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Target-induced self-assembly of DNA nanomachine on magnetic particle for multi-amplified biosensing of nucleic acid, protein, and cancer cell

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ABSTRACT

A biosensing system is established for the multi-amplified detection of DNA or specific substrates of aptamers under isothermal conditions, which combines nicked rolling circle amplification (N-RCA) and beacon assisted amplification (BAA) with sensitive colorimetric technique by using DNAzymes as reporter units. According to the configuration, the analysis of DNA is accomplished by recognizing the target to capture nucleic acid-functionalized magnetic particles, followed by the self-assembly of the other two nucleic acids into multicomponent DNA supramolecular structure on magnetic particles. After magnetic separation, the circularization with ligase and the fragmentation with polymerase activate N-RCA and BAA in the presence of polymerase, dNTPs, and the nicking endonuclease, successively producing horseradish peroxidase (HRP)-mimicking DNAzymes that act as colorimetric reporter to catalyze the oxidation of $ABTS^{2-}$ by H_2O_2 in the presence of hemin. Under the optimized conditions, we obtain a wide dynamic range for DNA analysis over 6 orders of magnitude from 1.0×10^{-14} to 1.0×10^{-9} M with a low limit of detection of 6.8×10^{-15} M. In the absence of a target, neither self-assembly of nucleic acids nor amplification process can be initiated, indicating an excellent selectivity of the proposed strategy. Similarly, an analogous system is activated by cancer cells or lysozyme through cooperative self-assembly of nucleic acids on magnetic particles in the presence of respective substrates of aptamers to synthesize HRP-mimicking DNAzymes that give the readout signal for the recognition events, achieving LODs of 81 Ramos cells and 7.2×10^{-15} M lysozyme, respectively.

1. Introduction

The development of sensitive and selective biosensors is important in biological studies, clinical diagnostics, and biodefense applications (Kirsch et al., 2013). Since biomolecules of interest may be present in very small amounts, substantially fundamental and applied research efforts have been devoted to develop amplification techniques which enable us to detect trace levels of a specific analyte (Willner et al., 2009). So far, various amplified biosensors have been reported. The amplification approaches include the development of amplifying labels (Bi et al., 2010; Lei and Ju, 2012; Pei et al., 2011), such as the conjugation of enzymes and the catalytic particles, and the employment of amplification techniques, such as polymerase chain reaction (PCR) (Cheglakov et al., 2006; Roloff and Seitz, 2013) and strand displacement reaction (Walker et al., 1992). Recently, catalytic nucleic acids have been isolated by the systematic evolution of ligands by exponential enrichment (SELEX), which are used as a new class of reporter units for amplified detection of various analytes (Willner et al., 2008a). For example, the hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking DNAzyme is extensively used for the colorimetric (Elbaz et al., 2009; Fu et al., 2011a, 2011b; Tang et al., 2012a), chemiluminescent (Niazov et al., 2004; Zheng et al., 2012), electrochemical (Dong et al., 2012; Tang et al., 2012b), or electrochemiluminescent (Zhou et al., 2013) biosensing events. In contrast to enzyme labels that are thermally unstable, and require tedious preparation and purification steps, DNAzymes are impressively stable under ambient and even elevated temperatures, which may be easily synthesized and modified. Additionally, to overcome the disadvantages of the existing PCR, the development of isothermal amplification systems that are activated upon sensing the analytes has attracted substantial research efforts

