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Colorimetric detection with aptamer-gold nanoparticle conjugates coupled to an android-based color analysis application for use in the field

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ABSTRACT

The feasibility of using aptamer-gold nanoparticle conjugates (Apt-AuNPs) to design colorimetric assays for in the field detection of small molecules was investigated. An assay to detect cocaine was designed using two clones of a known cocaine-binding aptamer. The assay was based on the AuNPs difference in affinity for single-stranded DNA (non-binding) and double stranded DNA (target bound). In the first assay, a commonly used design was followed, in which the aptamer and target were incubated to allow binding followed by exposure to the AuNPs. Interactions between the non-bound analytes and the AuNPs surface resulted in a number of false positives. The assay was redesigned by incubating the AuNPs and the aptamer prior to target addition to passivate the AuNPs surface. The adsorbed aptamer was able to bind the target while preventing non-specific interactions. The assay was validated with a number of masking and cutting agents and other controlled substances showing minimal false positives. Studies to improve the assay performance in the field were performed, showing that assay activity could be preserved for up to 2 months. To facilitate the assay analysis, an android application for automatic colorimetric characterization was developed. The application was validated by challenging the assay with cocaine standards of different concentrations, and comparing the results to a conventional plate reader, showing outstanding agreement. Finally, the rapid identification of cocaine in mixtures mimicking street samples was demonstrated. This work established that Apt-AuNPs can be used to design robust assays to be used in the field.

1. Introduction

Nanomaterials have revolutionized the area of sensor development, due to the ability to tune their properties based on the materials size and shape, offering multiple means to design sensing platforms for different applications [1,2]. The combination of biorecognition elements with nanomaterials allows the design of hybrid sensing materials with great target specificity and selectivity and tunable outputs including colorimetric, electronic and others [3]. Despite all these advantages and the great promise of nano-bio sensing systems, the feasibility of using these sensing platforms in-the-field remains to be demonstrated. In the case of colorimetric sensors, the integration of automated analysis tools that simplify data analysis for on-the-spot decision making is critical to help in the transition of this technology to the field.

Metal nanoparticles show size-dependent color change that can be exploited for the design of colorimetric detection systems. In particular, gold nanoparticles (AuNPs) show a red color when dispersed in solution, but change to blue when their aggregation is triggered [4]. The transition from a dispersed state (red) to an aggregated state (blue) has been engineered to result as a response to an external stimulus, creating colorimetric sensors for a myriad of targets [5].

Aptamer based sensing platforms for the detection of small molecules, peptides, proteins, and cells have gained a great deal of interest due to their high selectivity and sensitivity [6,7]. Aptamers are DNA/RNA molecules that bind a target with binding affinities

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in the nanomolar level [8,9]. Advantages specific to aptamers in sensing applications include ease of synthesis, controllable chemical modification, and chemical stability [10,11]. These properties make aptamers ideal candidates for recognition elements in selective target sensors [12].

The use of aptamers to control the aggregation state of AuNPs due to target binding has been realized for a number of aptamer/target pairs [13–15]. In general, an aptamer binding its target involves a conformational change that affects the AuNPs stability, and promotes AuNP aggregation. Despite the many reports on the use of these bio-nanomaterials for sensing different targets, no information is available on the feasibility of their use in field-related settings. Most of the sensing approaches reported are "proof-of-concept" studies that work in a laboratory setting and with a small set of hand-picked controls.

The main objective of this work was to investigate whether aptamers can be used in combination with AuNPs (Apt-AuNPs) to design a colorimetric assay to detect an analyte in field-relevant settings. To show the applicability of our approach to field-testing, an assay to detect cocaine was investigated. For this work, two well-characterized clones of a cocaine-binding aptamer (CBA) were used [16]. Both clones are known to bind cocaine, although with different affinities, but have not been demonstrated in AuNPs colorimetric sensing. In the case of Apt-AuNPs, a conformational change by the aptamer when binding its target has been proposed as critical to produce a colorimetric outcome [17]. The aptamer clone MN6 (short cocaine-binding aptamer) has been identified to transition from an open conformation (single stranded-like) to a more closed conformation (double stranded-like, with extensive base pairing) upon cocaine binding. On the other hand, the MN4 aptamer clone (long cocaine-binding aptamer) was demonstrated to maintain a closed structure in the presence or absence of cocaine, offering no significant conformational change upon binding. These properties of the CBAs were taken into consideration when designing the cocaine detection assays. Importantly, it was demonstrated that the assay design could be optimized to minimize responses to non-target analytes. It was proposed that these false positive responses were due to interactions between the analytes and the AuNPs′ surface which affected their stability, resulting in a color change similar to the one observed by target binding by the aptamer. These unwanted interactions were eliminated by protecting the AuNPs with the DNA prior to exposure to test analytes, resulting in an assay with minimal response to typical cutting agents, filler compounds or other controlled substances. Additionally, conditions that included storing the Apt-AuNPs for prolonged periods of time while maintaining the assay activity were investigated and optimized. Finally, to prevent ambiguities related to color determination by naked-eye analysis of the assay, a fully functional android-based color analysis application (app) was developed. This app was designed to analyze the color of an unknown sample and compared it to the color of positive references, to determine if the substance tested positive for cocaine.

