

APPLICATION NOTE

DNA Aptamers and Quantum Dots for Portable FRET Sensing of Small Molecules and Other Targets

BY BILL JACKSON PHD

In 2009 my colleagues and I began exploring the use of aptamers for diagnostic and environmental sensing applications. There is a continuing need for more reliable, more sensitive and more economical methods in “field-forward” and point-of-care applications. Because of their stability, low cost of synthesis, zero chemical variability, and the ability to be selected under non-physiological, conditions nucleic acid aptamers have a number of advantages over other molecular recognition elements such as antibodies.

Through a Phase I Small Business Innovation and Research (SBIR) grant, EP-D-08-024 from the EPA we began exploring a platform sensing chemistry based on rapidly responsive fluorescent resonance energy transfer, or FRET with DNA aptamers conjugated to quencher dyes. In particular, highly stable, highly fluorescent quantum dots were used in a sensing format compatible with readout by an inexpensive, portable, and yet highly sensitive fluorometer (Life Tech’s “Qubit™”).

The EPA was interested in developing methods and devices for monitoring any compound of interest in the public water system. There is mounting evidence that suggests an increasing presence of harmful or even toxic chemicals, including pharmaceuticals, in public drinking water. There are also potential terrorist threats to our water supply. Because aptamer production is not hindered by toxicity or immunogenicity issues they were chosen over antibodies.

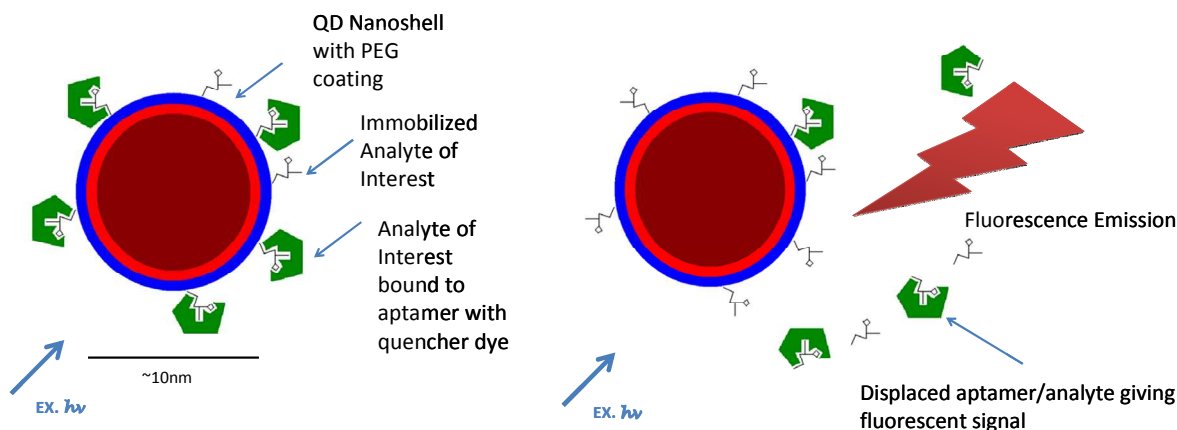


Figure 1: Schematic of aptamer sensing chemistry (approximate scale). In a competitive-binding fluorescence resonance energy transfer (FRET) assay, quantum dots (QDs) are conjugated to compounds of interest. Aptamers which bind these compounds with high specificity are identified and synthesized with a terminal fluorophore for quenching of the QD. Aptamers are then bound to immobilized compounds. Panel (B) When free environmental compound is exposed to this reagent mixture, QD-quenching aptamers are released from the QDs resulting in a fluorescence signal proportional to the compound concentration.