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recently since they can achieve amplified detection of various analytes with high specificity and efficiency, and rapidity at constant temperature (Guo et al., 2009; Zhang et al., 2013). For example, rolling circle amplification (RCA), a powerful isothermal amplification method, has been used as a signal amplification technique for DNA genotyping and protein microarray through autonomously synthesizing repeat units to generate signal (Dong et al., 2013; Zhao, et al., 2008). Recently, a variety of RCA methods have been developed, including hyper-branched RCA (Cao and Zhang, 2012; Cheng et al., 2006; Lizardi et al., 1998), primer generation RCA (PG-RCA) (Murakami et al., 2007; Murakami et al., 2012; Zeng et al., 2013), circle-to-circle amplification (C2CA) (Dahl et al., 2004), and multi-primed chain amplification (MCA) (Lee et al., 2012). Particularly, nicking endonuclease-assisted RCA has attracted intense interest lately due to its high sensitivity, excellent specificity, and great detection capacity, which has been used in selective isolation of DNA (Weissman and Lasken, 2001), nucleic acid detection (Lin et al, 2013; Schaerli et al., 2010; Xu et al., 2012), and cancer cell analysis (Li et al., 2012). Also, DNA-based molecular machine, a generic molecular system, has been widely applied as a useful tool for bioassays with highly amplified efficiency (Beissenhirtz and Willner, 2006; Teller and Willner, 2010). For instance, a typical DNA machine is the beacon assisted amplification (BAA) that performs autonomous replication-scission-displacement process to generate HRP-mimicking DNAzymes by a polymerase, dNTPs, and a nicking endonuclease, which employs as an effective amplified replication system as a result of the recognition of analyte on a predesigned nucleic acid track (Wang et al., 2013; Willner et al., 2008a; Zhu, et al., 2013).

For biosensors two essential components are required including a target recognition element and a signal transducer. For nucleic acids, recognition is achieved by hybridization to complementary strands through specific base pairing. For proteins and cancer cells, the traditional recognition events include the interactions of enzyme–substrate, protein–ligand, and antibody–antigen. Nowadays, a radically new kind of recognition oligonucleotides known as aptamers are selected in vitro by the SELEX procedure (Mayer, 2009). Aptamers can specifically recognize a broad range of targets, such as ions, low-molecular-weight substrates, and proteins, with antibody-like specificity and high affinity (Famulok et al., 2007). Specific aptamers selected by tumor cell-SELEX process have the ability to distinguish cancer cells from normal cells and further to differentiate between numerous types of cells by using tumor cells as targets (Fang and Tan, 2010; Zhu et al., 2012). Due to the unique advantages of aptamers, such as easy production and modification, thermal stability, and high selectivity and affinity, substantial efforts are directed toward the integration of aptamers as recognition matrices in the development of optical or electrochemical sensors (apatsensors or cytosensors) (Famulok and Mayer, 2011; Iliuk et al., 2011; Mascini et al., 2011; Tan et al., 2013). Sensing modules are important components of molecular devices. Optical, electronic, and microgravimetric transducer units provide readout signals for biomolecular recognition events (Li et al., 2010). Amongst them, optical biosensors attracted intense attention due to their advantages of easy operation, high sensitivity, good stability and reproducibility, and fast response (Ligler, 2009). The most prominent example for the development of optical sensor is the utilization of HRP-mimicking DNAzyme as catalytic label for colorimetric or chemiluminescent detection of DNA or enzyme activities (Cheglakov et al., 2007; Li et al., 2007; Willner et al., 2008b).

Herein, the present approach aims to utilize the structural and functional properties of DNA to fabricate biosensors, the aptamer to specifically recognize certain target, and HRP-mimicking DNAzyme as coupled amplifier to biocatalyze colorimetric signal, achieving multi-amplified schemes for bioanalysis of different targets. In this assay, nicked RCA (N-RCA) is performed by inserting a nicking site in circular template, in which many copies of products are successively yielded in pairs by multiple nicking-replication-displacement reactions. Moreover, multi-amplification is accomplished by simultaneous activation of N-RCA and beacon assisted amplification (BAA) to continuously synthesize HRP-mimicking DNAzymes as biocatalysts to transduce and amplify sensing events. Consequently, taking the advantages of the significant amplification efficiency achieved by the combination of N-RCA and BAA and the intrinsically high sensitivity of DNAzyme-driven colorimetric signal, a highly efficient, isothermal, and autocatalytic amplification platform is achieved for ultrasensitive and highly selective assays of nucleic acid, protein (lysozyme), and cancer cells (Ramos cells).

