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Amplified Analysis of DNA by the Autonomous Assembly of Polymers Consisting of DNAzyme Wires

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Supporting Information

ABSTRACT: A systematic study of the amplified optical detection of DNA by Mg^{2+}-dependent DNAzyme subunits is described. The use of two DNAzyme subunits and the respective fluorophore/quencher-modified substrate allows the detection of the target DNA with a sensitivity corresponding to 1×10^{-9} M. The use of two functional hairpin structures that include the DNAzyme subunits in a caged, inactive configuration leads, in the presence of the target DNA, to the opening of one of the hairpins and to the activation of an autonomous cross-opening process of the two hairpins, which affords polymer DNA wires consisting of the Mg^{2+}-dependent DNAzyme subunits. This amplification paradigm leads to the analysis of the target DNA with a sensitivity corresponding to 1×10^{-14} M. The amplification mixture composed of the two hairpins can be implemented as a versatile sensing platform for analyzing any gene in the presence of the appropriate hairpin probe. This is exemplified with the detection of the BRCA1 oncogene.

The amplified detection of DNA has attracted substantial research efforts, and numerous electrical,[1] optical,[2] and microgravimetric[3] amplified DNA sensors have been developed. These include the use of enzymes,[4] catalytic nanoparticles,[5] plasmonic nanoparticles,[6] and molecular catalysts[7] as amplifying labels. Catalytic nucleic acids (DNAzymes or ribozymes)[8] have found increasing interest as amplifying labels for sensing events.[9] The flexibility in mastering DNAzyme structures by encoding recognition functions into DNAzyme sequences and the reduced nonspecific absorption of these nucleic acids turn the DNAzymes into ideal candidates for the development of bioanalytical platforms. For example, the hemin/G-quadruplex horseradish peroxidase-mimicking DNAzyme[10] has been widely used to develop various DNA sensing platforms.[11] One of the challenging topics in DNA detection involves the development of new isothermal amplification schemes that could substitute the polymerase chain reaction (PCR). Different isothermal amplification methods have been developed. These include the rolling circle amplification (RCA) process, where the formation of a duplex between the analyte and a circular DNA is followed by isothermal polymerase/dNTPs replication of the circular template to yield long DNA chains.[12] Encoding into the circular template the information required for the synthesis of DNAzyme chains resulted in a 2-fold amplification of the recognition events by the replication and generation of the catalytic nucleic acids.[13] Similarly, analytical platforms implementing exonuclease III as a target-recycling biocatalyst have been reported to amplify DNA analysis.[14] Also, DNA machines, in which recognition events occurring on a DNA template trigger an isothermal replication/nicking process with the autonomous displacement of the horseradish peroxidase-mimicking DNAzyme, have been reported as an ultrasensitive method for detection of DNA.[15] Amplified DNA detection has also been accomplished by an autocatalytic and catabolic DNAzyme-mediated process.[16] In the present study, we introduce a new paradigm for the enzyme-free amplified detection of DNA that is based on the target-triggered autonomous cross-opening of functional DNA hairpin structures, which yields polymer DNA wires consisting of the Mg^{2+}-dependent DNAzyme[17] as a result of the primary recognition event.

One of the methods for detecting a target DNA involves self-organization of DNAzyme subunits by the analyte DNA. Figure 1A outlines the analysis of a target DNA by the Mg^{2+}-dependent DNAzyme subunits (**1**) and (**2**). While the domains I and I′ (brown) in the subunits correspond to the DNAzyme sequence, the domains II and II′ provide the recognition sites for the ribonucleobase (rA)-containing substrate (**3**), and the arms III and III′ of the subunits act as the recognition sequences for the analyte DNA (**4**). Substrate (**3**) is modified at its 5′ and 3′ ends with a fluorophore/quencher (F/Q) pair, resulting in quenching of the luminescence of the fluorophore. In the presence of the analyte (**4**) and the substrate (**3**), cooperative assembly of the active DNAzyme structure is stimulated, and in the presence of Mg^{2+} ions, the DNAzyme catalyzes the cleavage of the substrate and thus leads to the generation of fluorescence. This process was previously implemented for the development of logic DNA-computing circuits using a library of DNAzyme subunits.[18] DNAzyme subunits (**1**) and (**2**) and the substrate (**3**) were used to sense the target DNA (**4**) with a sensitivity corresponding to 1×10^{-9} M [Figure S1 in the Supporting Information (SI)]. This sensing platform amplifies the detection of the target DNA through the catalytic activity of the DNAzyme.

