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Dip-and-Read Method for Label-Free Renewable Sensing Enhanced Using Complex DNA Structures

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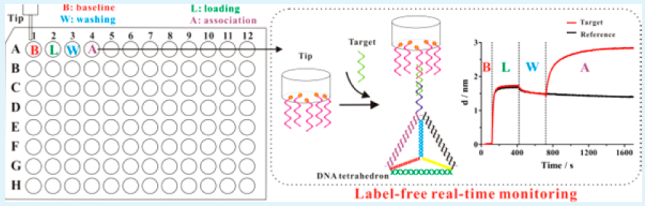
Supporting Information

ABSTRACT: A label-free assay is reported in this work for the detection of DNA with enhanced sensitivity using complex DNA structures (DNA tetrahedrons) based on the biolayer interferometry. The DNA tetrahedrons help to amplify the optical signals of the biolayer interferometry, thus improving the detection limit of DNA by about 100-fold. We further demonstrated that this method could be expanded to ATP detection by taking advantage of the target-dependent adaptability of aptamers. It appears to us that this new label-free assay promises new opportunities for developing novel biolayer interferometry assays.



KEYWORDS: *DNA tetrahedron, aptamer, DNA detection, signal amplification, dip-and-read, label-free*

INTRODUCTION

Detection of specific DNA sequences at trace concentrations is of great significance in pathogen detection, forensic analysis, environmental monitoring, food safety and clinic diagnosis.[1−3] Likewise, metabolites (i.e., small molecules) can have a wealth of biological functions, such as acting as cell signaling molecules, as tools in molecular biology and in many other roles.[4−6] To date, some universal detection platforms have been developed for the detection of a variety of molecular targets (e.g., DNA, small molecules).[7−11] Das and co-workers recently reported a universal electrochemical detector based on neutralizer displacement for the detection of a panel of molecules.[7] In our previous work, some versatile molecular beaconlike probes have been devised for the multiplex sensing of molecular targets such as DNA, protein, metal ions, and small molecule compounds.[8,9] Xia et al. reported a universal colorimetric assay for various analytes using unmodified gold nanoparticles and conjugated polyelectrolytes.[10] However, these methods may suffer certain drawbacks including tedious procedures, the need for labels, and specialized reagents, etc. Alternatively, label-free detection has been an extensively studied method in recent years.[12,13] In particular, a label-free platform based on biolayer interferometry has been developed by FortéBio's Octet to monitor the binding of proteins and other biomolecules to their partners directly in real time utilizing disposable fiber-optic tips that address samples from an open shaking microplate without any microfluidics.[14] The simple dip-and-read assay avoids labeling the protein and other biomolecules with fluorescent or chromogenic tags (e.g., dyes), thus reducing interferences. However, there is still much room for its further application upon integration with various technologies (e.g., DNA nanotechnology, aptamer, etc).

Herein, we harness a complex DNA structure (DNA tetrahedron) incorporated onto inexpensive disposable optical fiber biosensors to perform label-free, kinetic quantitation of sequence-specific DNA or ATP with an enhanced sensitivity (Scheme 1). DNA tetrahedron, as introduced by Goodman et al, is a complex DNA structure with edges composed of double-stranded DNA.[15,16] Recent years have witnessed the rapid development of DNA tetrahedron for biosensing.[17−19] DNA tetrahedral structure is a highly rigid scaffold, which can also offer a high surface density to its anchored surface.[18] From that, we assume in this work that DNA tetrahedron can act as a label-free signal enhancer in biolayer interferometry via enlargement of interfacial thickness or density.

