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Binding-induced collapse of DNA nano-assembly for naked-eye detection of ATP with plasmonic gold nanoparticles

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ABSTRACT

The detection of small molecules depends heavily on complicated GC–MS (Gas chromatography–mass spectrometry), HPLC (High-performance liquid chromatography) and some other complicated instruments that are not suitable for point of care detection. Here, we have demonstrated a fast (in 10 min), simple (instrument-free) and effective detection platform for small molecule-ATP. In our design, we engineered the hybridization region of aptamer and assembled it into a superstructure to avoid the exposed flexible ends. The binding of ATP triggered the collapse of the superstructures to produce single stranded DNA that can obviously tune the plasmonic coupling of unmodified gold nanoparticles (AuNPs). Compared to detection platforms based on fully hybridized aptamer double helix, the detection time was significantly decreased to 10 min. The resulting color change can be recognized by naked eyes. Our detection is highly specific and selective. Furthermore, a logic gate with multiplexed detection capability for ATP and DNA were demonstrated.

1. Introduction

Small molecules are involved in many biological processes including cell signaling, modulation of immune system and metabolic process (Esengil et al., 2007; Sreekumar et al., 2009). However, the detection of these small molecules usually depends on the GC–MS (Gas chromatography–mass spectrometry), HPLC (High-performance liquid chromatography), and some other complicated instruments which are expensive, complicated, time consuming and not suitable for point-of-care applications (Sreekumar et al., 2009). Here, we have developed a fast (in 10 min), instrument-free detection platform for small molecule-ATP, which could be accomplished in 10 minutes by persons without training experience.

There remains challenging in the development of simple, fast and instrument-free biosensors for applications in resource-limited settings (Chin et al., 2011; Ferguson et al., 2011; Park et al., 2014; Xiang and Lu, 2011). Recently, localized surface plasmon coupling of gold nanoparticles (AuNPs) has been widely employed to develop colorimetric detections. Especially, unmodified AuNPs based methods have attracted extensive interests because of their easy operation and simple readout(Arvizo et al., 2013; Du et al., 2011; Li and Rothberg, 2004; Li et al., 2012a; Liu et al., 2013; Liu et al., 2012; Nelson and Rothberg, 2011; Sener et al., 2014; Wang et al., 2007; Xia et al., 2010; Zhang et al., 2013; Zhang et al., 2012). The unmodified AuNPs can effectively discriminate double stranded or folded DNA from flexible, single stranded DNA, which leads to an obvious color change from red to purple or blue(Akhlaghi et al., 2012; Li and Rothberg, 2004; Nelson and Rothberg, 2011; Zhang et al., 2012). Based on this principle, aptamers are widely used as ligands to bind small molecules and the resulting conformational change of aptamer upon binding can be discriminated by the color change of unmodified AuNPs (Arvizo et al., 2013; Du et al., 2011; Li and Rothberg, 2004; Li et al., 2012a; Liu et al., 2013; Liu et al., 2012; Nelson and Rothberg, 2011; Sener et al., 2014; Wang et al., 2007; Xia et al., 2010; Zhang et al., 2013; Zhang et al., 2012). The above mentioned prototype detection platform were successfully used to detect small molecules such as ATP, cocaine and glucose as low as sub-micromole.

However, not all aptamer undergoes obvious conformational change, which hampers the generalizability of this strategy (Wang et al., 2007; Zuo et al., 2007; Zuo et al., 2009). As an alternative

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strategy, aptamers are hybridized to the complementary strands to form a rigid double stranded DNA(Cui et al., 2012; Das et al., 2012; Du et al., 2008; Guo et al., 2011; Huang et al., 2010; Li et al., 2012b; Lu et al., 2011; Wang et al., 2007; Wu et al., 2013; Yang et al., 2013; Zhou et al., 2011; Zuo et al., 2007; Zuo et al., 2009). The binding of small molecules de-hybridizes the double stranded DNA and releases original aptamers and single stranded DNA. This dehybridization process can be translated to the color change of unmodified AuNPs. However, the binding induced dehybridization of fully hybridized double helix is usually time-consuming (~3 hours)(Du et al., 2008; Wang et al., 2007; Wu et al., 2013; Zuo et al., 2007).