The present amplified sensing platform is based on the design of appropriate hairpin nanostructures that include DNAzyme subunits in a "caged", inactive configuration (Figure 1B). The system consists of the two hairpin nucleic acid structures (**5**) and (**6**) and the substrate (**3**). The two hairpins include the domains that correspond to the subunits of the Mg^{2+}-dependent DNAzyme. The participation of part of the DNAzyme subunits in the duplex structure of the stems of hairpins (**5**) and (**6**) prohibits the formation of the active DNAzyme structure. Hairpin (**5**)

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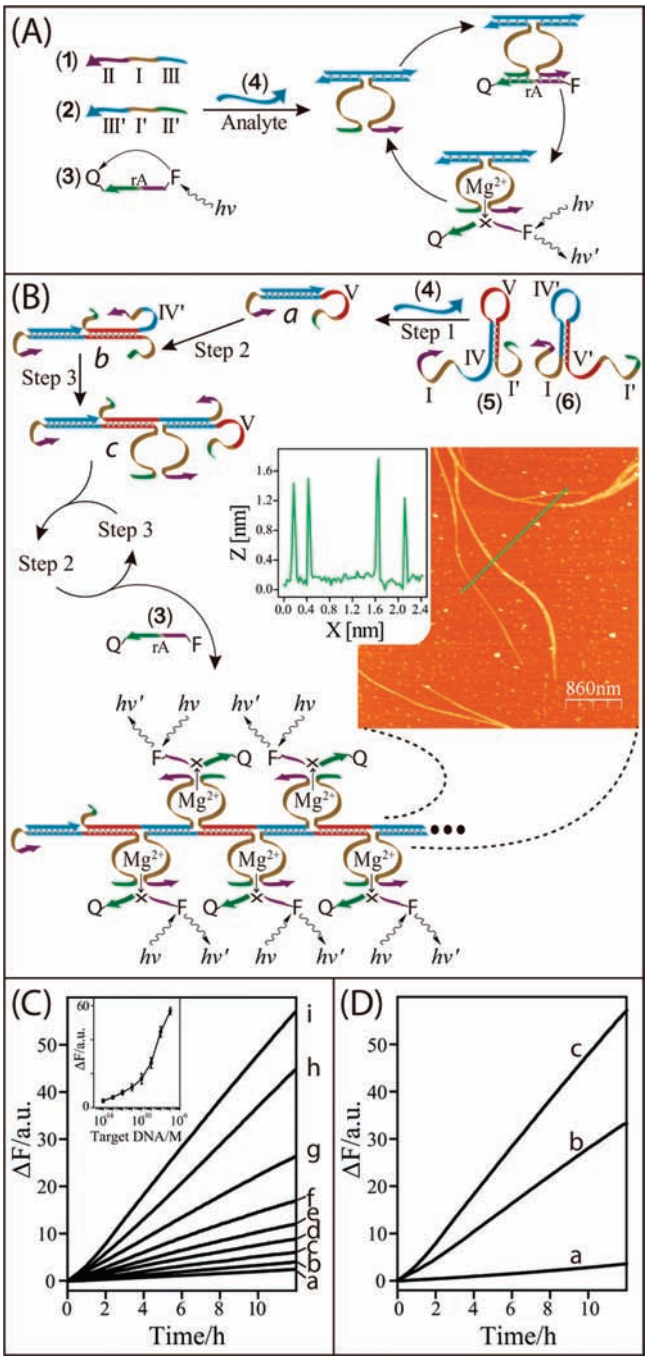