RESULTS AND DISCUSSION

As demonstrated in Scheme 1, a DNA tetrahedral structure was obtained by self-assembly (DNA oligos shown in Table S1 in the Supporting Information). Sequences in the same colors of the four DNA strands were hybridized to form six edges of the tetrahedron. The pendant probes in the as-prepared DNA tetrahedron could be further used for capturing target DNA with partially hybridization. The detection of target DNA was carried out via formation of a sandwiched structure of the pendant probes, target DNA and detection DNA probes saturated onto the surface of commercially available disposable optical fiber tips (see Figure S1, details in the Supporting Information). The hybridization reaction can be real-time monitored via dip-and-read optical fiber tips exploiting biolayer interferometry. Similarly, by taking advantage of the target-dependent adaptability of aptamers (artificial nucleic acids recognizing specific targets),[20,21] we splited a ATP aptamer into two parts according

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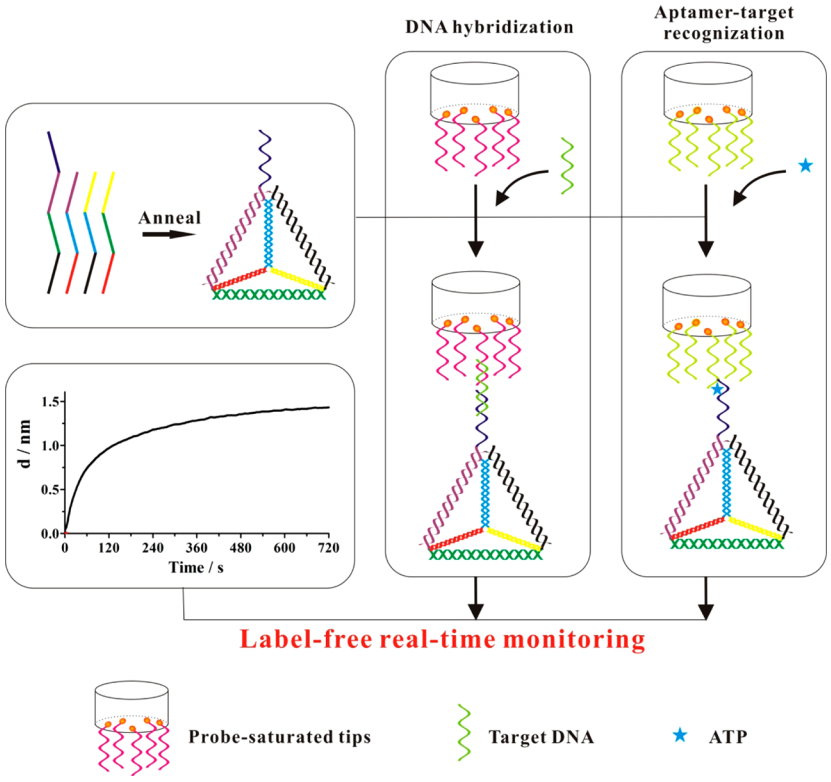
Scheme 1. Schematic Illustration of the Sandwich Assay for Label-Free Detection of Sequence-Specific DNA or ATP Enhanced via a Complex DNA Structure (DNA tetrahedron), Respectively



to our previous work,[22] one part immobilized onto streptavidin-coated disposable optical fiber tips and the other integrated into DNA tetrahedron as a pendant probe. The detection of ATP can also be carried out by using the aforesaid method.

Gel electrophoresis was applied to demonstrate the assembling of DNA tetrahedron. In Figure S2A, DNA tetrahedron moved more slowly than any other combinations lacking one strand, which confirmed the successful assembling of the complex DNA structure corresponding to previous reports.[17−19] The time-course of the target DNA-mediated hybridization reactions were monitored in the presence or absence of DNA tetrahedron, respectively (see Figure S2B in the Supporting Information). In the presence of 100 nM target DNA, the assay exhibited a nearly saturated signal within 12 min, and the introduction of DNA tetrahedron into the optical fiber tips can greatly improve the signal intensity in biolayer interferometry via enlargement of tip's interfacial thickness or density (see Figure S2C in the Supporting Information). Thus, we assume that an improved sensitivity for target DNA can be obtained employing DNA tetrahedron as a signal enhancer in the label-free detection assay.