2. Experimental

2.1. Materials

The nucleic acids were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), which were diluted in TE buffer (10 mM Tris–HCl, 1 mM EDTA, 12.5 mM $MgCl_2$, pH 8.0) to obtain the 0.1 mM stock solution. The sequences of the used oligonucleotides are listed in Table S1. The Klenow fragment exo- DNA polymerase and T4 DNA ligase were obtained from Fermentas (Canada), while Nt. BbvCI nicking endonuclease from New England Biolabs Inc. (USA). The deoxyribonucleoside triphosphates (dNTPs) mix was purchased from SBS Genetech Co., Ltd. (Beijing, China). Hemin, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) disodium salt ($ABTS^{2-}$), 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) were custom-ordered from Aladdin Chemistry Co. Ltd (China). Carboxyl group modified magnetic particles (1–2 μm) were ordered from BaseLine ChromTech Research Centre (Tianjin, China). Double-distilled, deionized ultrapure water was used in all experiments. All regents were of analytical grade and used without further purification. The UV–vis absorbance measurements were performed on a Cary 50 UV–vis spectrophotometer (Varian, USA).

2.2. Conjugation of nucleic acid (3) on magnetic particles

Firstly, 10 μL of carboxylated magnetic particles (10 mg/ml) was washed three times with PBS, which was further activated by 200 μL of 0.2 M EDC for 30 min. After magnetic separation, 10 μL of amino-modified nucleic acid (3) (1.0×10^{-8} M) was added to the activated magnetic particles, followed by the incubation of the mixture overnight at 37 °C. After washing with 200 μL of PBS three times and re-suspended in 10 μL of TE buffer, the resulting nucleic acid (3) conjugated magnetic particles were stored at 4 °C until use. The cell aptamer (5)-conjugated magnetic particles and nucleic acid (7)-conjugated magnetic particles were prepared in the same way.

2.3. DNA assay

According to Scheme 1 for the sensing assay of DNA, the mixture of 10 μL of (2) (1.0×10^{-8} M), 10 μL of (4) (1.0×10^{-8} M), 10 μL of the as-prepared (3)-conjugated magnetic particles, and 10 μL of different concentrations of target DNA (1) was incubated for 2 h to form the multicomponent supramolecular structure on magnetic particles. Note that (3) and (4) should be annealed respectively before use. Then, the circularization reaction was performed by the addition of 15 U T4 DNA ligase and 2 μL of H_2O in 5 μL of T4 buffer, followed by incubating at 25 °C for another 2 h. The beads were collected by magnetic field and washed with PBS. Then, the N-RCA and BAA were activated by

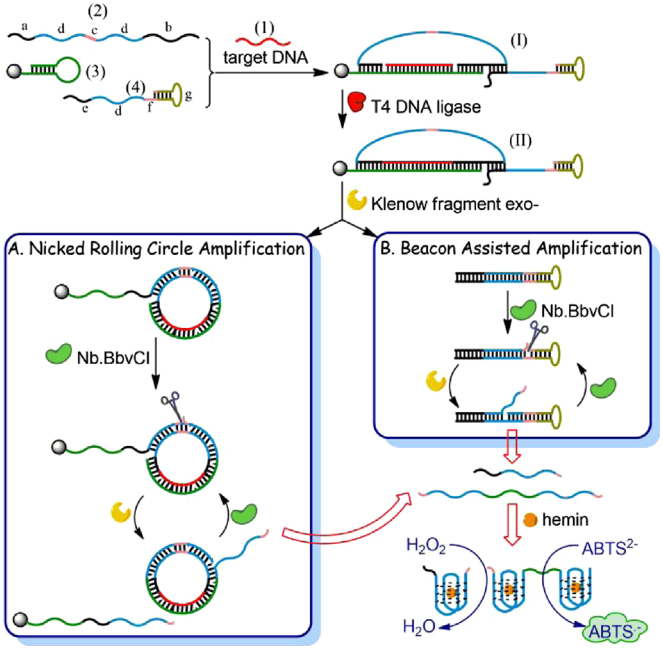
adding 30 μL of H_2O, 4 μL of NEBuffer, 4 μL of dNTPs, 5 U Klenow fragment exo- DNA polymerase, and 10 U Nb.BbvCI nicking endonuclease. After incubation at 37 °C for 90 min, the reaction was terminated by cooling down to 0 °C for 10 min to inactivate the enzymes followed by magnetic separation.

