Aptamer-Based Sensing Platform Using Three-Way DNA Junction-Driven Strand Displacement and Its Application in DNA Logic Circuit

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Supporting Information

ABSTRACT: We proposed a new three-way DNA junction-driven strand displacement mode and fabricated an aptamer-based label-free fluorescent sensing platform on the basis of this mechanism. Assembling the aptamer sequence into the three-way DNA junction makes the platform sensitive to the target of the aptamer. A label-free signal readout method, split G-quadruplex enhanced fluorescence of protoporphyrin IX (PPIX), was used to report the final signal. Here, adenosine triphosphatase (ATP) was taken as a model and detected through this approach, and DNA strand could also be detected by it. The mechanism was investigated by native polyacrylamide gel electrophoresis. Furthermore, on the basis of this molecular platform, we built a logic circuit with ATP and DNA strands as input. Aptamer played an important role in mediating the small molecule ATP to tune the DNA logic gate. Through altering the aptamer sequence, this molecular platform will be sensitive to various stimuli and applied in a wide field.

In recent years, DNA has been demonstrated as an excellent application prospect in wet computing, using biomolecules or living organisms to perform computing functions, for its outstanding data storage, parallel processing capabilities, and flexibility in design.1−8 Toehold-mediated strand displacement reaction (SDR) is a very useful tool in DNA computing to receive and process the DNA strand input signals.9−11 Since it can replace the DNA nucleases to release the output strands, it has been used to construct various logic gates, circuits, and even neural networks.12−20 However, the classic strand displacement mode is insufficient for building more powerful computing systems, which can proceed the information in a more efficient way and receive more input signals in different molecular styles, not merely DNA strands. To meet the growing needs of designing complex logic circuits and application in biological environments, new modes to tune the SDR are constantly emerging in recent years.21−23

DNA three-way junction is a Y-shaped DNA structure composed of three strands hybridized with each other.24,25 The multiarm structure gives us more choices to build sensing platforms. For toehold-mediated DNA strand displacement, the toehold end is essential for this reaction. Distributing binding and toehold domain on different strands will make the design flexible and the reaction easily tuned. DNA aptamer is an important separation and analysis tool in target molecule sensing.26,27 If the aptamer sequence is incorporated into the three-way junction structure, the molecular platform will be sensitive to its corresponding target. This strategy can be used to build a DNA logic circuit that can receive input signals in many other forms. Extension of the input range will promote the application of the logic circuit in a wide field.

Herein, we introduced a novel DNA three-way junction driven-strand displacement mode and built a label-free fluorescent sensing platform with the help of aptamer. The structure of the molecule and mechanism of the reaction are illustrated in Scheme 1. The binding and toehold domains are distributed on two different strands, respectively. The third strand hybridizes with them to form the Y-shaped junction

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through the toehold end and displace the output strand. Taking the adenosine triphosphatase (ATP)-binding aptamer (ABA) as the toehold domain-carrying strand, the molecular platform will be sensitive to the input ATP.28,29 At the same time, we can also use its complementary strand to hybridize ABA. Thus, ATP and DNA strand both can be used to tune the SDR. Through altering the aptamer strand, this sensing platform will be able to detect various other targets. Protoporphyrin IX (PPIX) usually aggregates into micelles with low fluorescence in aqueous solution, whereas its fluorescence can be dramatically enhanced upon binding to G-quadruplex.30,31 Split G-quadruplex enhanced fluorescence of PPIX has been utilized to capture the output strand and give off a final fluorescent signal in our previous works.12,32 We continued to use it as a signal reporter in this work, because this label-free fluorescence readout technique is very simple, economic, and easy to design. Furthermore, from another perspective, the whole system can work as a simple logic circuit with ATP and DNA as input. Since the gates in the circuit are all cascaded by DNA strands, this device has potential to expand into a complex computing system with specific function. Upon integrating different aptamer sequences into the circuit, this molecular platform will be sensitive to various stimuli and applied in more extensive areas.

The three-way DNA junction-driven SDR is the core reaction of the molecular platform, whose route is shown in Scheme 1. Strand S, which can draw G1 and G2 together to form the split G-quadruplex, hybridizes with strand I by its binding domain at first. A small part of the binding domain is placed on strand ABA, which can accelerate the hybridization of ABA and SI complex and help strand I capture the S in solution at the same time. Length of the toehold end is a crucial factor for the SDR.33 Taking the length and sequence of ABA into account, a toehold domain with a length of 8 bases is designed here to keep the reaction carrying out smoothly. Since binding domain and toehold domain are distributed on strand I and ABA, respectively, strand R fails to replace S from I without the mediation of toehold end. Strand ABA can bind to the SI complex to draw these two domains close and provide the toehold end for R. Formation of the three-way junction structure is a causative factor of the SDR. Upon the mediation of toehold end on ABA, R hybridizes with ABA and I to form the DNA junction and S is given off. Subsequently, the output strand S draws the two G-rich segments G1 and G2 together to induce the formation of split G-quadruplex, which can bind with PPIX and dramatically enhance its fluorescence. The fluorescence spectra are shown in Figure 1. Strand S was blocked by I, and the fluorescence intensity was at a low level at first. Adding either ABA or R was insufficient to release S (curve c, d), unless both of them were input. Strand S was replaced when both ABA and R were present and induced a high fluorescence signal (curve e). These results proved that the three-way DNA junction-driven SDR indeed happened as we designed.

