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DNA Zipper-Based Tweezers

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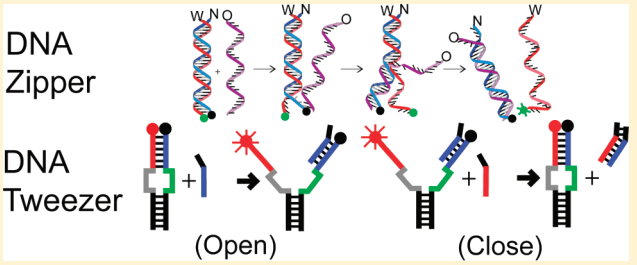
Supporting Information

ABSTRACT: Here we report the design and development of DNA zippers and tweezers. Essentially a zipper system consists of a normal strand (**N**), a weak strand (**W**), and an opening strand (**O**). **N** strand is made up of normal DNA bases, while **W** is engineered to have inosine substituting for guanine. By altering the number and order of inosine, **W** is engineered to provide less than natural bonding affinities to **N** in forming the [**N**:**W**] helix. When **O** is introduced (a natural complement of **N**), it competitively displaces **W** from [**N**:**W**] and forms [**N**:**O**]. This principle is incorporated in the development of a molecular device that can perform the functions of tweezers (sense, hold, and release). Tweezers were constructed by holding **N** and **W** together using a hinge at one end. Thus, when the tweezers open, **N** and **W** remain in the same vicinity. This allows the tweezers to cycle among open and close positions by their opening and closing strands. Control over their opening and closing kinetics is demonstrated. In contrast to the previously reported DNA tweezers, the zipper mechanism makes it possible to operate them with opening strands that do not contain single-stranded DNA overhangs. Our approach yields a robust, compact, and regenerative tweezer system that could potentially be integrated into complex nanomachines.



INTRODUCTION

DNA has been used to create a variety of molecular machines,[1−10] including tweezers,[1−3,9] sensors,[11−13] walkers,[14−18] and devices with properties mimicking logic-circuit operations.[1,18−24] They are promising because of their small size, high binding specificity, and ease of chemical synthesis and the commercial availability of inexpensive oligonucleotides. DNA tweezers are molecular devices that can sense, hold, and release a target DNA upon specific interaction. Different schemes of making DNA tweezers have been demonstrated, including an aptamer-based system that uses a nucleotide monophosphate and its deaminase as a mechanical actuator[25,26] and another with a catch and release function activated by pH sensitivity.[1,9,26−28] Structurally, these tweezers utilize a single-stranded DNA (ssDNA) overhang/toe to provide energy (fuel strands) to drive its function. Such configurations are not without limitations; for example, devices with ssDNA overhangs are more susceptible to nonspecific binding, are limited to using short fuel strands, and are more likely to trigger immune responses under in vivo conditions. In addition, to recycle back to the initial sensing position they require additional fuel strands that increase the complexity of the system and thus are less reliable.

Here, we report a novel tweezer system encompassing a DNA zipper mechanism at its core to achieve its function. First, we provide the physical construct and operation principle of zippers followed by tweezers. Essentially, a DNA zipper has three components (Figure 1): (i) a normal strand (**N**) containing only natural DNA bases [adenine (A), guanine (G), cytosine (C) and thymine (T)], (ii) a weak strand (**W**) containing inosine (I) substituted for guanine to provide less than natural bonding affinities to **N**, and (iii) an opening strand (**O**), which is a natural complement of **N**. DNA-based zippers using inosine had been proposed,[29] inosine preferentially hybridizes to C through two hydrogen bonds. I═C pair has a weaker energy of formation (∼21 kJ/mol) than G≡C pair (∼29 kJ/mol).[30] When **W** and **N** hybridize, there is less energy holding them together in the [**W**:**N**] helix, than [**N**:**O**]. Zipper action occurs when **O** strand (which for DNA tweezers is called the fuel strand) is introduced into the system. Stronger G≡C interaction between **O** and **N** outcompetes the I═C bonds and displaces **W** from [**N**:**W**] to form [**N**:**O**], resulting in the opening of the zipper. This can be summarized as: [**N**:**W**] + **O** → [**N**:**O**] + **W**.

THEORETICAL BACKGROUND

Physical Construct of a DNA Zipper. A simple comparison of the hydrogen-bond energies of [**N**:**W**] and [**N**:**O**] (see Table 1 for sequence) suggests approximately 140 kJ/mol is driving the reaction [**N**:**W**] + **O** → [**N**:**O**] + **W** (assuming ∼21 kJ/mol for the I═C bond and ∼29 kJ/mol for the C≡G bond).[30]

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Equally important, however, is the distribution of energy along the length of the strands: periodic spacing of I with a sufficient spatial frequency along the length of **W** is required for the operation of the zippers. Thermal stability, kinetics, and specificity are dependent on the number of I═C bonds, their order, and period of placement.

