Molecular Devices

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Switchable Motion of DNA on Solid Supports**

*Johann Elbaz, Ran Tel-Vered, Ronit Freeman, Huseyin Bekir Yildiz, and Itamar Willner**

The structural information encoded in nucleic acids leads to selective hybridization, specific recognition, and binding of low-molecular-weight substrates or biopolymers (for example, by aptamers),[1] and to specific catalytic properties (by DNAzymes or ribozymes).[2] Predesigned nucleic acid structures have recently been employed to perform mechanical or motor functions,[3] and different DNA structures have been implemented to conduct, for example, scissor,[4] molecular gear,[5] or "walker"[6] functions. Different applications of such DNA-based machines for sensing,[7] computing,[8] and nanomedicine[9] have been suggested. The activities of all the above-mentioned DNA machines were characterized only in solution. In the present study we have designed a DNA construct on solid supports, where the switchable motion of a nucleic acid strand is accomplished by external triggers.

Scheme 1 A outlines the paradigm of triggering the DNA motion between two distinct positions on the DNA "track", and demonstrates the electrochemical method that follows the translocation process. The thiolated nucleic acid **1** was assembled on a Au electrode and acted as the "track" for the triggered motion of the nucleic acid. The two nucleic acids **2** and **3** included complementary domains to the "track", and these were hybridized with the "track" to yield single-stranded nucleic acids, which acted as footholds for the translocated nucleic acid. The moving DNA **4** includes partial complementarity to the single-stranded tether of foothold **2** and foothold **3**. The number of complementary bases of **4** to **3** is, however, higher than the number of complementary bases of **4** to **2**, and hence, formation of the duplex structure between **4** and **3** is favored. Also, the 3′ end of **4** includes a methylene blue (MB^+) redox functionality, which acted as a redox label that follows, and electrochemically transduces, the switchable translocation process (see below). The single-stranded domain of foothold **3** is not only complementary to **4**, but it also includes the anti-adenosine monophosphate (AMP) sequence-specific aptamer.[10] Accordingly, treatment of the supramolecular nucleic acid composite foothold/track structure **4**/**3** with AMP (**6**) results in the formation of the energetically favored AMP–aptamer complex. This reduces the binding affinity of **4** to foothold **3** and, consequently, the nucleic acid **4** translocates and hybridizes with foothold **2** to form the less-stable duplex structure **4**/**2**. Reaction of the resulting structure with the enzyme adenosine deaminase converts AMP (**6**) into inosine monophosphate (IMP, **7**). The latter product lacks, however, affinity for the aptamer sequence, which leads to dissociation of the aptamer complex. The release of the free single-stranded foothold **3** results in the reverse translocation, and motion of **4** in the direction that forms the energetically favored structure between **4** and **3**. The redox label MB^+, tethered to the moving nucleic acid, acts as a reporter for the switchable motion. Previous studies have demonstrated that the electrical responses of redox labels tethered to nucleic acids are controlled by the relative position of the labels with respect to the electrode support. Redox labels tethered to nucleic acids in remote positions with respect to the electrode show poor electrical contact with the electrode, whereas the spatial localization of the redox label (for example, by hybridization) in proximity to the electrode improved the electron-transfer communication between the redox label and the electrode. These properties were used to develop electrochemical DNA sensors[11,12] and aptasensors.[13]