Figure 1. (A) Analysis of the target DNA (**4**) by the Mg^{2+}-dependent DNAzyme subunits (**1**) and (**2**) and catalyzed cleavage of the substrate (**3**). (B) Amplified analysis of the target DNA (**4**) using two functional hairpins (**5**) and (**6**), in which recognition of the target by (**5**) initiates the autonomous cross-opening of the hairpin structures, leading to the formation of polymer wires consisting of "two-sided" Mg^{2+}-dependent DNAzyme subunits that catalyze the cleavage of (**3**) and the generation of fluorescence. Inset: AFM image and cross-section analysis of the Mg^{2+}-dependent DNAzyme nanowires. (C) Time-dependent fluorescence changes upon analysis of different concentrations of (**4**) according to Figure 1B: (a) 0, (b) 1×10^{-14}, (c) 1×10^{-13}, (d) 1×10^{-12}, (e) 1×10^{-11}, (f) 1×10^{-10}, (g) 1×10^{-9}, (h) 1×10^{-8}, (i) 1×10^{-7} M. Inset: resulting calibration curve. (D) Fluorescence changes upon analysis of 1×10^{-7} M (**4**) according to (a) Figure 1A, (b) Figure S2, and (c) Figure 1B.

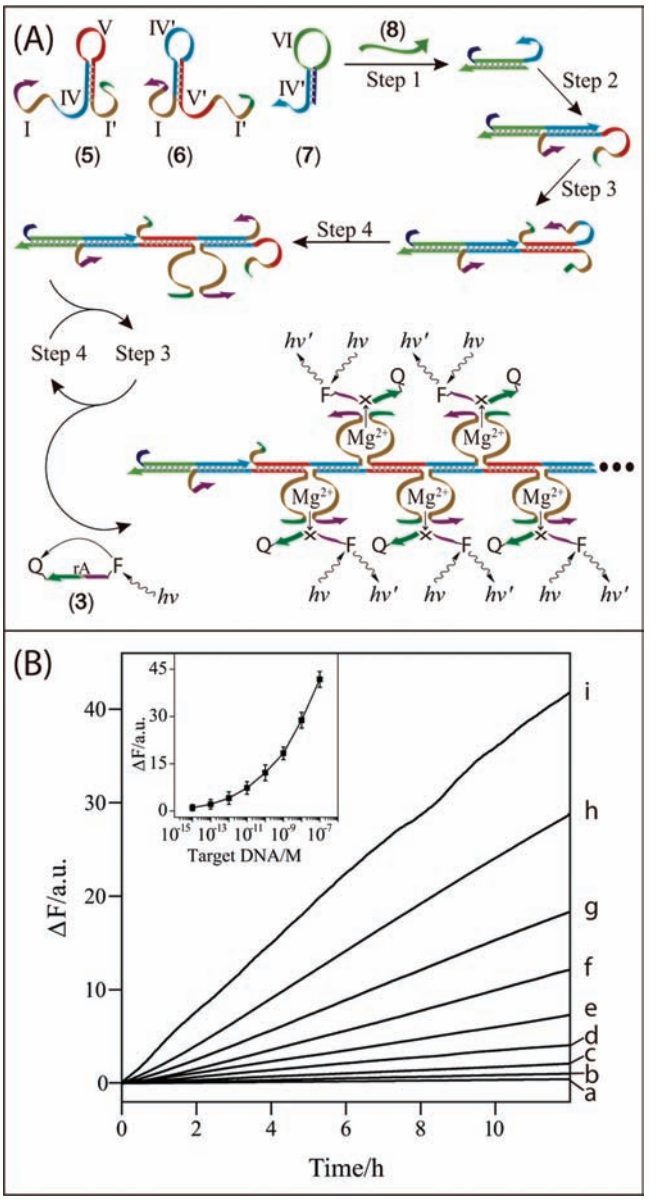

Figure 2. (A) Analysis of the BRCA1 oncogene (**8**) using probe hairpin (**7**) and the two functional hairpins (**5**) and (**6**) for the autonomous synthesis of the "two-sided" Mg^{2+}-dependent DNAzyme subunits polymer wires. (B) Fluorescence changes upon analysis of different concentrations of (**8**) according to the scheme outlined in Figure 2: (a) 0 M, (b) 1×10^{-14} M, (c) 1×10^{-13} M, (d) 1×10^{-12} M, (e) 1×10^{-11} M, (f) 1×10^{-10} M, (g) 1×10^{-9} M, (h) 1×10^{-8} M, (i) 1×10^{-7} M. Inset: Resulting calibration curve.