Physical Construct of Zipper-Based Tweezers. A schematic of the physical construct and operation of a tweezers is shown in Figure 2. It is a double helical structure and has three regions, namely, zippers, loops, and a hinge. It is constructed using $\mathbf{N_T}$ and $\mathbf{W_T}$ strands each made of 54 nt (nucleotide units) oligonucleotides (see Table 2 for sequences). The zippers consist of a 21 nt zipper section [red ($\mathbf{W_Z}$) and blue ($\mathbf{N_Z}$)] at one end and a 21 nt hinge section (marked in black) at the other end, with a 12 nt loop section (marked in green and gray) intervening them. They are functionalized at the zipper end with a fluorophore (Cy5.5) on $\mathbf{W_Z}$ and a quencher (IBRQ) on $\mathbf{N_Z}$ to monitor their activity. Closed tweezers are quenched (sensing, Figure 2a) and open tweezers are fluorescent (capture, Figure 2b). The loop $\mathbf{N_L}$ and $\mathbf{W_L}$ never hybridize, since they are noncomplementary. $\mathbf{N_H}$ and $\mathbf{W_H}$ remain hybridized all the time, since they are natural complements, and $\mathbf{N_Z}$ and $\mathbf{W_Z}$ are hybridized only in the closed position. Opening and closing of tweezers are driven by $\mathbf{O_i}$ and $\mathbf{C_i}$ (release, Figure 2c), respectively. The closing strand is complementary to the opening strand, and it would take a very long time to complete the closing reaction.[4] However, using locked nucleic acids (LNA's) in the closing strand (instead of normal bases) would significantly accelerate this process (described later in the paper). For the most part, $\mathbf{O_i}$ and $\mathbf{C_i}$ are complementary to each other; however, in some instances $\mathbf{O_i}$ contained more nucleotide units than $\mathbf{C_i}$ (Table 2).

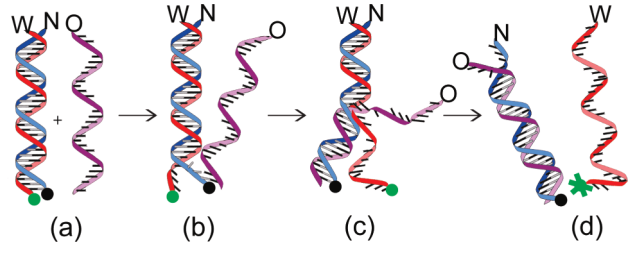

Figure 1. Schematic of a DNA zipper. (a) The zipper helix [**N**:**W**] is weakly bound together due to less hydrogen bonds between the I═C base pair. **W** and **N** are conjugated with fluorophores at both ends: FAM (green) and Iowa black FQ (IBFQ) on one end and Cy5 (red) and Iowa Black RQ (IBRQ) on the other end (not shown). In this configuration, the fluorescence is quenched due to colocalization of FAM and IBFQ as well as Cy5 and IBRQ. (b, c) Invasion of [**N**:**W**] by **O**. (d) **O** binds to N and forms a stable helix [**N**:**O**]. This interaction results in the loss of quenching and the FAM begins to fluoresce.

EXPERIMENTAL SECTION

Materials. All chemicals and buffer solutions were obtained from Sigma Aldrich (Saint Louis, MO) unless otherwise specified. All DNA constructs were obtained from IDT (Coralville, Iowa), DNA ladders were from Promega (Madison, WI) and DNA gels from Lonza (Walkersville, MD).

Measurement of the Melting Temperature (T_m). T_m values of the zipper helix [**N**:**W**] and the final state [**N**:**O**] were measured to be 54 and 71 °C, respectively, using an AVIV 202 circular dichroism spectrometer with a Peltier temperature controller and pH meter. Measurements were conducted using a double helix concentration of 20 μM suspended in a 10 mM PBS buffer (pH 7.4, 160 mM NaCl). T_m calculations of natural DNA pairs were performed using the IDT online calculator with 160 mM NaCl assuming an equal concentration of 0.1 μM for both strands. All DNA calculations of sequences containing deoxyinosine were performed using deoxyadenine in the place of deoxyinosine to obtain approximate values for zipper construction. Calculated values were found to be within a few degrees of our measured values.[31]

Fluorescence Observation of Zipper Activity. Zipper action was visualized by tagging **N** and **W** with fluorescent probes and observing the change in fluorescence with time. Fluorescent quenchers were placed at both ends of **N** (3′-IBFQ and 5′-IBRQ), and FAM and Cy5 were placed on **W** at 5′ and 3′ ends respectively, while **O** was left unlabeled. Fluorescence measurements were conducted using a Jobin Yvon FluoroMax-3 luminescence spectrometer. Fluorescent observations (Excitation/Emission) of FAM were performed at 495/520 nm, Cy5 at 648/688 nm, and Cy5.5 at 668/706 nm. All measurements were performed using quartz cuvettes with 40 μL sampling volume (Sterna Cell 16.40F-Q-10/Z15) filled with 100 μL of sample at the start of each experiment. The experiments were carried out on samples dissolved in nuclease free reaction buffer (30 mM Tris-HCl, 160 mM NaCl, pH 8.0). Basal fluorescence of the quenched zipper was measured on each sample prior to data collection. The data was collected typically at every second for ~90 min and at every 5 s for experiments involving more than 90 min. All zipper-opening experiments were conducted by adding 10 times more opening strands than zippers, unless stated otherwise. All initial tweezers-opening experiments were performed by adding 10 times more