The surface coverage of the MB^+-modified nucleic acid **4** was determined by chronocoulometry to be 2.55×10^{-12} mol cm^{-2}, and the surface coverage of the main DNA track **1** was determined by Tarlov's method[14] to be 3.4×10^{-12} mol cm^{-2}. Figure 1 A curve (a) shows the linear-sweep voltammogram of the system in which nucleic acid **4** on foothold **3** is in the remote position relative to the electrode. The electrical response of the MB^+ reporter label is observed. Treatment of the system with AMP (**6**) results in the linear-sweep voltammogram shown in Figure 1 A curve (b). The electrical response of MB^+ is intensified, consistent with the fact that the adenosine–aptamer complex is formed, and the translocation of **4** to foothold **2** had indeed occurred. The latter process orients the redox label close to the electrode, thereby leading to effective electrical communication between the redox label and the electrode. Treatment of the electrode with adenosine deaminase results in the linear-sweep voltammogram shown in Figure 1 A curve (c). The voltammetric response of the system is almost fully restored, indicating that the aptamer–AMP complex is dissociated (because of the biocatalyzed formation of IMP), and that the nucleic acid **4** is relocated on foothold **3**, positioning MB^+ away from the electrode. The AMP-induced movement of **4** to foothold **2** on the DNA scaffold, and the reverse translocation of **4** to foothold **3** stimulated by adenosine deaminase are switchable, and thus, the moving nucleic acid can be cycled between the two states (Figure 1 B). It should be noted that the current intensities associated with the two states slightly degrade upon cycling the moving nucleic acid. This is attributed to the partial desorption of **4** from the DNA template upon rinsing the surface after the enzyme-mediated process.

[*] J. Elbaz, Dr. R. Tel-Vered, R. Freeman, Dr. H. B. Yildiz, Prof. I. Willner
The Institute of Chemistry
The Hebrew University of Jerusalem
Jerusalem, 91904 (Israel)
Fax: (+972) 2-652-7715
E-mail: willnea@vms.huji.ac.il

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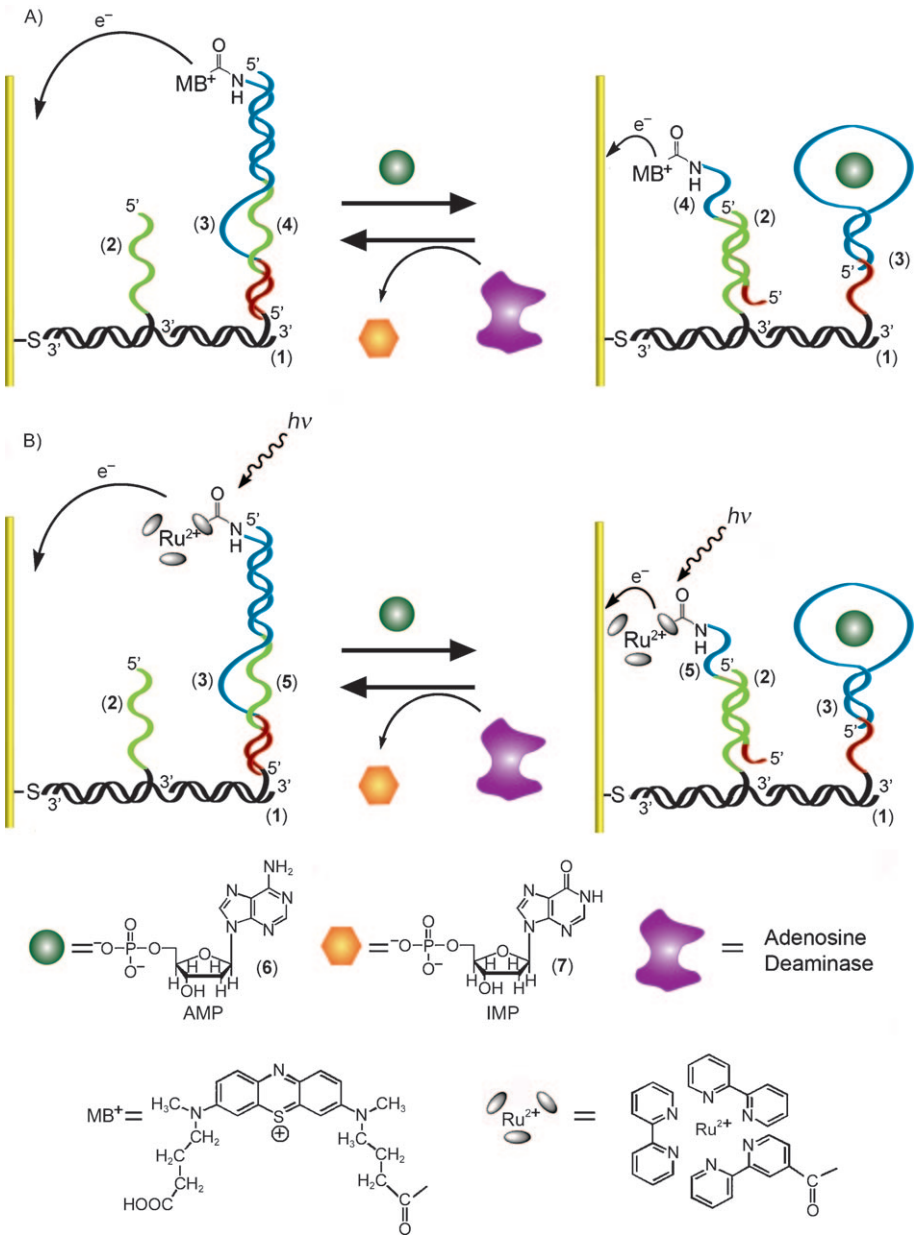