Assembling the aptamer sequence into the Y-junction enables the molecular platform to be sensitive to its corresponding target. ATP is chosen as a model, and its aptamer ABA is used in this work. As shown in Scheme 1, ABA binds to the SI complex to supply the toehold end for strand R. Since aptamer can selectively and tightly bind with its target, the binding between the SI complex and ABA can be easily
inhibited by ATP. Thus, the fluorescence intensity will drop down to a low level when enough ATP is added (Figure 1, curve f). In Figure 2, the fluorescence intensity decreases with the addition of ATP, and there is a linear relationship between the fluorescence intensity and ATP concentration from 80 to 480 μM with a detection limit of 40 μM (three times the standard deviation of the blank solution). This result proves that the more ATP is input the less S is output. To exclude other interference possibilities, we tested the effects of ATP itself for this system. The control experiment indicated that ATP in the concentration range used in this work had little effect on the fluorescence intensity enhanced by the split G-quadruplex (Figure S1, Supporting Information). Its selectivity was also investigated. As shown in Figure S2, Supporting Information, CTP, GTP, and UTP all failed to inhibit this SDR. Furthermore, due to the natural property of DNA, the input strand ABA can be blocked by its complementary strand cABA (Scheme 1). The presence of cABA also leads to a low output signal (Figure 1, curve g), and its effect on the SDR is greater than ATP. It should be attributed to the fully complementary duplex of cABA/ABA which is much stabler than ATP/ABA complex. The fluorescence intensity also linearly decreases with the addition of cABA in the range from 20 to 120 nM with a detection limit of 6.9 nM (three times the standard deviation of the blank solution) (Figure S3, Supporting Information). Analysis of the DNA strand cABA demonstrates that this sensing platform can be used to detect the specific sequence. Through altering the sequence of strand ABA, it will detect more other target complementary strands or some disease-related sequences.

Native polyacrylamide gel electrophoresis (PAGE) was used to identify the mechanism of this three-way DNA junction-driven SDR. Figure 3 shows the PAGE result, in which the DNAs contained in different lanes have been figured out in the table. Formation of the three-way DNA junction structure was first identified from lane 2 to 7. The mobility of the bands got lower after each addition of DNA strand, which was caused by the increase of weight and volume of the DNA complex. Through comparing the bands in these lanes, we can draw the conclusion that I, R, and ABA bind together to form the Y junction in lane 7. Strand S was tightly blocked by I, and the band of the SI double-stranded complex was obviously in lane 8. Addition of R affected this band a little in lane 11, which meant that only R was insufficient to replace S from I. However, when R and ABA were both added in lane 10, the band of SI complex got very weak and a new band appeared at the same position of Y junction in this lane. This phenomenon proved that SI complex was disbanded and the two input strands hybridized with the strand I to form the Y junction. Thus, the formation of the Y junction structure is a key factor of the SDR. Effects of cABA and ATP for this system were also investigated, respectively. In lane 13, the bands of SI and ABA/cABA complex were both stable, but no band of Y junction was observed. This phenomenon indicated that the SDR was inhibited by the absence of free ABA to form Y junction. A similar case occurred when ATP was input in lane 15. The differences were that a weak band of Y junction appeared, and the band of SI complex became thin in lane 15 compared with lane 11 and 13. It should be due to the binding between ABA and ATP being looser than ABA and its fully complementary strand cABA. Although part of SI complex disbanded and a small amount of Y junction was formed in lane 15, most S still hybridized with I and the fluorescence intensity was kept at a low level (Figure 1, curve f). Therefore, the PAGE results and fluorescence data match well for each other. It is indeed a reliable sensing platform that functions as we designed.

To further demonstrate the practicability of the three-way DNA junction-driven SDR mode, we built a simple logic circuit with ATP and DNA strands as input based on this platform. The schematic diagram is shown in Figure 4a. First, strand S and the G-rich segments worked as a YES gate. When free S
The data were collected from three independent experiments. The corresponding input concentrations of ATP and cABA are about 400 μM and 70 nM, respectively. These concentrations can be used to judge the input state. The fluorescent analysis was performed in the TEK buffer with a final concentration of 1.2 μM for PPIX, 0.16 μM for G1 and G2, 0.1 μM for S, 0.12 μM for I, ABA, cABA, and R, and 480 μM for ATP. Fluorescence intensity at 630 nm of different input modes of the circuit. The fluorescent analysis was performed in the TEK buffer with a final concentration of 1.2 μM for PPIX, 0.16 μM for G1 and G2, 0.1 μM for S, 0.12 μM for I, ABA, cABA, and R, and 480 μM for ATP. Fluorescence intensity at 630 nm of curve (c) in Figure 1 is set as 1. Threshold value is set at 0.45 to judge the positive and negative output signals. The three binary numbers represent the input status of R, ATP, and cABA, respectively. The data were collected from three independent experiments.

Figure 4. (a) Schematic diagram of the logic circuit with strands R, cABA, and ATP as input. (b) Normalized fluorescence intensity at 630 nm of different input modes of the circuit. The fluorescent analysis was performed in the TEK buffer with a final concentration of 1.2 μM for PPIX, 0.16 μM for G1 and G2, 0.1 μM for S, 0.12 μM for I, ABA, cABA, and R, and 480 μM for ATP. Fluorescence intensity at 630 nm of curve (c) in Figure 1 is set as 1. Threshold value is set at 0.45 to judge the positive and negative output signals. The three binary numbers represent the input status of R, ATP, and cABA, respectively. The data were collected from three independent experiments.

The authors declare no competing financial interest.

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