Letter

Computational lateral flow biosensor for proteins and small molecules: A new class of strip logic gates

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Computational lateral flow biosensor for proteins and small molecules: A new class of strip logic gates

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Abstract

The first example of strip logic gates ("OR" and "AND" functions) for proteins and small molecules has been constructed based on target-induced self-assembly of split aptamer fragments. Using thrombin and ATP as inputs, the corresponding split/integrated aptamers as molecular recognition elements, and gold nanoparticles as a tracer, the output signals can be directly visualized by observing the red bands on the test zones of the strips. The assay is simple, easy to perform, and cost-effective, allowing portable analysis at ambient temperature. The strip logic system is resistant to nonspecific interferants and can operate effectively even in human serum samples. Such logic strips hold great promise for application in intelligent point-of-care and in-field diagnostics.

Electronic logic gates, transistor-based computational devices which perform binary arithmetic processing and Boolean logic circuits, form the basis of conventional computer microprocessors. By analogy, molecular logic gates can be viewed as molecular-scale computers that process chemical or physical "inputs" to generate "outputs" based on a set of molecular computation.[1-3] Molecular logic designs aid chemical/biological sensing, small object recognition and intelligent diagnostics, which have attracted significant research interest.[4-7] In order to identify ideal candidates which satisfy logic operations, extensive efforts have been dedicated to design diverse logic gates, such as OR, AND, NOR, NAND, XOR, INHIBIT, half-adder, and half-subtractor.[8-21] Most of the molecular logic gates reported to date employ fluorescent, colorimetric, electrochemical, or electrochemiluminescent signals as their outputs, which often suffer from cumbersome handling procedures, instrument-dependent readout, and a lack of portability. In a step towards addressing these potential limitations, lateral flow strip biosensor (LFSB) is an economical and simple alternative approach, which has recently attracted considerable attention.[22-28] The LFSB-based methods possess several benefits including a user-friendly format, long-term stability, short assay time, and cost-effectiveness. Moreover, these strip biosensors eliminate complex analysis procedures that involve expensive instrumentation and minimize the requirements for highly qualified personnel. The semi-quantitative assay is easy to perform by visually observing the color intensity of the test zone, and the quantitative data can be obtained by recording the optical responses with a portable "strip reader". Recently, our group has successfully developed LFSB for the detection of nucleic acids,[29-31] proteins,[32] cancer cells,[33] and heavy metal ions.[34] To explore new dimensions in molecular logic gates, it

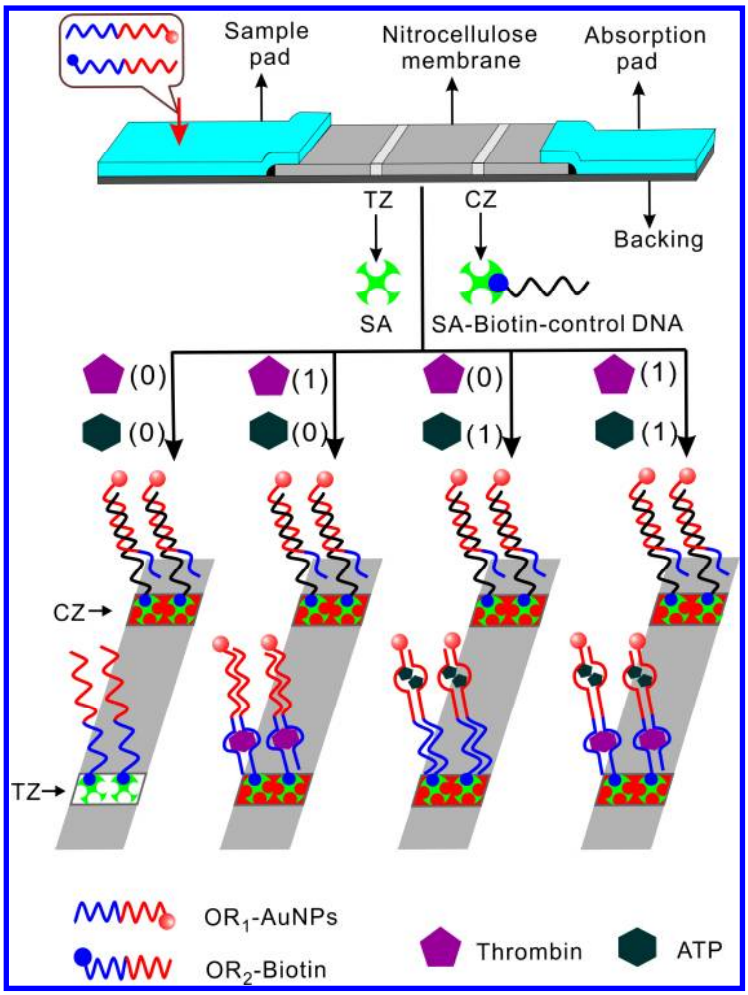
would be of great interest to combine strips with logic gates. Such combination is appealing in the fabrication of intelligent point-of-care devices and the design of molecular logic systems for on-site applications.

