Sensors

DOI: 10.1002/ange.200603106

A Simple and Sensitive "Dipstick" Test in Serum Based on Lateral Flow Separation of Aptamer-Linked Nanostructures**

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Aptamers are single-stranded nucleic acids that can be selected from a large random library to bind a number of molecules with high affinity and specificity. Therefore, they are considered to be the nucleic acid version of antibodies.[1,2] Since its first discovery in the early 1990s,[3,4] the use of aptamers for sensing and diagnostic applications has been extensively explored.[5–11] Many detection methods developed for antibodies have been successfully adapted to aptamer-based detections.[11–15] In many cases, aptamers show similar or even better performance when compared with antibodies in the laboratory.[12–14] However, practical applications of aptamer-based sensing and diagnostics, such as home and

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[**] This material is based upon work supported by the US Department of Energy (DE-FG02-01ER63179), the National Science Foundation through a Science and Technology Center of Advanced Materials for Purification of Water with Systems (WaterCAMPWS; CTS-0120978) and a Nanoscale Science and Engineering Center (NSEC; DMR-0117792) program, and by the US Army Research Laboratory and the US Army Research Office (DAAD19-03-1-0227).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

clinical tests, are still lagging behind antibody-based tests because aptamer-based sensors are not as yet user-friendly for average users who have limited training in chemical or biological laboratories.

Recent reports of colorimetric sensors based on signaling aptamers are a positive step towards improving user-friendliness as detection results can be observed with the naked eye without the need for sophisticated instruments.[16–20] For example, by using DNA aptamers to assemble DNA-functionalized gold nanoparticles, we have constructed colorimetric sensors with instantaneous color response for a wide range of analytes, such as adenosine, cocaine, K^+, and their combinations.[20,21] However, two disadvantages have prevented their practical applications in homes and in the field. The first disadvantage, common to all aptamer-based sensing methods reported so far, is that detection still requires professional laboratory-type operations, such as precise transfer of solutions, making it less useful for people who do not have a scientific background. Second, the sensors have low sensitivity for instrument-free observation. For example, to observe a distinct color change with the naked eye, a concentration of 0.5 mM or higher of adenosine was needed for the adenosine sensor, which was approximately 50-times higher than the K_d value ($\approx 10\ \mu M$) of this aptamer.[22] The low sensitivity is attributable to the multiplex connecting nature of the nanoparticle aggregates that gives a strong purple background. At low adenosine concentrations, only a small fraction of nanoparticles dissociate from the aggregates and change to a red color, which is likely to be masked by the strong purple background.

One of the most useful methods to convert antibody-based assays to user-friendly test kits is the lateral-flow technology, and a well-known example is the commercially available pregnancy test kit. Despite the wide applications in antibody assays, nucleic acid based lateral flow devices were only demonstrated for DNA detection.[23] To overcome the two limitations of aptamer-based sensors in the solution phase, we report herein, aptamer-based lateral flow devices that can be used as simple "dipsticks" or a litmus-test type of assay.[24] We show that these devices are not only simpler to operate, but also more sensitive than solution-based tests owing to the integration of binding, separation, and detection on a simple test-paper-like platform with no background interference.

We first chose the adenosine aptamer to build a model system to study aptamer-based lateral flow devices. Adenosine-responsive nanoparticle aggregates containing two kinds of DNA-functionalized gold nanoparticles (particles 1 and 2 in Figure 1a) and an aptamer DNA were prepared. Detailed DNA sequences, modifications, and linkages are shown in Figure 1b. Two kinds of thiol-modified DNA were used to functionalize particle 2: biotinylated and non-biotinylated. The biotin modifications (★) allowed the nanoparticles to be captured by streptavidin. The optimal ratio between the two DNA molecules was determined to be 1:1 because using 100% biotinylated DNA led to low yield of nanoparticle aggregates (<20%), whereas 10% led to inadequate streptavidin capture (data not shown). As the association rates of the two DNA molecules to gold nanoparticles were unknown,

