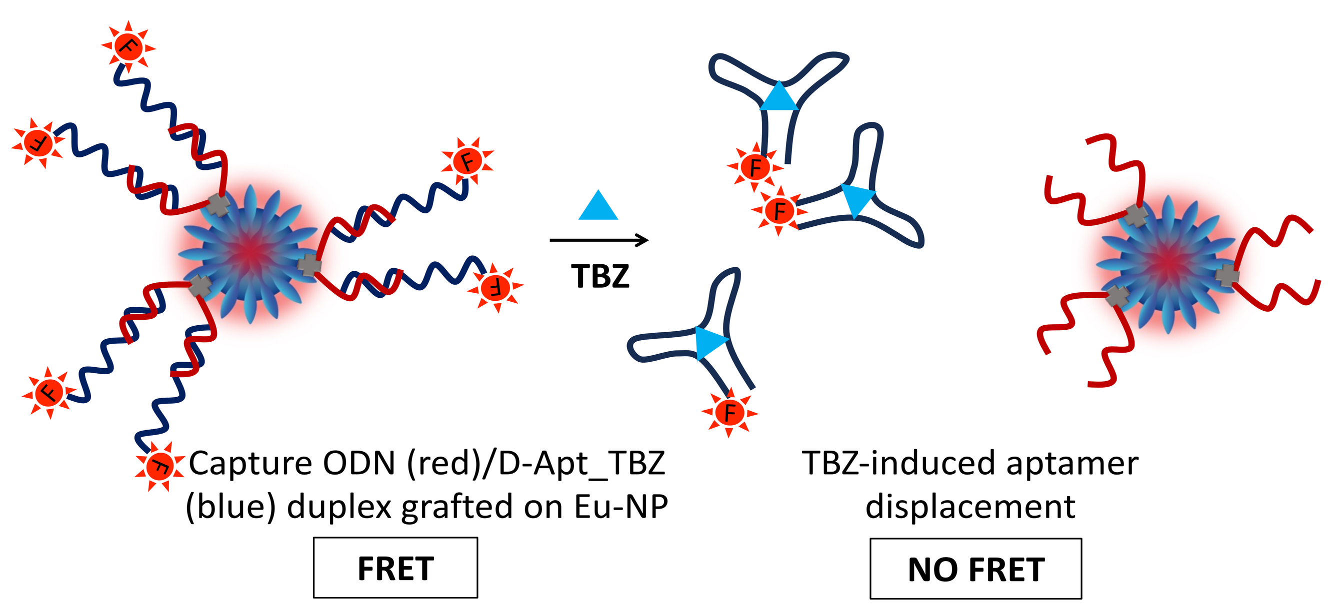


*Figure 2. Design and **colorimetric** response of the aptasensor for the TBZ detection.*

Apt_TBZ, adsorbed on the AuNPs, protected them from salt-induced aggregation. TBZ addition displaced Apt_TBZ from the nanoparticle, resulting in the AuNPs aggregation i.e., shift in the absorption spectrum with the corresponding colorimetric change. Quantitative TBZ detection with the LOD of **1.4 ppm** was achieved using IndiGo.

FRET-based aptasensor



Dye-labelled D-Apt_TBZ was grafted on the fluorescent lanthanide nanoparticle via biotinylated capture ODN, resulting in the FRET between the nanoparticle (donor) and the dye (acceptor). TBZ addition displaced D-Apt_TBZ from the nanoparticle, preventing the FRET thus allowing the nanoparticle emission.
The biosensor allowed the TBZ detection with the LOD of **5 ppb.** In addition, the use of a scrambled ODN sequence for TBZ detection didn't prevent FRET, indicating the selectivity of the assay.