Aptamers are artificial functional oligonucleotides that have high affinity and specificity toward their targets. It has been demonstrated that some aptamers can be split into two fragments without significant perturbation of their ligand-binding abilities.[35-37] In the current work, we split the 15-mer anti-thrombin aptamer and 27-mer anti-ATP aptamer into two fragments (subunits), respectively, according to the findings of Chen's and Plaxco's groups.[14,38,39] One subunit of thrombin aptamer was coupled with one subunit of ATP aptamer, and the other subunit of thrombin aptamer was combined with the other subunit of ATP aptamer, which obtained two integrated oligonucleotides. In the absence of targets, the integrated strands do not interact with each other. In the presence of respective or both targets, however, a tricomponent supramolecular aptamer complex is generated based on target-induced self-assembly of split aptamer fragments. By the appropriate labeling of the aptamer subunit with gold nanoparticles (AuNPs), aptamer subunits-target binding events can be directly visualized with the naked eye using the strip sensing platform. The molecular recognition elements of our constructed strip logic gates are split aptamers, which are different from previously published work. For example, Yin et al. reported aptamer-crosslinked hydrogels for logic gates operation visualized with AuNPs.[21] However, the detection mechanism is based on target-induced conformational changes of intact aptamers. Aptamer-based protein detection visualized with AuNPs in a lateral flow strip was reported by Xu et al.[28] However, the sandwich configuration requires special target molecule

which has two binding sites to interact with two intact aptamers simultaneously. So, our work is distinctive and a new exploration of aptamer-based logic gates. Herein, for the first time, we fabricate a strip logic system ("OR" and "AND" logic gates) for proteins and small molecules based on target-induced linkage of split aptamer fragments. Our work not only provides a "smart" and flexible logic platform for thrombin and ATP sensing, but also can expand the application for other ligand assays, such as adenosine monophosphate (AMP), theophylline, and cocaine.[40-42] It was reported that the aptamers of these ligands can also be split in halves that can self-assemble into a supramolecular aptamer fragments/target complex in the presence of the target.

As a first test we have designed a two-analyte "OR" logic gate (Scheme 1). The two integrated DNA are defined as OR_1 and OR_2, respectively (the split thrombin aptamer fragments are marked in blue; the split ATP aptamer fragments are marked in red). The two inputs are thrombin and ATP, with the absence and presence of each molecule defined as "0" and "1", respectively. The output is the color of the test zone, with the absence and presence of a red band on the test zone defined as "0" and "1", respectively. OR_1-conjugated gold nanoparticles (OR_1-AuNPs) are used as detection probes and biotin modified OR_2 (OR_2-Biotin) are used as capture probes. Relatively small AuNPs (~15 nm diameter) were used in the current work because they are stable and can increase the sensitivity and reproducibility of strip biosensors.[29] The LFSB consists of three components: sample pad, nitrocellulose membrane, and absorption pad. Conjugate pad was not used for the storage of DNA-AuNPs conjugates. If conjugate pad is used, the incubation time (< 3 min) wouldn't be enough for the target-induced self-assembly of the split aptamers and the targets, which

would then lower the detection sensitivity. As an alternative, we chose to store the DNA-AuNPs conjugates in centrifuge tubes at 4 ^{o}C before use. Streptavidin and streptavidin-biotin-control DNA (partially complementary to the OR_1) were dispensed on the nitrocellulose membrane to form the test and control zone, respectively. In the (0,0) state of the "OR" gate, the OR_1 and OR_2 exist predominantly in the dissociated form. The sample solution containing OR_1-AuNPs and OR_2-Biotin is applied on the sample pad. The solution migrates along the strip by capillary action and the OR_2-Biotin are captured on the test zone *via* the streptavidin-biotin interaction, while the OR_1-AuNPs are captured on the control zone *via* the hybridization between the OR_1 and the pre-immobilized streptavidin-biotin-control DNA. The accumulation of AuNPs on the control zone is visualized as a characteristic red band, indicating that the LFSB is working properly. In the absence of any inputs (0,0), no OR_1-AuNPs is captured on the test zone and no red band is observed on the test zone, indicating that the output is 0. In the presence of either (1,0; 0,1) or both inputs (1,1), the OR_1-AuNPs would combine with OR_2-Biotin to form G-quadruples structure at either or both sides of the integrated DNA strands to bind thrombin and/or ATP. Such target-induced conjunction of split aptamer fragments, in turn, allows the detection probes OR_1-AuNPs to be captured on the test zone through the OR_2-Biotin-streptavidin interaction. The accumulation of AuNPs on the test zone is visualized as a characteristic red band, indicating that the output is 1. The excess OR_1-AuNPs would be captured on the control zone by the hybridization between OR_1 and the control DNA probe, thus forming a second red band. Therefore, either or both inputs can induce a red band on the test zone and obtain a true value of output, giving rise to an "OR" logic gate.