2.4. Colorimetric measurement

The resulting supernatant was interacted with 4.0×10^{-7} M hemin to a total volumn of 1 mL in a buffer solution (25 mM HEPES, 20 mM KCl, 200 mM NaCl, Triton X-100 (0.05%, w/v), and DMSO (1%, v/v); pH 7.4) at 25 °C for 1 h. Finally, 400 μL of 10 mM $ABTS^{2-}$, 600 μL of 6.67 mM H_2O_2 were added and reacted for 10 min to allow the biocatalyzed oxidation of $ABTS^{2-}$. The UV–vis absorbance was recorded in the wavelength from 380 to 480 nm. The absorbance at ~419 nm that is the maximal absorption of the colored product $ABTS^{\bullet -}$ was used for quantitative analysis. Control experiments were carried out by detecting the products yielded in N-RCA and BAA, respectively.

2.5. Ramos cell assay

According to Scheme 2, the sensing assay for analyzing cancer cells was carried out by first hybridizing 20 μL of target DNA (1) (1.0×10^{-7} M) to aptamer (5) immobilized magnetic particles at 25 °C for 2 h to obtain the target DNA/aptamer/particle conjugates. Magnetic extraction was performed by adding different amounts of Ramos cells followed by incubating at 25 °C for 1 h. After magnetic separation, the supernatant containing the released DNA (1) was subjected to the same treatments as those described for colorimetric analysis of DNA.



Scheme 1. Multi-amplified detection of target DNA via cooperative self-assembly of a DNA nanomachine on magnetic particle to activate (A) nicked rolling circle amplification (N-RCA) and (B) beacon assisted amplification (BAA), yielding G-quadruplex DNAzyme structures those are activated in the presence of hemin, which catalyze the oxidation of $ABTS^{2-}$ by H_2O_2 to form colored $ABTS^{\bullet -}$ products. The sequences of respective nucleic acids are given as Table S1.

2.6. Lysozyme assay

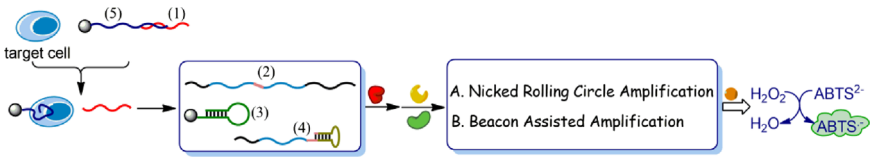
According to Scheme 3, the sensing assay for analyzing lysozyme was carried out by first mixing 10 μL of aptamer (6) (1.0×10^{-8} M) and 10 μL of different concentrations of lysozyme at 37 °C for 1 h, followed by adding 10 μL of (2) (1.0×10^{-8} M), 10 μL of (4) (1.0×10^{-8} M), and 10 μL of the as-prepared (7)-conjugated magnetic particles. After reaction at 25 °C for 2 h to form the multicomponent supramolecular structure on magnetic particles, the following procedures were the same as those described for colorimetric analysis of DNA.

3. Results and discussion

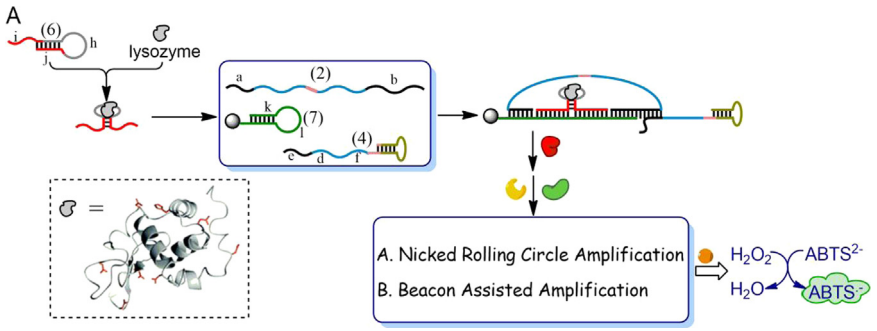
3.1. Principle of the proposed strategy for DNA detection

Scheme 1 depicts the self-assembly of nucleic acid construct for analyzing target DNA (1). The system consisted of padlock probe (2), molecular beacon (3) functionalized magnetic particle, and track DNA (4). Target DNA (1), served as a trigger, firstly binds to a complementary sequence in (3) causing it to "switch" conformation, thereby giving rise to the hybridization of (2) to the opened stem of (3) and (4) to a pre-defined segment of (2). As a result of activation, all of the predesigned components self-assemble into the supramolecular structure (I) on magnetic particle only in the presence of target DNA. Subsequently, (1) and (2) can be specifically ligated and circularized with (3) as the template by T4 DNA ligase, forming the supermolecular structure (II). Note that a blocker is terminated at the 5′-end of (4) with a domain non-complementary base (T4) to prevent undesired ligation of (4) with (3). Upon the addition of Klenow fragment exo- DNA polymerase and dNTPs, the multi-amplification processes, nicked rolling circle amplification (N-RCA) and beacon assisted amplification (BAA), are then activated. Here, each region of the components is designed to play a significant role in performing the amplification (vide infra).

