Biosensors

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Fast Colorimetric Sensing of Adenosine and Cocaine Based on a General Sensor Design Involving Aptamers and Nanoparticles**

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Aptamers are nucleic acid based binding molecules that are obtained through a combinatorial selection process known as systematic evolution of ligands by exponential enrichment (SELEX).[1,2] They are emerging as a new class of molecules that can rival antibodies in terms of the broad range of molecules they can selectively bind.[3,4] In comparison with antibodies, aptamers, particularly DNA aptamers, are relatively easy to obtain, more stable to biodegradation, and less vulnerable to denaturation. Therefore they are prime candidates as sensors in a number of applications, such as environmental monitoring and medical diagnostics. The key challenge to their successful application is transforming the aptamer-binding events into physically detectable signals. To meet the challenge, a number of methods have been developed, most of which involve fluorescence-based detection.[5–13] Simple colorimetric sensors can eliminate the use of analytical instruments and have attracted much attention recently.[14–17] For example, organic-dye replacement was employed to design a colorimetric cocaine sensor.[14] However, an appropriate dye has to be found for a designated aptamer, and a waiting time of 12 hours is needed to observe a color change. Cationic conjugated polymers form complexes of different color with aptamers in the presence or absence of a target analyte. A number of colorimetric sensors were made with this method.[15]

The high extinction coefficients and distance-dependent optical properties have made metallic nanoparticles very attractive in DNA-related colorimetric assays,[18–20] such as the detection of DNA with high sequence selectivity,[21,22] and the detection of metal ions[23,24] and other analytes.[16,17] Recently, aptamer-functionalized gold nanoparticles were employed to detect thrombin. This system took advantage of the fact that each thrombin molecule binds two aptamers, so nanoparticles

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could be cross-linked by thrombin. Subsequent catalytic growth of the gold nanoparticles was needed for detection with this sensor.[16a] We have previously reported the use of effector-regulated DNA enzymes (aptazymes) for colorimetric adenosine detection.[17] As the aptazyme-cleavage reaction and the assembly of nanoparticles had to be carried out separately, more than one hour was needed for detection.

With the initial success in designing aptamer-based colorimetric sensors, it remains desirable to have a more general method to construct sensors for any aptamer of interest. Preferably, the sensors should be simple to design, easy to operate, give a fast color change, and have a minimal consumption of materials. We herein describe a general method for sensor design that can meet all of the above criteria. To demonstrate the generality of this method, sensors for adenosine and cocaine were constructed, both of which can produce a color change within seconds at room temperature.

We first constructed an adenosine sensor as shown in Figure 1. The sensor was made of nanoparticle aggregates containing three components: gold nanoparticles (13 nm diameter) functionalized with 3′-thiol-modified DNA (3′Adap$_{Au}$), or 5′-thiol-modified DNA (5′Adap$_{Au}$), and a linker DNA (Linker$_{Adap}$) molecule. 3′Adap$_{Au}$ and 5′Adap$_{Au}$ were assembled with Linker$_{Adap}$ to form aggregates, which displayed a faint purple color when suspended in solution. Linker$_{Adap}$ can be divided into three segments. The first segment (in purple) hybridized with a 3′Adap$_{Au}$ nanoparticle. The second segment (in gray) hybridized with the last five nucleotides of a 5′Adap$_{Au}$ nanoparticle. The third segment (in green), the aptamer sequence for adenosine,[25] hybridized with the other seven nucleotides on the 5′Adap$_{Au}$ nanoparticle. In the presence of adenosine, the aptamer changed its structure to bind adenosine.[9,26–28] As a result, only five base pairs were left to hybridize with 5′Adap$_{Au}$, which was unstable at room temperature. Therefore, the 5′Adap$_{Au}$ particles dissociated from the 3′Adap$_{Au}$ particles, resulting in disassembly of the aggregates. Upon disassembly, the color of the system changed from purple to red. Niemeyer and coworkers employed DNA base-pairing interactions, rather than adenosine/aptamer interactions, to disassemble nanoparticle aggregates.[29] The method involving adenosine-induced disassembly was inspired by the recent reports from Nutiu and Li, who discovered that an aptamer underwent a structure-switching process upon binding to target molecules. Fluorescent sensors based on this behavior were developed.[9,30,31]

To study disassembly of nanoparticle aggregates, it is helpful to know the size of an aggregate and the number of linkages among particles. The nanoparticle aggregates assembled by Linker$_{Adap}$ were first characterized by transmission electron microscopy (TEM). Most aggregates were composed of thousands of nanoparticles forming a compact structure of several micrometers. The plasmon frequency of such aggregated particles decreased, which was responsible for the purple color. Typical TEM images are presented in the Supporting Information. The number of thiol-modified DNA molecules attached to each 13-nm-diameter nanoparticle was calculated to be 94 on the basis of the average results reported by two independent groups by assuming the same surface coverage.[32,33] The concentrations of Linker$_{Adap}$ and nanoparticles when preparing the aggregates were 100 nM and 12 nM (6 nM each of 5′Adap$_{Au}$ and 3′Adap$_{Au}$), respectively. As a result, each nanoparticle was connected by approximately 17 Linker$_{Adap}$ aptamers to other nanoparticles.