Scheme 1. Schematic illustration of the design strategy of the strip "OR" logic gate using thrombin and ATP as inputs. SA, streptavidin; CZ, the control zone; TZ, the test zone.

Figure 1A presents typical photo images of the "OR" gate, and Figure 1B and 1C show the corresponding calculation results. Much larger peak areas were obtained when the logic circuit was subjected to either or both inputs than that the original state (0,0). A truth table is given in Figure 1D.

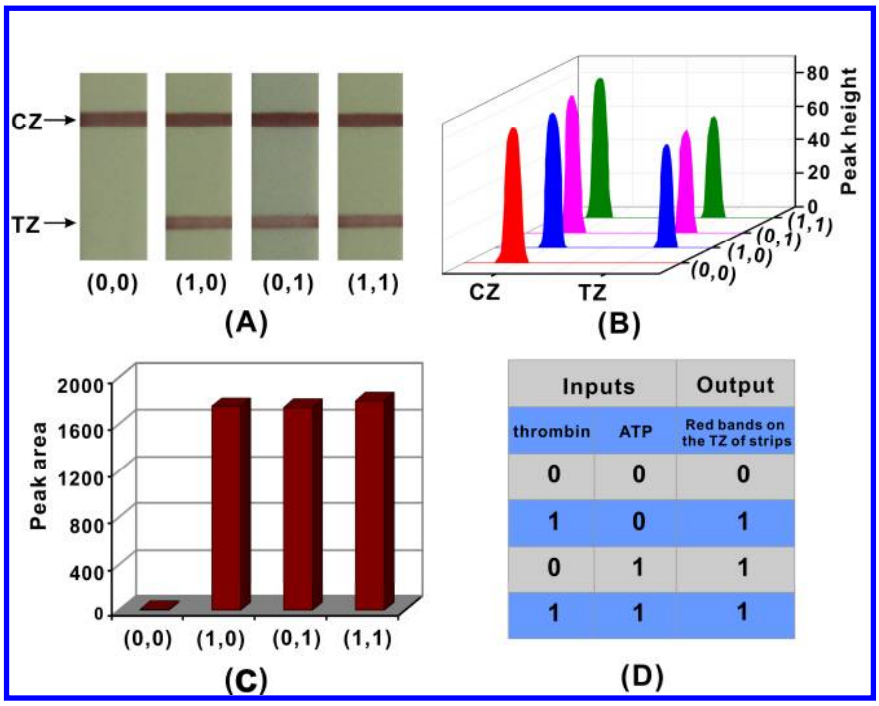
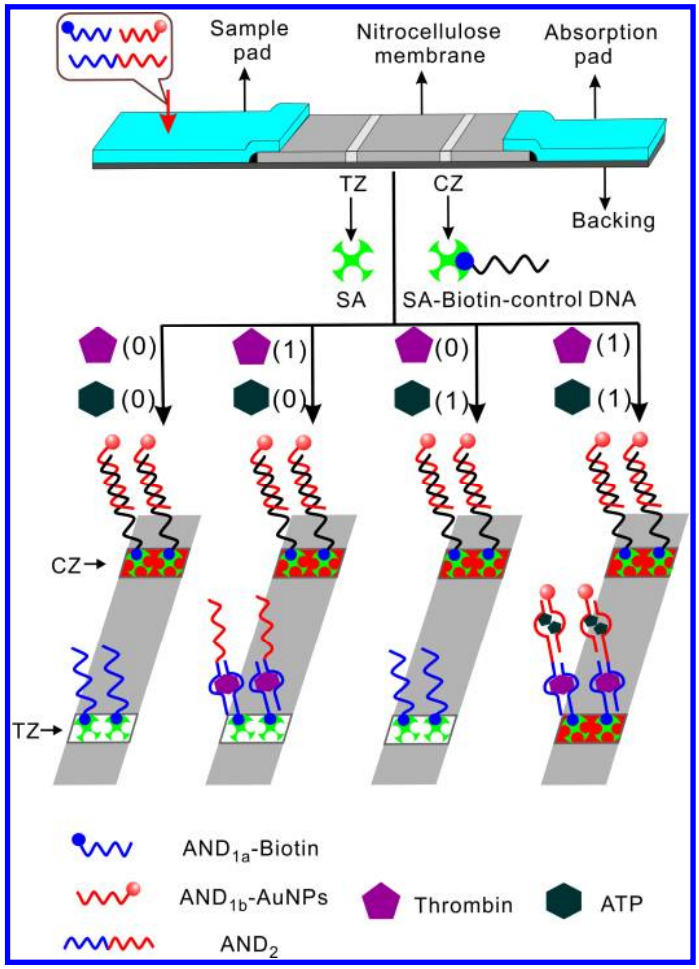


