Molecular Devices

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Coherent Activation of DNA Tweezers: A "SET–RESET" Logic System**

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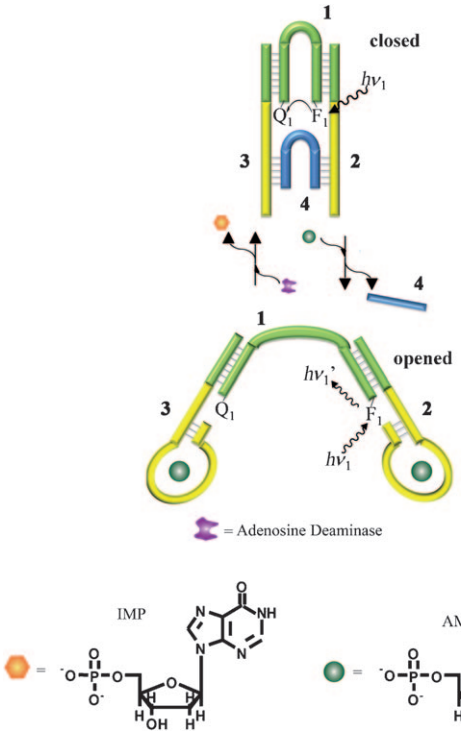
Recent research efforts have used the information encoded in base sequences in nucleic acids to develop functional biomolecules that perform mechanical functions, called "DNA machines".[1] Among the DNA structures that perform mechanical functions, nucleic acid tweezers,[2,3] walkers,[4,5] molecular gears,[6] and scissors[7] have been reported, and the use of such "DNA machines" for sensing,[8] nanotransporting,[9] nanomedicine,[10] and logic-gate operations[11] was suggested. For example, a DNA tweezer that captures, holds, and releases a molecular object was reported.[12] Herein, we introduce an aptamer-based DNA tweezer. We demonstrate the coherent operation of two DNA tweezers in opposite directions, and we show that the system performs SET–RESET logic functions. The previously reported DNA tweezers were activated by a "fuel" DNA molecule that eliminated a component of the tweezer structure by formation of a duplex waste product,[2] or by the cleavage of a nucleic acid component that is part of the tweezer by a DNAzyme unit integrated into the tweezer nanostructure.[3] In both of the systems, the cyclic operation of the tweezers yielded DNA as a waste product, and the cyclic operation of the machines required the constant addition of fuel nucleic acids. In the present system, the DNA sequences stay intact throughout the cyclic operations of the nucleic acid tweezers.

The tweezer's basic configuration and its mode of function are depicted in Scheme 1. It consists of a nucleic acid **1** that provides the tweezer frame (see Table 1 for sequence). This frame is functionalized at its 5′ and 3′ ends with a fluorophore F_1 (Cy5) and a quencher Q_1 (Iowa black RQ), respectively, as functional components that report on the tweezer state (open or closed). Parts of the nucleic acid **1** are hybridized with the nucleic acids **2** and **3** (marked in green), which include as part of their base sequences the anti-adenosine aptamer sequence[13] (marked in yellow). Parts of the aptamer domains are hybridized with the nucleic acid **4**, which acts as the activator that closes or opens the tweezer (marked in blue). The hybridization of **4** with the two domains of **2** and **3** preserves the frame in a "closed" configuration that is reflected by the quenching of the fluorophore F_1 (Cy5). Treatment of the "closed" configuration with adenosine monophosphate (AMP) results in the formation of the



Scheme 1. Cyclic activation of an aptamer-based tweezer using adenosine monophosphate (AMP) and adenosine deaminase as mechanical activators. IMP = inosine monophosphate.

adenosine–aptamer complex on the two "arms" of the tweezer and the release of the nucleic acid **4**. This process opens the tweezer, and the inefficient quenching of the fluorophore reflects the extended configuration of the nanostructure. Treatment of the open adenosine-functionalized-aptamer structure with adenosine deaminase (AD)[14] transforms adenosine monophosphate into inosine monophosphate (IMP), which is released by the aptamer sequence.