Scheme 1. A cyclic switchable motion of DNA on a nucleic acid track driven by AMP and a biocatalytic reaction stimulated by adenosine deaminase: A) Electrochemical detection of the process. B) Photo-electrochemical imaging of the process. The blue region corresponds to an aptamer sequence and its complementary blocker. The green sequences in **4** and **5** correspond to the complementary domains in foothold **2**. The red sequence is specific for hybridization on foothold **3** to favor the binding to this foothold prior to the reaction with AMP.

It should be noted that the "pure" switchable motion of the nucleic acid **4** between the two states is an over-simplified description. In reality, **4** hybridizes to both footholds, and its relative population on the footholds is controlled by either AMP or by the enhanced stabilization on foothold **3**. By using chronoamperometry,[15] we estimated the relative population of MB^+ on the footholds upon switching **4** between the two states. We found that in the initial state about 75% of **4** is linked to foothold **3**, and 25% is bound to foothold **2**. Interaction of the system with AMP resulted in approximately 59% of **4** being translocated from foothold **3** to foothold **2** (see the Supporting Information).

The switchable, triggered, translocation of the nucleic acid on the DNA scaffold can also be transduced by photoelectrochemical means (Scheme 1B). The moving nucleic acid was tethered to a $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine) derivative, and the labeled nucleic acid structure was hybridized with foothold **3** on the DNA scaffold. The photocurrent generated by the ruthenium(II) photosensitizer, in the presence of triethanolamine as an electron donor, is similarly controlled by the distance separating the photosensitizer from the electrode surface. Figure 2 curve (a) shows the photocurrent action spectrum when the moving nucleic acid **5** is positioned on foothold **3** away from the electrode. The photocurrent action spectrum is of low intensity, and it follows the absorption spectrum of the photosensitizer. This finding implies that the photocurrent is generated by the excitation of the Ru^{II} complex. Treatment of the system with AMP (**6**) results in the photocurrent action spectrum depicted in Figure 2 curve (b), where the photocurrent generated by the system is intensified. This result is consistent with the formation of the aptamer–AMP complex and the translocation of **5** to foothold **2**, thereby positioning the photosensitizer close to the electrode. This facilitates the injection of electrons from the photoexcited photosensitizer to the electrode and results in enhanced photocurrents. Treatment of the resulting electrode with adenosine deaminase transforms AMP (**6**) to IMP (**7**). This results in the photocurrent action spectrum depicted in Figure 2 curve (c). The original low-intensity photocurrent spectrum is almost fully restored. This result is consistent with the separation of the aptamer–AMP complex (because of the biocatalyzed formation of IMP), and the reverse movement of **5** to foothold **3**, which positions the photosensitizer away from the electrode. The "two-way" translocation of **5** on the DNA scaffold is stimulated by the reaction with AMP or adenosine deaminase, respectively, and the system can be cycled between the two states (Figure 2, inset).