To experimentally explore the potential of the proposed method for quantitative determination of target DNA with sensitivity enhanced by using DNA tetrahedron, various concentrations of target DNA (100 pM, 1 nM, 5 nM, 10 nM, 20 nM, 50 nM and 100 nM) were challenged to probe-saturated optical fiber tips (streptavidin coupling of biotinylated-DNA) upon in the presence or absence of DNA tetrahedron (TE) (Figure 1). The probe-saturated tips dipped to the solution containing target DNA resulted in time-dependent increases in the signal intensity in biolayer interferometry. As expected, DNA tetrahedron-assisted enhancement of sensitivity can be well demonstrated. Upon the addition of DNA tetrahedron, the signal of optical fiber tips can be obviously observed in the presence of 1 nM target DNA (Figure 1A, red line). However, there is no signal of optical fiber tips in the presence of 1 nM target DNA without the addition of DNA tetrahedron, whereas an observable signal can be monitored in the presence of 50 nM (Figure 1C, purple line). From the above data, the kinetic parameters for hybridization reaction of probe-target were determined. The GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA) was used to perform the data processing. To determine the initial velocities (v_0) for hybridization reaction of probe-target at different concentrations, the raw optical data of biolayer interferometry were collected with subtraction from a reference tip (the control sample without target DNA). The curves were fitted to a first-order exponential, and the initial velocities (v_0) were obtained from the slope at $t = 0$. Figure 1B revealed that the initial velocities (v_0) for hybridization reaction of probe-target were sensitive to target concentration and linearly increased with the increase of target DNA concentrations from 0 to 20 nM, and the present limit of detection ($3\sigma/S$, where σ is the standard deviation of the blank solution, $n = 8$) for this method is approximately calculated to be 200 pM for the perfectly matched complementary target DNA upon in the presence of DNA tetrahedron as a signal enhancer (Figure 1B). In a comparison, without DNA tetrahedron, the limit of detection is approximately 20 nM (Figure 1D). It was demonstrated that the detection sensitivity of the label-free detection method can be significantly improved by about 100-fold.

To evaluate the sequence discrimination ability of the proposed assay, we prepared four different target DNA oligos: PM (perfectly match), 1MM (single-base mismatch), 2MM (two-base mismatch), and 3MM (three-base mismatch). As shown in Figure 2B, the initial velocities for PM, 1MM, 2MM and 3MM in the same condition are appreciably discriminated. The results clearly demonstrated that the presented assay can provide a powerful alternative to specifically detect target DNA and

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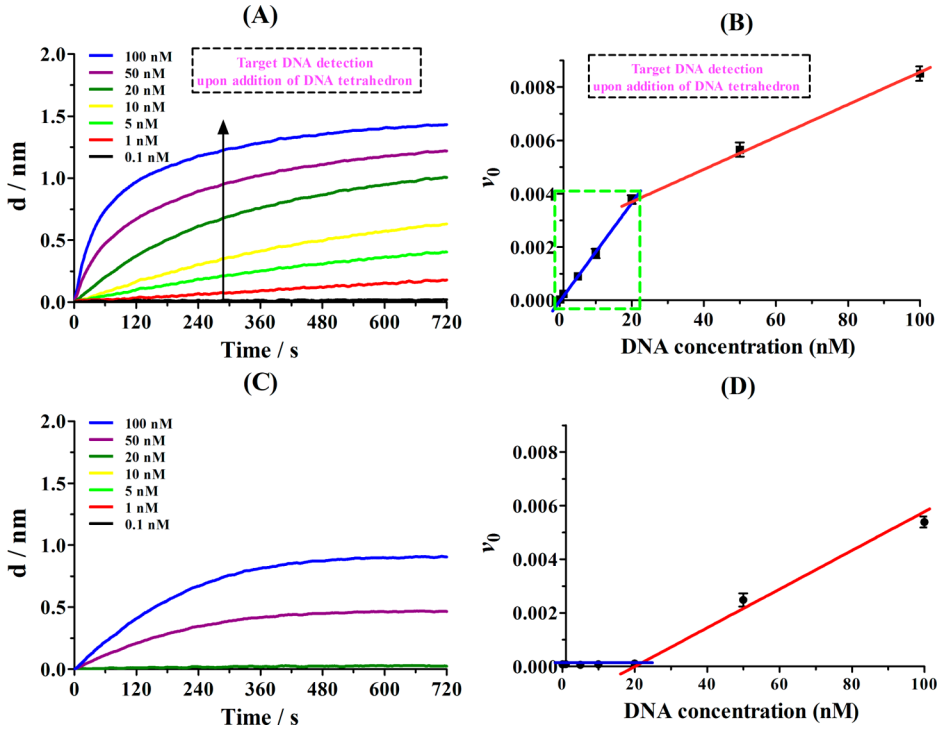