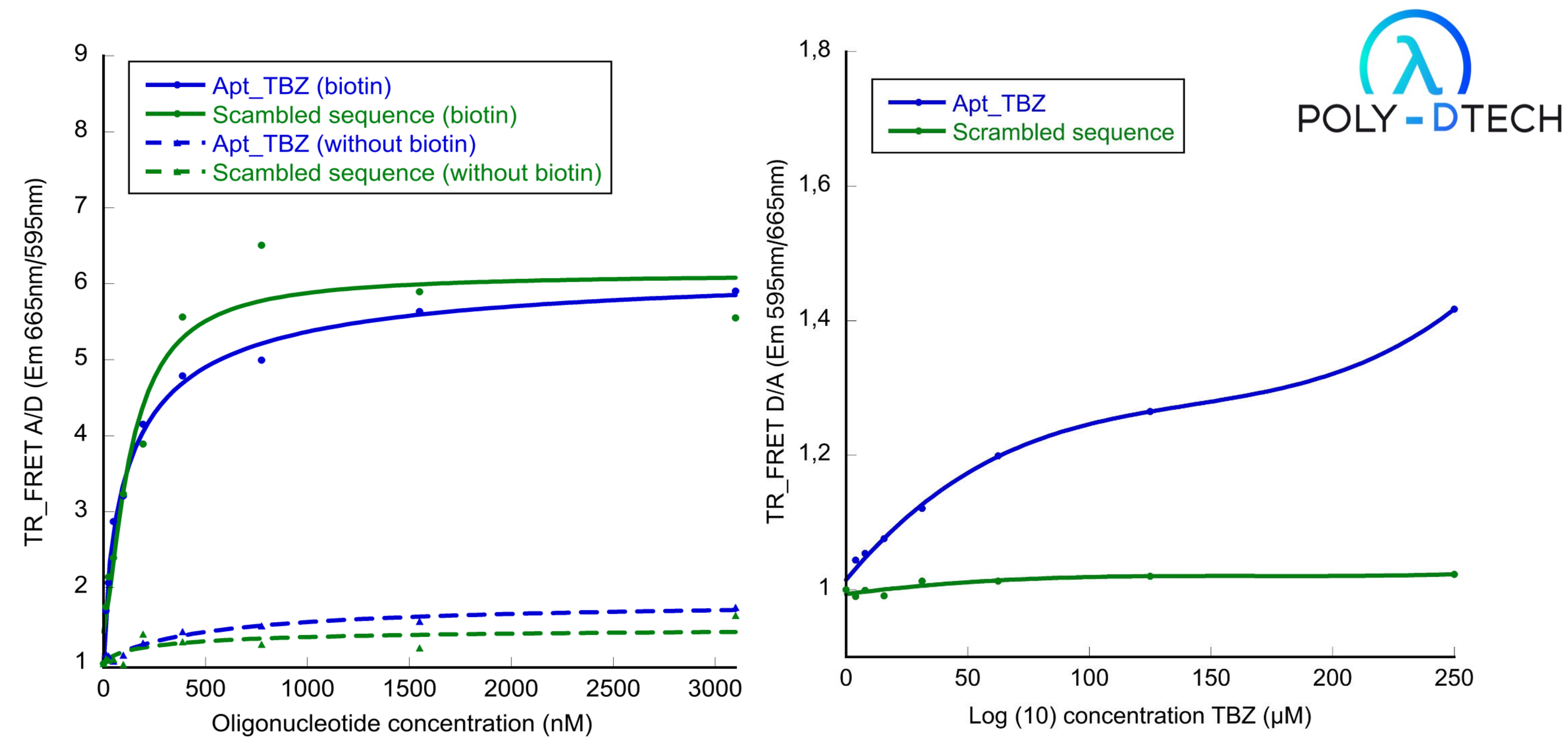


*Figure 3. Design and **TR-FRET binding assay** showing the succesfull grafting of aptamers on the nanoparticle (left) and the dose-response effect allowing the quantitative detection of TBZ (right).*

Conclusions

An aptamer against fungicide TBZ was selected, optimized and integrated in real-time, user-friendly biosensors for quantitative detection.
Several optical biosensors were developed. A fluorescence turn-on biosensing strategy based on molecular beacon and colorimetric biosensors based on AuNPs allowed the detection of TBZ in the low ppm range. Aptamers associated to lanthanides nanoparticles, generated optical biosensors with the LOD of 5 ppb. Measurements were carried out using Indigo, a simple, portable spectrophotometer, allowing the on-site detection of fungicides with transmission of the results to a smartphone.
Investigations are in progress in order to improve the sensitivity of our sensing systems.

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http://novaptech.com

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