Such aptamer-linked nanoparticle aggregates were used directly as a colorimetric sensor for the detection of adenosine. The absorbance spectrum of the purple-colored adenosine sensor is shown in Figure 2a (blue curve). The nanoparticle aggregates disassembled within 10 seconds of adding adenosine (2 mM) to give a red colored solution (red curve, Figure 2A). Upon disassembly of the aggregates, the absorbance at 522 nm increased, while the absorbance at 700 nm decreased. We chose to use the absorbance ratio at these two wavelengths to quantify the color of the system, with a high ratio associated with red colored dispersed particles and a low ratio associated with purple colored aggregates. The kinetics of the color change at various adenosine concentrations were monitored by UV/Vis spectroscopy. Faster rates of color change were observed at higher adenosine levels (Figure 2B). The color change was instantaneous in the presence of 2 mM adenosine. Quantitative analysis was performed by monitoring the absorbance ratio at one minute after the addition of adenosine

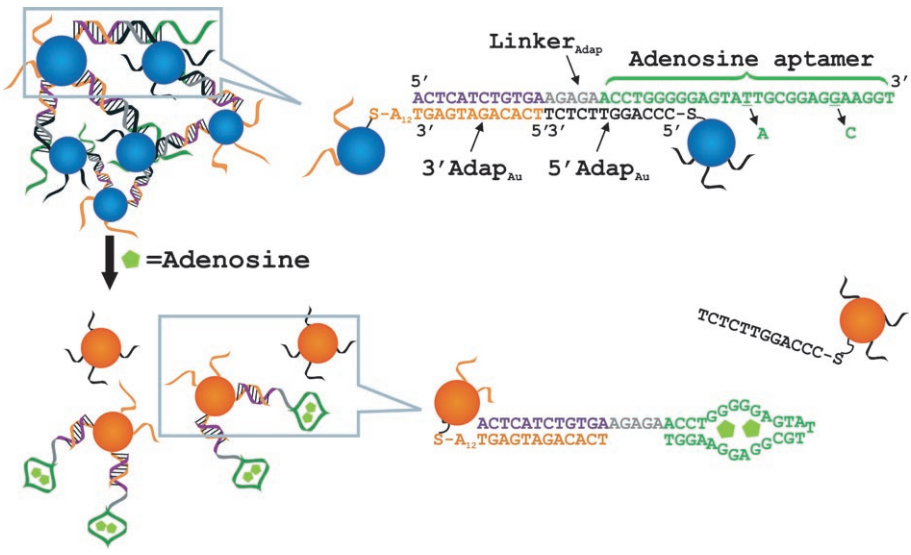

Figure 1. Schematic representation of colorimetric detection of adenosine. The DNA sequences are shown in the right side of the figure. The A_{12} in 3′Adap$_{Au}$ denotes a 12-mer polyadenine chain. In a control experiment, a mutated linker with the two mutations shown by the two short black arrows was used. Note: The drawing is not to scale.

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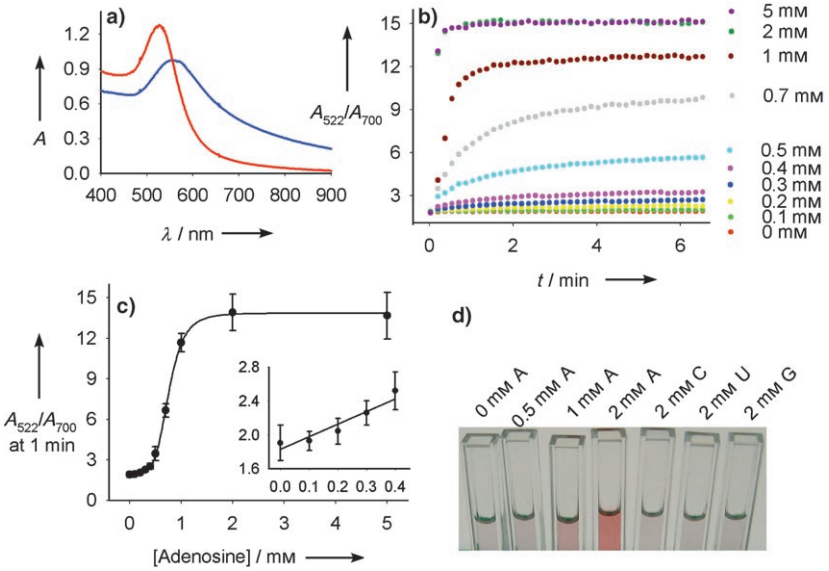