Figure 1. Kinetic analysis of various concentrations of target DNA (TA) (100 pM and 1, 5, 10, 20, 50, and 100 nM) hybridized to probe-saturated optical fiber tips (streptavidin coupling of biotinylated-DNA) upon in the (A) presence or (C) absence of DNA tetrahedron (TE), respectively. Note: the data were collected with subtraction from a reference tip (the control sample without target DNA). The kinetic parameters (v_0: initial velocity) for hybridization reaction of probe-target upon in the (B) presence or (D) absence of TE were determined, respectively.

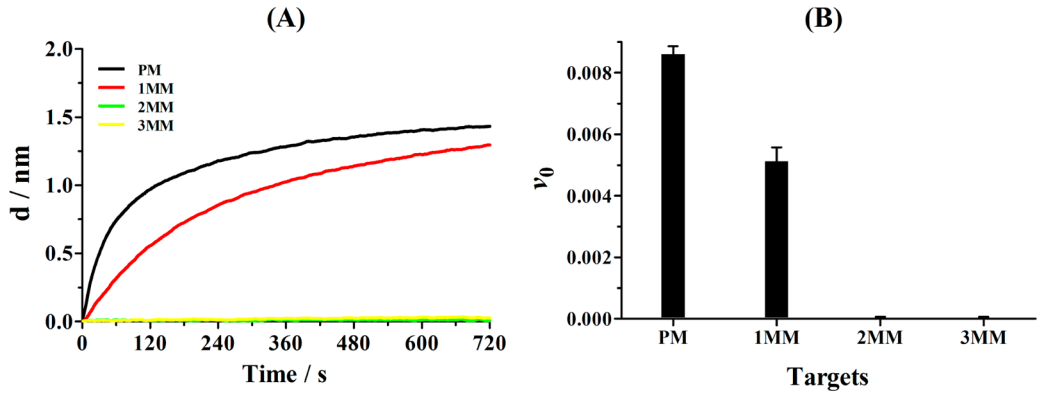

Figure 2. (A) Kinetic analysis of the label-free sensing strategy for DNA detection against the control DNA oligos including single-base mismatched (1MM), two-base mismatched (2MM) and three-base mismatched strands (3MM). The concentration of the DNA oligos: 100 nM. (B) The kinetic parameters (v_0: initial velocity) for hybridization reaction between probe and targets including perfect-matched (PM), single-base mismatched (1MM), two-base mismatched (2MM), and three-base mismatched strands (3MM).

discriminate single-base mismatches with specific responses. The identification of single-base mismatches is important for the single nucleotide polymorphisms (SNP) genotyping and routine screening of genetic mutations and diseases. The results demonstrated that the assay provides great potential for the quantification of nucleic acid as well as the detection of point mutation.

The development of renewable biosensors allowing multiple measurement cycles is highly favorable in the day-to-day practicality. Thus, the reusability of our presented sensor was evaluated. As shown in Figure 3, a new DNA sensor can be built for many cycles by denaturation of the resulting DNA duplex (step 1) incubated in sodium hydroxide solutions (step 2), leaving probes sensor ready for use in a subsequent recognition process without further loss of activity (step 3). Simpler and faster regeneration procedure makes our presented method hold promise for decentralized genetic testing.