This process, however, drives the accumulation of the previously released nucleic acid **4** that hybridizes with the free arms and induces the condensed closed structure of the tweezer. The cyclic opening and closing of the tweezer structure are depicted in Figure 1. In the closed configuration, the system shows low-intensity fluorescence as a result of the quenching of F_1 (Figure 1 A a). Upon treatment of the system with adenosine monophosphate, the extended configuration of the tweezer is obtained, as reflected by the high fluorescence intensity (Figure 1 A b). Treatment of the open structure with adenosine deaminase results in the formation of inosine monophosphate and the condensation of the tweezer arms to the closed configuration. This process is reflected by the quenching of the fluorophore (Figure 1 A c). By the cyclic

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Table 1: Different DNA sequences used to construct the tweezer systems.[a]

Number	Sequence
1	5′ Cy5- ATACGCTTATCGGTACATG AGCACTGCAGCACTGGACCAC-Iowa black RQ 3′
2	5′ CATGTACCGATAAGCGTAT TATGGAAGGAGGCGTTATGAGG GGT CCA 3′
3	5′ ACCTGGGGGAGTATTGCGGAG-GAAGGTGC GTGGTCCAGTGCTGCAGTG 3′
4	5′ CTCCTTCCATACG GTGCACCTTCCTC 3′
5	5′ FAM –TGCCTTGTAAGAGCGACCA TCAACCTGGAATGC TTCGGAA black hole 1 3′
6	5′ TGGTCGCTCTTACAAGGC ACGTATGGA 3′
7	5′ AGGTGCAC TTCCGAAGCATTCCAGGTT 3′

[a] Font colors correspond to the line colors in Schemes 1 and 2. Underlined sequences correspond to the hybridization domains of (**4**) in the different tweezers.

treatment of the functional DNA structure with adenosine monophosphate and adenosine deaminase, the system is cycled between the open and closed configurations (Figure 1 B; for additional experiments describing the opening of the tweezer by a single aptamer–AMP arm, see the Supporting Information, Figures S1 and S2).

With this basic DNA machine, the coherent opening and closing of two tweezers could also be accomplished (Scheme 2). The system consists of one tweezer **A** of the composition shown in Scheme 1. The second tweezer **B** consists of the frame sequence **5** modified at its 5′ and 3′ ends by the fluorophore F_2 (fluorescein) and the quencher Q_2 (black hole 1), respectively. The nucleic acids **6** and **7** include domains complementary to the frame **5** (marked in orange) and to the released nucleic acid **4**, but they lack the aptamer sequences. The sequence of the nucleic acids **6** and **7** are designed such that hybridization of **4** to the sequences **2** and **3** is energetically favored, but upon release of **4** by the formation of the aptamer–substrate complex, the released nucleic acid hybridizes with the less favored positions of tweezer **B** (marked in red), which is expected to close. Thus, treatment of the mixture of closed tweezer **A** and open tweezer **B** with adenosine monophosphate results in the formation of the adenosine–aptamer complexes and open tweezer **A**.

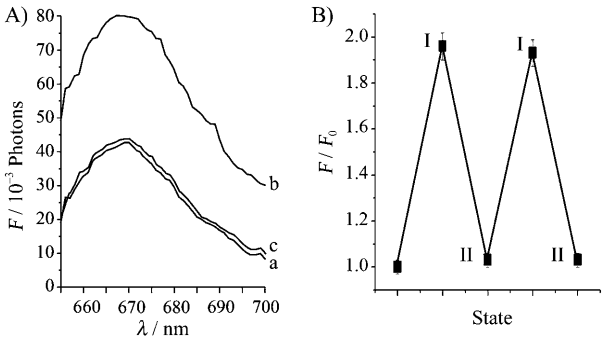
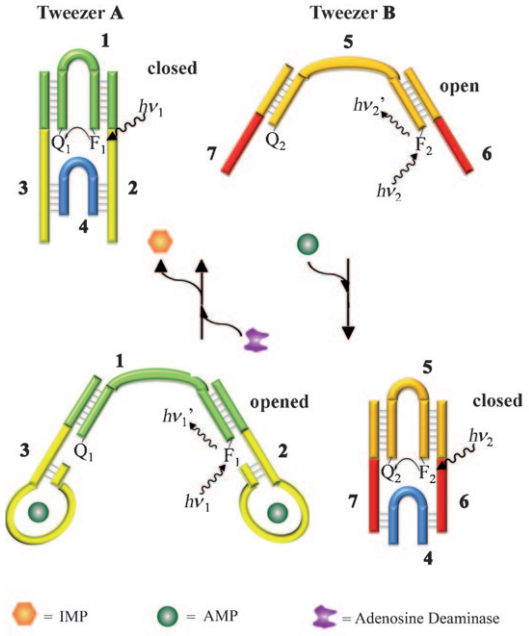