For N-RCA, as illustrated in Scheme 1A, the circularized (2) consists of five regions. Regions (a) and (b) are complementary to the opened region of (3) and a region of (4), which act as a bridge to fabricate DNA nanomachine on magnetic beads. Region (c) is the heart of the proposed N-RCA, which provides a nicking site for Nt.BbvCI restriction endonuclease upon the formation of a double strand of this region. Two regions (d) consist of sequences that are complementary to the HRP-mimicking DNAzymes, which are designed as the "products" that lead to the signal transduction. In the presence of Klenow fragment exo- DNA polymerase and dNTPs, a RCA is activated by employing strand (3) as primer and circularized (1) and (2) as template. Importantly, when primer replicates region (c) of the template, a nicking endonuclease Nt.BbvCI



Scheme 2. A cytosensor via self-assembly of a nucleic acid nanomachine on magnetic particle when the aptamers (5) recognize Ramos cells to release DNA (1) to trigger the proposed dual-amplification, N-RCA and BAA, followed by the autonomous synthesis of the reporter HRP-mimicking DNAzyme units.



Scheme 3. An atpasensor via self-assembly of a nucleic acid nanomachine on magnetic particle upon the formation of aptamer–substrate complexes. After circularization, the proposed dual-amplification process, N-RCA and BAA, is activated by using DNA polymerase, dNTPs and nicking endonuclease to synthesize DNAzyme units as the biocatalysts for the analysis of lysozyme. Inset: the tertiary structure of lysozyme (Santos-Silva et al., 2011).

recognition sequence is produced by double strand formation. As a result, a cleavage of the replicated single strand occurs at the marked position, which generates a new site for the initiation of replication. Thus, while the reactivated replication is promoted by DNA polymerase, the already synthesized strand on the circle template that contains two DNAzyme sequences is concomitantly displaced from magnetic beads to solution. Therefore, the introduction of a nicking site to the circle template ensures the generation of multiple complementary sequences of circle template to produce large numbers of DNAzymes, which enhances the amplification efficiency to a great extent. We call this mode of RCA as nicked rolling circle amplification (N-RCA).

For the beacon assisted amplification (BAA), as depicted in Scheme 1B, track DNA (4) contains four regions, including a recognition part (e) that is complementary to (2), a complementary sequence (d) to HRP-mimicking DNAzyme, a sequence (f) for the generation of a nicking site for Nb.BbvCI upon replication, and a hairpin region (g) for the initiation of the polymerization process. During the process of the above mentioned N-RCA, the replication of track DNA (4) is also activated with DNA polymerase and dNTPs, resulting in the release of (4) from magnetic bead to solution. As a "track", the replication/nicking process and displacement of the synthesized HRP-mimicking DNAzyme then autonomously occur in the presence of Klenow fragment exo- DNA polymerase, dNTPs, and Nt.BbvCI nicking endonuclease. Finally, a large amount of HRP-mimicking DNAzyme sequences are synthesized in solution, which fold into G-quadruplex structure in the presence of hemin and stimulate the generation of an enhanced colorimetric signal through catalyzing the oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid ($ABTS^{2-}$) by H_2O_2 to a colored product $ABTS^{\bullet-}$. Consequently, by combing the proposed N-RCA and BAA to synthesize HRP-mimicking DNAzyme units that act as the biocatalytic amplifiers, the readout signal is multi-amplified for sensitive detection of DNA target.