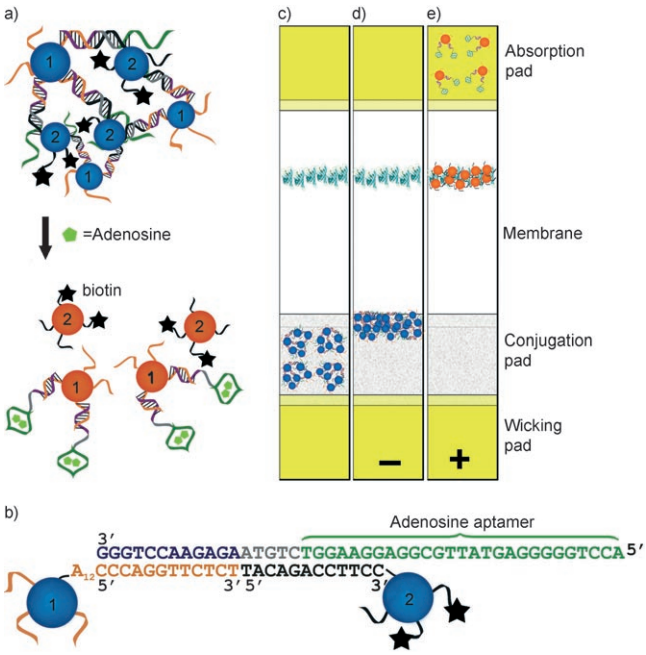

Figure 1. Aptamer/nanoparticle-based lateral flow device. a) Adenosine-induced disassembly of nanoparticle aggregates into red-colored dispersed nanoparticles. Biotin is denoted as black stars (★). b) DNA sequences and linkages in nanoparticle aggregates. Lateral flow devices loaded with the aggregates (on the conjugation pad) and streptavidin (on the membrane in cyan color) before use (c) and in a negative (d) or a positive (e) test.

the ratio of the two DNA molecules on particle 2 was only estimated to be 1:1.

The lateral flow devices consisted of four overlapping pads placed on a backing (Figure 1c,d,e). The four pads were as follows (from top to bottom): absorption pad (15 mm), HiFlow Plus membrane (25 mm), glass fiber conjugation pad (13 mm), and wicking pad (15 mm). The aptamer-linked nanoparticle aggregates were spotted on the conjugation pad, and streptavidin was applied on the membrane as a thin line (Figure 1c). The whole device was then dried overnight at room temperature before use. We hypothesize that nanoparticle aggregates are too large to migrate along the membrane, whereas dispersed nanoparticles can. If the wicking pad of the device is dipped into a solution, the solution will move up along the device and rehydrate the aggregates. In the absence of adenosine, the rehydrated aggregates will migrate to the bottom of the membrane where they stop because of their large size (Figure 1d). In the presence of adenosine, the nanoparticles would be disassembled owing to binding of adenosine by the aptamer (Figure 1a).[9,20] The dispersed nanoparticles can then migrate along the membrane and be captured by streptavidin to form a red line (Figure 1e).

To successfully carry out the detection, the first challenge is to preserve the aptamer activity and the connections between nanoparticles in the dry state. Each aggregate contains hundreds to thousands of DNA-linked nanoparticles. Direct drying in buffer solution could damage the aggregates. Sucrose is a commonly used additive to keep DNA in its

native state, and the effect of sucrose on drying was first studied. Five conditions with varying sucrose concentrations were tested (Figure 2a). At each sucrose concentration, three devices were used with the first one being an unused device, the second one being a negative test (without adenosine), and the third one being a positive test (with 0.5 mM adenosine). Direct drying (no sucrose) deactivated the aggregates, and no red band was observed in the presence of adenosine (Figure 2a, 0% sucrose). Interestingly, inclusion of 2% sucrose helped preserve the aggregates and a slight red band was observed on the membrane. With 8% sucrose, an intense red band was observed in the positive test, whereas no band was observed in the negative test. Instead, a dark band at the boundary between the conjugation pad and the membrane was observed. This observation supported our hypothesis that the aggregates cannot migrate along the membrane. The presence of aggregates on the boundary provides a useful control. If no such line is observed for a negative sample, the test is invalid, indicating poor rehydration or flow of the device. A further increase in the sucrose concentration up to 30% also showed intense red bands for positive tests, but slight bands in the negative tests could also be observed. Therefore, 8% sucrose was chosen for further experiments.

