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Cascade DNA nanomachine and exponential amplification biosensing

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ABSTRACT

DNA is a versatile scaffold for the assembly of multifunctional nanostructures, and potential applications of various DNA nanodevices have been recently demonstrated for disease diagnosis and treatment. In the current study, a powerful cascade DNA nanomachine was developed that can execute the exponential amplification of p53 tumor suppressor gene. During the operation of the newly-proposed DNA nanomachine, dual-cyclical nucleic acid strand-displacement polymerization (dual-CNDP) was ingeniously introduced, where the target trigger is repeatedly used as the fuel molecule and the nicked fragments are dramatically accumulated. Moreover, each displaced nicked fragment is able to activate the another type of cyclical strand-displacement amplification, increasing exponentially the value of fluorescence intensity. Essentially, one target binding event can induce considerable number of subsequent reactions, and the nanodevice was called cascade DNA nanomachine. It can implement several functions, including recognition element, signaling probe, polymerization primer and template. Using the developed autonomous operation of DNA nanomachine, the p53 gene can be quantified in the wide concentration range from 0.05 to 150 nM with the detection limit of 50 pM. If taking into account the final volume of mixture, the detection limit is calculated as lower as 6.2 pM, achieving an desirable assay ability. More strikingly, the mutant gene can be easily distinguished from the wild-type one. The proof-of-concept demonstrations reported herein is expected to promote the development and application of DNA nanomachine, showing great potential value in basic biology and medical diagnosis.

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1. Introduction

Due to the obvious advantages such as specific base-pairing, predictable assembly, and single-stranded flexibility, DNA is commonly employed as a smart building block in the design of DNA based nanomachines (Dittmer et al., 2005; Modi et al., 2009; Seeman, 2003; Simmel and Dittmer, 2005; Song et al., 2013; Wang et al., 2010), some of which have been applied as the useful tools for bioassays with highly amplified efficiency (Chen et al., 2015; Wen et al., 2012). The efficient construction of DNA nanomachine has become a more and more active research area in medical research and clinical diagnosis.

Generally, DNA nanomachine often consisted of one or more sequence-designed oligonucletide probes that can automatically produce a signal for readout through specific binding to the "fuel" (namely trigger molecule). In order to screen disease-related genotypes, mutations, phenotypes or karyotypes for clinical purposes as well as the improvement of life quality, a few of DNA nanomachines with a variety of functions have been developed and are playing an increasingly important role in biological research over the past decades (Bi et al., 2014; Chen et al., 2004, 2015; Zhao et al., 2009). However, although some molecular machines can imitate biological phenomena or perform certain functions, their practical use still remains a great challenge due to the less desirable structure-determined functions. It is necessary and urgent to design powerful DNA nanomachines.

Cancer is considered a worldwide mortal sickness and accounts for several millions of deaths every year, becoming a major public concern. Nevertheless, if accurate early diagnosis could be accomplished, there is a great chance of cure. For example, the 5-year survival rate is more than 90% when lung cancer is screened at its early stage. Thus, early diagnosis and prompt operation are essential for the successful treatment of cancers. In the clinical and basic biological research on the cancer-related diseases, the p53, also known as TP53, has been often highlighted as

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an ideal candidate maker due to its aberrant expression during the onset and progression of a variety of cancers (Nigro et al., 1989).

Based on the above considerations, in the current contribution, a cascade DNA nanomachine was developed to implement the exponential amplification of stimulus and used for the highly sensitive detection of p53 tumor suppressor gene. A rational designed recognition probe (RP) tagged with fluorophore and quencher acts as the "track" for the operating of machine, which was constructed to be capable of recognizing target DNA, serving as enzymatic replication template, polymerization primer and signaling probe in the presence of the target DNA "fuel". During the process for the hybridization event amplification, besides the target DNA acts as the trigger species to induce one cyclical strand-displacement polymerization (RT-CNDP) reaction and is repeatedly used, nicked strands are dramatically generated, each of which can in turn activate other cyclical strand-displacement polymerization (RN-CNDP)-based amplifications, resulting in the cascade operation of DNA nanomachines and exponential amplification effect. Simply stated, via coupling with dual-cyclical nucleic acid strand-displacement polymerization (RT-CNDP and RN-CNDP), a new-type of DNA nanomachine is for the first time designed as a proof-of-concept for the target binding exponential amplification. Moreover, this DNA nanomachine exhibits the desirable capability to discriminate the mutation point existing in p53 gene. Additionally, the hybridization event can be in situ signaled, circumventing the requirement for any additional reporting probe. In the text, the design of RP, molecular mechanism of signal conversion, characterization of cascade DNA nanomachine and assay capability are detailedly represented.