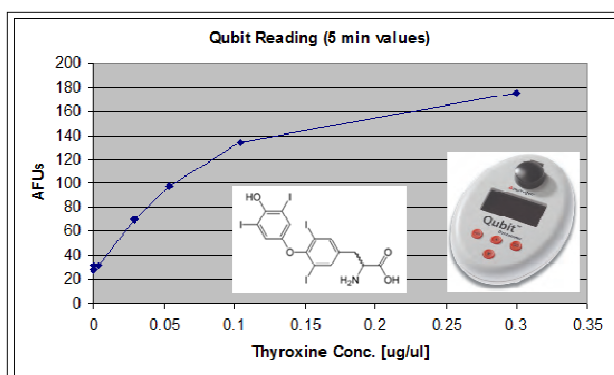
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Figure 1 depicts our modular FRET-aptamer sensing scheme. In a competitive FRET format, an analog to the free analyte is covalently conjugated to highly fluorescent quantum dot (QD) nanoparticles. QDs are semiconductor nanocrystals (~2-100 nm) with unique optical and electrical properties currently applied in biomedical imaging and electronics industries [1-3]. Fluorescent organic dyes are often used for FRET sensors, but their functional limitations (e.g., spectral cross-talk and non-uniform fluorophore photobleaching rates) complicate subsequent analyses [4]. QDs provide significant advantages over organic fluorophores because of their unique photophysical characteristics, such as size-tunable photoluminescence spectra, higher quantum yields, broad absorption, and narrow emission wavelengths [2,3]. Perhaps the most important advantage is a much increased resistance of QDs to photobleaching enabling development of more reproducible, stable assays.

DNA aptamers to the analyte of interest are conjugated to a strongly absorbing chromophore dye. Upon exposure of the sensing cocktail to free analyte, many of the quenching aptamers are released from the QDs resulting in restoration of fluorescence. In order to demonstrate the broad utility of this sensing platform we developed sensing cocktails for both the small molecule hormone, thyroxine (also known as T4) and the protein insulin. Using aptamers we developed we demonstrate the feasibility of this monitoring method.

Figure 2: Quantitative detection of the small molecule thyroxine (T4) via FRET. Insets show structure of T4 and portable (4.5 x 6.5 x 1.8 inch) Qubit™ fluorometer (Invitrogen) used to acquire data.



Thyroxine, which contains a primary amine (**Figure 2**), was covalently immobilized on carboxyl terminated quantum dots fluorescing at 655 nm (QD655, Invitrogen) using EDC-NHS chemistry. Conjugated QDs were then diluted as necessary to comprise various T4 sensor cocktails. A carboxyl version of the quenching chromophore, QSY21 (Invitrogen) was conjugated to a DNA aptamer synthesized with a 5'-amine. Similar methods were employed to create the necessary components of an insulin sensor. In that formulation, however, insulin-specific aptamers were conjugated to the QD reporter while insulin was conjugated to the quenching dye in order to compete with the free analyte.



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Once conjugations were complete, a typical sensing cocktail comprised approximately 4.0 μL QD/DNA, 1.0 μL QSY21/insulin, diluted in 400 μL PBS, pH 7.4. Dilutions of the sensing cocktails were adjusted to operate within the range of the portable Qubit fluorometer detector.

Figure 2 depicts the response of the sensing chemistry to free T4. While the sensor has not yet been optimized, we would expect to improve sensitivity by at least 2 orders of magnitude by better tailoring of both the absolute and relative amounts of cocktail components. However, even without any further optimization in sensitivity, the limit of detection depicted is presently ~ 10 ppm. With preconcentration steps often employed for environmental and clinical analytes such as methanol extraction, the current level of sensitivity may be acceptable for many applications. Separately, we have confirmed that this sensor is not responsive to the structurally similar compound, phenol, as well as DMSO (data not shown).