Supervior to the previously reported DNA machines that performed homogeneously to generate DNAzymes upon the introduction of targets, the proposed strategy achieves control over the activation of the system by hybridizing the components onto the functionalized magnetic beads. Although magnetic particles may increase the cost of the process, they can be easily produced and have been commercialized at a low price. It should be noted that magnetic particles used in this assay have important advantages. (i) The fabrication of DNA nanomachine on magnetic beads can facilitate thorough washing and efficiently simplify the procedure of purification, which decreases the background signal induced by non-complexed DNA construct. (ii) The large surface area-to-volume ratio of microsphere offers high binding capacity, which further enhances the assay sensitivity. It has been calculated that the surface coverage of molecular beacon (3) on magnetic particles is $\sim 1.8 \times 10^5$ (see Supporting information). (iii) Using magnetic beads in target recognition can eliminate the interference of any components in biological sample, which improves the anti-interference ability of the sensing system. (iv) The release of track (4) to solution enables its unperturbed activation of BAA towards the synthesis of the DNAzyme units. (v) The DNAzymes produced in the process of N-RCA can be easily incorporated with those generate in BAA through magnetic separation. Therefore, magnetic particles used in this assay play significant roles in not only simplifying the experimental operation but also increasing the sensing and amplification efficiency.

3.2. Sensitivity and specificity of DNA detection

To achieve the best sensing performance of the system, the effects of the amounts of T4 DNA ligase, Klenow fragment exo- DNA polymerase and Nt.BbvCI nicking endonuclease, and the reaction time of the amplification process are studied (see Supporting information for detail). After optimization the conditions, we investigated the analytical performance of the proposed multi-amplification strategy in DNA detection. In Fig. 1, the UV–vis absorbance in the absence of target DNA (1) with hemin (curve a) is similar to that observed in the presence of hemin only (curve b), which can be considered as the background. This phenomenon implys that the components are predesigned in such a way that the self-assembled construct is fabricated only in the presence of target analyte followed by the synthesis of DNAzyme sequences. The background absorbance is attributed to the inherent catalytic activity of hemin for the H_2O_2-mediated oxidation of $ABTS^{2-}$ (Elbaz et al., 2009). Fig. 1A shows the variance of UV–vis absorbance with the concentration of target DNA. As the concentration of the target DNA increases, the formation of $ABTS^{\bullet-}$ is enhanced, resulting in the increase of UV–vis signal correspondingly. In logarithmic scales, the UV–vis absorbance exhibits a linear correlation with the concentration of target DNA over a range of 6 orders of magnitude from 1.0×10^{-14} to 1.0×10^{-9} M. The limit of detection (LOD) is calculated to be 6.8×10^{-15} M by evaluating the average response of blank plus three times the standard deviation, which is comparable to or even lower than the sensitivities of recently reported isothermal amplification methods by using HRP-mimicking DNAzymes as reporters (Table S2). Reproducibility, accuracy and storage stability of the assay were further examined. The relative standard deviation (RSD) of UV–vis signal to 1.0×10^{-12} M target DNA is 5.6% for five parallel measurements, indicating the good precision and desirable reproducibility for DNA detection. After storage the fabricated DNA nanomachine on magnetic particle at 4 °C for 6 days over a period of 1 week with production lead time of ~12 h, the results show that the UV–vis response does not decrease with an RSC of 6.9%, showing good