Furthermore, we evaluated the analytical performance of the proposed method for ATP detection based on the aforementioned concept. As shown in Figure 4A, the probe-saturated tips inserted to the ATP-containing solution upon addition of DNA tetrahedron (TE) resulted in time-dependent increases in the signal intensity in biolayer interferometry. The kinetic parameters (v_0: initial velocity) for ATP-induced aptamer-target recognition in the dip-and-read assay were determined upon in the presence or absence of TE, respectively. The initial velocities (v_0) for recognition upon in the presence of TE were sensitive to ATP concentration and increased with the increase of ATP concentrations (Figure 4B). The control experiment was also conducted

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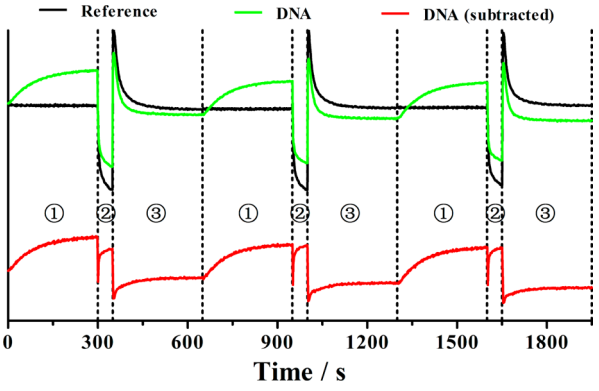

Figure 3. Evaluation of the reusability of our presented sensor for DNA detection. Kinetic monitoring of 100 nM target DNA hybridized to probe-saturated optical fiber tips in the presence of DNA tetrahedron (step 1) and denatured from tips in 0.1 M sodium hydroxide solutions (step 2) and washed and incubated in the hybridization buffer (step 3).

in the absence of TEstructure, whereas no observable signal can be measured even in the presence of 8 mM ATP (Figure 4C, black line). In this case, with the aid of TE structure, the detection sensitivity of the label-free detection method for ATP can be significantly improved (Figure 4B, D). To test selectivity, competing stimuli including UTP, CTP, GTP, and TTP at same molar levels were examined under the same conditions as in the case of ATP (Figure 4E). It was found that ATP results in an obvious change in initial velocities (v_0) for recognition, while there was nearly a negligible change in the presence of other stimuli (Figure 4F). The results demonstrated the excellent selectivity of this approach applied in ATP detection over competing stimuli. Moreover, by denaturation of the resulting ATP-induced aptamer-target DNA duplex incubated in sodium hydroxide solutions (0.1 M), the sensor was regenerated and readily reused to detection of additional ATP samples without further loss of activity, which further confirmed the reusability of our presented sensor (see Figure S3 in the Supporting Information).