Here, we designed a superstructure of DNA hybridization, in which a part of aptamer is hybridized to its complementary strand. The hybridization region contains only 17 base pairs in our study, which is shorter than fully hybridized aptamer double helix (27 base pairs). Then, to shield the exposed flexible end that can protect AuNPs from aggregation, we employed the partially hybridized complex as a basic unit to assemble the superstructure. The target binding disassembled this superstructure into aptamer and single stranded DNA in 10 minutes, which is fast. The surface plasmonic coupling of unmodified AuNPs can be tuned by the target binding induced disassembly of the rigid superstructure and the detection results can be read by naked eyes.

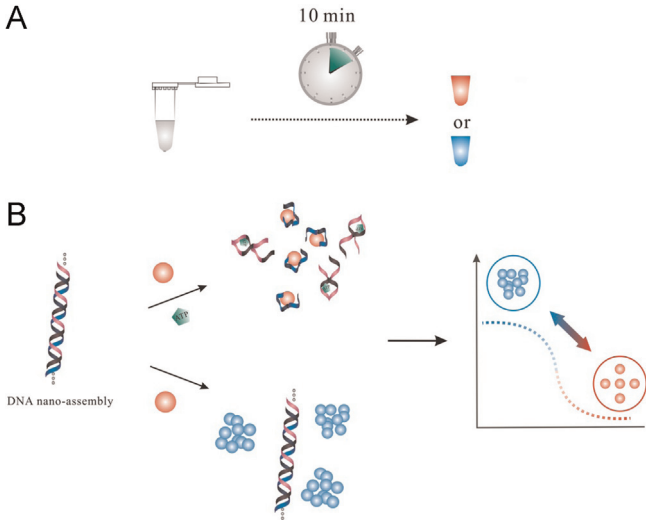

Fig. 1. (A) We have developed a fast detection platform for the selective detection of small molecule-ATP in 10 min and the resulting color change can be read by naked eyes. (B) In principle, our detection is based on the target binding-induced collapse of DNA nanoassembly. The generated single stranded DNA can effectively prevent the aggregation of unmodified AuNPs. Whereas, without target, the relatively rigid DNA nano-assembly does not possess the ability to protect the AuNPs.

2. Materials and methods

All oligonucleotides were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China) and dissolved in ultrapure water. The sequences are as following (Table 1):

ATP, TTP, CTP and GTP were obtained from Sigma-Aldrich. Gold nanoparticals (~20 nm in diameter, AuNP) were purchased from BBI Company. Ultrapure water that obtained from a Millipore water purification system (Mili-Q,Millipore) was used in all experiments. All other reagents were of analytical grade and used without further purification. The photographs were taken with Olympus C-5060 digital camera. The UV–vis absorption spectra were performed with a Hitachi UV-3010 spectrophotometer.

The original concentration of gold nanoparticles is about 1.16 nM. The gold nanoparticles were concentrated to 8nM in a centrifugation at 10620 g for 10min at 25 °C. The final concentration was determined by UV-visible spectroscopy.

The solution of ATP、TTP、CTP、GTP was prepared with Tris buffer (10mM, pH 7.4, 120 mM NaCl) and the concentration of stock solution is 5mM.

Typically, the DNA nanoassembly can be self-assembled through a heat-annealing process. ATP-aptamer and Anti-ATP-aptamer (10 μL each, 100 μM) were mixed in Tris–HCl buffer (10 mM, pH 7.4, 120 mM NaCl). At the same time, different concentration of ATP was added to the above solution to make the final volume of 100 μL. Firstly, the mixtures were heated to 80 °C for 5min, and allowed to cool at room temperature for another 5 min. Subsequently, 75 μL of the above-mentioned reaction mixture was transfered into a new tube. Then, the solution of AuNP (8 nM, 25 μL) was added to the tube. And the final concentration of AuNP was 2 nM. The resulting solution was detected by either visual observation or UV–vis characterization. To evaluate the specificity of ATP detection, we used the TTP、CTP and GTP as the control.

Table 1
The oligonucleotides that were used in our study.

