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Controlling the function of DNA nanostructures with specific trigger sequences

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Abstract

We report a hybridization-based switching mechanism with single-base specificity that can be readily integrated with functional DNA nanostructures. As an exemplar, we have developed a switchable DNAzyme (SDZ) that only becomes activated in the presence of a perfectly matched trigger sequence and operates effectively at room temperature.

Over the course of the past decade, DNA has proven to be an extraordinarily useful material for nanotechnology applications, as it can be chemically synthesized, easily modified and exhibits exceptional stability over a wide range of conditions, making it possible to construct a broad variety of functional nanoscale devices. For example, DNA-based biopolymers can be used for specific molecular recognition (*e.g.* aptamers¹), construction of complex 2D and 3D structures (*e.g.* origami^{2,3} and cages^{4,5}), computing,^{6,7} and nano-mechanical systems.^{8,9} Furthermore, seminal work by Breaker, Joyce and Santoro has shown that DNA-based ‘DNAzymes’ can perform enzymatic reactions.^{10,11} However, in order to maximize the usefulness of this wide range of DNA-based nanodevices, it is imperative to devise an effective strategy to control their functionality such that they can readily be switched on or off, on demand.

Towards this end, a number of approaches have been reported wherein specific DNA sequences are used as ‘triggers’. For example, Yurke and coworkers have shown that the mechanical motion of DNA tweezers can be controlled by specific trigger sequences that induce opening or closing of the nanostructure.¹² Similarly, Dirks and Pierce¹³ reported a hybridization chain reaction wherein a specific DNA sequence triggers the assembly of individual DNA monomers into multi-unit nanostructures. In this type of control scheme, it is critical that the switching mechanism is highly specific to the sequence of the trigger DNA, such that oligonucleotides containing mismatched bases are incapable of activating the molecular function. Ideally, the switching mechanism should only respond to a perfectly matched trigger sequence, and remain inactive in the presence of other sequences that contain even single-base mismatches. However, the development of a hybridization-based switching mechanism with single-base specificity has proven to be challenging, because the difference in hybridization energy between a given DNA sequence and a perfectly matched

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trigger sequence versus variants containing a single-base mismatch is usually minimal at room temperature.¹⁴

In this work, we report a hybridization-based switching mechanism with single-base fidelity that operates effectively at room temperature. Our mechanism is entirely composed of DNA and utilizes a 'triple-stem' structure that we have previously reported, wherein the difference in energy between self-hybridization versus hybridization to the trigger can be tuned.¹⁵ As an exemplar, we have synthesized a switchable DNzyme (SDZ) that exhibits exquisite specificity to a trigger sequence, with single-base fidelity. In its native state, the SDZ folds into a highly stable triple-stem structure, which locks the DNzyme moiety into an inactive state. Hybridization to the perfectly matched trigger sequence unlocks the structure, thereby activating the DNzyme motif. However, any differences within the trigger sequence, including single-base mismatches, prevent unlocking of the triple-stem structure, as the native self-hybridized 'triple-stem' state is more thermodynamically stable.¹⁵

The SDZ molecule consists of 76 DNA nucleotides (nt) containing the 10-23 DNzyme motif, and folds into a triple-stem conformation (**Experimental section, 1**) at room temperature (Scheme 1). The 10-23 DNzyme is an *in vitro*-selected DNA molecule with a 15-nt catalytic domain that exhibits sequence-specific RNA cleavage activity.^{10,11} This DNzyme is highly versatile, because the sequence of its recognition arms can be changed without loss of catalytic activity as long as the cleavage site contains an unpaired purine and a paired pyrimidine.¹⁶ Here, a 15-nt DNA/RNA chimeric substrate labeled with a fluorophore-quencher pair (**2**) was used to fluorescently measure DNzyme activity. At room temperature, the SDZ molecule folds into its native triple-stem structure, locking the DNzyme unit in an inactive state via intra-molecular hybridization with its two recognition arms. Hybridization to a perfectly matched trigger sequence (PM; **3**) opens the triple-stem structure, thereby activating the DNzyme motif (Scheme 1, top). In the presence of Mg²⁺, the activated 10-23 DNzyme cleaves the phosphodiester bond between the unpaired purine and the paired pyrimidine of the substrate, separating the fluorophore from the quencher to generate a fluorescence signal (Scheme 1, top right). Importantly, the activated DNzyme motif is capable of cleaving multiple substrate molecules, resulting in a significant increase in fluorescence (Fig. 1A, PM). In contrast, a single-base mismatched trigger sequence (MM; **4**) is unable to unlock the triple-stem nanostructure, as the state of the native self-hybridized SDZ molecule is more thermodynamically favorable (Scheme 1, bottom).¹⁵ As a result, the DNzyme motif remains inactive and thus incapable of cleaving the substrate, resulting in a minimal increase in the fluorescence signal (Fig. 1A, MM).