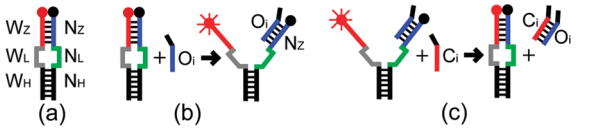

Figure 2. Schematic of the tweezers' action. (a) Closed tweezers and its three regions, namely, the zippers ($\mathbf{W_Z}$ and $\mathbf{N_Z}$), loops ($\mathbf{W_L}$ and $\mathbf{N_L}$), and hinges ($\mathbf{W_H}$ and $\mathbf{N_H}$). (b) Introduction of $\mathbf{O_i}$ opens the tweezers, creating the [$\mathbf{N_Z}$:$\mathbf{O_i}$] helix (capture). $\mathbf{O_i}$ contains a ssDNA overhang at its end (black). (c) Upon addition of a closing strand $\mathbf{C_i}$, it binds to $\mathbf{O_i}$ and thus aids in the closure of the tweezers (release). This regenerates the original closed position [$\mathbf{N_Z}$:$\mathbf{W_Z}$] (part a) and releases [$\mathbf{O_i}$:$\mathbf{C_i}$] helix.

Table 1. Sequences of the DNA Zipper Strands[a]

W	5′-FAM/IIT ITT ITT TIT TIT TII TTT IIT TTI TTI TII TTI II/Cy5−3′
N	5′-IBRQ/CCC AAC CAC AAC AAA CCA AAC CAA CAA CAA ACA ACA CC/IBFQ-3′
O	5′-GGT GTT GTT TGT TGT TGG TTT GGT TTG TTG TGG TTG GG-3′
$\mathbf{O_{M1}}$	5′-GaT GTT aTT TGT TaT TGG TTT aGT TTG TTa TGG TTa GG-3′
$\mathbf{O_{M2}}$	5′-aaT aTT GTT TaT TGT TaG TTT GaT TTG TTa TGaTTG aG-3′
$\mathbf{O_{M3}}$	5′-GaT GTT aTT TGT TaT TGa TTT aGT TTa TTG TGa TTG aG-3′
$\mathbf{O_{M4}}$	5′-GtT GTT tTT TGT TGT TGt TTT tGT TTt TTG TtG TTG tG-3′
$\mathbf{O_{M5}}$	5′-ttT GTT tTT TGT TtT TGG TTT tGT TTG TTt TGt TTG tt-3′

[a] Bases presented in lower case represent the sites of a base pair mismatch in the opening strand.

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Table 2. Sequences for DNA Tweezers[a]

$\mathbf{W_T}$ (54 nt)	5′-Cy5.5/TII ITT IIT ITT ITT TII TTT CTT CTT TCT TCT TGA CCA GTC GCA TGG ATC GGC-3′
$\mathbf{N_T}$ (54 nt)	5′-GCC GAT CCA TGC GAC TGG TCA TTT CCC TCT CCC AAA CCA AAC AAC ACC AAC CCA/IBRQ/-3′
$\mathbf{O_1}$ (28 nt)	5′-AGG AGA ATG GGT TGG TGT TGT TTG GTT T-3′
$\mathbf{C_{1\text{-}LNA}}$ (21 nt)	5′-ACA ACA C+CA A+CC +CA+T T+CT C+CT-3′
$\mathbf{C_{1\text{-}DNA}}$ (21 nt)	5′-ACA ACA CCA ACC CAT TCT CCT-3′
$\mathbf{O_2}$ (32 nt)	5′-GT GTT GTT TGG TTT GGG AGA GGG TCT CCT TTC-3′
$\mathbf{C_2}$ (32 nt)	5′-GAA AGG AGA CCC TCT CCC AAA CCA AAC AAC AC-3′
$\mathbf{O_3}$ (24 nt)	5′-GTG TTG TTT GGT TTG GGA GAG GGA-3′
$\mathbf{O_{3\text{-}FAM}}$ (24 nt)	5′-FAM/GTG TTG TTT GGT TTG GGA GAG GGA-3′
$\mathbf{C_{3\text{-}LNA}}$ (24 nt)	5′-T+CC +CT+C T+CC +CA+A A+CC AAA CAA CAC-3′
$\mathbf{C_{3\text{-}DNA}}$ (24 nt)	5′-TCC CTC TCC CAA ACC AAA CAA CAC-3′
$\mathbf{C_{4\text{-}LNA}}$ (24 nt)	5′-TCC +CT+C TC+C CA+A A+CC A+AA +CAA +CAC-3′

[a] Segments that are complementary to the weak side are red and the normal side is blue. A plus (+) in front of a base indicates that base is a LNA. Text highlighted in black represents ssDNA overhang.

Table 3. Kinetics of Opening Reaction with Different Constructs at 37°C

opening constructs (concentration)	time taken to complete 50% of the opening reaction ($t_{1/2}$) with different loop binding (L) or toe (T) lengths
zipper **O** (10×)	195 s
$\mathbf{O_1}$ (10×)	119 s/7 T
$\mathbf{O_2}$ (10×)	26 s/9 L/9T
$\mathbf{O_3}$ (10×)	10 s/10 L
$\mathbf{O_3}$ (1×)	15 s/10 L

opening strand, and successive opening and closing experiments were performed by consecutively adding 2 times more of each strand, unless stated otherwise (Tables 3 and 4). For example, after the initial opening of the tweezers, successive opening and closing cycles were conducted by adding 30 and 50 times $\mathbf{O_i}$ and 20 and 40 times $\mathbf{C_i}$, respectively.