ATP-aptamer	5′-CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG-3’
Anti-ATP-aptamer	5′-TACTCCCCCAGGTGCCGACGGCACCTTCCTCCGCA-3’
Capture-ATP-DNA	5′-ACCTGGGGGAGTATTGCGGAGGAAGGT-3’
Anti-ATP-DNA	5′-ACCTTCCTCCGCAATACTCCCCCAGGT -3’
Anti-ATP-aptamer2	5′-CGGCACCTTCCTCCGCA-3’

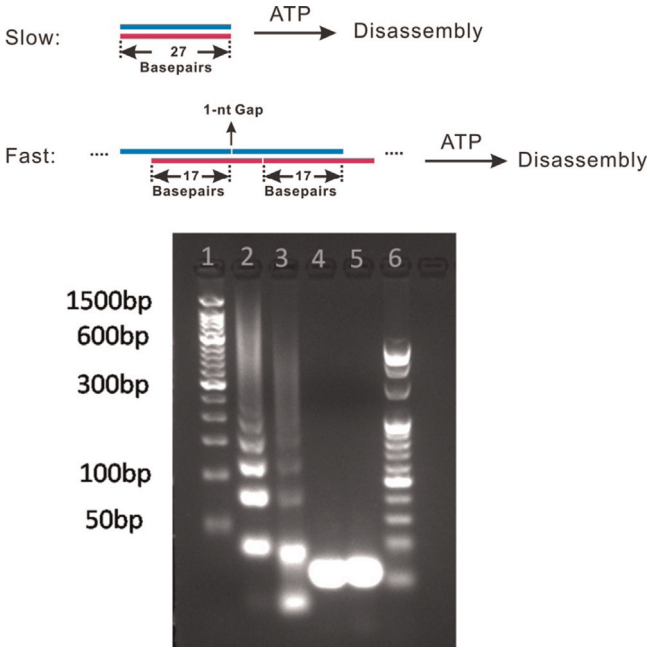

Fig. 2. The ATP binding induced the collapse of the superstructure in 10 min, which is proved by the gel analysis. As a contrast, the fully hybridized double helix cannot be destroyed in such a short time. (Lane 1: DNA ladder; Lane 2: superstructure; lane 3: mixture of superstructure and ATP; Lane 4: fully hybridized aptamer double helix; Lane 5: mixture of fully hybridized aptamer double helix and ATP; Lane 6: DNA ladder).

Agarose gel (3%) was prepared using a TBE (1X) buffer. The gel red was mixed with samples. The gel was run at 80V for 90 min in TBE (1X) buffer and was then scanned using the gel image analysis system.

In a typical target DNA assay, we used the Anti-ATP-apatmer (100 μM, 10 μL) as the capture DNA probe. Then different concentration of target DNA was added to the above mixture to make