We first characterized the activity of the SDZ at varying concentrations of trigger sequence, and observed dramatic differences in the responses to PM and MM triggers (Fig. 1B). At low concentrations, we observed a linear increase in fluorescence signal, wherein the slope for PM (1014 units/nM) was significantly higher than that for MM (45 units/nM) (Fig. 1B, inset). This difference in slope results in dramatic differences in fluorescence signal, enabling us to discriminate PM and MM triggers at concentrations as low as 750 pM. This detection limit represents a 10-fold improvement over the triple-stem structure lacking the amplification function reported in our earlier work.¹⁵

To quantify the SDZ's specificity for its PM trigger sequence, we defined the single-base mismatch discrimination factor (DF) as the ratio of the net fluorescence intensity observed with the PM trigger to that with a MM trigger after background subtraction. Thus, a larger DF is indicative of greater specificity. We challenged the SDZ molecule with trigger sequences containing single mismatches at different positions and measured the DF values. Using a fixed trigger concentration of 20 nM, we observed a DF of ~13 for various triggers containing different mismatched nucleotides at the same position as was modified in the

original MM sequence (Table 1, mismatched bases in red), which suggests that the specificity of the SDZ does not depend on the identity of the mismatched base pair. We also obtained DFs ranging from 2.2 to 7.2 for trigger sequences containing mismatched bases at various positions. We believe that the original MM trigger sequence (**4**) shows a higher DF compared to other trigger sequences because its nearest neighbors are A:T base pairs, which are less stable. We also observe that the trend in DF of internal mismatches is C:C > A:A $\approx$ T:T > G:G, which is fully in line with the inversely proportional trend in stabilities of the mismatches as reported by Peyret et al.¹⁷

To analyze the reaction rates of the SDZ, we performed time-course measurements at different concentrations of PM and MM trigger sequences (5, 10, 15, 20 and 40 nM) (Fig. S1). During the first 60 minutes, the DNAzyme-catalyzed reaction triggered by 40 nM PM yielded a considerably greater fluorescence signal gain (90 units/minute) in comparison with 40 nM MM (6 units/minute). The data also show that the rate of DNAzyme cleavage slowed with decreasing trigger concentrations. We used the Michaelis-Menten equation to calculate the rate constants of the SDZ for PM and MM trigger sequences from these data.^{18,19} For the PM trigger, we calculated a Michaelis-Menten constant (K_m) of 209 nM and apparent turn-over number (k_{cat}) of 0.18 min^{-1} , and therefore a reaction efficiency (k_{cat}/K_m) of $8.6 \times 10^5 \text{ M}^{-1}\text{min}^{-1}$. The k_{cat} of SDZ is close to that determined for the free 10-23 DNAzyme by Santoro and Joyce (0.1 min^{-1}),¹¹ suggesting that DNAzyme efficiency is not hampered by integration into the triple-stem structure nor by the room temperature reaction conditions. For the MM trigger, we obtained $K_m = 7.1 \text{ nM}$, $k_{cat} = 0.002 \text{ min}^{-1}$ and $k_{cat}/K_m = 2.9 \times 10^5 \text{ M}^{-1}\text{min}^{-1}$. The k_{cat} of the integrated DNAzyme with the MM trigger is 90 times lower than in the presence of the PM trigger, confirming the highly specific PM-triggered activation of the SDZ. This large difference in kinetic response enables the SDZ to exhibit excellent discrimination between PM and MM triggers (Fig. 2A) and sustain a large DF over an extended period of time (Fig. 2B).