Table 4. Kinetics of Tweezers Closing Reaction with Different Strands at 37°C

closing strand (concn)	tweezers opening constructs (concn)	time taken to complete 50% of closing reaction at 37 °C with different toe (T) lengths
$\mathbf{C_{1\text{-}LNA}}$ (20×)		10 s/7 T
$\mathbf{C_{1\text{-}DNA}}$ (20×)	$\mathbf{O_1}$ (10×)	320 s/7 T
$\mathbf{C_2}$ (20×)	$\mathbf{O_2}$ (10×)	32 s/9 T
$\mathbf{C_{3\text{-}LNA}}$ (10×)	$\mathbf{O_3}$ (2×)	1.2 h/n
$\mathbf{C_{4\text{-}LNA}}$ (10×)	$\mathbf{O_3}$ (2×)	6.7 h/n

The excessive concentrations ensured that the reactions stabilized with a terminating value and drove the reactions to completion significantly faster than equal concentrations.

Gel Electrophoresis Analysis of DNA Zippers and Tweezers. Initial and final states of the zippers were confirmed by DNA gel electrophoresis. The final double helix conformation [**N**:**O**] was created by thermal annealing of [**N**:**W**] in the presence of a 10-fold excess of **O** to ensure that the reaction was driven to completion and used as a control. Thermal annealing was accomplished using a custom program in a PCR thermocycler (Mastercycler personal, Eppendorf) to quickly raise the solution temperature to 94 °C, beyond the double-strand melting temperature ([**N**:**W**], 54 °C; [**N**:**O**], 71 °C), followed by a slow, controlled cooling at a rate of 1 °C/2 min to a final temperature of 4 °C.

DNA gel electrophoresis was performed with 4% agarose gel at 5 V/cm in TBE buffer while the solution temperature was monitored to remain less than 20 °C. In order to resolve single- and double-stranded DNA, the positions of the strands within the gel were determined using fluorescent gel imaging and ethidium bromide (EtBr) staining. Gels were imaged with a Bio-Rad FX-Imager Pro Plus and analyzed with the Quantity One software package (Bio-Rad).

RESULTS AND DISCUSSION

Fluorescence Observation of Zipper Activity. Time-lapse fluorescence of the initial zipper configuration [**N**:**W**] displayed a small but steady basal fluorescence due to colocalization of fluorescent markers and quenchers (Figure 3a, min). When **O** was added to the [**N**:**W**] helix, a continuous increase in fluorescence was observed that stabilized to a final steady state (Figure 3a, Opening exp). An increase in the fluorescence is due to delocalization of the fluorophores and quenchers (separation of **W** from **N**). Completion of the reaction was confirmed by comparing the peak signal produced by the thermal annealing of [**N**:**W**] with **O** (producing the highest fluorescence and lowest energy configuration of the system; Figure 3a, max). The results indicate that the zipper reaction was driven to its completion in about ∼42 min at 37 °C. Time required for 50% completion of zipper opening reactions ($t_{1/2}$) at 37 °C is presented in Table 3.

However, the observed increase in fluorescence could also result from (a) spontaneous strand dissociations, (b) random base pair mismatches, resulting in the formation of overhangs, and (c) slipping between the strands resulting in delocalization of fluorescent probes, due to weaker interactions in [**N**:**W**] helix. To rule out these possibilities, [**N**:**W**] helix was probed by observing any change in basal fluorescence after adding 10-fold higher concentration of $\mathbf{N_O}$ (**N** sequence without any quenchers). If any of the above possibilities should take place, then the formation of [$\mathbf{N_O}$:**W**] would result in an increase in the fluorescence. Absence of any such increase strongly suggests that such possibilities are

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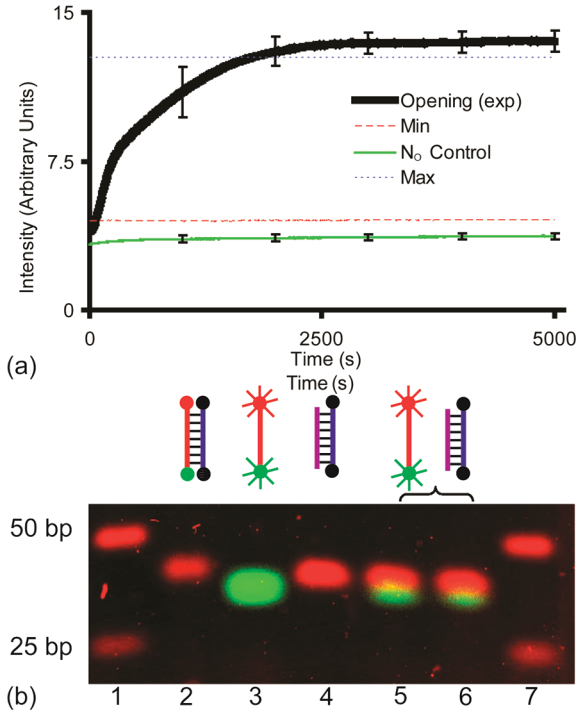

Figure 3. Demonstration of zipper action by fluorescence and gel electrophoresis. (a) The plot shows the time lapse fluorescence monitored by FAM tagged to the 5′ end of **W** (see Supporting Information Figure S1 for Cy5 data) and a quencher at the 3′ and 5′ ends of **N** (see the text for details). (b) Position of dsDNA in the gel was determined by EtBr staining (red) and **W** strand by Cy5 (ssDNA). Lane 2 is an initial zipper helix in its quenched state [**N**:**W**]. Lane 3 is single-stranded **W** with Cy5. Lane 4 is the opened zipper [**N**:**O**]. Lane 5 is the experimental reaction after incubation at 37 °C for 2 h. Lane 6 is the thermally annealed control.

either absent or insignificant (Figure 3a, $\mathbf{N}_o$ control). Figure S1 in Supporting Information includes the fluorescence from Cy5 on the other end of **W**.