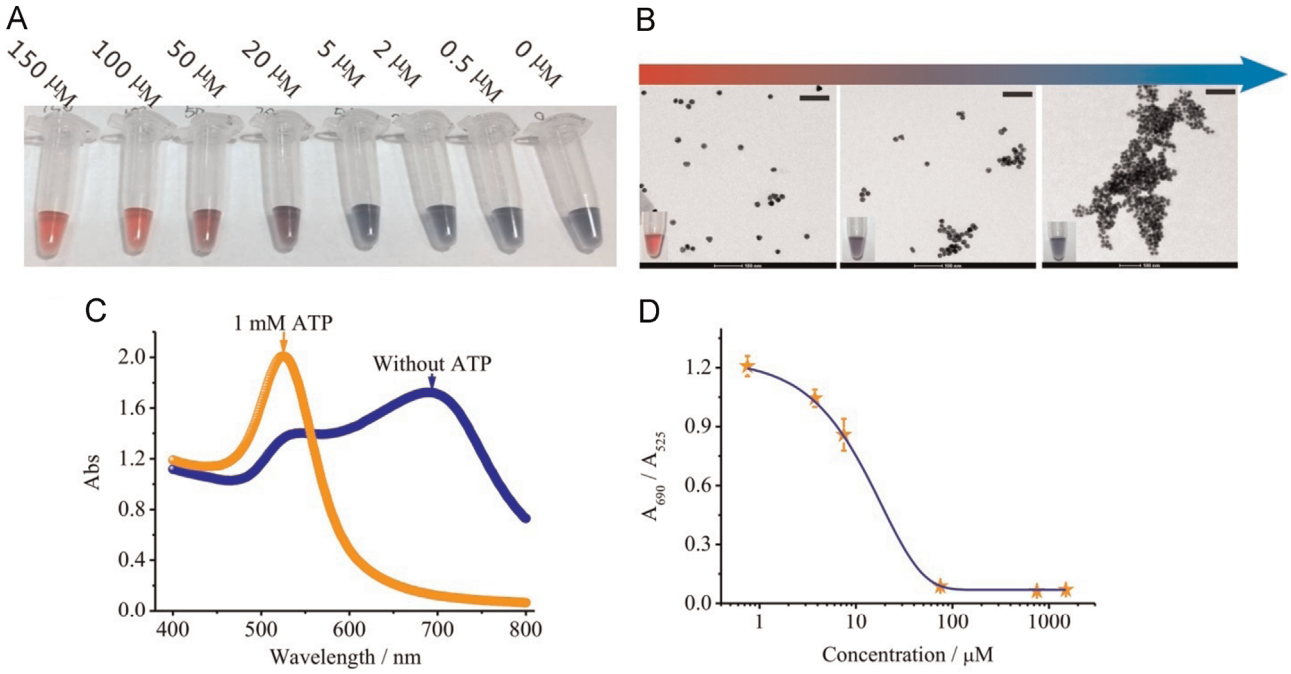

Fig. 3. (A) Color change of AuNPs at different concentration of ATP. (B) The status of AuNPs is well characterized by SEM (from left to right: 1 mM ATP, 10 μM ATP, without ATP. Scale bar: 100 nm.) (C) The plasmonic peaks of AuNPs with and without ATP. (D) The relationship between the concentration of ATP and the ratio of plasmonic peaks at 525 nm and 690 nm.

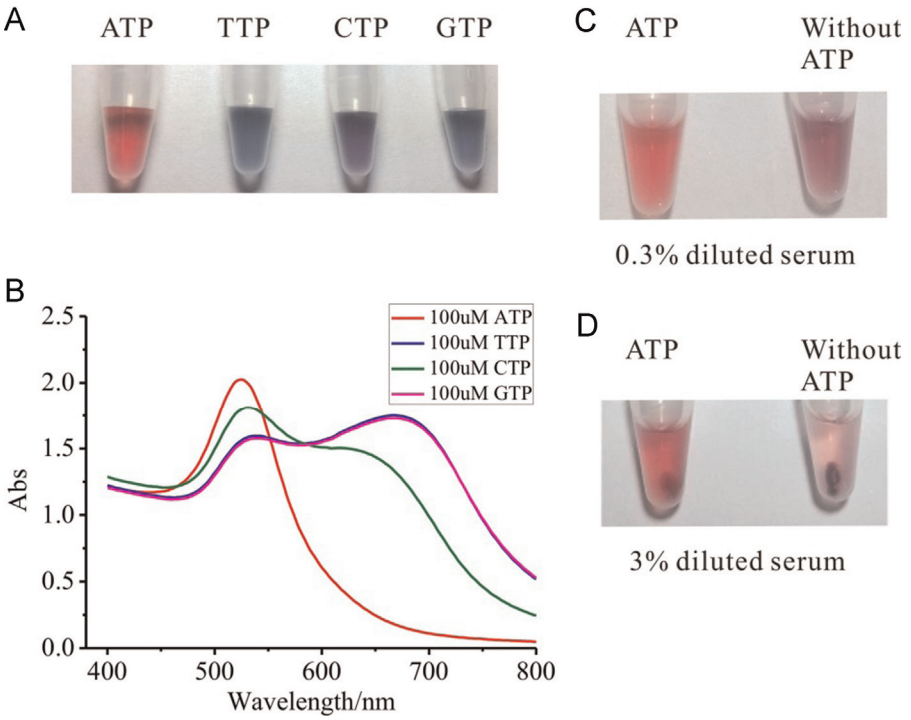

Fig. 4. (A) ATP effectively induced collapse of DNA superstructure. The AuNPs can be stabilized well by the produced single stranded DNA. The TTP, CTP and GTP have no influence on the DNA superstructure and the AuNPs cannot be protected. (B) The plasmonic peaks of AuNPs in the presence of ATP, TTP, CTP and GTP, respectively, which demonstrated excellent specificity of our detection. (C) In complicated sample (0.3% diluted serum), we can still selectively detect ATP through the color change of AuNPs. (D) In 3% diluted serum, due to the non-specific adsorption of proteins on the AuNPs (which can protect the AuNPs from aggregation), we need to centrifuge the sample to complete the detection. The concentration of ATP is 100 μM.

the final volume to be 100 μL. The solution was heated to 80 °C for 5 min, and then allowed to cool at room temperature for another 5 min. Subsequently, 75 μL of the above-mentioned reaction mixture was added into a new tube. Then, the solution of AuNP (8 nM, 25 μL) was added to the tube. And the final concentration of AuNP was 2 nM. The resulting solution was detected by either visual observation or UV–vis characterization.