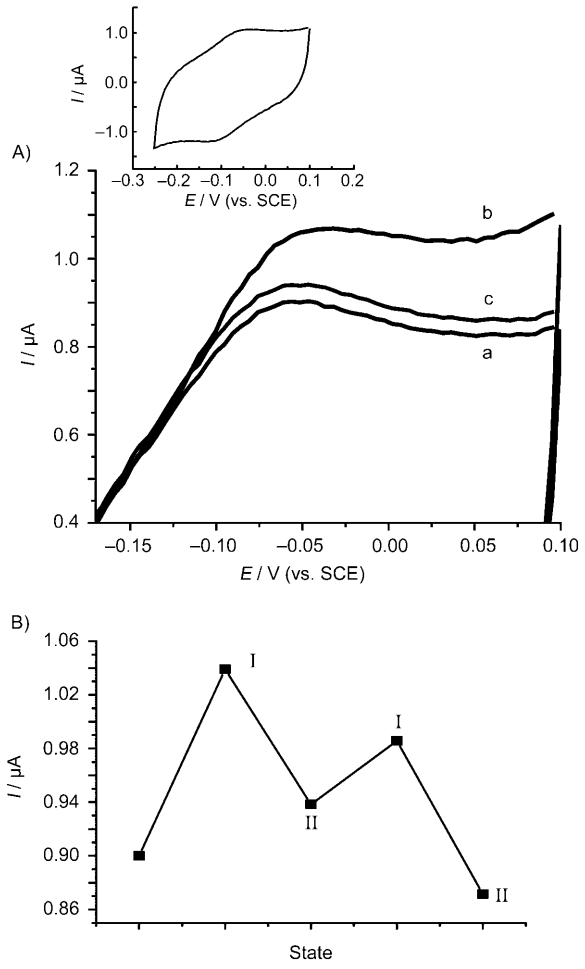


Figure 1. A) Linear-sweep voltammograms corresponding to: a) The MB^+-labeled nucleic acid **4** on foothold **3**. b) After treatment of the system with AMP, and the translocation of **4** to foothold **2**. c) After treatment of the resulting system with adenosine deaminase and translocation of **4** to foothold **3**. Inset: cyclic voltammogram of **4** linked to foothold **2**. B) Cyclic current responses, at $E = -0.05$ V versus saturated calomel electrode (SCE), upon the two-way translocation of **4** between footholds **2** and **3**: I) system treated with AMP, II) system treated with adenosine deaminase