includes the sequence IV (blue) that is complementary to the target (**4**). Hairpin (**6**) includes the domain V′ (red) that is complementary to the sequence V of hairpin (**5**). In the presence of the target (**4**), hairpin structure (**5**) opens by a strand displacement mechanism, leading to hybrid *a*. The resulting single-stranded domain V (red) opens hairpin (**6**) by a strand displacement mechanism (hybridizing with domain V′) to yield structure *b*. The resulting structure *b* opens hairpin (**5**) by displacement of strand IV with domain IV′ (blue) in structure *b*, yielding structure *c* that includes adjacent subunits of the Mg^{2+}-dependent DNAzyme or active DNAzyme. Structure *c* has the

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same exposed domain V as structure *a*. Thus, by the primary recognition of the target (**4**) by hairpin (**5**), the open hairpin structure *a* stimulates the autonomous cross-opening of hairpins (**6**) and (**5**) (steps 2 and 3, respectively), leading to the formation of chains of the active DNAzyme structure. The binding of the substrate to the DNAzyme in the presence of Mg^{2+} ions results in DNAzyme cleavage of substrate (**3**). While the fluorophore (F) is quenched in the substrate structure, the cleavage of (**3**) by the DNAzyme oligonucleotide leads to the generation of fluorescence that provides the readout signal. Figure 1C shows the time-dependent fluorescence changes upon analysis of different concentrations of the target (**4**) using hairpins (**5**) and (**6**) as DNAzyme-generating components. The sensitivity for analysis of (**4**) corresponds to 1×10^{-14} M. Figure 1B inset shows an atomic force microscopy (AFM) image of the resulting DNAzyme chains. Micrometer-long DNA chains are observed. The height of the chains is in the range of 0.8−1.5 nm. Some of the chains form bundles. While single-stranded DNA exhibits a height of ~0.6 nm, the nonlinear structure of the DNAzyme chains leads to the increased height value. The bundling of the chains presumably originates from cross-interaction of DNAzyme subunits associated with different chains. It should be noted that the development of the sensing platform in Figure 1B, which yields "two-sided" Mg^{2+}-dependent DNAzyme subunits, involved an intermediate phase, where a "single-sided" Mg^{2+}-dependent DNAzyme subunits polymer wire was developed (for details, see Figure S2). Figure 1D compares the time-dependent fluorescence changes upon analysis of the same target (**4**) at a concentration of 1×10^{-7} M using the different sensing methods. While the two separated subunits lead to very low fluorescence changes (sensitivity of 1×10^{-9} M), the "two-sided" Mg^{2+}-dependent DNAzyme nanowires lead to high fluorescence values (sensitivity of 1×10^{-14} M). The "one-sided" DNAzyme nanowires led to weaker fluorescence signals (sensitivity of 1×10^{-13} M). The sensing platform also exhibits selectivity, and one-, two-, or three-base mismatches in the target DNA can be discriminated (see Figure S3).

It should be noted that the amplified detection of the target DNA (**4**) through the autonomous target-triggered synthesis of the Mg^{2+}-dependent DNAzyme wires by cross-opening of the respective hairpin structures represents an optimized sensing platform that can be adapted to analyze any target DNA without significant further optimization of the established system (for details, see the SI). This was exemplified with the analysis of the BRCA1 oncogene (**8**) (Figure 2A). The "helper" hairpin (**7**) was introduced to recognize (**8**), and upon opening, it activates the cross-opening of hairpins (**5**) and (**6**), leading to the DNAzyme wires. Figure 2B depicts the time-dependent fluorescence changes upon analysis of different concentrations of oncogene (**8**) and the resulting calibration curve.

In conclusion, this study has introduced an enzyme-free DNAzyme-based amplification sensing platform with sensitivities comparable those of enzyme-based assays (e.g., Exo III[14] or nicking/polymerase enzymes[15]). The stability of DNAzyme against denaturation is certainly an important advantage of the system. The present disadvantage of the DNAzyme-based system is, however, the long duration of the analytical process. This limitation could be resolved in the future by using other DNAzymes exhibiting higher catalytic activities.

■ ASSOCIATED CONTENT

Supporting Information. DNA sequences, experimental procedures and methods, results for analysis of target (**4**) by the Mg^{2+}-dependent DNAzyme subunits (**1**) and (**2**), and discussion of the amplified analysis of target (**4**) using functional hairpins (**5**) and (**9**) via "one-sided" DNAzyme nanowires. This material is available free of charge via the Internet at http://pubs.acs.org.

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