To demonstrate that this method is able to recognize ATP in complicated matrices, we detected ATP in diluted serum (~3%, v/v). Then we centrifuged resulting solution for 2 min at 14,000 rcf.

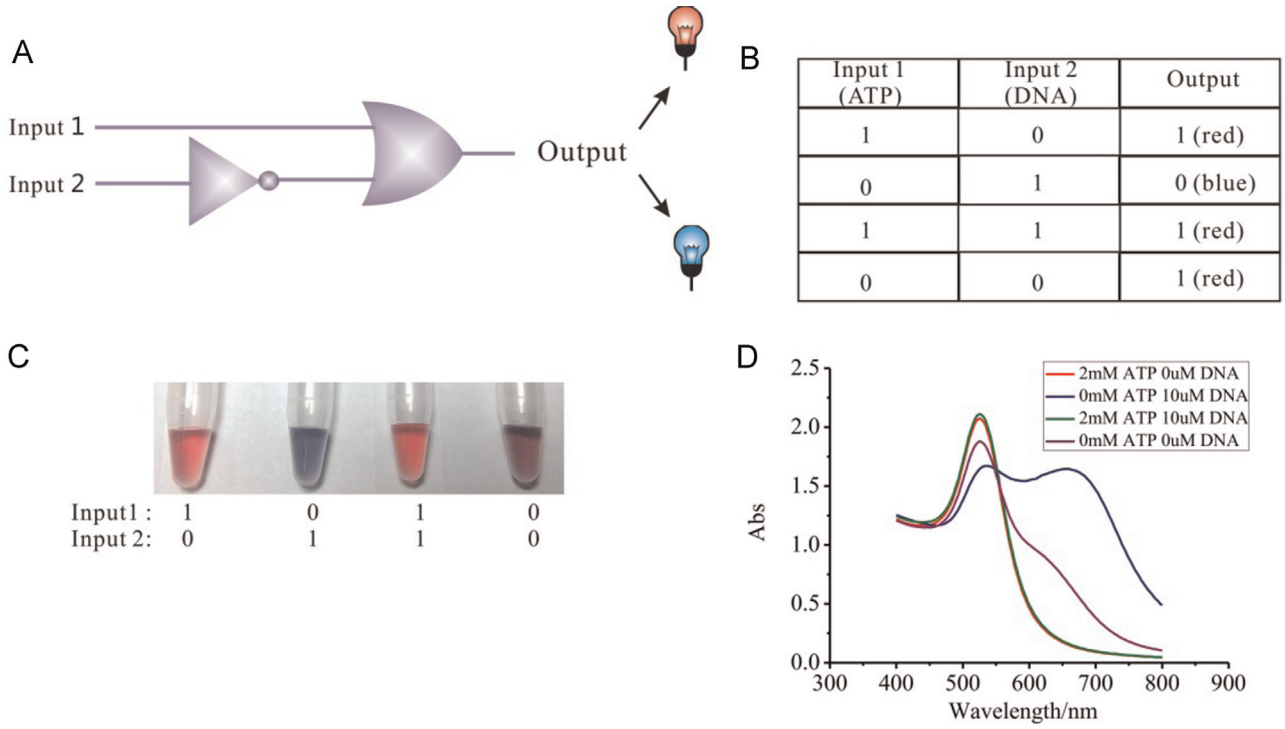

Fig. 5. (A) By using ATP and DNA as inputs and the color of AuNPs as output, we constructed the logic gate. (B) The truth table of the logic gate. (C) The resulting color of our logic gate with different combination of inputs. (D) The plasmonic peaks of AuNPs at different combination of inputs.

Finally, the sample with ATP (100 μM) was red, whereas, the sample without ATP turned to light blue.

3. Results and discussion

At first, to circumvent the problem of slow kinetics in the target binding induced displacement process (which is widely used as detection platform for ATP by our group and others(Du et al., 2008; Wang et al., 2007; Wu et al., 2013; Zuo et al., 2007)), we engineered the hybridization region of ATP's aptamer with its complementary strand. The hybridization region contains only 17 base pairs in our study, which is shorter than fully hybridized aptamer double helix (27 base pairs). Then, to shield the exposed flexible end that can protect AuNPs from aggregation(Liu et al., 2013), we employed the partially hybridized complex as a basic unit to assemble the superstructure (Fig. 2). In the superstructure, we designed a 1-nt gap between the basic units, which may facilitate the binding of ATP because of the thermodynamic change originated from the gapped DNA double helix (Lane et al., 1997). We characterized the ATP binding induced dehybridization process by gel analysis. We observed that the superstructure can be disassembled in a short time (10 min) upon the binding of ATP (Fig. 2 and figure S1, S2). Whereas, the fully hybridized double helix has no obvious change in such a short period (Fig. 2).