In this work, we have described a DNA hybridization-induced switching mechanism with exquisite sequence specificity to the trigger DNA molecule, which allows us to control the activity of functional DNA nanostructures. In our model, we show that only a perfectly-matched trigger oligonucleotide can activate the DNAzyme moiety embedded in the SDZ-DNA structure, while other sequences that differed by even a single base were unable to effectively unlock the nanostructure. Although we observed some differences in DF for the various mismatched trigger sequences we tested, we found that our switching mechanism can discriminate mismatches for all four nucleotides (A, T, C and G) at various positions of the trigger sequence over extended periods of time (> 5 hours). By measuring the kinetic performance, we also confirmed that the reaction efficiency (k_{cat}/K_m) of the SDZ is comparable to the native 10-23 DNAzyme, indicating that incorporation into our switchable triple-stem structure does not hamper catalytic activity. Given that this triple-stem structure operates effectively at room temperature and is entirely composed of DNA, we believe our switching mechanism could be broadly useful for regulating the activity of other functional DNA nanostructures beyond the DNAzyme example described here. Such control over the actuation of functional DNA machinery will be an important step towards fully harnessing the potential of DNA-based nanotechnology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Ellington A, Sozstak JW. *Nature*. 1999; 346:818. [PubMed: 1697402]
2. Seeman NC. *J Ther Biol*. 1982; 99:237.
3. Seeman NC. *Nature*. 2003; 421:427. [PubMed: 12540916]
4. Goodman RP, Heilemann M, Doose S, Erben CM, Kapanidis AN, Turberfield AJ. *Nat Nanotechnol*. 2008; 3:93. [PubMed: 18654468]
5. Andersen ES, Dong M, Nielsen MM, Jahn K, Subramani R, Mamdouh W, Golas MM, Sander B, Stark H, Oliveira CL, Pedersen JS, Birkedal V, Besenbacher FF, Gothelf KV, Kjems J. *Nature*. 2009; 459:73. [PubMed: 19424153]
6. Adleman LM. *Science*. 1994; 266:1021. [PubMed: 7973651]
7. Seelig G, Soloveichik D, Zhang DY, Winfree E. *Science*. 2006; 314:1585. [PubMed: 17158324]
8. Mao C, Sun W, Shen Z, Seeman NC. *Nature*. 1999; 397:144. [PubMed: 9923675]
9. Shin JS, Pierce NA. *J Am Chem Soc*. 2004; 126:10834. [PubMed: 15339155]
10. Breaker RR, Joyce GF. *Chem Biol*. 1994; 1:223. [PubMed: 9383394]
11. Santoro SW, Joyce FG. *Proc Natl Acad Sci USA*. 1997; 94:4262. [PubMed: 9113977]
12. Yurke B, Turberfield AJ, Mills AP Jr, Simmel FC, Neumann JL. *Nature*. 2000; 406:605. [PubMed: 10949296]
13. Dirks RM, Pierce NA. *Proc Natl Acad Sci USA*. 2004; 101:15275. [PubMed: 15492210]
14. Ikuta S, Takagi K, Wallace RB, Itakura K. *Nucleic Acids Res*. 1987; 15:797. [PubMed: 3822815]
15. Xiao Y, Plakos KJ, Lou X, White RJ, Qian J, Plaxco KW, Soh HT. *Angew Chem Int Ed Engl*. 2009; 48:4354. [PubMed: 19431180]
16. Cairns MJ, Hopkins TM, Witherington C, Sun LQ. *Antisense Nucleic Acid Drug Dev*. 2000; 10:323. [PubMed: 11079572]
17. Peyret N, Seneviratne PA, Allawi HT, SantaLucia J Jr. *Biochemistry*. 1999; 38:3468. [PubMed: 10090733]
18. Michaelis L, Menten M. *Biochem Z*. 1913; 49:333.
19. Lineweaver H, Burk DJ. *J Am Chem Soc*. 1934; 56:658.

Fig. 1. Proof-of-concept and dynamic range of the SDZ. (A) Fluorescence emission spectra following incubation of the SDZ molecule (15 nM) with 10 nM PM trigger, MM trigger or no trigger sequence. We incubated samples for three hours at room temperature with 400 nM substrate with excitation at $\lambda = 590$ nm. (B) Fluorescence signals with one sigma standard deviations from PM and MM trigger sequences at varying concentrations. Inset shows the initial linear increase in fluorescence observed at low concentrations of trigger DNA.

Fig. 2. Time-course measurements of the fluorescence (**A**) and DF (**B**) with one sigma standard deviations of our SDZ over 5 hours, obtained with 15 nM SDZ, 10 nM PM or MM trigger sequence and 400 nM substrate.

Scheme 1.

The SDZ molecule consists of a triple-stem probe integrated with a 10-23 DNAzyme motif, paired with a fluorescence/quencher-modified DNA/RNA chimeric substrate. In its native state, the probe renders the DNAzyme (blue) inactive by incorporating it into a thermodynamically stable triple-stem structure. (Top) Only a perfectly matched trigger sequence (PM trigger, green) can disrupt this structure and activate the DNAzyme, which cleaves the substrate and results in increased fluorescence. (Bottom) In contrast, trigger DNAs containing a single nucleotide mismatch (MM trigger, orange) cannot open the native structure and the DNAzyme remains inactive.

Table 1

SDZ performance with trigger sequences containing mismatched bases at various positions (mismatches marked in red).

Trigger sequence (5'-3')	Base-pair ^a	DF ^b
GGGACCCCCTGATTTTACC	PM C-G	
GGGACCCCCTCATTTTACC	MM C-C	13.2
GGGACCCCCTAATTTTACC	MM C-A	13.2
GGGACCCCCTTATTTTACC	MM C-T	13.3
GGGAGCCCCTGATTTTACC	MM G-G	2.2
GGGACGCCCTGATTTTACC	MM G-G	4.1
GGGACCGCCTGATTTTACC	MM G-G	3.8
GGGACCCGCTGATTTTACC	MM G-G	3.9
GGGACCCCGTGATTTTACC	MM G-G	3.2
GGGACCCCAGATTTTACC	MM A-A	5.4
GGGACCCCCTGTTTTTACC	MM T-T	6.2
GGGACCCCCTGAATTTTACC	MM A-A	5.6
GGGACCCCCTGATATTACC	MM A-A	7.2
GGGACCCCCTGATTATACC	MM A-A	6.3

^aPM = perfect match, MM = mismatch;

^bDF = discrimination factor.