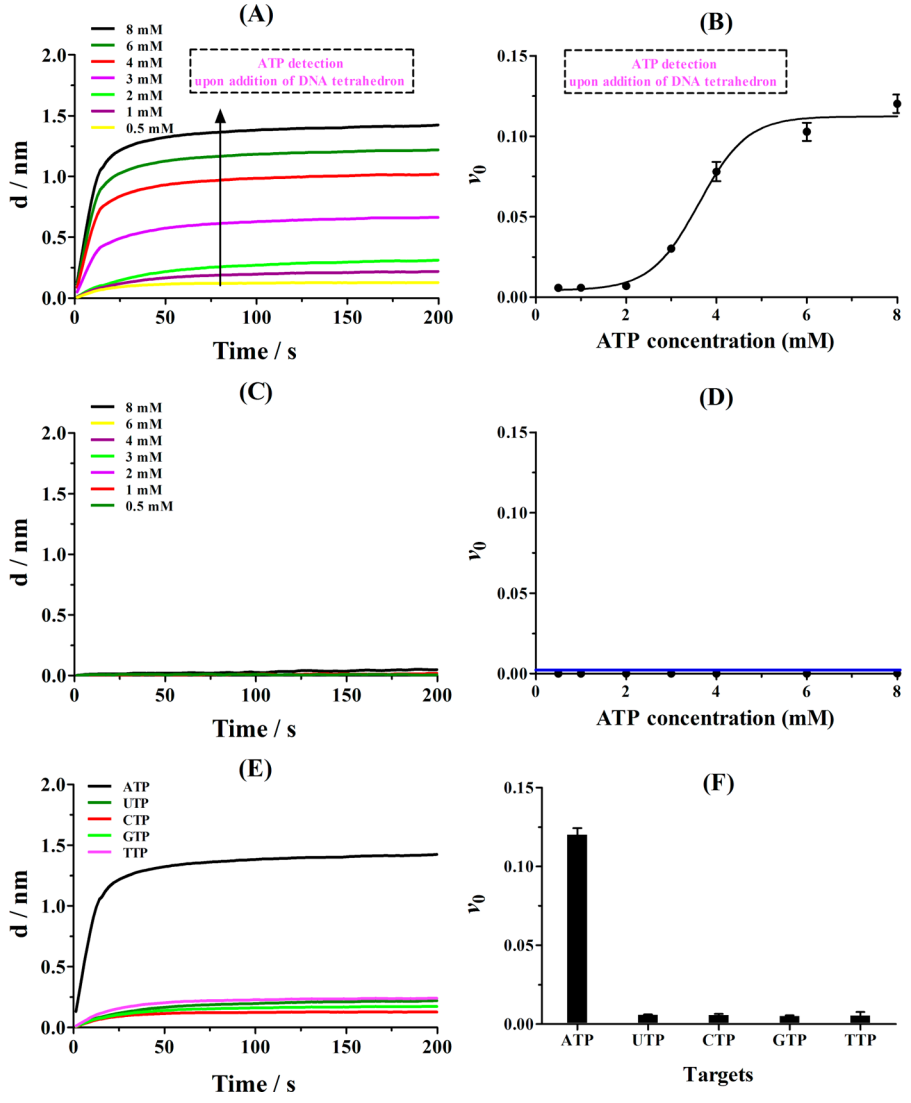

Figure 4. Kinetic analysis of various concentrations of ATP (0.5, 1, 2, 3, 4, 6, and 8 mM) binding to probe-saturated optical fiber tips (streptavidin coupling of biotinylated-DNA) upon in the (A) presence or (C) absence of DNA tetrahedron (TE), respectively. Note: the data were collected with subtraction from a reference tip (the control sample without ATP). The kinetic parameters (v_0: initial velocity) for hybridization reaction of probe-target in the dip-and-read assay were determined upon in the (B) presence or (D) absence of TE, respectively. (E) Kinetic analysis of the label-free sensing strategy for ATP detection against UTP, CTP, GTP, and TTP. The concentration of the ATP, UTP, CTP, GTP, and TTP: 8 mM. (F) The kinetic parameters (v_0: initial velocity) for hybridization reaction between probe and targets including ATP, UTP, CTP, GTP, and TTP.

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Additionally, in this work, the DNA tetrahedron-assisted signal enhancement in biolayer interferometry enabled the design of DNA-based AND logic systems based on Boolean logic (see Figure S4 in the Supporting Information). For input, we defined the presence of target DNA (TA) or DNA tetrahedron (TE) as 1 and their absence as 0 (the concentration of TA: 20 nM). The initial velocity (v_0) for hybridization reaction of probe-target in the dip-and-read biolayer interferometry was defined as the output (1 or 0) for the logic gate (see Figure S4B in the Supporting Information). With no input, the output was 0. When the system was subjected to the two inputs together (TA and TE), an obvious change in initial velocities (v_0) for recognition in the dip-and-read assay can be obtained to give an output signal of 1. In like manner, ATP/DNA tetrahedron (TE)-driven signal enhancement in biolayer interferometry was also showed to the design of DNA-based AND logic systems (see Figure S4C, D in the Supporting Information, the concentration of ATP: 8 mM).

CONCLUSIONS

In summary, we have successfully demonstrated that a complex DNA structure (DNA tetrahedron) can be used as a signal enhancer for improved sensitive detection of DNA in a label-free and renewable format. Furthermore, by taking advantage of the target-dependent adaptability of aptamers, we expanded the application of the presented method to ATP detection. This is a new concept for label-free assay, and opens opportunity for novel label-free assays based on the three-dimensional DNA structures-enhanced strategy.