2. Experimental section

2.1. Materials and chemicals

All oligonucleotide sequences designed in the study are listed in Table 1. The RP labeled with FAM and DABCYL was obtained via commercial synthesis by Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. (Shanghai, China) with HPLC purification, and its two italic fragments are complementary to each other. Other oligonucleotides were all supplied by Invitrogen Bio Inc. (Shanghai, China). All oligonucleotide stock solutions were prepared by dissolving DNA strands in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.6) and stored at 4 °C refrigerator before usage.

The Nt. BbvCI nicking endonuclease, Klenow Fragment (3′–5′ exo-) polymerase and low DNA ladder were purchased from New England Biolabs (USA) Ltd. The deoxynucleotide triphosphates (dNTPs) and Sybr Green I were obtained from Dingguo Changsheng Biotechnology Co., Ltd (Beijing, China). The 25-mM tris-buffer (pH 8.2, 100 mM NaCl, 50 mM KAc, 10 mM $MgAc_2$ and 1 mM DTT) was used as the reaction solution (Xie et al., 2013). All other chemical reagents were of analytical grade and used without further purification. Ultrapure water used to prepare all aqueous solutions throughout the experiments was obtained through a Kerton lab MINI water purification system (UK) (resistance > 18 MΩ/cm).

2.2. Operation of DNA machine for amplification detection of target DNA

RP was heated at 90 °C for 5 min, followed by slowly cooling down to room temperature. Subsequently, 3 μL of 5 μM RP and

Table 1
Oligonucleotide sequences designed in the current study.

Note	Sequence (5'-3')
RP (recognition probe)	5'-(DABCYL)***g****TCGCA****G***CACAAACACGCACCTCA**AAGC***CTGCGAC***t**(FAM)C-3'
PT1 (polymerization template 1)	5'-GTCGCAGCACAAACACGCACCTCAgctgaggAGTC-3'
PT2 (polymerization template 2)	5'-GTCGCAGCACAAACACGCACCTCAgctgaggAGTCG-3'
PT3 (polymerization template 3)	5'-GTCGCAGCACAAACACGCACCTCAgctgaggAGTCGC-3'
PT4 (polymerization template 4)	5'-GTCGCAGCACAAACACGCACCTCAgctgaggAGTCTT-3'
Nicked fragment	5'-TCAGCTGAGGTGCGTGTTTGTGCTGCGAC-3'
Target DNA (p53 gene)	5'-CA**GCTTTGAGGTGCGTGTTTGTGC**CTGTCCTG-3'
MT1 (mutant target DNA1)	5'-CAGCTTTGAGGTGC**a**TGTTTGTGCCTGTCCTG-3'
MT2 (mutant target DNA 2)	5'-CAGCTTT**t**AGGTGC**a**TGTTTGTGCCTGTCCTG-3'
MT3 (mutant target DNA 3)	5'-CAGCTTT**t**AGGTGC**a**TGTTTGT**t**CCTGTCCTG-3'
MT4 (mutant target DNA 4)	5'-CAGCTTT**t**AG**t**TGC**a**TGTTTGT**t**CCTGTCCTG-3'

For RP, both the FAM and DABCYL were attached onto the lowercase bases 't' and 'g', respectively, and the bold fragment can hybridize with the bold one indicated in target DNA. The self-hybridization of two italicized fragments helps RP fold into a hairpin structure, while the gray segment may serve as a primer when binding to polymerization template. PT1, PT2, and PT3 were projected individually with 5, 6, and 7-base fragment with gray background complementary to the primer at the 3′ terminus of RP. Except for two 'T' bases at the 3′ terminus, PT4 has the same sequence as PT1. Nicked fragment was designed to have the same base sequence as the nicked/displaced oligonucleotide strand resulting from RN-CNDP (seen in Scheme 1 and in the text), in which the boxed portion can hybridize to the boxed fragment of RP. Mutant target DNAs have the same sequence as the target DNA but with one or more point mutations indicated in bold lowercase in the middle region.

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