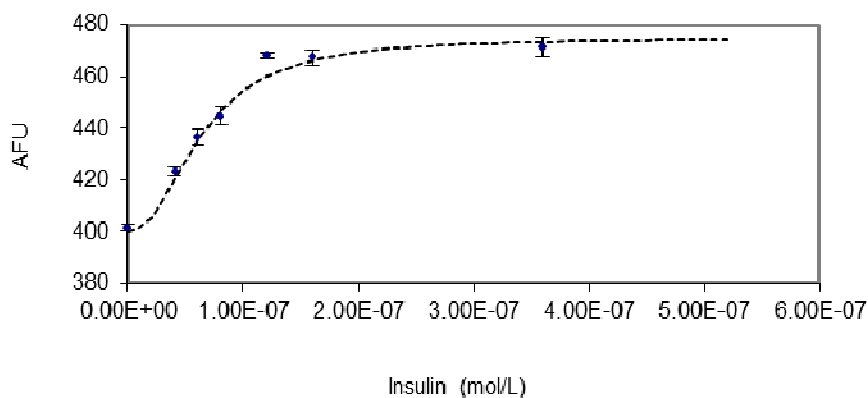


Figure 3: Response curve of quantum-dot-based sensor (Figure 4) to insulin as formulated with the insulin-specific aptamer #43 selected at Bio-Tex. (Panel A) The increase in fluorescence with increasing insulin concentrations is due to FRET.

Figure 3 shows the response of the sensor cocktail using insulin-specific aptamers. In this case, the limit of detection was approximately 20 nM. As a control experiment, we have confirmed that the sensor is not responsive to bovine serum albumin (BSA, data not shown).

Based on the preliminary results obtained thus far, the specific and sensitive quantitation of environmentally relevant compounds by aptamers is feasible. In the future we plan to address the challenge of detecting individual contaminants in complex samples, i.e. detect insulin in blood serum, or detect anatoxin-a in a lake water sample with aptamers we are currently developing. Anatoxin-a is of interest because is a naturally occurring toxin



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produced by some strains of *Anabaena* (particularly, *A. flos-aquae*) and at least four other genera of freshwater cyanobacteria (commonly referred to as blue-green algae). Algal blooms are increasingly common due to eutrophication of surface waters with the resulting potential to produce significant levels of the toxin in the environment. Anatoxin-a is a strong nicotinic acetylcholine receptor antagonist. Because it is not degraded by cholinesterase or any other known cellular enzymes, muscle cells continue to be stimulated, causing muscular twitching, fatigue and eventually paralysis. Severe overstimulation of respiratory muscles may result in respiratory arrest and rapid death [5].

ACKNOWLEDGMENTS

I wish to thank the EPA for their support through a Phase I Small Business Innovation and Research (SBIR) grant and my colleagues at BioTex, Inc., Houston, TX (Ed Frank, Ralph Ballerstadt, and Roger McNichols) and the University of Houston, Dept. of Chemical and Biomolecular Engineering, Houston, TX, USA (Uli Strych and Professor Richard C. Willson).

About *BILL JACKSON PHD*



Bill Jackson serves as President and Chief Scientist of Base Pair Biotechnologies and leads several aptamer selection projects against small molecule and other difficult targets. In the last 4 years he has served as PI on 11 SBIR grants, enjoyed service on a variety of review panels which cover a broad range of diagnostic, biosensing, and environmental applications, and is named as inventor on 4 issued patents and 10 pending applications. In 2010 Dr. Jackson co-authored an aptamer market research report, "Nucleic Acid Aptamers for Diagnostics and Therapeutics: Global Markets" for BCC Research.

Prior to formal spinout of aptamer discovery services under Base Pair, Bill guided numerous aptamer selections on both a service basis and for active research projects at parent company, BioTex, Inc. (also in Houston, TX). Dr. Jackson is still associated with BioTex where he serves as a Senior Scientist on a number of synergistic research projects involving aptamers and biosensing. At the University of Houston, his research focused primarily on the development of novel nucleic acid diagnostics using both mass spectrometry and nucleic acid microarrays. He has the somewhat rare distinction of funding his own doctoral dissertation serving as Principal Investigator on a NASA-funded SBIR grant. In previous employment Bill has been involved in the development of a wide variety of analytical chemistry equipment including instruments still in service aboard the International Space Station.

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