EXPERIMENTAL SECTION

Reagents and Materials. The oligonucleotides used in this study were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China) with the DNA oligos listed in Table S1 in the Supporting Information. Adenosine 5′-triphosphate (ATP), guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate (CTP), uridine 5′-triphosphate (UTP) and thymidine 5′-triphosphate (TTP) were purchased from Sigma-Aldrich (St. Louis, MO). Disposable fiber-optic streptavidin-coated tips were purchased from FortéBio Inc. (USA). TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0) were prepared using metal-free reagents in distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA) with an electrical resistance of 18.2 MΩ. All chemicals used in this work were of analytical reagent and obtained from commercial sources and directly used without additional purification.

Instrumentation. The QK^e Octet platform (ForteBio Inc., USA) was employed to monitor and quantify the DNA tetrahedron-assisted signal enhancement in biolayer interferometry in this work. The Octet platform was a label-free, real-time optical biosensor that handles samples without microfluidics, which utilizes a biolayer interferometry technology to monitor biological binding events on a fiber optic tip and provides output in the form of sensorgrams.

Synthesis of DNA Tetrahedron. Four DNA strands (A, B, C, and D) were diluted into a final concentration of 50 μM with TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). A quantity of 2 μL each strand was mixed with 42 μL of TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0), and the mixture was then heated to 95 °C for 2 min and cooled in ice bath for 0.5 min.

Electrophoretic Analysis of DNA Tetrahedron. The functional DNA tetrahedron was analyzed using polyacryamide gel electrophoresis (PAGE, 12.5%) at a constant current of 5 mA.

Real-Time Monitoring the DNA Tetrahedron-Assisted Signal Enhancement. We used the Octet platform (FortéBio Inc. USA) to monitor and quantify the DNA tetrahedron-assisted signal enhancement. The procedure includes four steps: (1) baseline; (2) loading; (3) washing; (4) association (see Figure S1 in the Supporting Information). The baseline solution (B, 200 μL, 18.4 mM Tris, 0.16 mM EDTA, 42 mM $MgCl_2$, pH 8.0), loading solution (L, 200 μL of 1.5 μM DNA-Probe in the baseline solution), washing solution (W, i.e. baseline solution) and association solution (A, 100 μL as-prepared DNA tetrahedron +100 μL target DNA or ATP with certain concentration) were added into the corresponding wells in a 96-well microtiter plate, respectively (see Figure S1A in the Supporting Information). A disposable fiber-optic streptavidin-coated tip was dipped into the baseline solution B for 120 s with gentle automated shaking, then moved to the next well for loading DNA-Probe (streptavidin coupling of biotinylated-DNA) in the loading solution L to form a probe-saturated optical fiber tip within 300s, with that, the probe-saturated tip was washed to remove certain nonspecific-adsorbed DNA-Probe onto the surface within 300 s and finally used to capure target DNA in the format of a sandwich-type among the detection DNA probe onto the surface of tip, the pendant probe in the DNA tetrahedron and target DNA in the association solution A within 720 s.

Design of DNA-Based AND Logic Systems. The DNA tetrahedron-assisted signal enhancement in biolayer interferometry enabled the design of DNA-based AND logic systems based on Boolean logic (see Figure S4 in the Supporting Information). For input, we defined the presence of DNA tetrahedron (TE) or target DNA (TA) as 1 and their absence as 0. The initial velocities (v_0) for hybridization reaction of probe-target was defined as the output (1 or 0) for the logic gate (see Figure S4B in the Supporting Information). The four possible input combinations were (0, 0), (1, 0), (0, 1) and (1, 1) listed in the truth table (see Figure S4B in the Supporting Information). With no input, or with TE or TA input alone, the output was 0. With TE and TA input, a time-dependent increases in the signal intensity in biolayer interferometry can be obtained (see Figure S4A in the Supporting Information, black line), which resulted a higher initial velocities (v_0) for hybridization reaction of probe-target, giving an output signal of 1 (see Figure S4B in the Supporting Information). Similarly, ATP-induced aptamer-target recognization with the above method was also demonstrated to design a DNA-based AND logic system based on Boolean logic (see Figure S4C, D in the Supporting Information).

Data Analysis. The GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA) was employed to perform the data processing.

ASSOCIATED CONTENT

Supporting Information

The DNA oligos used in this study and additional experimental data. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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