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Real-time and label-free analyte detection in a flow-through mode using immobilized fluorescent aptamer/quantum dots molecular switches

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ABSTRACT

Inspired by the goal to create a biosensor with designer specificity for real-time detection of unlabeled analytes in a flow-through mode, we designed a miniature flow cell with interchangeable quartz window carrying immobilized aptamer/quantum dot molecular switches as a part of a portable fluorescent setup. The inner surface of the 1.5 mm ID, 12 μl flow cell quartz window has been modified with the aptamer sensing complexes containing highly-fluorescent quantum dots. The aptamer complexes were designed as molecular switches to undergo conformational change and release fluorescent label upon interaction with the flow of the analyte, causing fluorescence decrease. The specificity of the sensor was designed to address the light chain of Botulinum Neurotoxin A and Ricin Toxin A chain, which could be specifically and repeatedly detected in the flow of 60 μl/min with sensitivity comparable to other real-time detection methods. The specifics of quantum dots use as fluorescent labels for continuous monitoring under constant UV illumination were outlined. The possibility for multispecific sensing was explored by testing of bi-specific sensor. This work shows the possibility of surface-bound aptamer sensing for flow-through analyte detection and provides a useful tool to perform surface fluorescent studies in real-time. The flexibility of the described design allows for sensor specificity change through altering the specificity of the aptamer. Future work should address response quantification. The described sensing approach can be adapted to a number of environmental or clinical targets.

1. Introduction

Specific recognition of biomolecules is the essence of all communications in Nature. Biosensors combine the principle of biological recognition with signal transduction, producing measurable and quantifiable output, which can be analyzed. Obtaining a biosensor output in real-time gives an important advantage of providing a rapid response, which is especially relevant for environmental outbreaks and medical emergencies.

Exponential growth of biosensing publications, currently exceeding 4000 research papers annually characterizes high need and numerous applications of biosensors. Real-time biosensors constitute less than 10% of that due to complexity of the task to combine adequate sensitivity with high detection speed. While PCR-based methods are unsurpassed in sensitivity and are used in more than half of the real-time biosensing methods, they can only detect the living matter, possessing the genetic material, which leaves out individual molecules, proteins, toxins, heavy metals and other potential analytes. Contrary to PCR-based methods, biorecognition events with key-lock specificity, such as between antigen and antibody or aptamer and its target can be used to directly detect a wider range of analytes of organic and inorganic nature. Used in conjunction with electrochemical or optical signal output, it can result in a biosensor suitable for point-of-care or field diagnostic.

Stability of DNA aptamers in a wide range of temperature conditions makes them a popular choice over antibodies for a number of biosensing applications. A number of aptamers specific to environmental and food pathogens, including viruses, bacteria, spores, toxins and heavy metals have been used for biosensor development and recently reviewed (Amaya-González et al., 2013; Sekhon et al., 2013; Kiilerich-Pedersen et al., 2013). The aptamers also attract researchers by the possibility of rational design, such as, for example, molecular switches used in biosensors (Plaxco and Soh, 2011; Tang et al., 2014) and smart materials (Mastronardi et al., 2014). Another possibility is detecting an aptamer binding event through nucleic acid amplification (Ma et al., 2012), which

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efficiently converts detection of non-genetic material into nucleic-acid detection method with all the advantages of down-to-one-molecule sensitivity potential.

The kinetics of aptamer binding allows for real-time monitoring. While real-time aptasensors can have electrochemical (Chang et al., 2014; Zheng et al., 2014) or optical (Yildirim et al., 2012) signal output, they are typically based on folding of the aptamer upon analyte binding, which causes the signal. In case of aptamer beacons, the quencher on a complementary strand can be displaced by an analyte, allowing monitoring of signal accumulation in a matter of seconds (Tuleuova and Revzin, 2010). The aptamers can be also surface-bound and even anchored on cell surface, registering chemical transmitter release in real-time (Tokunaga et al., 2012).

Real-time aptamer-based detection can be performed in a flow-through mode. Thus, a microfluidic electrochemical aptasensor capable of real-time measurement of therapeutic agents in blood in living animals has been recently reported (Ferguson et al., 2013), significantly advancing the sensing capacity to the desirable point-of care diagnostics.

With multiple existing aptamers specific to environmental pollutants (Long et al., 2013), one can expect a development of a family of real-time flow sensors for on-site monitoring. Thus developing a universal platform with interchangeable specificity is highly desirable.

We have been focusing on a modular approach for fluorescent real-time aptasensor, aiming at simplicity and portability. We have previously reported a concept of real-time flow aptasensor using stable quantum dots (QD) fluorescence detection (Bogomolova and Aldissi, 2011). The versatility of QDs has been especially popular for intra-cell and tissue monitoring, as well as for many other biosensing applications. The idea of combining aptamer sensing and quantum dot fluorescence is entering the real-time biosensing field (Yang et al., 2014). With a potential for multispecific detection, QD can also become a useful tool for expansion of existing real-time sensing approaches. Exploring the performance of QDs in a flow sensor format, we have developed a miniature flow aptasensor, suitable for modular assembly, rapid exchange of sensor functionality and multispecific detection.