Under optimized drying conditions, sensitivity and selectivity of the devices were tested. The devices were dipped into buffer solutions containing various nucleoside species at different concentrations (Figure 2b). No red band was observed in the absence of adenosine. With increasing adenosine concentrations, more intense red bands were observed, and the detection limit was ca. 20 μM. For the other ribonucleosides, no red bands were observed with 1 mM cytidine or uridine, suggesting that the high selectivity of the aptamer was maintained. Guanosine was not tested because of its poor solubility at room temperature. Because the detections are solely based on intensity of the same color instead of color change, such sensors can provide qualitative or semiquantitative results. In fact, for most lateral flow based detections, such as the pregnancy test, only a yes or no answer is needed.

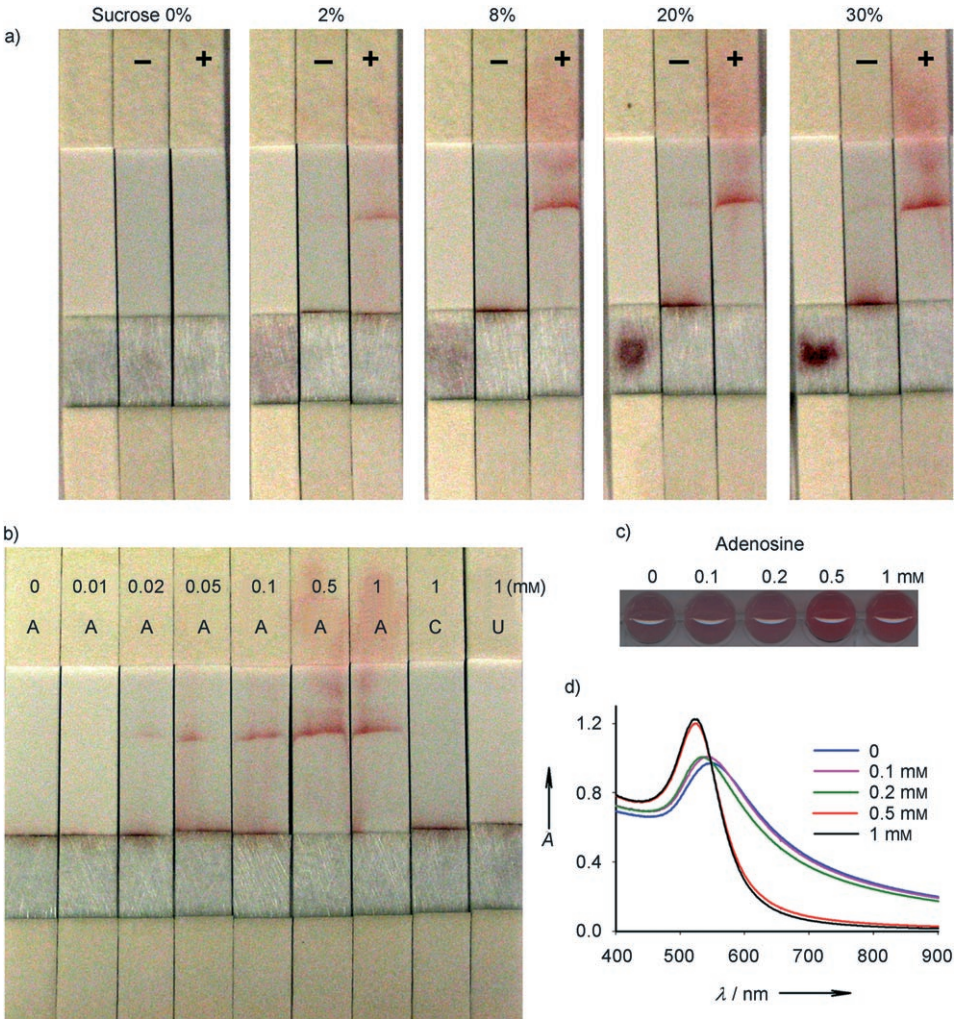

Figure 2. Lateral flow based detection of adenosine. a) The effect of sucrose concentration during drying. b) Test of the adenosine sensing lateral flow device with varying concentrations of nucleosides. A = adenosine, C = cytidine, U = uridine. c) Scanned image of adenosine-dependent color change of the aptamer-linked aggregates in solution phase. d) UV/Vis spectra of the samples in (c).