Figure 1. (A) Photograph of the LFSB in "OR" gate. (B) Their corresponding optical response features. (C) Peak areas of the red bands on the TZ. (D) A truth table of the "OR" logic gate. The concentrations of thrombin and ATP are 2 μM and 2 mM (Figure S1 and S2, Supporting Information), respectively.

Encouraged by these results, we sought to create an "AND" logic gate using similar design strategies (Scheme 2). Biotinylated AND_{1a} (AND_{1a}-Biotin) and AuNPs modified AND_{1b} (AND_{1b}-AuNPs) were used as capture probes and detection probes, respectively. Streptavidin and streptavidin-biotin-control DNA (partially complementary to the AND_{1b}) were dispensed on the nitrocellulose membrane to form the test and control zone, respectively. Only the presence of both inputs (1,1) could cause the capture probes (AND_{1a}-Biotin) to combine with the detection probes (AND_{1b}-AuNPs) through AND_2 based on target-induced conjunction of split aptamer fragments. The formed complexes (AND_{1a}-Biotin-AND_2-AND_{1b}-AuNPs) are captured on the test zone through the reaction between the biotin and the pre-immobilized streptavidin. The excess of AND_{1b}-AuNPs are captured on the control zone by the hybridization between the AND_{1b} and the streptavidin-biotin-control DNA. Thus, red bands are formed on the test zone and control zone due to the accumulation of AuNPs. Accordingly, the output reads 1. In the absence of any inputs (0,0) or in the presence of either input (1,0; 0,1), the AND_{1a}-Biotin and the AND_{1b}-AuNPs exist mainly in the dissociated state and can hardly interact with each other. No red bands would be observed on the test zone in the pattern of such input combinations due to the failure of capturing the AND_{1b}-AuNPs on the test zone and the output reads 0. A red band is always formed on the control zone of the strip to confirm the proper functioning of the test. The system performs the "AND" logic circuit, and the output signal (a red band on the test zone) is generated (true value "1") only if the system is subjected to the both inputs simultaneously.



Scheme 2. Schematic illustration of the design strategy of the strip "AND" logic gate using thrombin and ATP as inputs. SA, streptavidin; CZ, the control zone; TZ, the test zone.

Figure 2A presents typical photo images of the "AND" gate, and Figure 2B and 2C show the corresponding calculation results. Much larger peak areas were obtained when the logic circuit was subjected to both inputs than other input combinations. A truth table is given in Figure 2D.