2. Experimental section

2.1. Materials

All the materials were purchased as analytical grade and used without further purification from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Standard 1 mg/mL methanol solutions of ecgonine methyl ester hydrochloride (EME) and cocaine hydrochloride were purchased from Lipomed Inc. (Cambridge, MA). DNAse/RNAse Free water and Quant-iT® OliGreen® ssDNA Reagent and Kit were purchased from Invitrogen Corporation (Carlsbad, CA). HEPES buffer was purchased from Amresco Inc. (Solon, OH). The substances listed in Table 2 were obtained from the United States Army Criminal Investigation Laboratory (USACIL-Forest Park, GA). Centrifuge tubes were purchased from Axygen, Inc. (Union City, CA). The aptamers were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). DNA batches were purified by standard desalting. The aptamer sequences were 5′-GGC GAC AAG GAA AAT CCT TCA ACG AAG TGG GTC GCC-3′ (long cocaine-binding aptamer, MN4) and 5′-GAC AAG GAA AAT CCT TCA ATG AAG TGG GTC-3′ (short cocaine-binding aptamer, MN6).

2.2. Gold nanoparticle synthesis

A 100 mL solution of 1 mM $HAuCl_4$ was heated and refluxed at its boiling point with stirring, and 10 mL of a 38.8 mM sodium citrate solution was added. The solution continued to boil with mixing for 20–25 min. The sample was cooled to room temperature, kept in the dark, and filtered using a 250 mL Corning Filter System with 0.22 μm pore size. The sample was stored at RT wrapped in aluminum foil until used.

The AuNPs were determined to be 15 nm in diameter by dynamic light scattering (DLS). DLS measurements were performed in a Zetasizer Nano-instrument (Malvern Instruments, Westborough, MA) utilized in backscatter mode (173° detection angle) with the temperature set at 20.0 ± 0.1 °C. The final AuNPs concentration was determined to be 10 nM using a Cary 300 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA) based on the extinction measured at 520 nm, using $\varepsilon = 2.4 \times 10^8$ $Lmol^{-1}$ cm^{-1}.

2.3. Free aptamer cocaine colorimetric assay

The AuNPs were diluted with buffer by mixing 7.5 mL of AuNPs synthesized in sodium citrate, as described, with 7.5 mL of 20 mM HEPES 2 mM $MgCl_2$ pH 7.4 buffer in a 50 mL conical vial, and stored in the dark at room temperature, overnight. Test solutions were prepared by adding 1.8 μL of 30 μM CBA, dissolved in water, to 18.2 μL of the desired concentration of the analyte dissolved in buffer. After incubation for 30 min, these solutions were added to 180 μL of the buffer treated AuNPs, and incubated for 30 min. Under these conditions, the aptamers were loaded at a ratio of 60 DNA/AuNPs. Finally, NaCl was added to promote AuNPs aggregation followed by quantification of the color change. Typical NaCl concentrations used in the assay were 25 mM and 40 mM for MN4 and MN6, respectively. The exact NaCl concentration varied slightly on a daily basis and was adjusted to obtain consistent background values.

The assay response was monitored by measuring the AuNPs extinction at 650 nm (aggregated AuNPs, blue color) and 530 nm (dispersed AuNPs, red color) 150 s after NaCl addition in a Spectra Max M5 plate reader (Molecular Devices, Sunnyvale, CA). The data was plotted as the ratio of aggregated-to-dispersed AuNPs (E_{650}/E_{530}, blue/red), and a calibration curve was obtained. The standard deviation (SD) was used to represent the error in the measurements of four replicates. The detection limit (DL) was calculated as three times SD above the blank for all calibration curves. The DL was used as a measure of the sensitivity of the assay. The data was normalized to the blank for ease of comparison and to account for batch variations. Images were taken 150 s after the addition of NaCl with a Canon SLR camera and a smartphone.

2.4. Adsorbed aptamer cocaine colorimetric assay

The CBAs were adsorbed on the AuNPs simply by mixing them and allowing them to incubate in buffer. Two different DNA densities were used 60 and 300 DNA/AuNPs, CBA-60-AuNPs and