Further, we probed the specificity and efficiency of zippers for seven different opening strands with significant (16−24%) sequence mismatches $\mathbf{O}_{M1}$−$\mathbf{O}_{M5}$ (Table 1) measured at 37 °C (see Supporting Information, Figure S2a,b). The results suggest that zippers have relatively high degree of binding specificity to the opening strands. They remained relatively stable after the addition of opening strands that contained six to nine base pair mismatches (see Table 1 for sequence details) distributed along their length.

DNA Gel Electrophoresis of Zipper Action. Initial and final states of zippers were confirmed by DNA gel electrophoresis. The products and reactants of the zipper reaction along with thermally annealed sample [**N**:**O**] as a control were analyzed. Since the mass-charge ratio of double and single stranded DNA are the same, all products and reactants run collinear on the gel electrophoresis. To resolve this, we identified the double strands with EtBr and single strands with fluorophores. The results (Figure 3b) confirm the findings of time-lapse fluorescence observation of zipper action.

Tweezers' Activity. We then examined tweezers by activity adopting a similar strategy as for the zippers by tagging **W** with Cy5.5 and **N** with IBRQ, while both opening and closing strands remain unlabeled. Time lapse fluorescence measurements and

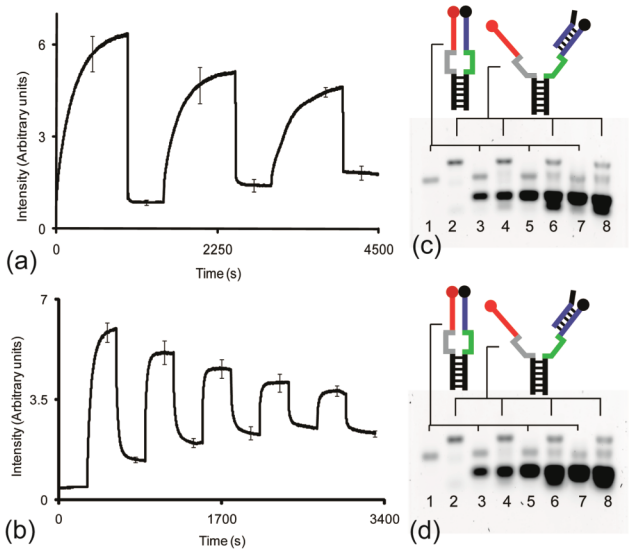

Figure 4. Observation of tweezers action using $\mathbf{O}_1$ and $\mathbf{C}_{1\text{-LNA}}$. (a) Time lapse fluorescence observation from three cycles of the tweezers. The closed tweezers [$\mathbf{N}_Z$:$\mathbf{W}_Z$] were opened by $\mathbf{O}_1$ (0−1000 s) and reclosed by $\mathbf{C}_{1\text{-LNA}}$ (1000−1500 s). (b) Time lapse fluorescence measurements from five successive cycles using $\mathbf{O}_2$ and $\mathbf{C}_2$ conducted as described, except that $\mathbf{O}_2$ was hybridized to 9 nt of $\mathbf{W}_L$ and $\mathbf{C}_2$ hybridized to 9 nt of an overhang on $\mathbf{O}_2$, to speed up the process. (c) DNA gel electrophoresis containing the products from four successive cycles using $\mathbf{O}_2$ and $\mathbf{C}_2$ imaged using EtBr. The thick bands at the bottom of lanes are excess double helixes, the waste product from the reversing of the tweezers. (d) The same as above in part c except that each reaction was thermally annealed prior to running on the gel. This confirms that the loss of functionality is due to excess waste product.

fluorescence gel imaging from three successive opening and closing cycles of the tweezers using the $\mathbf{O}_1$, $\mathbf{C}_{1\text{-LNA}}$ pair are presented in Figure 4b. (The reaction is shown schematically in Figure 2.)

To begin with, the tweezers are in closed configuration [$\mathbf{W}_Z$:$\mathbf{N}_Z$] before addition of $\mathbf{O}_1$. Since the quencher and Cy5.5 are colocalized, there is no significant fluorescence. After the addition of $\mathbf{O}_1$, the tweezers switch to the capture position [$\mathbf{N}_Z$:$\mathbf{O}_1$], where the fluorescence from Cy5.5 almost immediately begins to rise. The increasing fluorescence can be seen in Figure 4a from 0 to 1000, 1500 to 2500, and 3000 to 4000 s. Almost immediately after adding $\mathbf{C}_{1\text{-LNA}}$, it switches to the release position [$\mathbf{O}_1$:$\mathbf{C}_{1\text{-LNA}}$]. This release resets the tweezers back to the closed position [$\mathbf{W}_Z$:$\mathbf{N}_Z$] and the fluorescence signal rapidly decreases (Figure 4a from 1000 to 1500, 2500 to 3000, and 4000−4500 s).