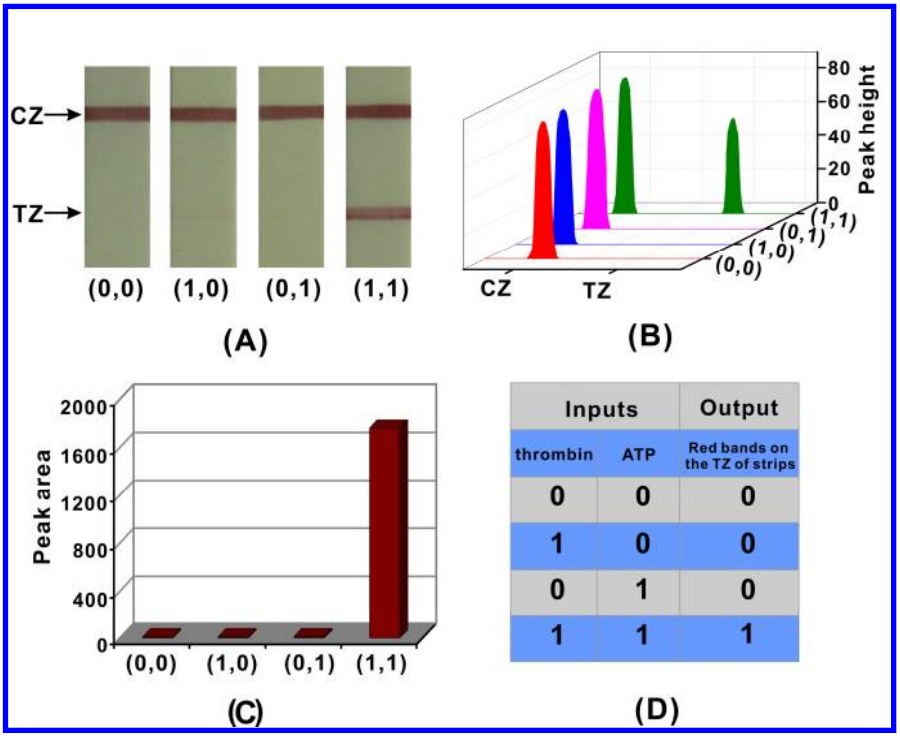


Figure 2. (A) Photograph of the LFSB in "AND" gate. (B) Their corresponding optical response features. (C) Peak areas of the red bands on the TZ. (D) A truth table of the "AND" logic gate. The concentrations of thrombin and ATP are 2 μM and 2 mM, respectively.

To validate the specificity of the "OR" and "AND" logic gates, non-target molecules, such as cytidine triphosphate (CTP), uridine triphosphate (UTP), hemoglobin, and lysozyme were used as negative control inputs. As shown in Figure S3 (Supporting Information), the "OR" logic gate obtained a red band on the test zone (output = 1) using thrombin or ATP as stimulus input but no red band was visualized on the test zone (output = 0) using any of the control inputs. As shown in Figure S4 (Supporting Information), the "AND" logic gate yielded a red band on the test zone (output = 1) in the presence of both thrombin and ATP inputs but no red band was observed on the test zone (output = 0) in the presence of other input combinations. These results demonstrate that the logic gates based on target-induced conjunction of split aptamer fragments are resistant to nonspecific interferants.

The practical applicability of the proposed logic system was investigated by operating the "OR" and "AND" logic gates in human serum samples. Serum samples were prepared by diluting to 10% in dilution buffer (20 mM Tris-HCl, 150 mM NaCl, 10 mM $MgCl_2$, pH 7.5). Thrombin and ATP spiked in 10% human serum were used as inputs to perform the logic operation for real biological samples. Sample solution containing the targets, the detection probes, and the capture probes was incubated for 30 min at room temperature and then applied to the sample pad of the LFSB for logic analysis. As shown in Figure S5 (Supporting Information), without the spiked thrombin and ATP (0,0), no red band is observed on the test zone in the "OR" gate (output = 0). While serum samples spiked with either thrombin (1,0) or ATP (0,1), or both the inputs (1,1), a red band can be visualized by the naked eye on the test zone (output = 1). As shown in Figure S6 (Supporting Information), a red band is formed on the test zone only if the "AND" gate is subjected to the two spiked inputs together (1,1). In

the absence of both inputs (0,0) or in the presence of either input (1,0; 0,1), the system fails to produce a red band on the test zone. It indicates that the strip logic system performs well even in relatively complex sample matrices and is not affected when the inputs existed in clinical samples. The results also definitely illuminate the potential application of the logic design in clinical diagnostics.

In summary, we have introduced, for the first time, a strip logic system ("OR" and "AND" logic gates) for proteins and small molecules based on target-induced self-assembly of split aptamer fragments. Using the split/integrated aptamers as molecular recognition elements, thrombin and ATP as inputs, and gold nanoparticles as a tracer, the outputs can be directly visualized by observing the red bands on the test zone of the strips. Such logic gate system possesses several distinctive advantages that are listed below. (1) The assay is simple, easy to perform, cost-effective and does not demand either technical expertise or expensive sophisticated instruments. The logic operation with a user-friendly format is readily performed in local clinical laboratories. (2) The strip outputs can be unambiguously determined by the presence or the absence of visible red bands on the test zone, and each measurement can be readily finished within 10 min. This strip logic system can be developed into rapid test kits, which allows portable analysis at ambient temperature. (3) Colored samples can also be used as inputs in the strip logic operations, and do not affect the detectability and performance of the biosensor. While such colored inputs are questionable for use in colorimetric logic gates. (4) The logic strips also exhibit excellent selectivity and are capable of monitoring the targets in human serum solutions, demonstrating the robustness of the logic system. In view of these advantages, our work not only introduces a new concept

for devising strip logic gates, but also provides a "smart" and flexible logic sensing platform for point-of-care and in-field diagnostics.