A solution-phase reaction was also carried out for comparison. To insure a fair comparison, the same batch of aggregates was used for both flow-based and solution-phase reactions under optimized conditions. As can be observed in Figure 2c, 0.5 mM adenosine was needed to observe a red color. The extinction spectra of the solution-phase samples were also recorded on a spectrophotometer (Figure 2d), and a small shift was observable with only 0.1 mM adenosine. This difference, however, cannot be distinguished by the naked eye. Compared with solution-phase results, the flow device had at least 10-fold higher sensitivity if the naked eye was used as a detector. Further improvement of sensitivity could be realized by color-enhancing methods (e.g., gold nanoparticles can be used as a catalyst to grow metallic silver to drastically improve the sensitivity for visual detection.[25])

To demonstrate the generality of the method described herein, we further prepared nanoparticle aggregates linked by a cocaine aptamer,[16,20,26] as shown in Figure 3a. The aggregates were loaded in lateral flow devices, and the device

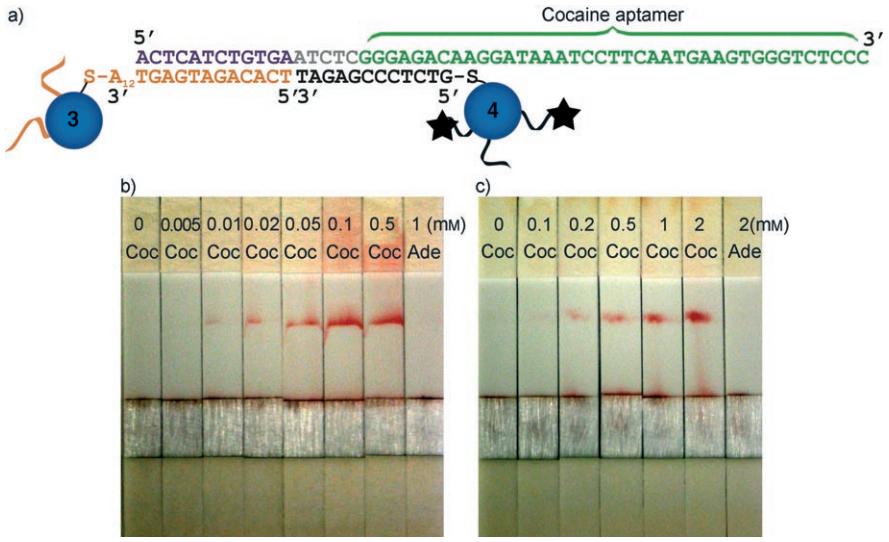

Figure 3. Lateral flow based detection of cocaine. a) DNA sequences and linkages in cocaine aptamer-linked nanoparticle aggregates. Test of the cocaine-sensing lateral flow device with varying concentrations of cocaine in buffer solution (b) and in undiluted human blood serum (c). Coc = cocaine, Ade = adenosine.

responses to varying concentration of cocaine were tested (Figure 3b). The intensity of the red bands increased with increasing cocaine concentration, and the detection limit was ca. 10 μM. As a negative control, the device did not show a red line with 1 mM adenosine (the last strip in Figure 3b).

Finally, we investigated the possibility of using such devices to detect analytes in human blood serum. Cocaine was spiked into untreated serum, and 10 μL of the serum samples were added directly to the conjugation pads to rehydrate and react with the nanoparticle aggregates. After 20 seconds, the wicking pad part of the device was dipped into a running buffer solution. As can be seen in Figure 3c, a distinct red line can be observed when the serum contained 0.2 mM cocaine and the color intensity increased with increasing cocaine concentration; adenosine failed to produce a red line. These results demonstrate that the device is compatible with biological samples, making applications in medical diagnostics possible. The sensitivity in serum was about 20-fold lower compared with that in buffer solution, which was mainly attributed to the difference in buffer-solution conditions and possible cocaine degradation in serum. The serum sample was directly used without any dilution or desalting and, as a result, the ionic strength appeared to be higher than the optimal conditions for the sensor, which leaves us with room for further optimization to increase sensitivity.