$\mathbf{O}_1$ strand contained 28 nt and was complementary to $\mathbf{N}_Z$ (21 nt), the additional 7 nt formed a DNA overhang, enabling $\mathbf{O}_1$ to be removed by $\mathbf{C}_{1\text{-LNA}}$ (Figure 4a). $\mathbf{C}_{1\text{-LNA}}$ had 21 nt and contained six LNA base modifications (Table 2). $\mathbf{C}_{1\text{-LNA}}$ was complementary to the entire 7 nt overhang of $\mathbf{O}_1$ and its remaining 14 nt. Since $\mathbf{C}_{1\text{-LNA}}$ and $\mathbf{W}_Z$ are complements (see Table 2 for sequences), $\mathbf{C}_{1\text{-LNA}}$ was made shorter than $\mathbf{O}_1$ to reduce the affinity between them. This necessitates the condition that the T_m of [$\mathbf{W}_Z$:$\mathbf{C}_{1\text{-LNA}}$] be sufficiently less than the operating temperature of the tweezers. Otherwise, $\mathbf{W}_Z$ will hybridize with $\mathbf{C}_{1\text{-LNA}}$, preventing the tweezers from closing [$\mathbf{W}_Z$:$\mathbf{C}_{1\text{-LNA}}$]. The six LNA bases were positioned near the overhang binding end of $\mathbf{C}_{1\text{-LNA}}$ in order to preferentially increase the binding affinity between $\mathbf{C}_{1\text{-LNA}}$ and $\mathbf{O}_1$.

To test the robustness of the tweezers, they were driven further for three cycles (Figure 4a) by adding $\mathbf{O}_1$ and $\mathbf{C}_{1\text{-LNA}}$.

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The results show a strong robustness; they cycled efficiently among closed, capture, release, and back to closed positions. Peak fluorescence from each of the successive opening cycles, however, can be seen to decrease relative to the prior peaks. This is due to dilution of the sample by the addition of the opening and closing strands (10 μL each) at each step and is consistent with earlier observations.[32] For the demonstration of this effect, a time lapse fluorescence measurement from a dilution control sample is included (Figure S3, Supporting Information).

Basal fluorescence from the closed tweezers is a measure of the degree of colocalization of the quencher and Cy5.5. This is the minimum fluorescence of the system prior to any dilution effects. As the peaks presented in Figure 4a decreased from the dilution effects, the minimum fluorescence from the closed tweezers was expected to remain the same or to decrease as well. However, it increased during these cycles (Figure 4a). It is reasonable to argue that the elevated basal fluorescence with successive cycles result from increased competition from the waste products. It is worth noting that after completion of three cycles (Figure 4a) there are 90 times more opening strands and 120 times more closing strands present in the solution than the zippers. A loss of functionality with increasing cycles has been reported, and the underlying cause was attributed to excessive waste product poisoning the functional DNA systems.[4,33] To confirm that the loss of functionality was due to the excess waste product and not from the destruction of the tweezers, reactions from four successive opening and closing cycles were subjected to DNA gel electrophoresis. To rule out dilution effects, the concentrations of the tweezers in each cycle were kept the same. The gels show that the opening efficiency of the gates goes down with successive cycles. There is no visible difference between the gel containing the experiment (Figure 4c) and thermally annealed control (Figure 4d). If the tweezers were to fail, one would expect them to come apart at the lower hinge holding the two sides of the device together, but this portion is relatively stable and has a calculated T_m of ~67 °C. If the tweezers did dissociate with successive cycles, then thermal annealing would heal the system, and that would be revealed as a visible difference in the gel. Lack of such difference substantiates that the loss of functionality is due to excess waste product and not otherwise.

Tweezers' Kinetics: Role of Overhangs and LNA Bases. We examined the role of overhangs and LNA bases in the tweezers' kinetics. LNA bases are known to be highly selective and capable of single nucleotide discrimination when hybridizing and have increased target specificity.[34,35] Results shown in Figure 4a indicate that the tweezers closed about 10 times faster than it opened. $\mathbf{O_1}$ opened the tweezers using the zipper mechanism alone and $\mathbf{C_{1\text{-}LNA}}$ removed $\mathbf{O_1}$ by taking advantage of a 7 nt overhang on $\mathbf{O_1}$ (Table 2). To investigate the opening rates of the tweezers using the zipper mechanism together with an overhang, $\mathbf{O_2}$ was constructed. $\mathbf{O_2}$ bound to 9 nt of $\mathbf{N_L}$ and 14 nt of $\mathbf{N_Z}$. It also contained a 9 nt overhang to facilitate its removal by $\mathbf{C_2}$. The combination of the two overhangs allowed the tweezers to be cycled more quickly. Using the $\mathbf{O_2}$, $\mathbf{C_2}$ pair, the tweezers cycled in ~600 s (Figure 4b) compared to ~1200 s for $\mathbf{O_1}$ and $\mathbf{C_{1\text{-}LNA}}$.

A major disadvantage of DNA devices relying on fuel strands containing overhangs to supply the driving energy is that the length of the fuel strand and the action rate of the device are limited by entropy. The overhangs are made of ssDNA, and when the fuel strand binds to it, the unbound portion of the fuel strand attempts to outcompete the other DNA strand in the helix.