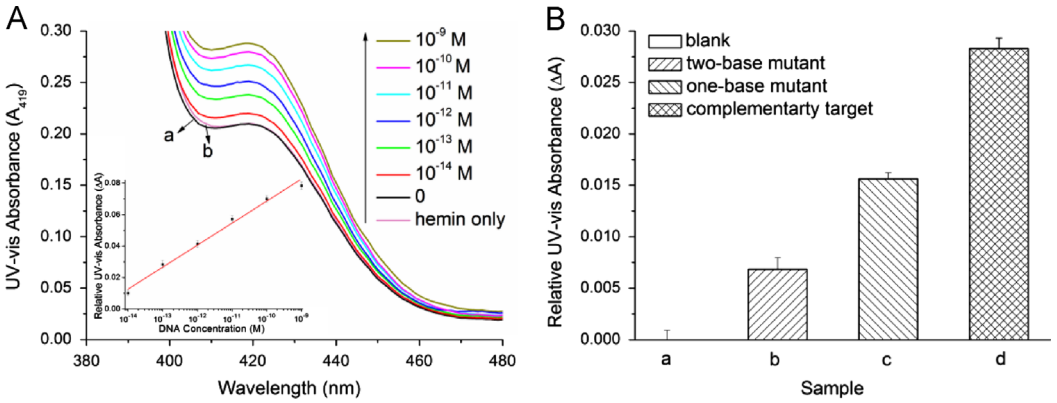


Fig. 1. (A) UV–vis absorbance of the synthesized DNAzymes obtained from different concentrations of target DNA. Inset is the corresponding calibration curve. The regression equation is expressed as $\Delta A = 0.0140 \log C + 0.2087$ with a correlation coefficient of 0.9952, where ΔA and C represent the relative UV–vis absorbance ($A - A_0$) and the concentration of target DNA (M), respectively. (B) Selectivity of the proposed method. The concentration of each analyte is 1.0×10^{-13} M. Error bars represent the standard deviation calculated from three independent experiments.

stability of the sensor. It should be noted that to ensure the precision and accuracy efficiently, the experimental conditions, such as the amount of enzymes (T4 DNA ligase, DNA polymerase, and nicking endonuclease), and the reaction time should be precisely controlled.

To confirm the amplification efficiency of this assay, the system was challenged by detecting DNAzyme products yielded by N-RCA or BAA, respectively. The UV–vis signals generated by the different products were then recorded (Fig. S3). Notably, for the analysis of 1.0×10^{-13} M target DNA a significant UV–vis absorbance is observed when the whole products are detected, which is higher than either of that produced by BAA or N-RCA, thus suggesting that the smart design by detecting the whole DNAzyme products could achieve high amplification efficiency to improve the performance of this sensing platform. Accordingly, the LODs obtained by detecting DNAzyme products yielded by N-RCA or BAA are 5.0×10^{-14} M and 1.0×10^{-13} M, respectively, which indicate the sensitivity of the proposed method by detecting the whole products has improved as much as 5- and 10-fold. The high sensitivity and large dynamic range of the proposed assay can be attributed to the high amplification efficiency of the proposed multi-amplification strategy, the excellent detection sensitivity of UV–vis detector, and the employment of magnetic beads.

Control experiments were further carried out to demonstrate the specificity of the proposed method. Fig. 1B shows that small signals are detected for two-base mutant, even a single base mismatch, while a significant signal is observed only in the presence of target analyte. Thus, the system is selectively activated by the target substrate and no active DNAzyme is formed for the mismatched.

3.3. Ramos cell assay

Inspired by the successful self-assembly of nucleic acids into functional nanostructure for multi-amplified detection of DNA, this strategy was further applied to determinate cancer cells using cell aptamer recognition (Scheme 2). Ramos cells were used as a model target cell to demonstrate the proof of principle. Firstly, the amino-modified cell aptamers TE02 (5) are immobilized on magnetic particles, which further hybridize with nucleic acid (1) that is the same as the target DNA in Scheme 1 with 14-mer complementary to the aptamer. In the presence of Ramos cells, the aptamers (5) functionalized magnetic particles specifically bind onto the cell surface through the conformational change of aptamers from a DNA/DNA duplex to a DNA/target complex. As a result, DNA (1) releases from magnetic carrier to solution. After magnetic separation, the released (1) is then acted as the target DNA to initiate the self-assembly of nucleic acid into functional DNA nanomachine on magnetic particles as described in DNA detection, followed by the activation of dual-amplification process, N-RCA and BAA, to synthesize DNAzyme units. Through intercalating with hemin, the activated HRP-mimicking DNAzymes biocatalyze the oxidation of $ABTS^{2-}$ by H_2O_2 to the colored product $ABTS^{\bullet -}$, providing an optical readout signal for the amplified sensing of Ramos cells.

The derived calibration curve that corresponds to the colorimetric detection of Ramos cells by the proposed multi-amplification strategy is shown in Fig. S4A. Ramos cells could be analyzed with a detection limit that corresponded to 81 cells. To evaluate the selectivity of the proposed system for cancer cell detection, control cells, MCF cells, are introduced as interferences. Due to the specific recognition of aptamer TE02 towards Ramos cells, the method can easily discriminate Ramos cells from MCF cells. As shown in Fig. S4B, a significant UV–vis absorbance is detected in the presence of Ramos cells. In contrast, no distinct signal is observed for control samples. Thus, the proposed cytosensor exhibits high selectivity for target cells and only the targets can trigger the amplification process efficiently.