Based on this engineering, we tuned the surface plasmonic coupling of AuNPs through the target binding induced collapse of DNA superstructure (Figs. 1 and 3). The DNA superstructures with relatively rigid backbone cannot adsorb on the AuNPs. Hence, these superstructures have no ability to protect AuNPs from aggregation. As a result, the color of AuNPs turned blue (Fig. 3A). After mixing the superstructures with ATP, the target binding induced the collapse of the superstructures, which produced folded aptamers and single stranded DNA. The resulted products adsorbed on the AuNPs and stabilized the AuNPs very well (Fig. 3A, the detection limit for naked eye detection is ~5 μM). The status of AuNPs at different concentration of ATP can be well characterized by SEM (scanning electron microscope). With 1mM ATP, the AuNPs that were fully protected by single stranded DNA dispersed well (Fig. 3B, left). At the ATP concentration of 10 μM, the AuNPs with limited protection and aggregated with a purple color (Fig. 3B, middle). Without ATP, the AuNPs aggregated heavily by losing the protection of single stranded DNA (Fig. 3B, right). Gold nanoparticle aggregation induced the combination of the plasmon resonances. Then the aggregation appeared as one large particle rather than small individual nanoparticles. Plasmon resonance associated-absorption wavelengths shifted to longer wavelength, in turn, reflected light shifted from red to blue. Therefore the color changed from red to blue on aggregation. We compared the plasmonic peaks of AuNPs with ATP (1 mM) and without ATP, the plasmonic peak at 525 nm decreased in intensity, while the plasmonic peak at 690 nm increased dramatically. Finally, we quantified the ATP by using the ratio of plasmonic peaks at 525 nm and 690 nm. We can sensitively detect ATP as low as 0.75 μM using the UV–vis spectrometer (based on the 3σ standard). The sensitivity is comparable to the sensitivity of biosensors with electrochemical or fluorescent readout (table S1). The AuNPs with various diameters can be used to realize the ATP detection (figure S3).

The specificity and selectivity of our detection platform are excellent. We challenged our detection with the analogs of ATP (TTP, CTP, GTP). We concluded that our detection platform is highly specific. Only with ATP, the AuNPs can be protected in red color, which indicated that only ATP can induce the collapse of DNA superstructure. The analogs have no obvious interferences on the detection of ATP (Fig. 4A and B). We can also selectively detect ATP in complicated medium such as diluted serum (Figs. 4C and D for 0.3% diluted serum and 3% diluted serum, respectively). As demonstrated, the serum sample containing ATP induced the collapse of DNA superstructure and protected the AuNPs from aggregation effectively. Whereas, the blank serum sample kept the DNA superstructure untouched resulting the obvious aggregation of AuNPs. However, we found that when we increased the serum

concentration and employed 5% diluted serum, the large number of non-specific proteins began to affect the detection (figure S4).

As an expansion of our detection platform with fast response, a DNA detection method was developed by using the assembly of the DNA nanoassembly (figure S5). As a combination, we further developed a logic gate system to do multiplexed detection of ATP and DNA (Fig. 5). We defined ATP and one strand of DNA superstructure as input 1 and input 2, respectively. The output of our logic gate system is the color of AuNPs that is simple to read. For input, the presence of ATP at concentration of 2 mM and DNA strand at 10 μM was defined as "1" states. The absence of these molecules was defined as "0" states. Thus, we realized a logic operation that combines an "Inhibit" gate and an "OR" gate. The truth table and outputs with different color were demonstrated in Fig. 5.

4. Conclusion

We have demonstrated a fast (in 10 min) and simple (instrument-free) detection for small molecule-ATP. We achieved the detection limit of 5 μM and 0.75 μM by using the naked eyes and UV–vis spectrometer, respectively. Our detection is specific enough to differentiate the ATP from its analogs GTP, CTP and UTP, and selective enough to detect ATP in diluted serum. By re-engineering the hybridization of aptamer, we decreased the detection time from hours to 10 min. By coupling the plasmonic effect of gold nanoparticles, the detection results can be identified by unaided eyes. With these characters, our platform could provide a promising tool for the point-of-care applications and in instrument-limited areas.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.10.031.

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