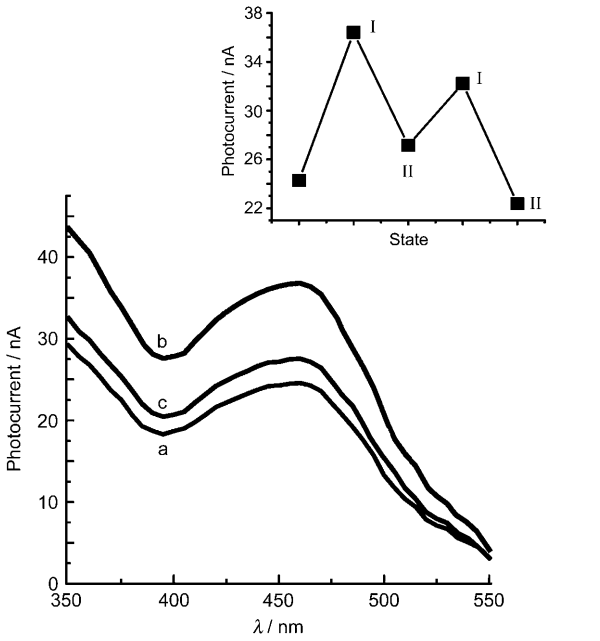
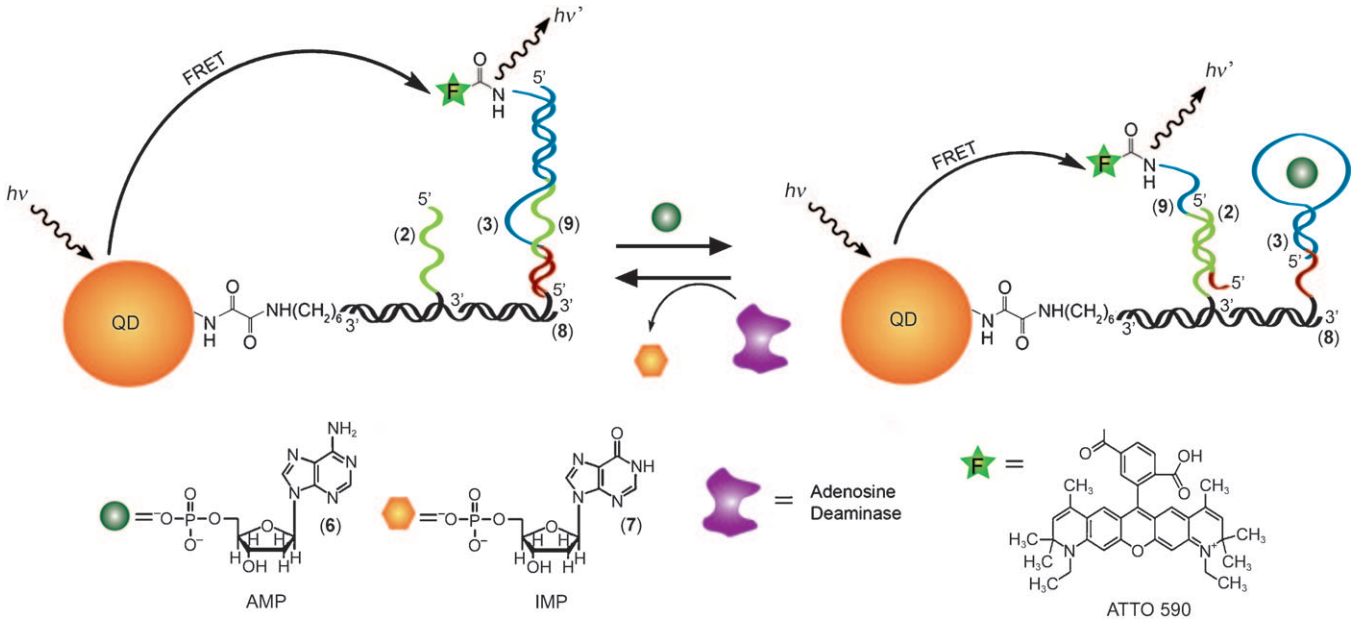


Figure 2. Photocurrents corresponding to: a) The Ru^{II}-labeled nucleic acid **5** associated with foothold **3**. b) After treatment of the system with AMP and the translocation of **5** to foothold **2**. c) After treatment of the resulting system with adenosine deaminase and translocation of **5** to foothold **3**. Inset: cyclic current responses, at $\lambda = 450$ nm, upon cyclic translocation of **5** between footholds **2** and **3**: I) system treated with AMP, II) system treated with adenosine deaminase.