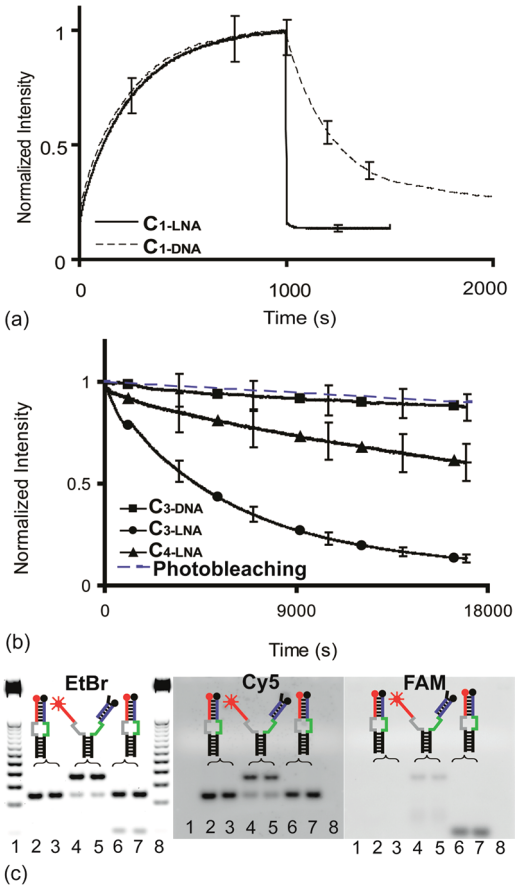

Figure 5. (a) Comparison of the closing kinetics of $\mathbf{C_{1\text{-}LNA}}$ and $\mathbf{C_{1\text{-}DNA}}$. (b) Comparison of the closing kinetics of different LNA constructs. $\mathbf{C_{3\text{-}DNA}}$ is included as a stability control to measure the rate of spontaneous dissociation and is shown to be negligible compared to the photobleaching. (c) DNA gel electrophoresis of the tweezers opened using $\mathbf{O_{3\text{-}FAM}}$ followed by closing with $\mathbf{C_{3\text{-}LNA}}$. The tweezers were opened using only 80% of $\mathbf{O_{3\text{-}FAM}}$ required to open all of the tweezers. The three images verify that $\mathbf{O_{3\text{-}FAM}}$ hybridized to the tweezers and that $\mathbf{C_{3\text{-}LNA}}$ hybridized to $\mathbf{O_{3\text{-}FAM}}$. The contents of each lane are as follows: lanes 1 and 8 contain a 25 nt DNA stepladder, lanes 2 and 3 contain the closed tweezers, lanes 4 and 5 contain the tweezers opened by $\mathbf{O_{3\text{-}FAM}}$, and lanes 6 and 7 contain the tweezers closed by $\mathbf{C_{3\text{-}LNA}}$.

However, there is no energy driving this competing activity (Figure 1). This part of the process relies entirely on entropy, and thus, the probability for the strand exchange is a function of the temperature and the strand length. Thus, in overhang-based devices, once the fuel strand reaches a certain length, the probability of it displacing the other strand will be minimal, if any.

Examination of the closing rates using $\mathbf{O_1}$, $\mathbf{C_{1\text{-}LNA}}$ (Figure 4a) compared to $\mathbf{O_2}$, $\mathbf{C_2}$ (Figure 4b) indicates that $\mathbf{C_{1\text{-}LNA}}$ removed $\mathbf{O_1}$ considerably faster compared to the rate at which $\mathbf{C_2}$ removed $\mathbf{O_2}$ (Figure 4b). While $\mathbf{C_{1\text{-}LNA}}$ and $\mathbf{C_2}$ have many subtle differences between their modes of operation, a major difference is the six LNA base modifications concentrated at the overhang portion of $\mathbf{C_{1\text{-}LNA}}$. The effects of the LNA base modifications on the closing rate were determined by comparing the closing rates of $\mathbf{C_{1\text{-}LNA}}$ with $\mathbf{C_{1\text{-}DNA}}$, a natural DNA strand with the identical sequence as $\mathbf{C_{1\text{-}LNA}}$ (to assess the effect of LNA), plotted together (Figure 5a). There are three possible factors responsible for increasing the closing rate of tweezers with LNA, namely, (i) increased hybridization

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energy between a LNA/DNA helix, (ii) structural conformation of $\mathbf{C_{1\text{-}LNA}}$ enabling it to hybridize to the overhang more quickly, and (iii) LNA bases lowering the binding affinity of $\mathbf{C_{1\text{-}LNA}}$ to $\mathbf{W_Z}$.

In addition, tweezers with overhangs are more prone to random hybridizations. In situations where overhangs are undesirable, LNAs could be employed. LNA/DNA helixes have higher T_m than DNA/DNA helixes, for a given sequence,[34,35] and this energy difference can be used to invade small DNA duplex. However, such reactions are relatively slow. One such system is demonstrated below with $\mathbf{O_3}$ and $\mathbf{C_{3\text{-}LNA}}$. $\mathbf{O_3}$ contained only natural bases and it did not contain any overhangs to facilitate its removal (Figure 5b). The binding interactions of the strands were as follows: $\mathbf{O_3}$ hybridized with lower 14 nt of $\mathbf{N_Z}$ and to the first 10 nt of the loop. $\mathbf{C_{3\text{-}LNA}}$ was complementary to $\mathbf{O_3}$ and it contained seven LNA modifications, most of which were positioned in the loop binding portion. The $\mathbf{O_3}$, $\mathbf{C_{3\text{-}LNA}}$ pair opened the tweezers in less than 300 s and closed it in about 18000 s (5 h) (Figure 5b). A control strand $\mathbf{C_{3\text{-}DNA}}$ containing identical sequence as $\mathbf{C_{3\text{-}LNA}}$, but only natural DNA bases, did not reclose the tweezers. However, the observed decay in the signal was commensurate with photobleaching of the sample (Figure 5b).