Acknowledgments

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Supporting Information Available

Experimental details, sensitivity analysis, and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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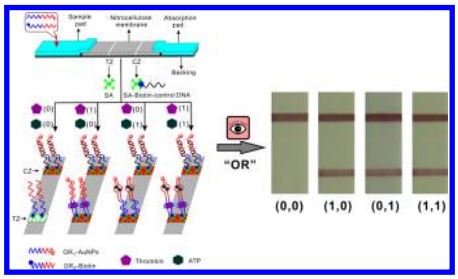
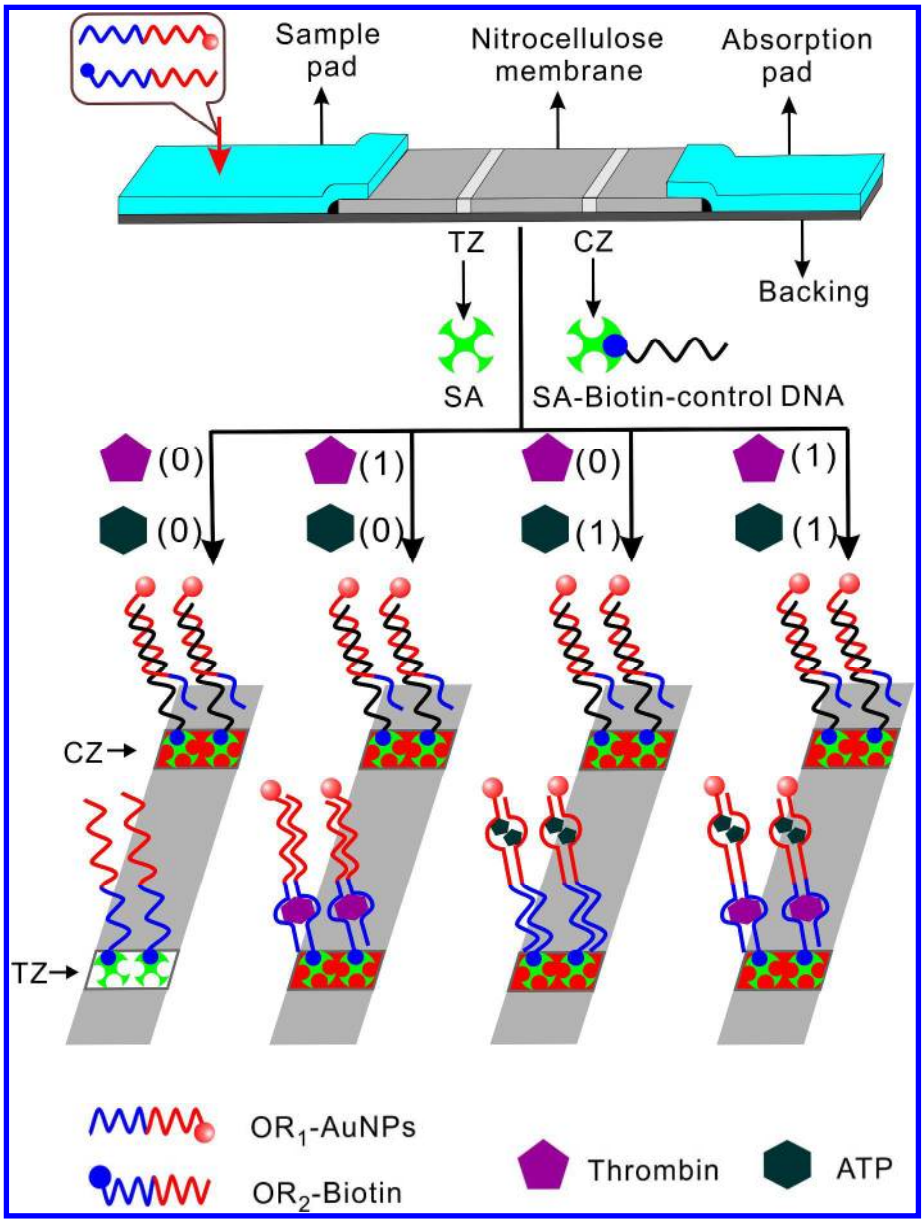
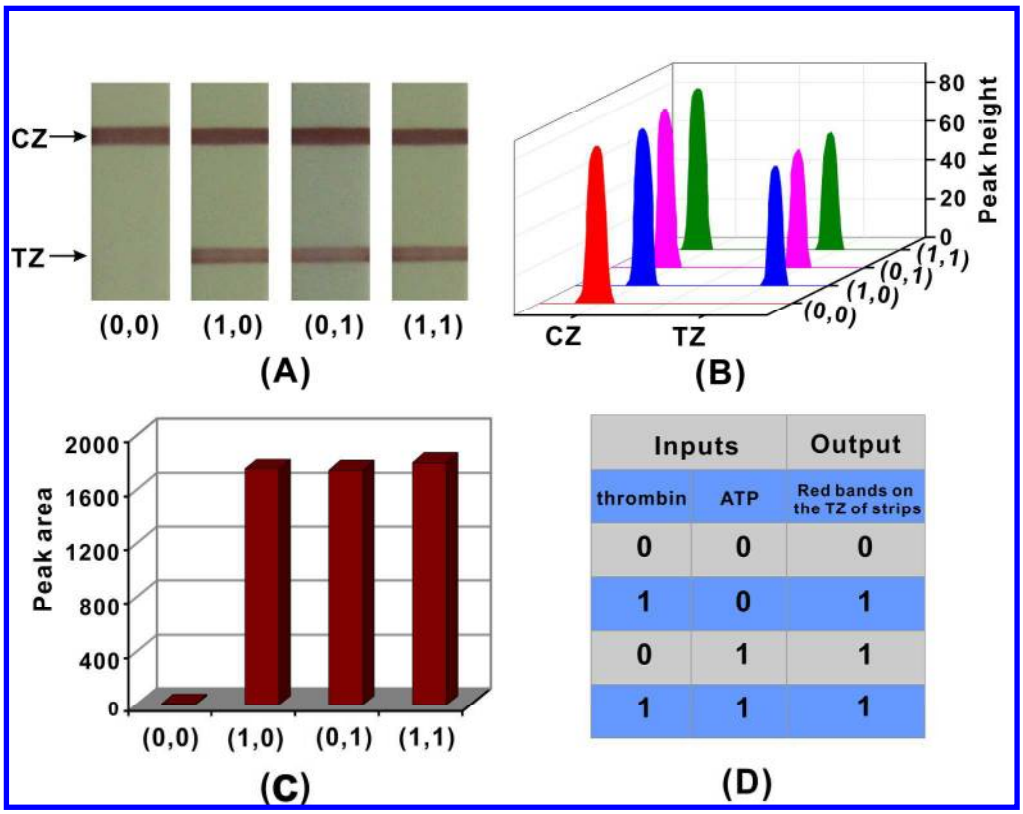