2. Materials and methods

Commercially available QD Conjugates were used in this study. Semiconductor nanocrystals with CdSe core, encapsulated in ZnS and polymer shells, modified with covalently attached streptavidin, "Qdot® ITK™ Streptavidin Conjugates" (15–20 nm for QD 565-QD705 conjugates, correspondingly) were purchased from Invitrogen Life technologies. Phosphate-buffered saline (PBS) and deionized water were purchased from Invitrogen Life technologies. Ricin Toxin Chain A (RTA) and other chemicals were purchased from Sigma-Aldrich. Botulinum Neurotoxin Type B Light Chain (BoNTA-LC) was purchased from List Biological Laboratories (CA, USA). Custom oligonucleotide synthesis and HPLC purification was performed by Eurofins Genomics (formerly Operon).

Flow Cell components: Quartz tubes (1.5 mm ID, 2.5 mm OD × 7 mm) were custom-cut by Donghai Xuri Lighting Electric Apparatus Co. (China) and (1.5 mm ID, 2.5 mm OD × 6.5 mm) by Polymicro Technologies, Molex Inc. (AZ, USA); silicone tubing was purchased from Altec Products LTD (UK), and custom flow cell body was designed and manufactured by ALine Inc. (CA, USA), Fig. 1.

The portable fluorescent setup included LED 400 nm light source, two fiber optic cables, cuvette holder and miniature USB2000 fiber optic spectrophotometer, all from Ocean Optics (FL, USA) and a variable speed peristaltic pump from Fisher Scientific. The fluorescence spectra and real-time intensity data were recorded using Spectra Suite acquisition software (Ocean Optics) on a laptop computer. (Fig. A1).

2.1. Oligonucleotide probe design

1) AT-Anchor oligonucleotide (used to coat silanized quartz and immobilize the aptamers): 5′-AAAATAAAATAAAATAAAA-TAAAATAAAAT-3′
2) BLC oligonucleotide (aptamer specific to the light chain of botulinum neurotoxin A (Lou et al., 2009) with added 5′ T-rich sequence complementary to AT-anchor for immobilization). Aptamer sequence is shown in bold. Positions of complementary detector oligonucleotides are underlined.
5′-TTTTATTTTATTTTATTTT-CTTGAGTGTCATGG
ACGTTCCGGTCTTGGGCGGGATATTTGTTTGTTTTCTGCCTATGTT-
3′ LC1 LC2 LC3 LC4
3) Detector oligonucleotides (end-biotinylated and partially complementary to BLC aptamer, designed to disrupt possible stem-loop conformations, shown in Fig. A2, appendix A). Complementary portions are shown in bold.
Detector LC1: 5′ biotin-(A_{12})CCATGACACT 3′
Detector LC2: 5′ biotin-(A_{12})ACCGGAACGT 3′

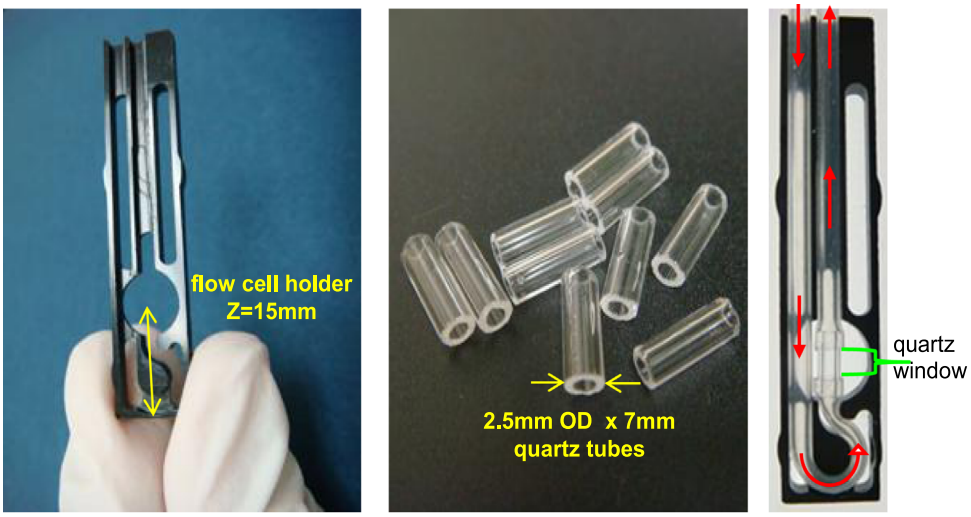

Fig. 1. "Interchangeable Window" flow cell (IWFC). Quartz tube inner volume = 12 μl. The inner surface of quartz tubes is modified with a sensing complex. The tubes are easily interchanged using flexible silicon tubing. IWFC fits diagonally into a cuvette.