Figure 1. A) Fluorescence spectra corresponding to: a) The closed tweezer. b) The open tweezer generated upon treatment of the closed tweezer with adenosine monophosphate, 2×10^{-3} M for 15 min. c) The closed tweezer generated by treatment of the opened tweezer with adenosine deaminase (five units, 1 h). B) Cyclic activation of the tweezer between the open (I) and the closed (II) forms by adenosine monophosphate and adenosine deaminase, respectively. All measurements were performed in 0.01 M 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES) buffer, pH 7.4, 400 mM NaCl.

The released nucleic acid **4**, however, hybridizes with tweezer **B** and transforms it into the closed configuration. The coherent opening and closing of the tweezers **A** and **B** are, then, imaged by the switching on of the fluorescence of F_1 and the concomitant quenching of F_2. The system is initiated with tweezer **A** in the closed state (F_1 is quenched) and tweezer **B** in the open state (highly fluorescent F_2; Figure 2 A a, fluorescence of F_1 and F_2 monitored at 650–700 nm and 510–560 nm, respectively). Treating the system with adenosine monophosphate results in the formation of the aptamer complexes, the release of **4**, and the opening of tweezer **A** (high fluorescence of F_1; Figure 2 A b, monitored at 650–700 nm). The released **4** hybridizes with tweezer **B**, which is thereby closed (low fluorescence of F_2; Figure 2 A b, monitored at 510–560 nm). Treatment of the system with adenosine deaminase degrades the aptamer complexes and translocates **4** to the favored tweezer **A**. This transfer results in closure of tweezer **A** (fluorescence of F_1 declines) and the re-opening of tweezer **B** (highly fluorescent F_2; Figure 2 A c). It should be noted that in order to perform the cyclic operation of the single-tweezer system and the two-tweezer assembly, the systems were subjected to a thermal treatment (70 °C, 20 min) to denature the enzyme (adenosine deaminase). One may argue, however, that this thermal treatment disassembles all of the duplex structures of the different tweezers, and hence, the two-state systems depicted in Schemes 1 and 2 are not accurate. To reveal that the two-state configurations are generated under isothermal conditions, we performed a single-cycle operation of the systems at



Scheme 2. Coherent activation of two aptamer-based tweezers using adenosine monophosphate and adenosine deaminase as activators.

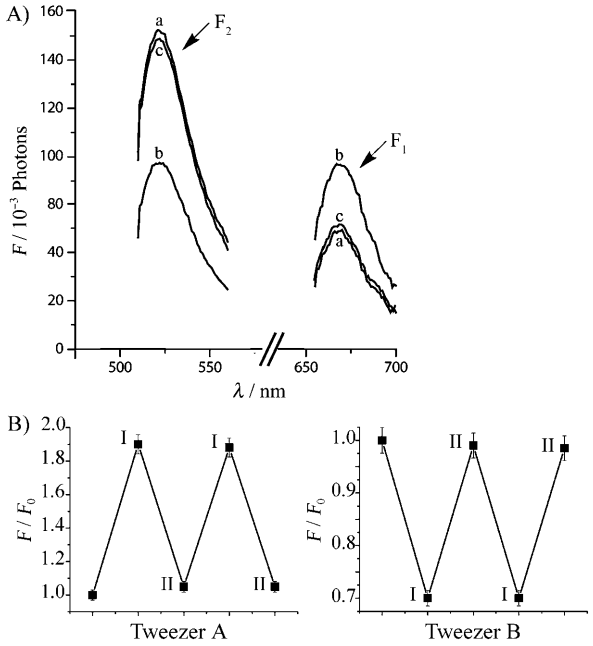


Figure 2. A) Fluorescence spectra corresponding to the states of tweezers **A** and **B** in the presence of adenosine monophosphate and adenosine deaminase as activators (tweezer **A** is monitored at $\lambda = 650$–700 nm and tweezer **B** is monitored at $\lambda = 510$–560 nm): a) Starting configuration of the system: tweezer **A** closed, tweezer **B** open. b) After treatment of the system with adenosine monophosphate. c) After treating the resulting system in (b) with adenosine deaminase. B) Cyclic coherent activation of the two tweezers by adenosine monophosphate (I) and adenosine deaminase (II).