Further support for the translocation process of the nucleic acid on the DNA "track" was obtained by FRET experiments with CdSe/ZnS quantum dots (QDs), used as the solid support and ATTO 590 functionalized nucleic acid **9** as the moving DNA (Scheme 2). The QDs were modified with "track" **8**, and the two footholds **2** and **3** were hybridized with the corresponding complementary sequences of **8**. Foothold **3** was blocked with the ATTO 590 modified nucleic acid **9**, and this acted as the moving DNA with optical readout capabilities. The photophysical properties of the dye permit FRET from the QDs to the chromophore. Figure 3 curve (a) depicts the fluorescence spectrum of the QDs functionalized with the moving nucleic acid **9** on foothold **3**. A high-intensity luminescence band of the CdSe/ZnS QDs at $\lambda = 570$ nm is observed, and a low-intensity fluorescence signal of ATTO 590 at $\lambda = 620$ nm is detected. These results indicate an inefficient FRET, because of the spatial separation of the acceptor dye from the QDs. Figure 3 curve (b) shows the fluorescence spectrum of the system after treatment with AMP (**6**). The luminescence of the QDs decreases in intensity, while the fluorescence of ATTO 590, at $\lambda = 620$ nm, is intensified. These results are consistent with the translocation of **9** to foothold **2**, as a result of the formation of the AMP–aptamer complex. The proximity of the dye to the QDs results in enhanced FRET, and the intensified fluorescence of the acceptor dye. Figure 3 curve (c) shows the fluorescence spectrum of the system upon treatment with adenosine deaminase, which transforms AMP (**6**) into IMP (**7**). This process is accompanied by the release of IMP from the aptamer site, followed by the reverse motion of **9** to foothold **3**, where hybridization is energetically favored. This process is accompanied, as expected, by an increase in the CdSe/ZnS luminescence, and a decrease in the fluorescence intensity of ATTO 590. Control experiments indicated that the fluorescence of the free acceptor dye is unaffected by the addition of AMP, which implies that the cyclic fluorescence properties of the system originate from structural (distance) changes of the acceptor dye with respect to the QDs. It should be noted, however, that the biocatalyzed transformation of AMP to IMP does not fully restore the original low-intensity luminescence of the CdSe/ZnS QDs, thus implying an incomplete translocation of **9** to the aptamer foothold. This situation is attributed to the partial nonspecific adsorption of the dye to the QDs, which inhibits the translocation to the aptamer foothold.[16]

In conclusion, the present study has demonstrated the switchable motion of nucleic acid units on a DNA scaffold



Scheme 2. Monitoring the switchable translocation of a dye-labeled nucleic acid on a DNA track associated with a CdSe/ZnS QD by fluorescence resonance energy transfer (FRET).

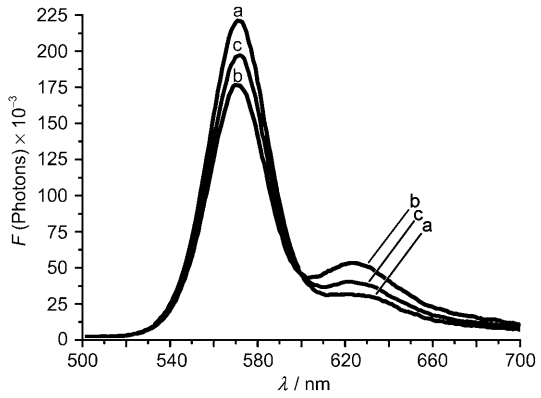


Figure 3. Fluorescence intensities corresponding to: a) The dye-labeled nucleic acid **9** on foothold **3**. b) After treatment of the system with AMP and the translocation of **9** to foothold **2**. c) After treatment of the resulting system with adenosine deaminase, and translocation of **9** to foothold **3**.

associated with different surfaces, and the electrochemical, photoelectrochemical, or optical transduction of the dynamic translocation processes. The systems advance the topic of DNA machines through the construction of cyclic, externally triggered, dynamically moving DNA structures, by demonstrating the immobilization of the systems on different surfaces, and by revealing the electrochemical, photoelectrochemical or optical readout of the mechanical processes.

Experimental Section

Chemicals: Hops Yellow Core Shell EviDots, CdSe/ZnS quantum dots in toluene were purchased from Evident Technologies. Bis(sulfosuccinimidyl) suberate (BS^3) was purchased from Pierce Biotechnologies. All other reagents were purchased from Sigma–Aldrich Inc.