3.4. Lysozyme assay

To further demonstrate the feasibility of our cooperative self-assembly of functional DNA nanomachine on magnetic particle for multi-amplified assay of various targets, another sensing system is designed to organize functional aptasensor constructs for the detection of protein. Scheme 3 depicts the method for analyzing lysozyme. The self-assembley of aptamer constructs activate dual-amplification, leading to the catalyzed optical sensing of the substrates. Here, a hairpin structure (6) is designed that consists of lysozyme aptamer sequence (domain h) and the target recognition regions (domains i and j) for nucleic acid (7). The aptamer is partially protected in the stem and exhibits partial sequence in the loop. The aptamer is tethered at its two ends to two additionally short nucleic acid sequences (i and j), which bridge the aptamer to regions a and b. This composition ensures that parts of the aptamer and target recognition region (j) are caged in the duplex structure of the stem. Thus, the target recognition regions cannot open the hairpin (6) in the absence of lysozyme. Upon the addition of lysozyme, the lysozyme–aptamer complex is formed, resulting in the separation of the stem of the hairpin structure (6) and hybridization of the two short DNA sequences. This allows the

released target recognition region (j) hybridize to domain (l) in strand (7) together with the other recognition region (i) to domain (k) in (7). As a result, molecular beacon (7) is opened, which further initiate the self-assemble of nucleic acids into DNA nanomachine on magnetic beads, followed by circularization by T4 DNA ligase and dual-amplification by DNA polymerase and nicking endonuclease. After execution the N-RCA and BAA, the DNAzyme sequences greatly yielded assemble into the active G-quadruplex HRP-mimicking DNAzymes in the presence of hemin. The DNAzymes catalyze the oxidation of $ABTS^{2-}$ by H_2O_2 to $ABTS^{\bullet -}$, which enables the amplified colorimetric readout for the detection of lysozyme.

The UV–vis absorbance of different concentrations of lysozyme is shown in Fig. S5A. As the concentration of lysozyme increases, the UV–vis absorbance intensifies. The system enables the detection of lysozyme with a linear range from 1.0×10^{-14} to 1.0×10^{-9} M and a LOD corresponded to 7.2×10^{-15} M. To investigate the specificity of the study, the proposed aptasensor was challenged against other several possible interferences. From the results of Fig. S5B, a significant UV–vis signal is observed for lysozyme. In contrast, the UV–vis signals obtained by analyzing BSA and thrombin are similar to that of the blank, indicating the coincidental activation of self-assembly and the following amplification process do not occur in the absence of the lysozyme. Therefore, the proposed method exhibits highly selectivity toward target protein.

4. Conclusion

In the present study, various configurations for multi-amplified optical biosensors are developed for the analysis of nucleic acids, cancer cell (Ramos), or protein (lysozyme). The system consists of three nucleic acids, which stably coexist in solution until the target is introduced. The targets act as the initiators, allowing the cooperative self-assembly of multicomponent functional supramolecular structure on magnetic particle that simultaneously activate N-RCA and BAA for amplified sensing of DNA or the specific substrate of aptamer (Ramos cell or lysozyme). The resulting HRP-mimicking DNAzymes provide biocatalytic labels for amplified colorimetric readout of the recognition events, achieving a significant improvement of the detection limit for DNA, Ramos cell, and lysozyme down to 6.8×10^{-15} M, 81 cells, and 7.2×10^{-15} M, respectively, with excellent specificities. Although the present analytical strategy is relative complicated, the operation is easy, robust and cost-effective. Moreover, the present study exhibits many distinct advantages. Firstly, comparing with other isothermal amplification reactions, this strategy combines RCA and BBA together for the first time, achieving a remarkably high sensitivity and wide dynamic range, which are comparable to those of PCR. More importantly, the design of this system is simple and ingenious by inserting a nicking sequence on templates (circular or linear track) to generate the products, thereby being free from troublesome design and usage of tedious inputs. Secondly, magnetic particles are used to not only simplify experimental operation but also increase the sensing and amplification efficiency. Considering the availability of a broad library of aptamers and the existence of various DNA/biomolecular interactions, our smart system is applicable to various molecular diagnostic assays by simply designing nucleic acid probes to initiate the multi-amplification strategy upon target recognition.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.09.066.

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