In summary, we have immobilized both adenosine and cocaine aptamer-linked nanoparticle aggregates onto a lateral flow device, resulting in a simpler and more excitingly, a more sensitive "dipstick" test than the corresponding test in solution. Significantly, the device can perform in complex sample matrix such as in human blood serum. A novel aspect of the lateral flow device described herein is that it takes advantage of the physical size difference of nanoparticles in various assembly states, and the fact that aggregated nanostructures do not move along the membrane, which provide a critical control for the performance of the device. As aptamers for a broad range of molecules have been obtained,[1,2] this lateral flow method should be general enough so that it can be adapted to develop dipstick tests for any analyte that is compatible with the nanoparticle/aptamer-based assay.

Experimental Section

Materials: All DNA samples were purchased from Integrated DNA Technologies Inc. Nucleosides, cocaine, avidin from egg white, and human blood serum were purchased from Aldrich. Streptavidin was purchased from Promega. Gold nanoparticles were prepared following literature procedures.[27]

Device preparation: The Millipore Hi-Flow Plus Assembly Kit (Millipore Corporation, Bedford, MA) was used. The kit contains a Hi-Flow Plus Cellulose Ester Membrane with a nominal capillary flow time of 90 seconds/4 cm, an absorption pad, a wicking, and a glass fiber conjugation pad. The device assembly was shown in Figure 1c (see also the Supporting Information). Nanoparticle preparation, functionalization, and assembly have been described in detail previously.[20,28] Note that particles 2 and 4 (Figure 1a,b and Figure 3a) were functionalized with two kinds of thiol-modified DNA with an equal molar ratio. One DNA contained a biotin moiety at one end, whereas the other did not contain a biotin. For functionalization, 1.5 μM of each DNA was added to the nanoparticles. Particles 1 or 3 were functionalized with only a thiol-modified DNA. After forming nanoparticle aggregates, the aggregates were separated from dispersed nanoparticles and free DNA by centrifugation. To allow a fast disassembly of nanoparticle aggregates, very brief centrifugation was used to avoid formation of very large aggregates and the supernatant was removed by a pipette. The purified nanoparticle aggregates were dispersed in designated buffer solutions (in most experiments: 8% sucrose, 200 mM NaCl, 25 mM Tris acetate; pH 8.2) and agitated vigorously with a pipette. The color of the aggregates should be dark purple. Nanoparticle aggregates (6 μL) were spotted on each conjugation pad, and 10 mg mL^{-1} streptavidin (2 μL) was applied on the membrane by a 2 μL pipet to form a line. The loaded strips were stored in a drawer overnight before use. To detect cocaine in serum, 100 mg mL^{-1} egg white avidin (3 μL) was applied on the membrane for each device.

Detection: For adenosine detection, varying concentrations of nucleosides were dissolved in a buffer solution containing 100 mM NaCl, 25 mM Tris acetate, pH 8.2. For cocaine detection in buffer solution, the NaCl concentration was 50 mM. The wicking pad part of each device was dipped into the solutions for ca. 20 seconds when the conjugation pad was fully hydrated and the liquid started to migrate on the membrane. Then the device was placed horizontally on a plastic surface for the flow to continue. For cocaine detection in serum, 10 μL of serum was added on the conjugation pad. After 20 seconds, the wicking pad was placed in a running buffer solution containing 100 mM NaCl, 25 mM Tris acetate, pH 8.2 to allow flow to happen. A digital camera was used to take pictures of the devices after ca. 5 minutes. For solution-phase reactions, the same aggregates were dispersed in 200 mM NaCl, 25 mM Tris acetate, pH 8.2. Varying concentration of adenosine was added and the color of each solution was acquired by a scanner and quantified by a UV/Vis spectrometer (Hewlett-Packard 8453).

Received: August 1, 2006
Revised: September 19, 2006
Published online: November 9, 2006

Keywords: aptamers · cocaine · lateral flow · sensors · trace analysis

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