Oligonucleotides: The following oligonucleotide sequences were purchased from Sigma–Genosys:
1: 5′-AAAAAGTGAGTGAGTGAACCTCAGCAATA-3′
2: 5′-GAGATACATCGAAAACACTCACTCACT-3′
3: 5′-TTGGAAGGAGGCGTTATGAGGGGGTCCACGATAAT-TAAAATATTGCTGAGGT-3′
8: 5′-$NH_2(CH_2)_6$AAAAAAAAAAAAAGTGAGTGAGTGAA-CCTCAGCAATA-3′.
The nucleic acid sequence 5′-AATTATCGATGTATCTCCTT-CCAA$(CH_2)_6NH_2$-3′ (0.5 mM) was modified for 1 h in a 10 mM phosphate buffer solution, pH 7.4, using an excess of: ruthenium(II)-(bis(2,2′-bipyridine)-4′-methyl-4-carboxybipyridine)-NHS (NHS = *N*-hydroxy succinimide) to form **5**, methylene blue-NHS to form **4**, or ATTO 590-NHS to form **9**. The resulting products were purified by separation on a microspin (G-25) column.

Preparation of the switchable DNA translocation systems: The different systems were prepared in a phosphate buffer solution (20 mM, pH 7.4, 300 mM NaCl; 500 μL) that included the mixture of sequences to yield the respective systems: **1**, **2**, **3**, and **4** for the electrochemical assay; **1**, **2**, **3**, and **5** for the photocurrent assay; and **8**, **2**, **3**, and **9** for the optical assay. The concentration of each nucleic acid in the different systems was 1 μM. The resulting nucleic acid mixtures were heated to 70 °C for 5 min, and then cooled to room temperature for 25 min.

Photocurrent or electrochemical assays: The resulting hybridized DNA systems were added to a clean Au wire electrode or on a Au-coated glass slide for 2 h. This stimulated the binding of the thiol functionalities associated with the DNA scaffolds to the electrodes. The electrodes were washed with phosphate buffer to ensure the removal of any nonspecifically adsorbed DNA. The movement of the nucleic acid **4** or **5** to foothold **2** was performed by adding AMP (1 mM) to the systems, and allowing the rearrangement of the systems for 1 h. The reverse movement of **4** or **5** was triggered by the addition of adenosine deaminase (5 U) and allowing the systems to react for 1 h.

Preparation of reduced glutathione (GSH) capped QDs: QDs were precipitated from a toluene solution by addition of methanol (2 mL) to a suspension of the QDs in toluene (0.5 mL), followed by centrifugation for 5 min at 3000 rpm. The resulting precipitate was dissolved in chloroform (1 mL), to which a solution (200 μL,

containing 0.139 gr GSH and 60 mg KOH in 1 mL methanol) was added, and the resulting mixture was shaken for 1 min. Subsequently, 1 mM aqueous solution of NaOH (1 mL) was added to the mixture, thus resulting in the transfer of the QDs to the water phase. The solution of QDs was separated from the chloroform phase. The excess of GSH in the water phase was removed by two successive precipitation steps of the QDs, using NaCl and methanol, followed by centrifugation. The resulting QDs were dissolved in 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (1 mL) at pH 7.2.

Optical assay: A BS^3 stock solution (1 mg mL^{-1} in 10 mM HEPES buffer, pH 8; 20 μL) was added to a solution of the GSH-capped QDs in HEPES (150 μL), and the mixture was shaken for 20 min. The QDs were then purified by precipitation. The particles were subsequently dissolved in the nucleic acids mixture consisting of the hybridized and purified **10**, **2**, **3**, and **9**, and allowed to react for 1 h. The excess DNA was removed by one precipitation of the QDs, and the purified particles were dissolved in 20 mM phosphate buffer, pH 7.4, containing 500 mM NaCl (0.5 mL). The movement of the nucleic acid **9** to **2** was stimulated by adding AMP (1 mM) for 1 h and the reverse translocation was triggered by the addition of adenosine deaminase (5 U) for 1 h.

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