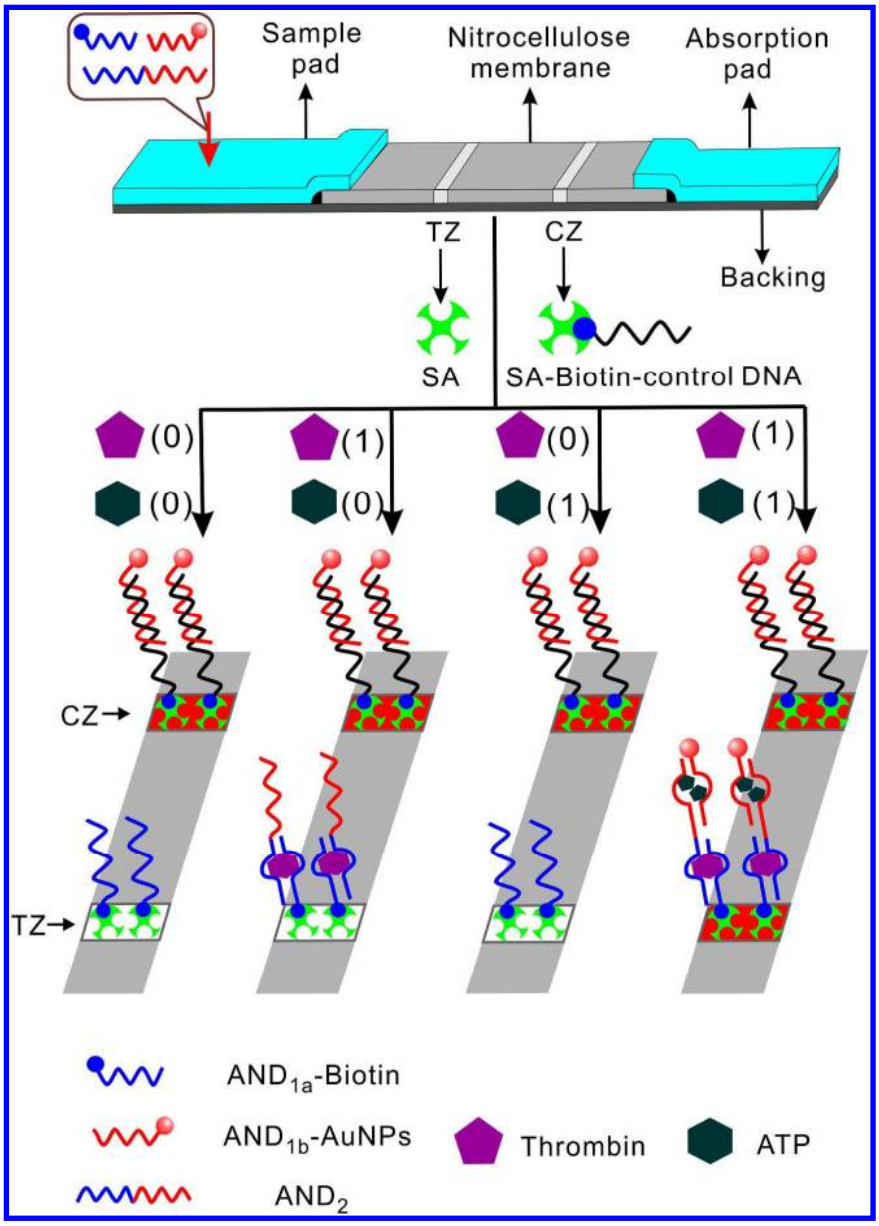


Scheme 1. Schematic illustration of the design strategy of the strip "OR" logic gate using thrombin and ATP as inputs. SA, streptavidin; CZ, the control zone; TZ, the test zone.
296x394mm (300 x 300 DPI)