25 °C, indicating that the fundamental mechanical translocations do, indeed, proceed without the application of a thermal cycle (see the Supporting Information, Figure S4). Further evidence that the tweezer systems depicted in Schemes 1 and 2 operated under isothermal conditions was obtained by analyzing the gel electrophoresis results of the two systems (see Figure S5 in the Supporting Information).

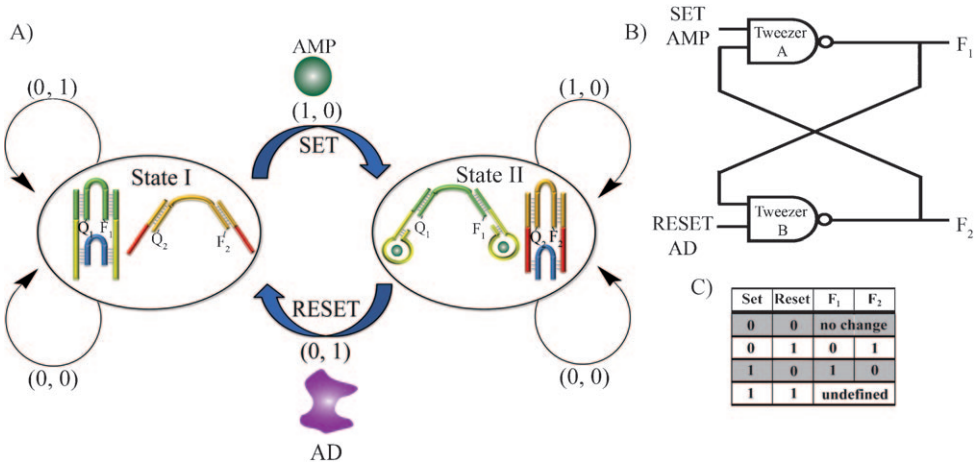
The dual opening and closing of the two tweezers is reversible (Figure 2B). It should be noted that the precise tailoring of the nucleic acid **4** and its degree of hybridization with sequences **2**/**3** and **6**/**7** is essential to allow the effective two-way operation of the tweezers. It is essential that the number of complementary bases of **4** to tweezer **A** have an enhanced duplex stability as compared to tweezer **B** to induce the formation of the closed tweezer **A** prior to the interaction with adenosine monophosphate. Also, the number of complementary bases of **4** to tweezer **A** should be limited, so that **4** can be separated by the formation of the adenosine monophosphate–aptamer complex. Furthermore, it is essential to maintain sufficient base complementarity between **4** and tweezer **B** to induce hybridization and closure of tweezer **B** upon the release of **4** from tweezer **A**. These considerations imply that the stability of the duplex structure in tweezer **A**, consisting of 11 base pairs on each arm ($\Delta G = -21$ kcal mol^{-1} for each arm), should be higher than the eight base-pair duplexes on the two arms of tweezer **B** ($\Delta G = -14$ kcal mol^{-1} for each arm). The base pairing of tweezer **B** must include, however, more than six base pairs ($\Delta G = -11$ kcal mol^{-1} for each arm) to suppress spontaneous dissociation at room temperature. Table 2 lists a series of sequences that were examined to optimize the cyclic operation of the two tweezers. We find that sequence **4c** is the optimal structure to fulfill these three critical requirements.

Table 2: Optimization of the nucleic acid sequence **4** for the activation of the two tweezers.[a]

Name	Sequence	F_1/F_0[b]	F_2/F_0[c]
4a	TCCTTCCATACG GTGCACCTTCCT	1.75	1.11
4b	TCCTTCCATACG GTGCACCTTCCT	1.75	1.32
4c	CTCCTTCCATACG GTGCACCTTCCTC	1.90	1.65
4d	CCTCCTTCCATACG GTGCACCTTCCTCC	1.45	1.22

[a] The red color corresponds to the base sequence that hybridizes with the tweezer **B** system. The underlined portion corresponds to the bases that hybridize with the tweezer **A** system. The kinetics features of the formation of the aptamer–AMP complex on tweezer **A** and hybridization of **4** with tweezer **B** are discussed in the Supporting Information, Figure S3, A, B. [b] F_1/F_0 corresponds to the ratio of fluorescence intensities between the open and closed states of tweezer **A**. [c] F_2/F_0 corresponds to the ratio of fluorescence intensities between the opened and closed state of tweezer **B**.