Figure 2. A) Absorbance spectra of the adenosine sensor before (blue) and 10 seconds after (red) the addition of adenosine (2 mM). B) Kinetics of the color change at various concentrations of adenosine. C) Quantification of adenosine concentration by monitoring the absorbance ratio one minute after the addition of adenosine. The data were obtained from three independent measurements and the error bars indicate the standard deviation. D) Photograph of solutions of the sensor one minute after the addition of adenosine or other nucleosides at room temperature. A = adenosine, C = cytidine, U = uridine, G = guanosine.

(Figure 2C), from which adenosine can be detected in concentrations from 0.3 to 2 mM. At low adenosine concentration, only a small fraction of the gold nanoparticles was released from the aggregates. This could be attributed to the fact that there were around 17 linkages for each nanoparticle. All of these linkages need to be broken before a nanoparticle can be released. Therefore, the change in absorbance was very small in the presence of the high background absorbance of the remaining aggregates. As a result, the sensitivity of the current detection method is not as high as that of fluorescence-based detection methods.[9,30,31] The color change was directly observed by the naked eye and allowed qualitative or semiquantitative analysis. An image taken one minute after the addition of nucleosides at different concentration levels is shown in Figure 2D. The color changed from faint purple to red with increasing concentrations of adenosine, whereas the color remained purple for samples with other nucleosides, which suggests that the sensor has high selectivity.

A control experiment was performed to support the mechanism of disassembly shown in Figure 1. Instead of the original linker ($Linker_{Adap}$), a mutated linker was employed to assemble nanoparticles. The sites of mutation are marked in Figure 1; aptamers with this mutated sequence had been shown to be incapable of binding ATP.[9] No color change was observed for aggregates linked by the mutated linker after adding adenosine (see Supporting Information), which strongly suggested that the observed color change was caused by adenosine/aptamer interactions rather than being an artifact. Structural studies by NMR spectroscopy indicated that each adenosine aptamer binds two AMP molecules.[26] As the aptamer has similar affinity for ATP, AMP, and adenosine, two adenosine molecules were drawn for each aptamer in Figure 1.

To test the generality of the method, we constructed a colorimetric sensor for cocaine based on a reported cocaine aptamer, with slight modifications.[7,8] The 3′ and 5′ ends did not form perfect Watson–Crick pairs in the reported aptamer, and no cocaine-induced color change was observed. When these bases were paired, the nanoparticle aggregates assembled with the new aptamer linker ($Linker_{Coc}$, Figure 3) showed a cocaine-induced color change (Figure 4). The rate of color change increased with increasing cocaine concentration (Figure 4A), from which cocaine could be quantified in the range from 50 to 500 μM. An image of the sensor alone and with either adenosine (1 mM) or cocaine (1 mM) is also shown (Figure 4B, inset). Similarly to the adenosine sensor, the colorimetric cocaine sensor can also produce an instantaneous color change.

In conclusion, we have demonstrated a general design of colorimetric sensors based on the disassembly of nanoparticle aggregates linked by aptamers. The sensors are easy to use