Another closing strand, $\mathbf{C_{4\text{-}LNA}}$, with the same base pair sequence as $\mathbf{C_{3\text{-}LNA}}$ containing eight LNA modifications evenly distributed along its length was constructed. The even distribution of the LNA modifications along $\mathbf{C_{4\text{-}LNA}}$ resulted in a significant decrease in the opening rate of the tweezers (~3 times). This decreased opening rate is expected to be caused by the higher affinity between the $\mathbf{C_{4\text{-}LNA}}$ and $\mathbf{W_Z}$ portion of the tweezers (because the LNA bases are positioned along the section that is complementary to $\mathbf{W_Z}$) (Figure 5b). This demonstrates that it is feasible to construct complex DNA-based nanomachines without overhangs by using LNAs together with zippers and is consistent with earlier work.[34,35] Different states of the tweezers and their action was verified by fluorescent DNA gel electrophoresis (Figure 5c) for $\mathbf{O_3}$ and $\mathbf{C_{3\text{-}LNA}}$ strands; the results verified their different states, namely, closed, capture, release, and closed positions.

In summary, two different opening schemes (zipper alone and $\mathbf{N_L}$ hybridizing overhang) and three different closing schemes (overhang, overhang with LNA's, and LNA's only) of tweezers have been presented thus far. For comparing their kinetics, time required for the 50% completion of the opening and closing reaction ($t_{1/2}$) with different strand configurations are presented below in Tables 3 and 4, respectively.

Techniques and Principles for Creating Zipper-Based Devices. On the basis of the present observations, the most important features of a functional zipper appear to be the total driving energy and how this energy is distributed along the length of the strands. The bases providing the driving energy must occur with a sufficient frequency along the length of the weak strand in order for a favorable displacement reaction. If too many natural DNA bases occur between the driving bases (inosine), the reaction may terminate.

The entropy-induced statistical fluctuations between the bases appear to enable the reaction to progress along sufficiently small sections of natural base pairs. The length of the natural section that could be overcome by the statistical fluctuations is unclear; however, it is a temperature- and sequence-dependent property. The bases used to supply the driving energy need not be inosine; there are actually varieties of commercially available synthetic bases that hybridize with less or more than natural affinity that could be utilized. We have used other bases (data not shown); however, inosine is currently several times cheaper than any other commercially available alternative such as 2-aminopurine.

When making a device such as the tweezers, it is important to keep the cross-binding nature of the closing strands in mind. The cross binding creates a challenging window of functionality for this type of device. In general, there must be a difference between the energies of the hybridization of $[\mathbf{C_i}:\mathbf{W_Z}]$ and $[\mathbf{C_i}:\mathbf{O_i}]$. In more practical terms, this means that there is a temperature window in which the tweezers can function. The operating temperature of the tweezers must be significantly below the T_m of the zipper portions of the tweezers $[\mathbf{W_Z}:\mathbf{N_Z}]$ and significantly above the T_m of $[\mathbf{C_i}:\mathbf{W_z}]$. We were able to increase the operating temperature range of the tweezers in three ways: (1) DNA overhangs, (2) truncating the length of $\mathbf{C_i}$ relative to $\mathbf{O_i}$, and (3) using LNA base modifications concentrated at sequence portions that are uncommon between $\mathbf{C_i}$ and $\mathbf{W_Z}$. DNA strands naturally self-assemble into energetically stable configurations. It is important to control the interaction energies of the systems constituents to minimize unwanted self-assembly from DNA.[36] If semistable unwanted hybridization between the different system elements occurs it can significantly affect the kinetics of the system, and if stable hybridizations occur (unwanted self-assembly), the function of the system can completely cease.[1,26,27]

The zipper-based tweezers have the following advantages: (a) their driving energy is distributed over the entire length of the fuel strands, allowing more driving energy to be employed. This translates into devices that should be able to sense and capture longer DNA strands with an additional way to tune the kinetics (open/close) compared to nonzipper-based tweezers that contain all of their driving energy at short overhangs or loops. This also allows the use of longer fuel strands because they do not have any sticky ssDNA overhangs dangling from the ends of the tweezers in the sensing (closed) position. This enables them to be opened without the use of overhangs, thus allowing the construction of a device that spontaneously regenerates to its closed position.[37] These unique properties of the zippers could be useful in the development of new therapeutics and nanoscale machines.

CONCLUSION

We successfully created DNA zipper based tweezers that do not require a fuel strand with overhangs for its operation. This provides more flexibility in designing tweezers with easily adjustable kinetics, better stability, high recognition specificity, and less inclination for attracting to immune reactions. These properties also make the tweezers compact and modular so that they could be easily integrated with complex hierarchical DNA nanomachines. In particular, they could be employed as a renewable component in a lab-on-a-chip device for detection of DNA sequences.[1−3,10,38]

ASSOCIATED CONTENT

Supporting Information. Figures displaying time-lapsed fluorescence of Cy5 on the DNA zipper, change in intensity with dilution, and the base pair specificity of the zipper. This material is available free of charge via the Internet at http://pubs.acs.org.

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