The application of nucleic acids for the activation of logic-gate operations has attracted recent research effort, and different computational paradigms have been reported.[11] One interesting logic operation is represented by the SET–RESET function, where a system undergoes a flip-flop transformation between two states upon its activation by one or another signal input.[15] In fact, such a SET–RESET



Scheme 3. A) The use of the coherent mechanical activators of two tweezers as a SET–RESET logic device. AD = adenosine deaminase. B) Schematic depiction of the SET–RESET system. C) Truth table corresponding to the SET–RESET system.

logic operation was reported at the molecular level using light and electrical stimuli as inputs.[16] The activation of the two states I and II by the two inputs (adenosine monophosphate and adenosine deaminase) represents a SET–RESET logic operation. That is, the activation of state I (0, 1) with adenosine monophosphate (1, 0) leads to state II (1, 0), whereas reaction of state II (1, 0), with adenosine deaminase (0, 1) regenerates state I (0, 1) (Scheme 3 A). From the truth table for the SET–RESET operation, it is evident that the two-tweezer DNA machines follow this logic operation (Scheme 3 B, C).

In conclusion, our study demonstrated the concurrent activation of two DNA tweezer nanostructures. It should be noted that the "coherent" notation of the process is not precise and is used mainly phenomenologically to describe the transformation. A true "coherent" process would require the full hybridization of the released **4** to tweezer **B** by a coupled configuration of the two states. This is naturally not the case, owing to the lower extent of base pairing in tweezer **B** and the transfer of **4** through the solution. However, such DNA machines hold great promise as transport-and-release molecular devices and as smart materials for future logic operations.

Experimental Section

Materials: Adenosine deaminase, adenosine 5′-monophosphate, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES) and sodium chloride were purchased from Sigma–Aldrich Inc. Unlabeled DNA oligonucleotides (**1** and **5**) were purchased from Integrated DNA Technologies Inc. (Coralville, IA).

All other oligonucleotide sequences were purchased from Sigma-Genosys. Ultrapure water from a NANOpure Diamond (Barnstead) source was used in all of the experiments.

Instrumentation: Light-emission measurements were performed using a photon-counting spectrometer (Edinburgh Instruments, FLS 920) equipped with a cooled photomultiplier detection system connected to a computer (F900 v 6.3 software). The excitation of Cy5 and fluorescein was performed at 648 and 495 nm, respectively.

Assay: The tweezer system in Scheme 1 was studied in a solution consisting of **1**–**4** (250 nM each) in HEPES buffer (400 mM NaCl, pH 7.4). The solution was incubated at 80 °C for 5 min and slowly cooled to room temperature to hybridize the components. The open tweezer was generated by adding adenosine 5′-monophosphate (2 mM) for 15 min. The closed tweezer was generated by adding adenosine deaminase (5 units, where 1 unit $= 6 \times 10^{-3}$ mg, and 1 unit will transform 1 mM of substrate to product in 1 minute), allowing the mixture to react for 1 h at 25 °C, and subsequently incubating at 70 °C for 20 min to denature the enzyme. The system was then cooled slowly to room temperature for 30 min to hybridize the tweezer system.

The two-tweezer system depicted in Scheme 2 was examined in a solution consisting of **1**–**7** (250 nM each) in HEPES buffer (400 mM NaCl, pH 7.4). The solution was incubated at 80 °C for 5 min and slowly cooled to room temperature to hybridize the components. The open tweezer **A** and the closed tweezer **B** were generated by adding adenosine 5′-monophosphate (2 mM) for 1 h. The closed tweezer **A** and the open tweezer **B** were generated by adding adenosine deaminase (5 units), allowing the system to react for 1 h at 25 °C, and subsequently incubating the mixture at 70 °C for 20 min to denature the enzyme. Then the system was cooled slowly to room temperature for 30 min to hybridize the tweezers system.

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