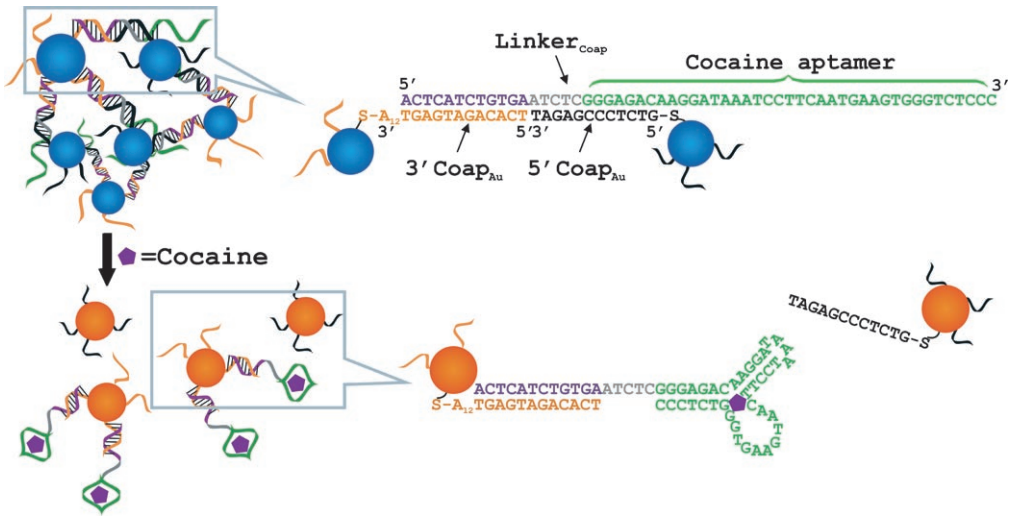

Figure 3. Schematic representation of the colorimetric detection of cocaine based on cocaine-induced disassembly of nanoparticle aggregates linked by a cocaine aptamer.

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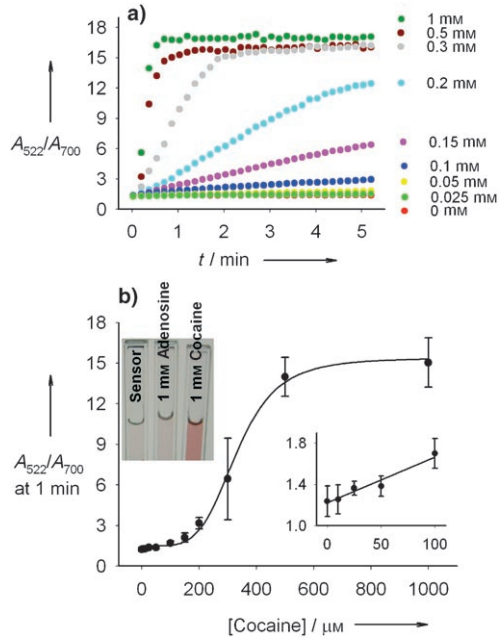

Figure 4. A) Kinetics of the color change of the cocaine sensor at various concentrations of cocaine. B) Quantification of cocaine concentration by monitoring the absorbance ratio one minute after the addition of cocaine. The data were obtained from three independent measurements and the error bars indicate the standard deviation. Inset: Photograph of a solution of the sensor one minute after the addition of cocaine or adenosine at room temperature.

and show a fast color change. As no special features on the aptamers are required, the design should be applicable to any aptamer of choice. Such simple colorimetric sensors should find applications in a diverse range of areas, such as medical diagnostics, environmental monitoring, and the electronic industry.

Experimental Section

Materials: All DNA samples were purchased from Integrated DNA Technologies Inc. (Coralville, IA). The linker DNA molecules were purified by gel electrophoresis, whereas the thiol-modified DNA molecules were purified by the standard desalting method. Adenosine, cytidine, uridine, guanosine, and cocaine hydrochloride were purchased from Aldrich (St. Louis, MO). Gold nanoparticles (13 nm diameter) were synthesized by the citrate reduction method.[22] Thiol-modified DNA was activated with two equivalents of tris(2-carboxyethyl)phosphine hydrochloride (TCEP). TCEP-activated thiol-modified DNA and gold nanoparticles were mixed at room temperature for 16 h or longer, and NaCl (100 mM) and tris(hydroxymethyl)aminomethane (tris) acetate (5 mM) were added to the solution to give a pH value of 8.2. The solution was left at room temperature for another 25 days, and the DNA-functionalized nanoparticles were purified by centrifugation and removal of supernatant before use.

Sensor preparation: Solutions of 5′-thiol-modified DNA (5′$Adap_{Au}$ or 5′$Coap_{Au}$; 500 μL; $A_{522\,nm} = 2.2$; ca. 12 nM) and 3′-thiol-modified DNA (3′$Adap_{Au}$ or 3′$Coap_{Au}$; 500 μL; $A_{522\,nm} = 2.2$; ca. 12 nM) were mixed in the presence of NaCl (300 mM), tris acetate buffer (25 mM; pH 8.2), and linker ($Linker_{Adap}$ or $Linker_{Coap}$; 100 nM). The samples were stored at 4 °C for 90 minutes. Nanoparticles aggregated and changed color from red to purple in this process. The samples were centrifuged, and the precipitates were collected and dispersed in the same buffer (300 mM NaCl; 25 mM tris acetate; pH 8.2).

Detection: To detect adenosine or cocaine, 100 μL of the sensor suspension described above was added to a small volume of concentrated adenosine solution. The buffer contained 300 mM NaCl for adenosine detection and 150 mM NaCl for cocaine detection. The color change was monitored with a UV/Vis spectrophotometer (Hewlett–Packard 8453) at 28 °C for adenosine detection and at 30 °C for cocaine detection. To monitor the color change with the naked eye, the experiments were performed at room temperature (ca. 23 °C).

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