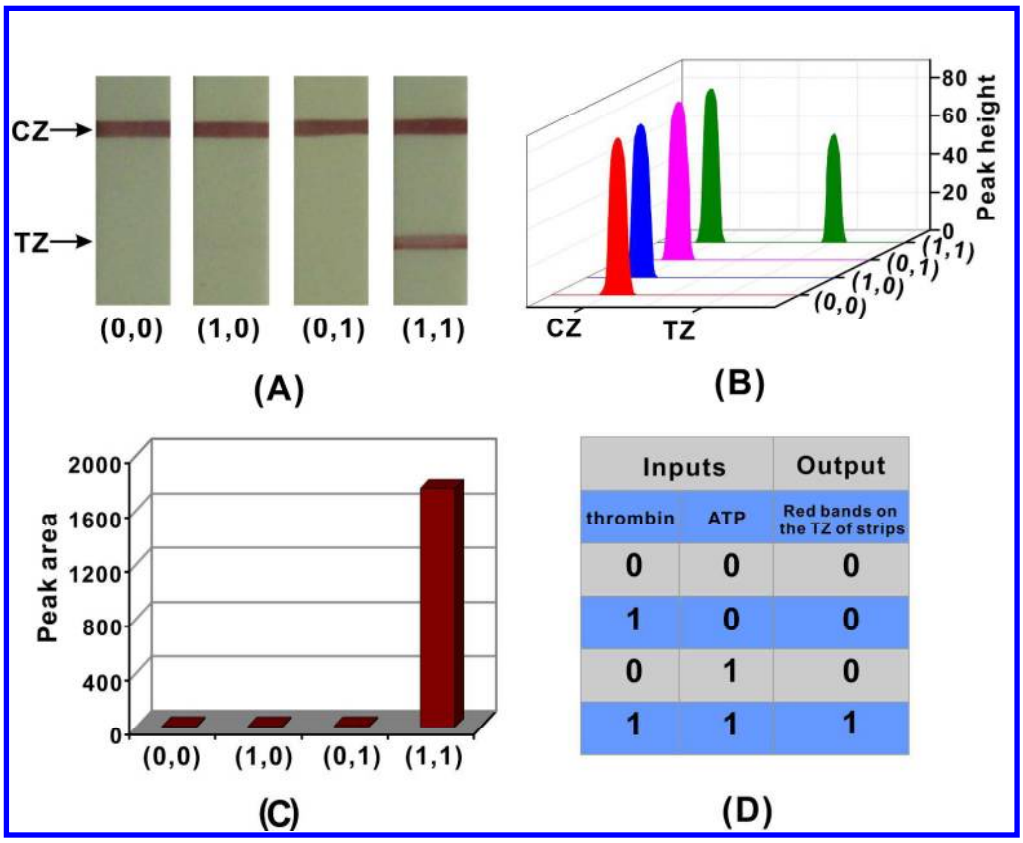


Inputs		Output
thrombin	ATP	Red bands on the TZ of strips
0	0	0
1	0	1
0	1	1
1	1	1

Figure 1. (A) Photograph of the LFSB in "OR" gate. (B) Their corresponding optical responses features. (C) Peak areas of the red bands on the TZ. (D) A truth table of the "OR" logic gate. The concentrations of thrombin and ATP are 2 uM and 2 mM (Figure S1 and S2, Supporting Information), respectively.
234x185mm (300 x 300 DPI)



Scheme 2. Schematic illustration of the design strategy of the strip "AND" logic gate using thrombin and ATP as inputs. SA, streptavidin; CZ, the control zone; TZ, the test zone.
296x415mm (300 x 300 DPI)



Inputs		Output
thrombin	ATP	Red bands on the TZ of strips
0	0	0
1	0	0
0	1	0
1	1	1

Figure 2. (A) Photograph of the LFSB in "AND" gate. (B) Their corresponding optical responses features. (C) Peak areas of the red bands on the TZ. (D) A truth table of the "AND" logic gate. The concentrations of thrombin and ATP are 2 uM and 2 mM, respectively.
241x197mm (300 x 300 DPI)